THE RELATIONSHIPS AMONG ENDOGENOUS CORTISOL, SUBJECTIVE STRESS, AND BONE MINERAL DENSITY IN NON-ELDERLY WOMEN

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November 15, 2002

To the Dean of Graduate Studies and Research:

I am submitting herewith a dissertation written by Sherry Carter M.S., W.H.C.N.P., entitled "The Relationship Between Endogenous Cortisol, Bone Mineral Density and Subjective Stress in Non-Elderly Women." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of PhD with a major in Health Studies.

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We have read this dissertation and recommend its acceptance:

Department Chair or Administrative Equivalent

Accepted:

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ABSTRACT

THE RELATIONSHIPS AMONG ENDOGENOUS CORTISOL, SUBJECTIVE STRESS AND BONE MINERAL DENSITY IN NON-ELDERLY WOMEN

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December, 2002

The study was conducted to determine the relationships among endogenous cortisol (determined by salivary cortisol), subjective (perceived) stress and BMD in non-elderly women. A sample of 38 women between the ages of 30 and 65 years, without a previous diagnosis of osteoporosis, and who were not taking glucocorticoids was obtained to examine the relationships among endogenous cortisol, subjective stress and bone mineral density (BMD). This study also sought to determine whether salivary cortisol or subjective stress were predictive of bone mineral density in this population. These determinations were based on Pearson product moment correlation coefficients. Step-wise multiple correlation analysis was used to determine the variables that are significant predictors of BMD. Independent variables considered in the equation were age, race, family history of osteoporosis, body mass index, alcohol use, cigarette smoking, caffeine intake, dietary calcium

intake, calcium supplementation, use of hormone replacement therapy, and weight bearing physical activity.

Endogenous cortisol was found to positively correlate with BMD; however, the association was modest. The relationship between subjective stress and BMD was not statistically significant. After controlling for selected extraneous variables, the relationship between cortisol and BMD remained statistically significant. Regression analysis indicated that 20% of the variance of BMD can be attributed to the combined effects of caffeine and alcohol intake; however, caffeine had a greater association.

Cortisol levels were not associated with scores on the Index of Clinical Stress, a measure of subjective stress. Additional findings included a modest but statistically significant inverse relationship between caffeine intake and BMD, and a weak but statistically significant positive relationship between alcohol consumption and BMD. A final finding, though not pertaining to BMD, was a statistically significant inverse association between calcium intake and blood pressure.

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CHAPTER I

INTRODUCTION

Osteoporosis is the most common metabolic bone disease, affecting 200 million individuals worldwide and more than 28 million individuals in the United States (Greenspan et al., 2000). Osteoporosis is a major public health concern because of its association with fracture. It is estimated that around 40% of Caucasian women in the United States aged 50 years experience at least one clinically apparent fragility fracture in their lifetime (Walker-Bone, Dennison, & Cooper, 2001). The most serious of the fractures are those occurring in the hip because of the associated morbidity and mortality.

Osteoporosis is a condition in which bone mass is depleted through a cycle of bone formation and resorption, made less efficient by physiologic changes that herald the onset of mid-life. All adults begin to lose bone mass in mid-life; however, bone loss is greater in women than in men of the same age. Bone mineral density (BMD) is the major predictor of increased risk of fracture and is a function of peak bone mass minus any subsequent losses to bone mass (Cobbs & Ralapati, 1998).

In the United States, more than one million fractures occur annually in women with osteoporosis. Every five years, from the age of 70 years, the incidence of hip fracture doubles. Hip fractures have an associated mortality rate of 12-15%, and

are the second leading cause of death in people aged 47-74 years. The lifetime risk of hip, spine, and wrist fractures in the United States population has been estimated at 40% in women and 13% in men (Melton, Chrischilles, Cooper, Lane, & Riggs, 1992). The overall public health consequences of osteoporosis are staggering. Expenditures for treatment of osteoporotic fracture in the United States are estimated at $10 billion to $15 billion annually (Anonymous, 2001). By the year 2040, there is estimated to be 80,000,000 postmenopausal women in the U.S. many of whom will be at risk for suffering the consequences of osteoporosis (Miller, 2000). With the increase in expected longevity and population growth, within 50 years the costs of treating hip fractures alone has been estimated to exceed $240 billion per year (Barrett, Baron, Karagas, & Beach, 1999).

Peak bone mass is largely determined by genetic factors; however, other important factors influencing BMD include the following: (a) age, (b) race/ethnicity, (c) family history of osteoporosis, (d) body mass index (BMI), (e) alcohol use, (f) cigarette smoking, (g) caffeine intake, (h) calcium intake, and (i) use of hormone replacement therapy, and (j) physical activity.

One risk factor for low BMD is exposure to exogenous glucocorticoids (Adachi & Ioannidis, 2000). Over 40% of one's bone mass may be lost during the first six months of glucocorticoid therapy. However, less is known about the effects of endogenous circulating cortisol levels.

Dennison (1999) and Raff et al. (1999) reported an inverse relationship between endogenous cortisol levels and bone density in elderly men and women. Only one study was found that reported a positive relationship between endogenous cortisol and BMD in non-elderly women (Brooke-Wavell, Clow, Noori, Evans, & Hucklebridge 2002).

Psychological stress evokes a neuroendocrine response that, when repeatedly activated or activated over a prolonged period of time, places a person at risk for a range of physical disorders (Cohen, Kessler, & Underwood-Gordon, 1997). Cortisol is one of several stress hormones released when individuals encounter stress and chronic stress may result in sustained levels of cortisol. However, the stress response is complex and questions arise as to the validity of this assumption. For example, one theory is that with prolonged stress, individuals adapt with an attenuated response and rather than having increased cortisol, cortisol level is actually suppressed, resulting in a net decrease of cortisol output (Rosmond, 2000).

That exogenous cortisol is associated with decreased bone density; it is reasonable to assume that elevated levels of endogenous cortisol may also adversely affect bone density. This study hopes to add light on the issue of the relationship of endogenous cortisol to BMD and determine the relationship of endogenous cortisol and a measure of subjective stress.

Purpose of the Study

The study was conducted to determine if relationships existed among endogenous cortisol (determined by salivary cortisol), subjective stress, and BMD in non-elderly women. The study also sought to determine whether endogenous cortisol or subjective stress were predictive of BMD in this population. This determination was based on Pearson product moment correlation analysis of salivary cortisol, levels of subjective stress, and BMD. In addition, step-wise multiple correlation analysis was used to examine selected extraneous variables in predicting BMD. The variables examined were the following: (a) age, (b) race, (c) family history of osteoporosis, (d) body mass index, (e) alcohol use, (f) cigarette smoking, (g) caffeine intake, (h) dietary calcium intake, (i) calcium supplementation, (j) use of hormone replacement therapy, and (k) weight-bearing physical activity level. Finally, the relationship of endogenous cortisol and a measure of stress was determined.

Hypotheses

This study was designed to test the following null hypotheses at the .05 level of significance:

Ho1 There is no relationship between salivary cortisol level and bone mineral density measured by dual energy x-ray absorptiometry DXA) in non-elderly women.

Ho2 There is no relationship between salivary cortisol level and subjective stress as measured by *The Index of Clinical Stress* in non-elderly women.

Definition of Terms

For the purpose of the study the following terms were established:

Allostasis. The physiological responses initiated by various systems in the body in an attempt to maintain homeostasis (McEwen, 2000).

Allostatic Load. The adverse effects that the body experiences due to repeated cycles of allostasis as well as the inefficient turning-on or off these responses (McEwen, 200).

Bone Mineral Density (BMD). An indication of bone strength. BMD is measured in grams per square centimeter (gm/cm2) using Dual Energy X-ray Absorptiometry (DXA) (Miller & Zapalowski, 2000).

Bone metabolism. The process of laying down healthy new bone to replace damaged bone (Merckmedicus, 2001)

Bone remodeling. Replacing old bone with new bone tissue (Nelson, 1999).

Bone resorption. The removal of old bone by osteoclasts (Rodan, Raisz, & Bilezikian, 1996).

Glucocorticoids. Natural or synthetic hormones produced in the adrenal cortex which influence or control key processes of the body (Adachi & Ioannidis, 2000).

Corticol bone. The bone that forms the outer shell around cancellous bone and is mainly located in the diaphyses of long bones (University of Michigan, 2001).

Dual-energy x-ray absorptiometry (DXA). A method of measuring bone density based on the proportion of a beam of photons that passes through the bone (Miller & Zapalowski, 2000).

Endogenous cortisol. Hormone secreted by the adrenal gland in response to Corticotropin Releasing Hormone, secreted by the anterior pituitary gland (Dennison et al., 1999).

Exogenous cortisol. Synthetic hormone, usually prescribed for some physical disorder (Adachi & Ioannidis, 2000).

General Adaptation Syndrome (GAS). A model of stress, developed by Hans Selye, consisting of three stages of response to stressful situations: (1) the alarm phase, (2) the resistance phase, and (3) the exhaustion phase (Selye, 1976).

Osteoporosis. A disease in which bones become brittle due to a loss of bone mass (density) and a change in bone structure, measured by a T-score below -2.5 with the presence of fractures (Miller & Zapalowski, 2000).

Peak bone mass. The point at which bones reach their maximum strength and density. Bone mass usually peaks around the age of 30 (Madlock & Allison, 2000).

Limitations

The study was subject to the following limitations:

1. Data were from a sample of convenience and may not be representative of the population.
2. Endogenous cortisol levels are known to be secreted in a circadian pattern with highs and lows over a 24 -hour period.
3. Data on the Index of Clinical Stress may reflect current stress and may be less valid in measuring chronic stress.
4. The Hypothalamic Pituitary Axis (HPA), which initiates the hormonal stress response, is complex and multiple factors influence the secretion of cortisol.

Delimitations

The study was subject to the following delimitations:

1. Data was not obtained from a random sample.
2. The study was limited to a sample of women scheduled for bone density testing, which indicates access to health care and a general predisposition for positive health related behaviors. The results may not be generalizable to multicultural populations of women without access to health care or those with more negative health-related behaviors.

3. Cortisol levels were obtained from a single sample, collected at various times of the day.
4. Data used for BMD analyses were from a single body site.
5. There are multiple aspects of stress and the measurement tool for subjective stress may not cover all available definitions.
6. Exclusion criteria for the study included women with diagnosed osteoporosis and study participants may have had undiagnosed osteoporosis.

Assumptions

For the purpose of this study, the following assumptions were made:

1. The study sample was representative of the population.
2. Measurement of lumbar spine and hip reflected BMD in other sites.
3. Content validity and reliability were established for the tool measuring subjective stress.

Justification

Osteoporosis affects over 28 million individuals in the United States (Greenspan et al., 2000). The National Osteoporosis Foundation (2002) estimates that by the year 2010 over 52 million women and men will be affected by the condition. The number of women age 50 years and older at risk for developing the disease by the year 2020 will increase to approximately 41 million.

Though much is known about the risk factors for osteoporosis, studies of bone mass in non-elderly women are needed (Heaney, 2000), for only when all risks factors for the condition are elucidated will populations at risk be appropriately screened, identified, and targeted for health promotion interventions. Evidence exists that endogenous cortisol and psychological stress are positively correlated and that both inversely correlate with BMD in elderly men and women (Dennsion, et al., 1999). Determining whether or not these same relationships exist in non-elderly women would be beneficial. In addition, knowledge gained by examining the relationship of endogenous cortisol to a measure of subjective stress would be beneficial to researchers with limited resources for or access to physiological assays measuring stress.

CHAPTER II

REVIEW OF THE LITERATURE

The purpose of the study was two-fold: to determine the relationship of endogenous cortisol to bone mineral density (BMD) in non-elderly women and to determine the relationship between salivary cortisol and a measure of subjective stress. In order to better understand the relationship of endogenous cortisol on BMD, research on the following topics was reviewed: (a) bone physiology, (b) bone mineral density, (c) osteoporosis, (d) measurement of bone mineral density, (e) the stress response, (f) cortisol and BMD, and (g) salivary measures of endogenous cortisol.

Bone Physiology

Bone is living tissue made of calcium, phosphorous, and collagen. Integrated throughout bone is a network of blood vessels that supply oxygen, nutrients, and other products vital for bone metabolism. Collagen gives bone a suppleness and flexibility to absorb physical stress. Bone hardness comes from crystals of calcium and phosphorus. Bones also contain minerals such as magnesium, phosphorus, fluoride, and sulfate. Bone is dynamic in the sense that it is continually being broken down and reformed, a process exquisitely controlled by a host of mechanical stimuli, as well as endocrine and metabolic factors.

The three functions of bone are to support the body, protect internal organs, and store essential minerals (Nelson, 1999). Bones of the human skeleton are generally classified into two types of tissue: cortical bone, also known as compact bone, and trabecular bone, also known as cancellous or spongy bone. Cortical bone makes up about 80% of the human skeleton, while trabecular bone comprises the remaining 20%. However, trabecular bone has a much greater surface area, compared with cortical bone (Buckwalter, Glimcher, Cooper, & Recker, 1996). These two types of bone are classified on the basis of porosity and microstructure. Cortical bone is dense, with a porosity ranging between 5% and 10%. Cortical bone forms the outer shell around trabecular bone and is illustrated in Figure 1.

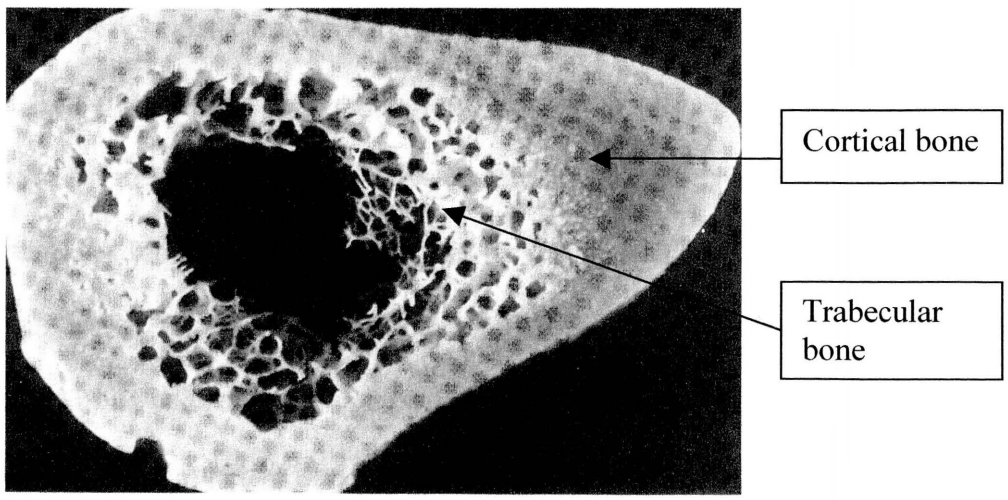

Figure 1. Cross section of cortical and trabecular bone.

Note. From Lee, C.A., & Einhorn, T.A. (2001). The bone organ system. In R. Marcus, D. Feldman, & J. Kelsey (Eds.), *Osteoporosis: Vol 2.* (2nd ed., pp. 3-20). Reprinted with permission.

Trabecular bone has a porosity of about 50-90% and is found mainly in the vertebrae, in small bones, and the epiphyses of the long bones. Both types of bone have the same chemical composition and are metabolically active. The major metabolic activity of bone is the ongoing cycle of bone resorption and formation known as bone remodeling. It is a complex process regulated by many factors (Hotta, Fukuda, Sato, Hizuka, Shibasaki, & Takano, 2000; Masi et al., 2001; Power et al., 1999).

There are four major types of bone cells: Osteoblasts, osteocytes, lining cells, and osteoclasts. Each cell type has a particular function in the process of bone metabolism (Merckmedicus, 2001).

Osteoblasts are primarily responsible for bone formation. They form a contiguous layer of cells situated on the surface of bone and are either active or inactive. The purpose of active osteoblasts is to lay down collagen to form the foundation for new bone. Osteoblasts use calcium and other minerals that they obtain from the blood to form crystals on the collagen. The collagen then hardens to form bone tissue. At the end of this cycle, 10-20% of the osteoblasts are transformed into mature bone cells and become part of the new bone (Aubin, 2000). At this point, they are considered inactive and transform in approximately 3-5 days to become osteocytes.

Osteocytes are osteoblasts that become entrapped in newly formed bone matrix. They are connected to adjacent cells by long cytoplasmic projections,

enriched micro filaments (Lian & Stein, 2001). Though the role of osteocytes is not clearly defined, they have been shown to synthesize new bone matrix and, release mineral from bone tissue. In addition, they receive mechanical and metabolic signals and transmit these signals to other cells in the bone (Lee & Einhorn, 2001). Osteocytes and other bone components are destroyed by osteoclasts during the process of bone resorption.

Lining cells are also derived from osteoblasts and are found on the surface of bone. Their purpose is to prime the surface of bone so osteoclasts can begin bone removal.

Osteoclasts are multinucleated cells located in slight indentations of the bone surface. Osteoclasts secrete acidic enzymes, which digest bone and create a cavity in bone matrix in a process known as bone resorption. Under normal circumstances, bone resorption releases approximately 500 mg of calcium a day into the systemic circulation (Heersche & Manolson, 2000). Osteoclastic activity and inhibition is controlled by numerous mechanical, metabolic and endocrine factors. Osteoclasts have a lifespan of about seven days, after which time they undergo apoptosis, or programmed cellular death (Weinstein, Nicholas, & Manolagas, 2000).

There are three major ways that bone tissue may be altered: osteogenesis, modeling, and remodeling. Osteogenesis begins in the embryonic period and continues throughout adulthood. Osteogenesis occurs in two ways: by intramembranous ossification and endochondral ossification. Both types form bone

by replacing existing cartilage. In intramembranous ossification, osteoblasts deposit calcium into the protein matrix of bone (collagen). Endochondral ossification occurs as osteoclasts dissolve calcium previously stored away in bone and carries it to tissues whenever needed (Merckmedicus, 2001).

The process of osteogenesis appears to be closely linked to the mechanical demands encountered with normal function. In other words, bone formation peaks at sites of high mechanical stress and ebbs at sites with less mechanical load. Wolff's Law explains the normal phenomenon of bone's ability to adapt to changes in chronic mechanical loading in order to hold up to future loads of the same nature (Beck, Shaw, & Snow, 2001). This assumption posits that where bone is needed it is laid down and where it is not, it is resorped (Rubin, Judex, McLeod, & Qin, 2001).

The normal response of bone to accommodate to normal wear and tear may be altered as a consequence of aging or in response to a health related disorder. For example, in the elderly, increased stress at certain skeletal sites may have the negative affect of increasing the risk for bone fracture. In addition, calcium deficiency or a hormonal imbalance will dramatically affect the interaction of biophysical stimuli involved in the modeling response. Moreover, mechanical signals that are osteogenic in the children and young adults may not have the same effects on bones in the elderly (Rubin et a., 2001).

Bone modeling and osteogenesis are closely linked in that bones grow in diameter and length by the process of modeling. Bone modeling is an adaptive

process and distinct from bone "remodeling," which is the term used to describe the resorption and formation of mineralized tissue (Chen, Zhao, Oyajobi, & Mundy, 2000). With bone modeling, osteoblasts manufacture and secrete structural proteins that are deposited between bone cells to form a bone matrix. Eventually these proteins crystallize to form new bone.

The process of bone remodeling is the body's way of repairing microfractures sustained from everyday wear and tear of physical stress. In addition, remodeling frees calcium stores to help maintain blood calcium levels (Morgan, 2001). Bone remodeling can be conceptualized as consisting of bone remodeling units (BMU), composed of osteoclasts and osteoblasts. Through an ongoing complex physiologic process, osteoblasts form new bone that replaces old bone resorped by osteoclasts (American Medical Association, 2001).

The process of bone remodeling is ongoing throughout life, proceeds from a quiescent phase to an active resorption phase, and is believed to occur in five stages: (a) Quiescence, (b) Activation, (c) Resorption, (d) Reversal, and (e) Formation (University of Michigan, 2001). The first stage refers to the resting state of the bone surface.

During activation, osteoclasts are recruited to a quiescent bone surface through a complex biochemical process. The resorption stage begins as the osteoclasts erode a cavity (lacunae) about 100 mm wide and 50 mm deep into the bone surface. This is illustrated in Figure 2. This process takes about about 10 days.

During the reversal phase, resorption stops and osteoblasts are recruited to the site. Formation begins as osteoblasts deposit osteoid (collagen) matrix at the site of the cavity, forming the base for new bone material. The matrix calcifies and becomes new bone. This formation process constitutes the majority of the 90- to 120-day bone remodeling cycle.

Bone Mineral Density

In the healthy female, bone mass is acquired throughout growth in childhood and into early adult life to reach peak bone mass around age 30 to 40 years. Bone mass then stabilizes until the age of menopause, when estrogen deficiency triggers an imbalance in the remodeling cycle, with a net loss of bone mass. At the time of menopause, women begin a period of accelerated bone loss, averaging from 2-5% per year over the next 10 years (Kenny & Prestwood, 2000).

It is estimated that 46% to 62% BMD is attributable to genetic factors (Koller et al., 2000). From 38% to 54% of BMD may be affected by physical disorders and lifestyle factors.

The relationship of cigarette smoking and BMD is unclear and there exist many inconsistencies. For example, one study found a inverse relationship between smoking and BMD and fracture risk in older women, but found no association in premenopausal and early postmenopausal women (Law & Hackshaw, 1997).

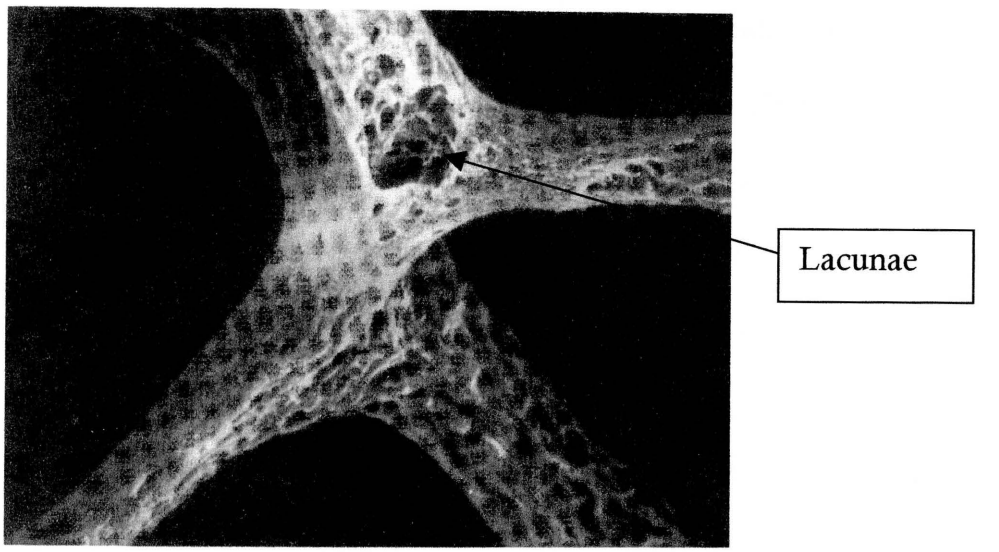

Figure 2. Trabecular bone.

Note. From Lee, C.A., & Einhorn, T.A. (2001). The bone organ system. In R. Marcus, D. Feldman, & J. Kelsey (Eds.), *Osteoporosis: Vol 2.* (2nd ed., pp. 3-20). Reprinted with permission.

Similarly, studies of the relationship between alcohol consumption and BMD are controversial. Felson, Zhang, Hannan & Kannel (1995) reported results from the Framingham study that found a positive correlation between alcohol intake and BMD among women in the highest drinking category. In contrast, a study of Chinese women indicated no significant association between alcohol intake and BMD for either duration or quantity of alcohol consumed (Hu, Zhao, Fitzpatrick, Barpia, & Campbell, 1994). Alcoholism, though, is associated with liver damage, nutritional deficiencies and intestinal mal-absorption, all of which contribute to bone loss (Bonnick, 2000).

Studies of caffeine as a risk factor for low BMD are conflicting, as well. Caffeine intake has been reported to decrease BMD (Massey, 1998; Rapuri, Gallagher, Kinyamu, & Ryschon, 2001). However, Hegarty, May, and Khaw (2000) reported a positive association between tea drinking and BMD in older women. Caffeine's negative effects on bone may be linked to its propensity to increase calcium excretion (Bonnick, 2000).

Though the effects of physical activity on BMD remains undetermined, the positive effects of weight-bearing physical activity appears to support a positive effect. Pruitt, Taaffe, and Marcus (1995) reported that lumbar spine BMD was maintained in a group of menopausal women who participated in resistance training.

The results of studies of the association between calcium intake and BMD are inconsistent. One study found that the rate of cortical bone loss seemed to be inversely related to calcium intake in perimenopausal women whose daily calcium intake ranged from 564-2,580 mg (Chiu, 1999). However, a study by Elders, Netelenbos, and Lips (1991) indicated that calcium supplementation slowed the rate of bone loss in perimenopausal women.

Since bone density is one of the main determinants of fracture risk, high bone mass at skeletal maturity (peak bone mass) is considered the best protection against age-related bone loss. Small differences of even 5-10% in bone mass and BMD at maturity could contribute to substantial differences in the incidence of osteoporotic

fractures (Miller & Zapalowski, 2000; Slemenda, Reister, Hui, Miller, Christian, & Johnston, 1994).

Bone loss can occur in several ways: (a) osteoclasts may create an excessively deep cavity, which cannot be filled by the action of the osteoblasts; (b) the function of the osteoblasts may be compromised, leaving an unfilled cavity, or (c) an increased number of bone remodeling units can be activated which, when combined with either of the above two processes, may result in increased bone loss (American Medical Association, 2001).

Osteoporosis

Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (Anonymous, 2001; Beck & Shoemaker, 2000). The progression of osteoporosis is illustrated in Figure 3.

According to the World Health Organization (WHO) guidelines, an individual with bone mass measuring 1 to 2.5 standard deviations (SD) below the mean value of a young reference sample is classified as having low bone mass (osteopenia), and individual with a bone mass 2.5 or more standard deviations below the reference sample is diagnosed as having osteoporosis (Barrett, Baron, Karagas, & Beach, 1999). The age-matched SD score is commonly referred to as the "Z-score," and the young normal SD score has been labeled as the "T-score."

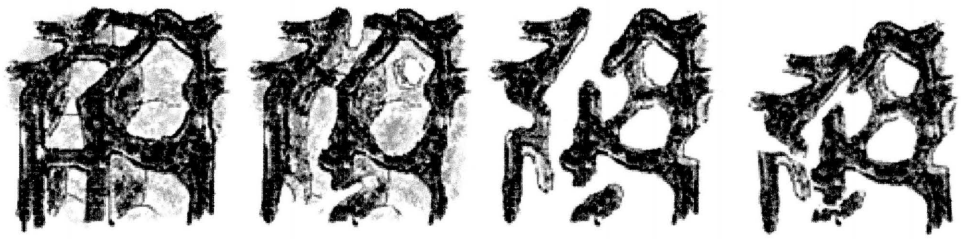

Figure 3. Progression of osteoporosis.

Note. From Ott, S. (2002) Standarization of BMD. Retrieved May 21, 2002 at http://courses.washington.edu/bonephys/opop/opop.html Reprined with permission.

The prevalence of low bone mass in white women living in the United States is estimated to be 34-50% (12-17 million) and up to 13-18% (4-6 million) of postmenopausal aged have osteoporosis (Looker et al., 1995). The incidence of osteoporotic fractures is increasing in most Western societies. As bone density is one of the main determinants of fracture risk, maximizing BMD at skeletal maturity (peak bone mass) is a logical strategy to protect against osteoporosis (Rodan, Raisz, & Bilezikian, 1996).

Risk factors for osteoporosis can be categorized as either modifiable or non-modifiable (WHO, 1994). Non-modifiable factors associated with osteoporosis are advanced age, being a Caucasian or Asian female, a family history of osteoporosis, and having a genetic predisposition. Important modifiable risk factors for osteoporosis include cigarette smoking, alcohol consumption, sedentary lifestyle, inadequate calcium intake, and vitamin D deficiency.

Osteoporosis can be classified as primary or secondary, based on the etiologic factors (Nelson, 1999). Primary osteoporosis occurs as part of normal human aging and is linked to the estrogen deficiency state of menopause. Secondary osteoporosis is caused by a host of factors, including a large number of physical disorders, medications, and lifestyle factors. Sixty-four percent of osteoporosis is attributable to secondary etiologies. Early diagnosis of osteoporosis is essential to assess the severity of the condition and implement interventions to help control its progression.

Measuring Bone Mineral Density

According to McKay, Petit, Schultz, Prior, Barr and Khan (2000), there is confusion in the literature regarding the use of the term "bone mass," which is often used interchangeably with bone mineral content (BMC) or BMD. Bone mineral content is the absolute amount of mineral present in the region of bone being measured. Bone mineral density is the relative amount of bone mineral per measured area of bone. Current measurement systems do not measure bone mass per se. What is measured is the bone mineral content contained in the area scanned or "apparent" BMD (McKay et al., 2000). The results are "apparent" in that the measurement is a combined value of bone, marrow, and other tissues. A measurement of the true density would require an isolated three-dimensional sample of a pure bone, excluding any marrow components (Faulkner, 2001). Even spinal quantitative computed tomography (QCT), which is a volumetric measure of vertebral trabecular

bone (usually expressed in milligrams per cubic centimeter), is a measure of apparent density, as it includes the marrow space of the vertebral body (Faulkner, 2001).

The earliest measures of bone density were obtained from conventional X-rays by comparing the brightness of the skeleton to the surrounding tissues. However, this technique proved to be unsatisfactory because bone mineral losses of at least 30% are required before visually detected on a conventional X-ray (Resnick, 1987). Because of the problems of using radiographs for measuring bone mass, bone densitometers were developed.

The first densitometers used the technique of single photon absorptiometry (SPA) (Faulkner, 2001). With absorptiometry, a beam of radiation (incoming radiation) is directed through a bone. The BMD was calculated by dividing the bone content, related to the attenuation of the radiation, by the bone area (Faulkner, 2001). The amount of outgoing radiation is measured for attenuation. The higher the bone mineral content, the greater the attenuation. The necessity to place the region to be scanned in a water bath with SPA proved limiting and the SPA was eventually replaced with dual-photon absorptiometry (DPA).

With DPA, regions of the body not amenable to immersion in a water bath, such as the spine and hip could be tested. This was particularly important since the areas of the body most vulnerable to fracture occur in the spine and hip (Truscott, 2000). However, DPA also had limitations. For example, the measurement

procedure was slow and maintenance of the equipment was expensive (Bonnick, 2000).

The procedure most frequently used today for measuring BMD is dual energy/X-ray absorptiometry (DXA). The DXA procedure is obtained quickly, involves minimal radiation exposure ($1/30^{th}$ that of a chest x-ray), and is precise, with the ability to detect changes in bone density as small as 1% to 4% per year (Bonnick, 2000). Dual energy/X-ray absorptiometry is not without its limitations, though. Because DXA is a projection method and measurements take place through both cortisol and trabecular bone, changes in the content of trabecular bone, which is the most metabolically active, may be obscured (Truscott, 2000).

Computed tomography (CT) is capable of producing a cross-section view through bone; however, the increased radiation compared to DXA and the cost make the procedure less practical. Bone mineral density can also be measured by ultrasound, which uses sound waves instead of radiation.

The World Health Organization (WHO) has established diagnostic criteria for osteoporosis based on dual-energy x-ray absorptiometry (DXA). Though the relative contribution of BMD to subsequent fractures is somewhat controversial, measurement of BMD remains the gold standard in the diagnosis of osteoporosis (Madlock & Allison, 2000). Prospective studies have shown that bone mass, regardless of how it is measured, is inversely related to risk of fracture.

For an individual patient, osteopenia (T-score between -1 and -2.5) carries a two-fold increase in risk for fracture compared with normal BMD, and osteoporosis (T-score less than -2.5, without the presence of a fracture) carries a four to five-fold increase in fracture risk. Severe osteoporosis (T-score less than -2.5 plus the presence of a fracture) is associated with a 20-fold increased risk for further fractures.

The Stress Response

The evolution of the stress response was an adaptation to protect early human beings from harm, to prepare one for "fight or flight." The hormonal cascade initiated by stress is regulated by the hypothalamus, pituitary gland, and adrenal cortex (HPA axis) and responds to signals from the Sympathetic Nervous System (Sapolsky, 1998). The hypothalamus produces corticotropin-releasing hormone (CRH), which is released in small pulses into the pituitary portal circulation. The anterior pituitary responds to CRH with adrenocorticotropic hormone (ACTH) synthesis and its subsequent pulsatile secretion into the peripheral circulation. The adrenal cortex, in turn, responds to plasma ACTH with generation and secretion of cortisol.

The modern definition of stress is a process in which environmental demands exceed the adaptive capacity of an organism to maintain equilibrium (Sapolsky, 1998). Episodic levels of moderate stress can enhance performance, but stress experienced repeatedly can impede performance, over time. The ability to effectively

deal with stress is a function of perception and capacity to control the stressor. Uncontrolled stress can cause psychological and biological changes that may increase the risk of disease (Sapolsky, 1998).

The perception that one is experiencing stress (subjective stress) is the outcome of both the interpretation of the meaning of an event and the evaluation of the adequacy of coping resources (McEwen and Lasley, 2001). The intensity of a stress response and its ultimate effect on the body is complex and is primarily determined by genetics and characteristics that are formed from early developmental experiences (Singh, Petrides, Gold, Chrousos, & Deuster, 1999). Though stress is experienced in many forms, both positive and negative, the non-specific effect on the body is the same (Selye, 1976; Wann, Schrader, & Wilson, 1999).

Much of what is known today about stress and its affect on the body is based on the work, in the 1930's, of Hans Selye, whose research made the connection between stress and physiological conditions. He described the cascade of physiological reactions to stress known as the General Adaptation Syndrome (GAS) (Selye, 1976). According to Selye, the stress reaction occurs in three phases. In the first phase, the body releases epinephrine to combat a stressor and to stay in control (alarm reaction). If the stressor is not removed, the second phase of resistance or adaptation follows. In this phase, glucocorticoids (cortisol) and glucagon are secreted to raise levels of circulating sugar glucose and increase blood pressure, in an effort to gain stabilization. In the third and final phase, exhaustion, the body is depleted of

reserves and organ systems begin to break down and finally succumb to what Selye called "diseases of adaptation." Irrespective of the type of stress encountered by the rats on which he was experimenting, he found changes in three distinct body systems. The rats developed peptic ulcers, hypertrophy of the adrenal glands, and atrophy of the immune tissues.

The third phase of Selye's theory (exhaustion) has come under question more recently, as researchers show that stress hormones are rarely depleted, during even the most sustained circumstances (Saplosky, 1998). A more contemporary theory posits that the stress response does not become exhausted, but rather, the stress-response itself becomes damaging. "This is not so much because the adrenal runs out of glucocorticoids (or the pituitary out of ACTH) as much as the fact that organisms habituate to all sorts of horrific stressors to a much greater extent than one would have anticipated" (R. Sapolsky, personal communication, September 3, 2001).

The HPA axis appears to have some plasticity and can become sensitized to chronic stressors, responding with less vigor upon repeated exposures to the same stressor (Gerra et al., 2001). Johnson, O'Conner, Deak, Spencer, Watkins, and Maier (2001) found the sensitization that occurs with exposure to a chronic stressor is selective and can actually prime the HPA for enhanced reactivity to a new and distinct stressor.

The mechanisms that trigger the stress response and initiate the secretion of cortisol are complex and varied, according to interacting genetic, developmental, and

experiential factors. Though influenced by many factors, cortisol is characteristically secreted in a diurnal rhythm, coordinated by the light-dark cycle and sleep-waking, with peaks in the early morning and nadirs in the evening (McEwen, 2000). The physiological and psychological reactions mounted by the body, in concert with the stress response, are a cascading effect of early developmental events that predispose individuals to react in specific ways. Interestingly, the experience of subjective stress does not always appear to increase the secretion of stress mediators such as cortisol (Kirschbaum & Kudielka, 1999).

McEwen (2000) describes the stress response as a system that protects the body in the short term but causes damage if there are many adverse stressors over a long period of time. In McEwen's model, environmental and psychosocial stressors are replaced with two terms: allostasis and allostatic load. Allostasis is the body's response to challenge in order to maintain homeostasis. The HPA axis responds to stress by initiating the adaptive response, which includes the secretion of stress hormones such as cortisol, sustaining it until the stress ceases, and then turning it off (recovery). This process causes changes in the structure and function of a variety of cells and tissues. Importantly, these changes cause both rapid effects and changes in gene expression that have long-lasting consequences for cell function. For example, the effects of cortisol on the body are wide-ranging because cortisol receptors are widely distributed and can be found in almost all body tissues and organs (McEwen,

2000). Controlling the release of stress hormones is essential if allostasis is to be maintained.

Allostatic load refers to the adverse impact or wear and tear that the body experiences due to repeated cycles of allostasis (the system's adaptation to various psychological and environmental challenges), as well as the inefficient turning-on or shutting off of these responses (McEwen, 2000). Genetic makeup, early development, and lifestyle behaviors are among the factors that contribute to allostatic load by influencing the reactivity of the systems that produce physiological stress mediators. There are four subtypes of allostatic load: a) too many repeated stressors (economic hardship), b) failure to adapt to the same stressor (failure to habituate to a particular stressor such as public speaking), c) failure to turn off the stress response or express the normal nadir of the diurnal cortisol pattern (sleep disorders and depression), and d) inadequate hormonal stress response allowing other systems to become overactive (autoimmune disorders) (McEwen, 2000). Allostatic load is illustrated in Figure 4.

Compatible with the latter subtype of allostatic load is the phenomenon of hypocortisolism in individuals exposed to chronic stress. Hypocortisolism results from reduced adrenocortical secretion, reduced adrencortical reactivity, or enhanced negative feedback inhibition of the HPA axis (Heim, Ehlert, & Hellhammer, 1999). Several researchers have found unusually low cortisol values in individuals with conditions related to chronic stress such as Post Traumatic Stress Syndrome and

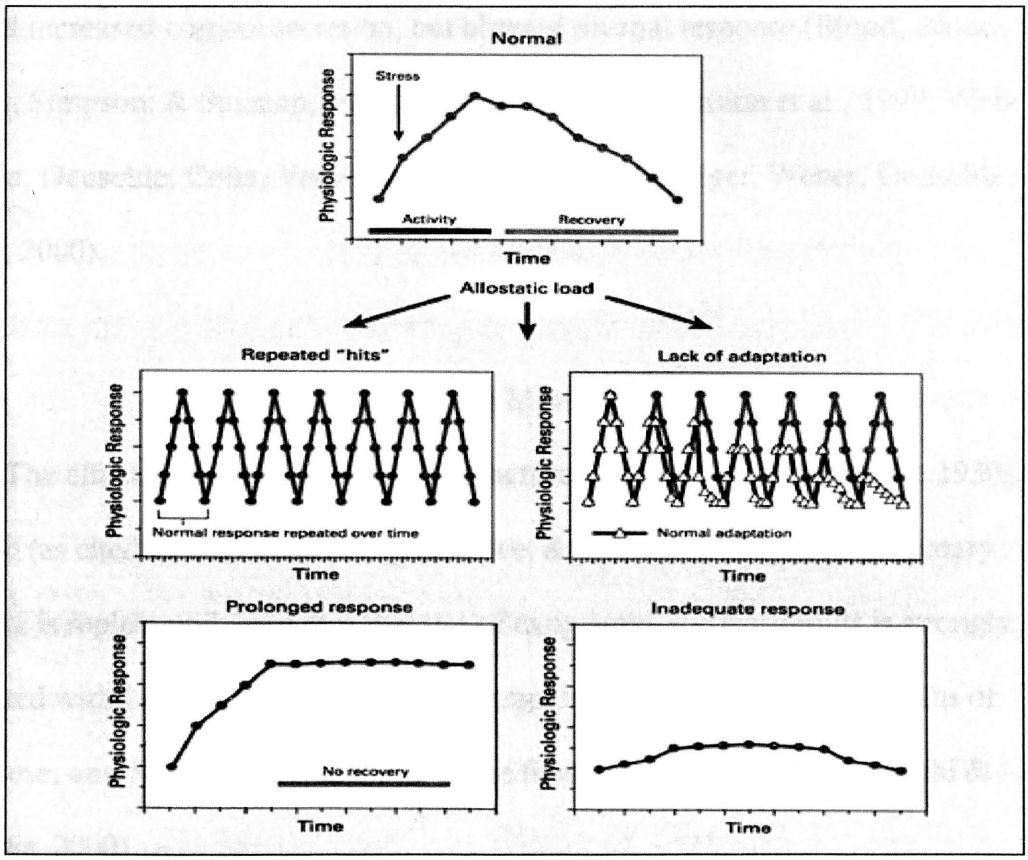

Figure 4. Allostatic load.

Note. From McEwen, B.S. (1998). Seminars in medicine of the beth israel deaconess medical center: Protective and damaging effects of stress mediators. *New England Journal of Medicine, 338(3)*, 171-179. Reprinted with permission.

women with a history of childhood sexual abuse (Thaller, Vrkljan, Hotujac, & Thakore, 1999; Habib, Gold, & Chrousos, 2001; Chrousos, 2000; & Stein, Yehuda, Koverola, & Hanna, 1997).

Depression has been shown to be associated with hyperactivity of the HPA axis and increased cortisol secretion, but blunted diurnal response (Blood, Blood, Bennett, Simpson, & Susman, 1994; Chrousos, 1998; Dennsion et al., 1999; Weber, Lewicka, Deuschle, Colla, Vecsei, & Heuser, 2000; Schweiger, Weber, Deuschle, & Heuser, 2000).

Cortisol and Bone Mineral Density

The effect of excessive cortisol on fractures was first described in the 1930s by Cushing (as cited in Greendale, Unger, Rowe, & Seeman, 1999). Contemporary literature is replete with evidence that use of exogenous corticosteroids is strongly associated with bone loss. Bone loss occurs rapidly during the first six months of steroid use, and 5% of bone is lost within the first year of menopause (Adachi & Ioannidis, 2000).

Hypercortisolism is known to decrease bone density by inhibiting osteoblastic activity and altering responses to parathyroid hormone, prostaglandins, cytokines, growth factors, and Vitamin D (Adachi & Ioannidis, 2000). High cortisol levels are associated with bone loss, reduced osteoblastic activity, and increased osteoclastic activity (Delany, Dong, & Canalis, 1994). High circulating cortisol also acts on osteoclasts by increasing parathyroid hormone and accelerating bone resorption (Keenan, 1997; Lane, Sanchez, Modin, Genant, & Arnaud, 1998). Finally, cortisol promotes negative calcium balance by reducing intestinal calcium absorption and

renal tubular calcium reabsorption (Dennison et al., 1999). Trabecular bone such as the vertebral bodies, femoral necks, and distal radii are most susceptible to steroid induced alterations in bone metabolism (Adachi & Ioannidis, 2000).

Less is known about the effects of levels of endogenous cortisol and BMD. What evidence that is known pertains the most to studies involving elderly populations and in populations suffering from depression. The majority of published studies was conducted among elderly men and women and shows a significant inverse correlation between endogenous cortisol and BMD (Dennison et al., 1999; Raff, Raff, Duthie, & Wilson, 1999). However, one study indicated a positive correlation between awakening cortisol levels and BMD in a sample of premenopausal women (Brooke-Wavell et al., 2002). Another study reported a positive association between evening cortisol levels and BMD at two appendicular sites, but not at other sites, in elderly men (Raff et al., 1999).

Support for the link between psychological processes and heath parameters are increasingly being reported in contemporary literature. Excessive and sustained cortisol secretion have been associated with depression, memory loss, hypertension, osteoporosis, irritable bowel syndrome, immunosuppression, metabolic syndrome X, hemodynamic abnormalities, abdominal obesity, atherosclerosis, hypertension, and cardiovascular disease, (Chrousos, 1998; Greendale, Unger, Rowe, & Seeman, 1999; Greendale, Kritz-Silverstein, Seeman, & Barrett-Connor, 2000; Heitkemper et al., 1996; Heshmati, Riggs, Burritt, McAlistewr, Wollan, & Khosla, 1998; Rozanski,

Blumenthal, & Kaplan, 1999; Weber et al., 2000; Wickrama, Lorenz, & Wallace, 2001).

Salivary Measures of Endogenous Cortisol

Salivary cortisol is an acceptable method to measure levels of circulating cortisol because the measures obtained by salivary cortisol are highly correlated to the amount of biologically active (free) fraction of the hormone found in the plasma (Umeda, Hiramatsu, Iwaoka, Shimada, Miura, & Sato, 1981; Castro, Elias, Martinelli, Antonini, Santiago, & Moreira, 2000). Correlations between salivary and blood measures are reported to reach or exceed .9 (80% of total variance share). Moreover, salivary cortisol has even been shown to be more helpful than plasma cortisol (Guechot et al., 1982). However, caution is advised when comparing results among samples analyzed by different techniques, because values differ between enzyme–immunoassay (EIA) and radioimmunoassay (RIA) (Raff, Homar, & Burns, 2002).

Salivary cortisol is non-invasive, easy to collect, and stores easily. Cortisol is highly lipid soluble and can readily diffuse through cell membranes into saliva (Kirschbaum & Helhammer, 1989). Saliva flow has little or no effect on salivary cortisol levels. Though the cotton used in saliva sampling techniques has been reported to interfere with some saliva biomarkers, cortisol is not altered by the cotton collection procedure (Shirtcliff, Granger, Schwartz, & Curran, 2001).

Though Stone et al. (2001) reported flattened cortisol responses in 15% of a group of individuals, cortisol is generally believed to be secreted in a diurnal pattern, related to the sleep-wake cycle. Upon awakening, there is a burst of cortisol secretion. Cortisol levels peak at around 8:00 a.m., decline throughout the day, then fall to reach a nadir in the evening. Within the first 30 minutes of awakening, free cortisol level rises by 50-60% and is independent of time of awakening, sleep duration, sleep quality, physical activity, or morning routines (Pruessner, 1999). Variations in this pattern of cortisol secretion are a trait characteristic (Gerra et al., 2001). In other words, it is either genetically predetermined or a physiological trait formed through early developmental processes.

A number of factors can influence the response magnitude and time course of cortisol secretion, including gender, use of oral contraceptives, seasonal variations, and the menstrual cycle (Pruessner, 1999; McCormick & Teillon, 2001; & Maes et al., 1997). The cortisol secretory response does not appear to vary significantly; however, between perimenopausal and postmenopausal women (Parker et al., 2000). During times of increased stress, cortisol output may increase 10 times the basal rate (Smith, Muir, & Hall, 1997; Migeon & Wisniewski, 2001).

CHAPTER III

METHODOLOGY

The study was conducted using a descriptive, correlational design. This design was appropriate because there was no manipulation of variables by the researcher. The purpose of this study was to determine if correlations existed among endogenous cortisol (determined by salivary cortisol), subjective stress, and bone mineral density (BMD) in non-elderly women. The study also sought to determine whether endogenous cortisol or subjective stress were predictive of BMD in this population. This determination was based on a Pearson product moment correlation analysis of salivary cortisol and BMD. In addition, step-wise multiple correlation analysis was used to examine selected extraneous variables in predicting BMD. The variables examined were the following: (a) age, (b) race (c) family history of osteoporosis, (d) body mass index, (e) alcohol use, (f) cigarette smoking, (g) caffeine intake, (h) dietary calcium intake, (i) calcium supplementation, (j) use of hormone replacement therapy, and (k) weight-bearing physical activity level. Finally, the relationship of endogenous cortisol and a measure of stress was determined. The methodology for the study is presented in this chapter under the following headings: (a) Population and Sample, (b) Procedures, (c) Instrumentation, and (d) Treatment of the Data.

Population and Sample

The population of interest for this study was women between 30 and 65 years of age, without a previous diagnosis of osteoporosis, who were not taking glucocorticoids. The accessible population for this study was women within two weeks of obtaining BMD testing at an ambulatory health care setting located next to a large teaching institution in North Central Texas. Women eligible for the study had obtained or planned to obtain a BMD test within two weeks of data collection, were not taking exogenous corticosteroids, and were able to read, write, and speak the English language. Women were not eligible if they had a previous diagnosis of osteoporosis, had eaten or exercised within two hours of saliva sampling, or were currently taking exogenous corticosteroids.

Initially, the sample was to include only women between 30 and 60 years of age. However, the age of inclusion was increased to 65 on the assumption that the definition of elderly is subjective and women between 60 and 65 years of age were expressing interest in being included in the study. A sample size of 60 was desired, but after 10 months, data collection ceased; a sample of 38 had been obtained.

Protection of Human Participants

Protection of human participants' rights was in accordance with current policies of Texas Woman's University. The study was exempt from review by the Institutional Review Board at Texas Woman's University because the research

protocol had been reviewed and approved by the Institutional Review Board (IRB) at the study site (Appendix A). Prior to data collection, each potential participant met with the researcher and received a verbal explanation of the study. The researcher explained that the investigation would involve obtaining a sample of saliva, responding to two short questionnaires, and releasing to the researcher the results of her BMD test.

Potential benefit to participants included increased awareness of the effects of stress in their life; increased awareness of modifiable health behaviors related to diet, activity, and BMD; and knowledge of any abnormally high cortisol levels, as determined by the saliva sample. The only risk involved with participation in the study was that answering the stress questionnaire may have caused some level of unease as participants thought about things in their lives that were stressful. Participants' confidentiality was protected by the assignment of a code number when they volunteered for the study, which was then used as an identifier in the data base. Participants were told that they could withdraw from the study at any time. They were also told that results of the study would be available upon request.

Procedures

Volunteers for the study were recruited by two separate mechanisms. The researcher distributed recruitment brochures (Appendix B) in the reception areas of the study site. In addition, a recruitment letter (Appendix C) was mailed to clientele

30-65 years of age, scheduled for BMD testing at the study site. Upon being contacted, the researcher confirmed that the potential participant was eligible for the study. The researcher then provided an overview of the study. A meeting was then scheduled within 2 weeks of the appointment of the BMD test to obtain informed consent. Initially, the researcher planned to collect data immediately following the BMD, however, the researcher was not always available at that time. This variation in data collection was unlikely to have affected results because, according to Steinweg (2002), bone remodeling occurs over several months. It was therefore determined that data collection within 2 weeks of the BMD was acceptable.

After the purpose of the study and the data collection procedures was explained to potential participants, informed consent was obtained. Those who agreed to participate were then asked to provide a sample of saliva. Next, participants responded to two short questionnaires. The first questionnaire gathered information about socio-demographic characteristics and health-related behaviors known to be associated with BMD, such as age; race/ethnicity; family history of osteoporosis; use of hormone replacement; calcium, alcohol and caffeine intake; cigarette smoking; weight-bearing physical activity level; and dietary calcium supplementation. Though not primary variables of interest, they are known factors pertinent to BMD and their measurement allowed their control as extraneous variables.

The second questionnaire measured the participants self-reported personal stress levels (Abell, 1991). Because responding to a stress questionnaire may be inherently stressful, with the effect of artificially raising cortisol levels, the stress questionnaire was administered after collecting the saliva sample.

Upon completion of data collection, volunteers were given a token of appreciation in the amount of $25. Initially, no incentive was planned. However, recruitment was difficult and the researcher believed offering an incentive would yield more volunteers. Instruments will be discussed in two sections, according to the two independent variables, the dependent variable, and selected extraneous variables.

Instruments

Independent Measures

Endogenous Cortisol

The primary independent variable in the study was salivary cortisol. To obtain saliva samples, a collection device known as a salivette® was used. The device is a plastic cylinder with an internal chamber that holds a cotton swab (3 cm X .75 cm in size). Each volunteer placed the cotton swab in her mouth for two minutes. After each sample was collected, it was stored in a freezer, pending collection of additional samples. The samples were then sent for analysis at the Behavioral Endocrinology Laboratory at Pennsylvania State University, where a highly sensitive enzyme immunoassay (EIA) test by Salimetrics was performed. Samples of cortisol

were sent for analysis using recommendations of the Centers for Disease Control (CDC) guidelines for transport of biological specimens. The CDC does not consider saliva a biohazardous agent unless samples are visibly contaminated with blood. Recommendations (Appendix D) included packing the samples in dry ice and labeling the shipment as a biological agent. Each sample was measured in duplicate and averaged for reporting. Cortisol is measured in micrograms per deciliter (ug/dl). A Pearson product-moment correlation analysis was calculated between cortisol level and BMD. In addition, salivary cortisol levels were examined for an association with subjective stress.

Subjective Stress

The *Index of Clinical Stress* (ICS) (Appendix E) was administered to each participant. The ICS is considered a global measure of stress-related personal discomfort (Hudson, 1997). This scale measures stress levels in general, as opposed to stress related to a specific setting or condition. The scale contains 25 items that produce scores ranging from 0 to 100. The scores can be regarded as true ratio scale values. A score of 0 indicates the respondent has none of the attribute and a score of 100 represents the highest possible distress level that the scale is capable of measuring (Hudson, 1997). A clinical cutting score has not yet been established for this scale. In internal consistency reliability testing, the scale consistently achieved an Alpha coefficient of .90 or larger (Hudson, 1997). Moreover, the scale consistently achieved validity coefficients of .60 or better in testing for content, construct, and factorial

validity (Hudson, 1997). However, the psychometric research that has been conducted to validate the scale has relied predominately on self-report from people who have grown up in the United States or have lived there for a significant period of time. It is designed for use by individuals over the age of 12 years and those not mentally impaired or with low literacy skills.

The measure of interest was chronic stress; therefore, participants were instructed to respond to questions based on how they felt, in general, over the past year or so, rather than in the more recent past. The questionnaire includes 25 items that are answered using a Likert scale from 1-7, with a reply of 1 meaning "none of the time," and 7 meaning "all of the time." The higher the score, the greater is the stress. The first step in scoring the ICS was to reverse-score each of the positively worded items. The procedure for reverse-scoring the appropriate items involved calculating Y, where Y = K+1-X, where K is the largest possible score value for an item and X is the original item score. After reverse scoring the appropriate items and denoting the item responses as Y, the total score, S, was computed for each scale as S = (Sum (Y)-N)(100)/[(N)(K)-1)] where N is the number of items that were properly completed by the respondent and K is the largest possible score value for an item. No items were left blank by any participant. According to Hudson (1997), the scale's readability has a Flesch-Kincaid grade level of four, which was appropriate for the participants of the study. The Pearson product-moment correlation analysis was

calculated for cortisol levels and scores from the *Index of Clinical Stress*. Scores from the ICS were also correlated with BMD.

Extraneous Variables

Selected anthropometric measures and personal demographic data were recorded on the Demographic and Practices Questionnaire (Appendix F). The Demographic and Practices Questionnaire was designed by the researcher to explain the effects of confounding variables on BMD. Information was collected and recorded on age, race, blood pressure, BMI, family history of osteoporosis and selective health behaviors reported to be related to BMD (hormone replacement therapy; intake of calcium, alcohol and caffeine; cigarette smoking; and weight-bearing physical activity) (Steinweg, 2002; Cornuz, Feskanich, Willett & Colditz, 1999; Morgan, 2001, Ravn et al., 1999; Ward & Klesges, 2001).

For purposes of calculating Pearson correlation, frequency counts were calculated, based on weekly consumption, for calcium, alcohol, caffeine intake, the degree of cigarette smoking, and amount of weight bearing physical activity levels. Current use of hormone replacement therapy was recorded as a dichotomous variable, as was family history of osteoporosis and calcium supplementation. Body Mass Index (BMI) was calculated as $wt(kg)/ht^2(m)$.

Dependent Variable

Bone mineral density was determined by dual-energy x-ray absorptiometry (DXA) measurement of either the lumbar spine or the proximal femur by a Lunar DPX IQ. DPX-MD (Lunar Radiation Corporation, Madison, WI) or Hologic QDR 4500W bone densitometer (Hologic Inc, Bedford, Mass). DXA delivers precise BMD results in two minutes for the spine and femur. To measure BMD, a radiation source is collimated to a beam directed at a radiation detector placed directly opposite the site to be measured. Individuals undergoing a DXA lie on a table in the path of the radiation beam. The source/detector assemble is then scanned back and forth across the measurement site. The attenuation of the radiation beam is determined and is related to the bone mineral content. The bone area of the scanned region is determined by a computer, and the BMD is calculated as the ratio of the bone content to the measured area. Measurements were made by trained and qualified technicians. The amount of radiation of a DXA is approximately 1-5 muSv. This compares with 5-8 muSv and 60 muSV for natural background radiation per day and the radiation exposure from an 8 hour airplane flight, respectively (Faulkner, 2001).

Three measures were recorded for BMD: g/cm3, T scores and Z scores. The aged-matched SD score is commonly referred to as the Z-score, and the young normal SD score has been labeled the T-score. For most BMD tests, -1 SD equals a 10-12% decrease in bone density (National Osteoporosis Foundation, 2002).

The WHO bases the assessment of osteoporosis on the T-score and is represented as follows: (a) Normal: T-score > -1, (b) Low bone mass (osteopenia): T-score between -1 and -2.5, and (c) Osteoporosis: T-score < -2.5. Only the total BMD measurement was used for the analysis.

Treatment of the Data

This study was designed to determine the relationship of endogenous cortisol, subjective stress and BMD. Descriptive statistics (frequencies, percentages, and measures of central tendency) were calculated for the demographic data collected. Variability (range, variance, S.D., SEM) and measures of central tendency (mean, median, minimum, maximum) were calculated for salivary cortisol levels, subjective stress scores, and BMD. Statistical analysis using the Pearson correlation measured the degree and direction of linear relationships between salivary cortisol, subjective stress scores, and BMD. The strength of the relationship was measured by the coefficient of determination (r^2). The coefficient of determination assesses the proportion of variability in one variable that can be determined from the relationship with the other variable.

In addition, multiple correlation analysis was used to examine (a) age, (b) race (c) family history of osteoporosis, (d) body mass index, (e) alcohol use, (f) cigarette smoking, (g) caffeine intake, (h) dietary calcium intake, (i) calcium supplementation, (j) use of hormone replacement therapy, and (k) weight-bearing

physical activity level in predicting BMD. Data analysis was done using the SPSS 10.0 PC version statistical package. A statistical significance level of $p<.05$ was used.

CHAPTER IV

FINDINGS

The study was conducted to determine if correlations existed among endogenous cortisol (determined by salivary cortisol), subjective stress and bone mineral density (BMD) in non-elderly women. The study also sought to determine whether endogenous cortisol or subjective stress were predictive of BMD in this population. This determination was based on Pearson product moment correlation analysis between salivary cortisol and BMD. In addition, multiple correlation was used to examine selected extraneous variables in predicting BMD. The variables examined were the following: (a) age, (b) race, (c) family history of osteoporosis, (d) body mass index, (e) alcohol use, (f) cigarette smoking, (g) caffeine intake, (h) dietary calcium intake, (i) calcium supplementation, (j) use of hormone replacement therapy, and (k) weight bearing physical activity level. Step-wise multiple correlation regression analysis was used to examine each of the extraneous variables of age, race, family history of osteoporosis, body mass index, alcohol use, cigarette smoking, caffeine intake, dietary calcium intake, calcium supplementation, use of hormone replacement therapy, and weight bearing physical activity level for predicting BMD. Finally, the relationship of endogenous cortisol and a measure of stress was determined.

Description of the Participants

The population of interest for this study was women between 30 and 65 years of age, without a previous diagnosis of osteoporosis, who were not taking glucocorticoids. The accessible population for this study was women within two weeks of obtaining BMD testing at an ambulatory health care setting located next to a large teaching institution in North Central Texas.

Initially, a sample size of 60 was desired; however, the researcher's status as a full-time employee proved to be too limiting. In addition, the remuneration provided each participant, as a token of appreciation for their volunteering for the study, was provided solely by the researcher and was limited. After 10 months, data collection ceased; a sample of 38 had been obtained.

Demographic and anthropometric characteristics are indicated in Table 1. The participants (n = 38) were between the ages of 30-63 years of age and the mean age of the group was 49. Twenty-seven participants (71.1%) reported their race as Caucasian, seven (18%) stated they were African American or Black, two (5%) reported their race as Hispanic or Latino, one (2.6%) stated they were Asian, and one (2.6%) stated they were Native American.

Table 1

Demographic and Anthropometric Characteristics of the Study Participants (n = 38)

Variable	M	SD	SEM
Age (years)	49.3	8.56	1.39
Race			
• White	27(71.1%)		
• Black	7(18.4%)		
• Asian	1(2.6%)		
• Hispanic	2(5.3%)		
• American Indian	1(2.6%)		
BMI (kg/m^2)	24.9	4.80	.788
Systolic BP	127.61	18.26	2.96
Diastolic BP	76.11	11.78	1.91
Gravidity	2.08	1.59	.26
Parity	1.54	1.26	.21

Note. BMI = body mass index; BP = blood pressure

The mean BMI for the participants was 24.94 (range 19-38). Twenty-one (55.3%) of the participants reported that they were menopausal. Of these women, 20

were currently taking hormone replacement therapy of either estrogen alone (ERT) or estrogen plus progestin (HRT). Fourteen (37%) of the women were currently taking HRT, six (16%) were on ERT. The mean number of years of HRT use was 6 (range <1 to 15) years. The mean number of years of ERT use was 6 (range <1 to 20) years. Eleven (29%) participants reported a family history of osteoporosis. Eighteen (47%) reported that they did not have a family history of osteoporosis and a family history of osteoporosis was unknown for 9 (24%) participants.

Analysis of Data

Statistical analyses were directed at addressing the following null hypotheses:

- Ho1 There is no relationship between salivary cortisol level and BMD measured by dual energy x-ray absorptiometry in non-elderly women.
- Ho2 There is no relationship between salivary cortisol level and subjective stress as measured by *The Index of Clinical Stress* in non-elderly women.

The relationship between cortisol and BMD was examined using Pearson product moment correlation, and partial correlation analyses. Cortisol levels were logarithmically transformed to achieve normality prior to analysis. Descriptive statistics for cortisol levels are presented in Table 2. Mean level of cortisol was

.13 ug/dL ± 6.54 ug/dL and ranged from .042 ug/dL to .326 ug/dL. All cortisol samples were collected between the hours of 08:20 to 19:00.

The correlation between cortisol levels and BMD was statistically significant ($r = .378$, $p < .05$). As cortisol levels increased, so did measures of BMD. A scatter plot of the correlation is illustrated in Figure 5.

Table 2

Descriptive Statistics for Cortisol Levels and BMD

Partial Variable	Min	Max	M	SD	SEM	Variance
Cortisol Level (ug/dL)	.042	.326	.136	.065	.0106	.0428
BMD (g/cm^2)	.722	1.877	1.125	.219	.0355	.0479

n= 38

Cortisol is known to be secreted in a distinct circadian rhythm, peaking at around 08:00 hours with a nadir around 23:00 hours. When examining the association between cortisol and BMD, while controlling for time of day the sample was obtained, the correlation increased in significance ($r = .384$, $p < .009$).

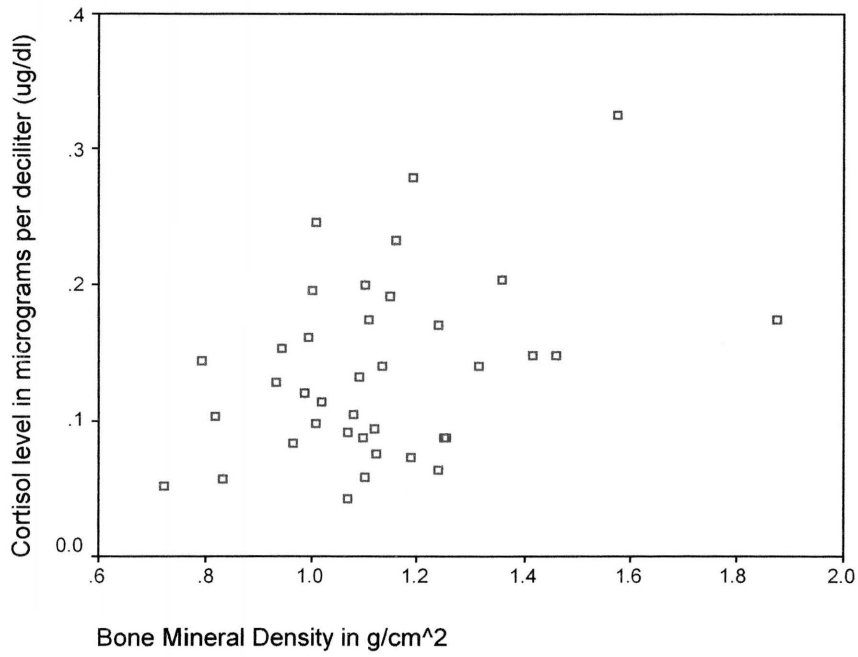

Figure 5. Scatter plot for cortisol levels and BMD

Partial correlations were also obtained for age, race, family history of osteoporosis, body mass index, alcohol use, cigarette smoking, caffeine intake, dietary calcium intake, calcium supplementation, use of HRT and weight-bearing physical activity level. The zero-order correlation and partial correlations are presented in Table 3. Regardless of the partialling variable employed, the relationship between cortisol and BMD remained statistically significant, though the association was modest.

Table 3

Zero-Order and Partial Correlations for BMD and Cortisol

Variable		r	p
1.	None	.378	.010
2.	Age	.370	.012
3.	Race (White or Black)	.402	.007
4.	Family History of Osteoporosis	.399	.018
5.	Body Mass Index (BMI)	.374	.025
6.	Alcohol Use	.347	.036
7.	Cigarette Smoking	.378	.021
8.	Caffeine Intake	.345	.037
9.	Dietary Calcium Intake	.378	.021
10.	Calcium Supplementation	.380	.024
11.	Currently Taking HRT	.378	.011
12.	Weight bearing physical activity	.392	.017

Note. One-tailed significance

Scores on the *Index of Clinical Stress* ranged from 8 to 63.3. Mean score was 28.9 $\pm$ 14.9. Cortisol levels were not significantly associated with subjective stress scores. Descriptive statistics for cortisol and stress scores are presented in Table 4. Of interest was the modest, though statistically significant, inverse correlation between subjective stress and age ($r = -.278$, $p. < .05$). Also of interest was the finding that

weight-bearing physical activity levels inversely correlated with subjective stress. However, this relationship was not statistically significant. The correlation matrix for selected extraneous variables is presented in Table 5.

Step-wise multiple correlation analysis was used to determine the variables that are significant predictors of BMD. Independent variables considered in the equation were age, race, family history of osteoporosis, body mass index, alcohol use, cigarette smoking, caffeine intake, dietary calcium intake, calcium supplementation, use of hormone replacement therapy, and weight-bearing physical activity level. The beta weight (-.325) for caffeine intake was influential in contributing negatively to BMD, with 10% of the variance of BMD being attributed to caffeine intake. When combined with caffeine intake, the beta weight (.307) for alcohol consumption was influential in positively contributing to BMD. Twenty percent (20%) of the variance of BMD can be attributed to the combined effects of caffeine and alcohol intake; however, caffeine has a greater association (Tables 6 and 7).

Table 4

Descriptive Statistics for Cortisol Levels and Stress Scores

Variable	Min	Max	M	SD	SEM	Variance
Cortisol level (ug/dL)	.042	.326	.136	.0654	.011	.0428
Stress Scores	8.0	63.3	28.95	14.93	2.422	222.89

Table 5

Correlation Matrix for Selected Variables

Variable	1	2	3	4	5	6	7	8	9	10
1. BMD	1.00									
2. Cortisol Level	.378*	1.00								
3. Age	-.208	-.081	1.00							
4. Body mass Index (BMI)	-.064	-.205	-.085	1.00						
5. Dietary Calcium Intake	-.021	-.013	-.177	-.419*	1.00					
6. Cigarette Smoking	.216	-.002	-.320*	.014	-.102	1.00				
7. Alcohol Intake	.272*	.184	-.123	-.184	.152	.204	1.00			
8. Caffeine Intake	-.299*	-.183	.157	.057	.163	-.044	-.015	1.00		
9. Weight bearing activity levels	-.035	.184	.094	-.238	.112	-.084	.122	.277*	1.00	
10. Subjective Stress	-.021	.066	-.278*	-.197	.222	-.177	.095	.109	-.229	1.00

Note. *p= <.05; BMD=Bone mineral density

Table 6

Summary of Multiple Correlation Regression Analysis for Variables Predicting BMD: Model 1
(n=38)

Variable	*B*	*SE B*	β	T	p
Model 1					
TCaffeine	-.007	.004	-.327	-2.020	.051

Note. r=.327, r^2=.107, Adj-r^2=.081, SE=.215, F(1,34)=4.079, p<.05. TCaffeine=total caffeine intake

Though they were not included as primary variables of interest, additional findings included statistically significant correlations for several of the other variables examined in the study. A moderate, positive correlation was found between calcium intake and BMI (r = -.419, p < .05). Modest, inverse correlations were found between age and cigarette smoking (r = -.320, p = < .05) and between caffeine intake and BMD (r = -.299, p = < .05). In addition, modest inverse correlations were found between calcium intake and systolic (r = -.382, p = < .05) and diastolic blood pressure (r= -.379, p = < .05).

Table 7

Summary of Multiple Correlation Regression Analysis for Variables Predicting BMD: Model 2
(n=38)

Variable	*B*	*SE B*	*β*	T	p
Model 2					
TCaffeine	-.007	.003	-.325	-2.091	.044
TAlcohol	.027	.013	.307	1.975	.057

Note. r=.449, r^2=.202, Adj-r^2=.153, SE=.206, F(2,33)=4.165, p.<05. TCaffeine=total caffeine intake, weekly; TAlcohol=total alcohol intake, weekly

CHAPTER V

SUMMARY/DISCUSSION

Osteoporosis is the most common metabolic bone disease, affecting 200 million individuals worldwide and more than 28 million individuals in the United States (Greenspan et al., 2000). This condition is a major public health concern because of its association with fracture. Complications of hip fractures are the second leading cause of death in people 47-74 years of age.

In the United States, more than one million fractures occur annually in women with osteoporosis, with hip fractures exceeding other types of fractures. Every five years, from the age of 70 years, the incidence of hip fracture doubles. Hip fractures have an associated mortality rate of 12-15%, and are the second leading cause of death in people age 47-74 years. Expenditures for treatment of osteoporotic fracture in the United States are estimated at $10 billion to $15 billion annually (Anonymous, 2001). By the year 2040, it is estimated that these will be 80,000,000 postmenopausal women in the U.S., many of whom will be at risk for suffering the consequences of osteoporosis (Miller, 2000). Both the increase in expected longevity and population growth, within 50 years the costs of treating hip fractures alone has been estimated to exceed $240 billion per year (Barrett, Baron, Karagas, & Beach, 1999).

Peak bone mass is largely determined by genetic factors; however, other important factors influencing BMD include being Caucasian, low BMI, early menopause, cigarette smoking, alcohol consumption, sedentary lifestyle, inadequate calcium intake, vitamin D deficiency, and cigarette smoking. Another risk factor for low BMD is exposure to exogenous glucocorticoids. Over 40% of one's bone mass may be lost during the first six months of glucocorticoid therapy (Adachi & Ioannidis, 2000).

The general assumption is that psychological stress evokes a neuroendocrine response that, when repeatedly activated or activated over a prolonged period of time, places a person at risk for a range of physical disorders (Cohen, Kessler, & Gordon, 1997). Cortisol is one of several stress hormones released when individuals experience stress. Since exogenous cortisol is associated with decreased bone density, it is reasonable to assume that elevated levels of endogenous cortisol may also adversely affect bone density.

The study was conducted to determine if correlations existed among endogenous cortisol (determined by salivary cortisol), subjective stress, and BMD in non-elderly women. The study also sought to determine whether endogenous cortisol or subjective stress were predictive of BMD in this population. This determination was based on Pearson product moment correlation analysis of salivary cortisol and BMD. In addition, step-wise multiple correlation analysis was used to examine selected extraneous variables in predicting BMD. The extraneous variables were the

following: (a) age, (b) race, (c) family history of osteoporosis, (d) body mass index, (e) alcohol use, (f) cigarette smoking, (g) caffeine intake, (h) dietary calcium intake, (i) calcium supplementation, (j) use of hormone replacement therapy, and (k) weight-bearing physical activity level. Finally, the relationship of endogenous cortisol and a measure of subjective stress was examined. This chapter includes the study design, discussion of the findings, conclusions and implications, and limitations and recommendations.

Study Design

The study was conducted using a descriptive, correlational design. A sample of convenience was obtained from a group of women within two weeks of having a BMD test at a university affiliated clinical setting, to determine the relationships among endogenous cortisol, subjective stress, and BMD. This determination was based on Pearson product moment correlation analysis of cortisol levels and BMD, followed by a determination of the relationship between cortisol levels and subjective stress scores.

Each woman provided a sample of saliva and responded to two short questionnaires. The first questionnaire gathered information on selected anthropometrics, demographic characteristics and health-related practices pertaining to diet and physical activity. The second questionnaire was a measure of perceived stress.

Bone density was determined by dual-energy x-ray absorptiometry (DXA) measurement of either the lumbar spine or the proximal femur. The total BMD in g/cm^3 measurement was used for the analysis.

Descriptive statistics were obtained as well as correlation analysis to examine relationships among cortisol level, subjective stress and BMD. In addition, multiple correlations were used to examine selected extraneous variables reported to impact bone mineral density. Data analysis was done using the SPSS 10>0 PC version statistical package. A statistical significance level of $p<.05$ was used.

Discussion of the Findings

Hypothesis One

The first null hypothesis stated that, "there is no relationship between salivary cortisol level and BMD measured by dual x-ray absorptiometry in non-elderly women." This null hypothesis was rejected. The Pearson correlation coefficient indicated a modest but statistically significant ($r = .378$, $p < .05$) positive correlation between cortisol levels and BMD. The relationship between cortisol and BMD remained statistically significant after controlling for age, race, family history of osteoporosis, body mass index, alcohol use, cigarette smoking, caffeine intake, dietary calcium intake, calcium supplementation, use of hormone replacement therapy, and weight-bearing physical activity.

Hypothesis Two

The second null hypothesis stated that, "there is no relationship between salivary cortisol level and subjective stress as measured by *The Index of Clinical Stress.*" This null hypothesis was accepted. The Pearson correlation coefficient indicated no statistically significant ($p < .05$) relationship.

Additional findings of interest pertaining to subjective stress were the modest, though statistically significant ($r = -.278$, $p<.05$) inverse correlation between subjective stress and age. Step-wise multiple correlation analysis determined that of the variables age, race, family history of osteoporosis, body mass index, alcohol use, cigarette smoking, caffeine intake, dietary calcium intake, calcium supplementation, use of hormone replacement therapy, and weight-bearing physical activity, only caffeine and alcohol intake could predict BMD. Twenty percent (20%) of the variance of BMD can be attributed to the combined effects of caffeine and alcohol intake; however, caffeine has a greater association.

Though not included as primary variables of interest, additional statistically significant findings included moderate, positive correlation between calcium and BMI ($r = -.419$, $p < .05$), a modest inverse correlation between age and cigarette smoking ($r = -.320$, $p < .05$), and a inverse correlation between caffeine intake and BMD ($r = -.299$, $p < .05$). In addition, a statistically significant inverse correlation, was found between calcium intake and systolic ($r = -.382$, $p < .05$) and diastolic blood pressure ($r= -.379$, $p < .05$).

Conclusions/Implications

The study sought to examine the relationships among endogenous cortisol, subjective stress and BMD. The sample for this study was small and, therefore, caution must be used in generalizing the results to a broad population.

The major finding of this study was a modest positive correlation between cortisol and BMD. Cortisol levels were not associated with subjective stress. A major limitation of this study was that only one saliva specimen, to be analyzed for levels of cortisol, was obtained over various times during the day, and cortisol is known to be secreted in a distinct circadian rhythm, peaking at around 08:00 hours with a nadir around 23:00 hours. This pattern could be seen in the inverse though not statistically significant correlation between cortisol level and time of day the sample was obtained. However, when controlling for the time of day the sample was obtained, the relationship between cortisol and BMD remained statistically significant, though the association was modest.

These findings are consistent with a recent study that found a positive correlation between cortisol levels at early morning awakening and BMD in a sample of premenopausal women (Brooke-Wavell, Clow, Noori, Evans, and Hucklebridge, 2002). The findings of this study are also consistent with the positive association between evening cortisol levels and radial and trochanteric BMD in elderly men, but conflicts that of a inverse association between lumbar spine BMD

and morning salivary cortisol in elderly men in this same study (Raff et al., 1999).

These findings suggest that there may be different relationships among different age groups and between genders, as well as at different bone sites. Moreover, the positive association supports the suggestion of Nielson et al. (1992) that cortisol may in some way be linked to osteocalcin, a protein found exclusively in bone. Osteocalcin is thought to have a role in bone formation by decreasing osteoclastic activity (Li, Srivastava, Gu, Masinde, Mohan, & Baylink, 2002). Osteocalcin is secreted in a circadian rhythm, as is cortisol, but with zeniths at night and nadirs in the morning. This is the opposite pattern of cortisol secretion. According to Brook-Wavell et al. (2002) this interconnectedness may suggest that peaks in morning cortisol levels in healthy individuals may have some role in the life cycle of bone forming osteoblasts.

The finding that cortisol level was not associated with subjective stress was somewhat unexpected, but could be explained within the context of allostatic load. Allostatic load, as well as the reactivity of the HPA, is complex, and both are highly influenced by genetics, as well as individual characteristics and internal and external environments (McEwen, 2000). Therefore, the use of cortisol as a specific indicator of stress is limited, especially when considering individuals who show inverted or flattened responses (Cohen, Kessler, & Gordon, 1997). Moreover, caution must be used when relying on any self-reported measures of stress. For example, negative affectivity bias, acquiescence response styles, extreme response styles, and social

desirability bias may alone or in combination threaten the validity of information gathered by self-report (Anastasi & Urbinia, 1997).

Though not a primary variable of interest, other outcomes of this study were a statistically significant inverse correlation between caffeine intake and BMD, and a positive correlation between alcohol intake and BMD. These findings are consistent with studies that found caffeine was inversely associated with BMD, but conflict with others that reported a positive association between tea drinking and BMD in older women. (Massey, 1998; Rapuri, Gallagher, Kinyamu, & Ryschon, 2001; Hegarty, May, & Khaw 2000; Wu, Yang, Yao, Lu, Wu, & Chang, 2002).

Studies of the relationship between alcohol consumption and BMD are ambiguous. Felson et al. (1995) reported results from the Framingham study that indicated a positive correlation between alcohol intake and BMD among women in the highest drinking category. In contrast, researchers who examined the association between alcohol and BMD in a group of Chinese women reported no significant association for either duration or quantity of alcohol consumed (Hu, Zhao, Fitzpatrick, Barpia, & Campbell, 1994).

Additional findings in this study included a negative correlation between calcium intake and blood pressure. This finding is consistent with findings of several other researchers that reported an inverse association between dietary calcium and blood pressure (Appel et al., 1997; Morikawa et al., 2002).

Clearly, bone health is a quality of life issue. Though much of the propensity for developing osteoporosis is genetic, modifiable risk factors exist. New knowledge of modifiable risk factors provides the foundation for strategies to increase awareness of bone health and motivate people to engage in behaviors that will have a positive impact on their health and well-being.

Limitations and Recommendations

The sample for this study was small and, therefore, caution must be used in generalizing the results to a broad population. Moreover, data was from a sample of convenience and may not be representative of the population. The cortisol sample obtained was a single sample only, and cortisol levels are known to have a diurnal pattern of secretion and vary over a 24-hour period. Data on the *Index of Clinical Stress* may reflect current stress and may be less valid in measuring chronic stress. Reporting bias may have interfered with validity of the tool for measuring stress. The hormonal stress response is complex, and multiple factors influence the secretion of cortisol.

The study was limited to a sample of women with access to health care and a general predisposition for positive health-related behaviors. The results may not be generalized to women without access to health care or those with more negative health-related behaviors.

Based on the conclusions of this study, the following recommendations are made:

1. Replicate the study using a larger sample size.
2. Another study should be conducted that includes multiple measures of cortisol over a period of 24 hours.
3. Replicate the study using a different measure of subjective stress.
4. Replicate the study using a different measure of physiological stress.
5. A study could be conducted that compares cortisol levels in women experiencing acute stress verus chronic stress.
6. Another study of interest would be to examine the relationship of endogenous cortisol and biomarkers of bone turnover.

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APPENDICES

APPENDIX A

Institutional Review Board Approval

TEXAS WOMAN'S
UNIVERSITY

INSTITUTIONAL REVIEW BOARD

P.O. Box 425619
Denton, TX 76204-5619
Phone: (940) 898-3375
Fax: (940) 898-3416

November 26, 2001

Ms. Sherry Carter
2702 Sunrise Dr.
Arlington, TX 76006

Dear Ms. Carter:

Re: The Relationship of Endogenous Cortisol, Subjective Stress, and Bone Mineral Density in Non-Elderly Women

The above referenced study has been reviewed by a committee of the Institutional Review Board (IRB). This study was determined to be exempt from further TWU IRB review as it has been reviewed and approved by an IRB at The University of Texas Southwestern Medical Center at Dallas and all participants in the study will be located at UT Southwestern.

Another review by the TWU IRB is required if your project is changed in any way. If you have any questions, please feel free to call the TWU Institutional Review Board at the phone number listed above.

Sincerely,

Dr. Linda Rubin, Chair
Institutional Review Board - Denton

cc. Dr. Susan Ward, Department of Health Studies
Dr. William Cissell, Department of Health Studies
Graduate School

APPENDIX B

Recruitment Brochure

There are no costs to you.

You will receive $25. 00 cash, if you agree to be in the study.

You are invited to be a part of this study because you have had a bone mineral density test within the past 2 weeks or you have an appointment for one in the near future.

If you decide to be in the study, the time you will spend for the study will be about 20 minutes.

Please read flip side

No information that can be identified with you will be disclosed to any outside sources.

Being in this study is voluntary. You may withdraw from the study at any time.

You may not be in the study if you:

- have been previously diagnosed with osteoporosis,
- are taking glucocorticoids (steroids),
- have eaten a meal or exercised 2 hours before collection of saliva sample.

SOUTHWESTERN

Women Volunteers Needed to Study

THE EFFECT OF STRESS ON BONE MINERAL DENSITY

"A Research Study to Help Women"

Stress can cause increased levels of a hormone called cortisol. High levels of cortisol have been shown by researchers to decrease bone mineral density. Cortisol can be detected easily in saliva (spit). I would greatly appreciate your assistance by volunteering to be in this study.

For women, age 30-65 years

If you volunteer for the study, you will be asked to do the following after your bone mineral density test:

- sign an informed consent form
- collect a sample of saliva (spit) by placing a small cotton pad in your mouth for about 2 minutes
- answer two short questionnaires, the first one is about things like age, race, exercise, and calcium intake, the second one measures the degree of problems you may have with personal stress
- release to the researcher the results of your bone mineral density test.

Risks/Benefits

The only risk involved by being in the study is that answering the stress questionnaire may cause some level of unease as you think about things in your life that are stressful.

Answering the stress questionnaire may be beneficial as you become more aware of the effects of stress in your life. Being aware of stress in your life can be beneficial because it may motivate you to look for ways to decrease your stress.

Researcher: Sherry Carter, MS, WHCNP, Doctoral Candidate, Texas Woman's University and Faculty Associate at The University Texas Southwestern Medical Center Dallas, **(metro) 817-691-5210**, 214-905-2116 or 214-905-2129.

APPENDIX C

Recruitment Letter

TEXAS WOMAN'S UNIVERSITY
DEPARTMENT OF HEALTH STUDIES
And
THE UNIVERSITY OF TEXAS SOUTHWESTERN
MEDICAL CENTER AT DALLAS

Women volunteers needed for a study of

THE RELATIONSHIP BETWEEN ENDOGENOUS CORTISOL, SUBJECTIVE STRESS, AND BONE MINERAL DENSITY IN NON-ELDERLY WOMEN

My name is Sherry Carter. I am a women's health care nurse practitioner and a doctoral candidate at Texas Woman's University. I am conducting a research study that will determine the relationship between salivary cortisol, stress and bone mineral density.

Stress has been associated with increased levels of a hormone called cortisol. Prolonged high levels of cortisol have been shown by researchers to decrease bone mineral density. Cortisol can be detected easily in saliva.

I am looking for about 60 women aged 30-65, to participate in this study. To be included in the study, volunteers must recently have had or plan to have a bone mineral density test.

If you decide to volunteer for the study, the additional time you will spend for the study will be about 20 minutes. If you volunteer for the study, you will be asked to do the following after your bone mineral density test:

- sign an informed consent form
- collect a sample of saliva by placing a small cotton pad in your mouth for about 2 minutes,

- answer two short questionnaires, the first one is about personal characteristics (age, race, exercise, etc.), the second one measures the degree of problems you may have with personal stress, and
- release to me, the results of your bone density test.

No information that can be identified with you will be disclosed in publication or to any outside sources. Signed consent forms will be kept in a locked file cabinet in my office of. The saliva samples will be coded to maintain confidentiality and stored in a locked freezer until all samples have been collected. After you complete the questionnaires, they will be placed in a sealed envelope and stored in a locked file cabinet at my office.

The procedure for the bone mineral density testing by dual energy x-ray absorptiometry (DXA) will not be altered in any way. The only risk involved by participating in the study is that answering the stress questionnaire may cause some level of unease as you think about things in your life that are stressful.

Potential benefit to you may include increased awareness of the effects of stress in your life, increased awareness of modifiable health behaviors related to diet and activity, and knowledge of an abnormally high cortisol level, as determined by the saliva sample..

Your participation in the study will benefit society by adding to the knowledge of factors related to low bone mineral density in non-elderly women. The information will contribute to the development of health education and other interventions that promote health for women.

The findings from this study will be used in my dissertation as partial fulfillment for a doctor of philosophy degree. It is anticipated that the study findings will be presented at research conferences and published in professional journals.

Participation in this study is voluntary, refusal to participate will involve no additional charge and no change in your care or your previously scheduled bone mineral density test.

You may withdraw from the study at any time. If you have questions or concerns during the study, you may reach me at metro-817-691-5210 or 214-905-2129. If you would like a copy of the study results, indicate so and a copy will be sent to you.
Certain things might interfere with the study results. Therefore, you cannot participate in the study if you:

- have previously been diagnosed with osteoporosis
- are currently taking glucocorticoids (steroids)
- will have eaten or exercised 2 hours previous to the appointment for the bone mineral density test

You will receive $25. cash as my appreciation of your time and effort.

Having heard this description of the study, if you qualify and want to participate in the study, please contact me by telephone at one of the following phone numbers: work (214-905-2116), home- (metro 817-691-5210) or by email at sherry.carter@utsouthwestern.edu and I will arrange to meet you after your bone mineral density test. If you wish, we can reschedule a time that is more convenient for you. The collection of saliva and answering the questionnaires will take about 20 minutes.

After we obtain informed consent, you will be weighed and your blood pressure will be obtained. Next, we will collect a sample of saliva and give you the questionnaires. If you do NOT want to participate in the study, you do not need to do anything more. Your consideration is greatly appreciated.

Sherry Carter, MS, WHCNP

APPENDIX D

Recommendations for Shipping

Saliva Sample Shipping Advice

These recommendations follow the U.S. Centers for Disease Control (CDC) guidelines for transport of biological specimens. The CDC does not consider saliva a biohazardous agent unless samples are visibly contaminated with blood.

Supplies needed:

1. Shipping container
2. Small ziplock freezer bags (quart size)
3. Large ziplock freezer bags (gallon size)
4. Newspaper
5. Paper towels or other absorbent material
6. Sample submittal sheet (provided by Salimetrics)
7. Dry ice

Shipping Container: We recommend Freez Safe® insulated containers manufactured by Polyfoam Packers Corporation. These containers are available from VWR Scientific Products (1-800-932-5000):

Size	Polyfoam No.	VWR Cat. No.	Inside Dimensions (cm/in)
small	324	15713-621	20.3 (8) x 14.9 (6) x 29.2(11.5)
medium	355	15714-500	27.9 (11) x 27.9 (11) x 29.5 (11.6)
large	398	15714-703	47.6 (18.8) x 27.9 (11) x 31.1 (12.3)

Packaging Saliva Samples:

1. Organize individual samples in numerical order and place them in groups in the small ziplock freezer bags. Place paper towels or other absorbent material in each bag in case of leakage. If samples are contained in a cryostorage box, skip this step.
2. Place one or more bagged groups of samples or a single cryostorage box inside a large freezer bag.
3. Place dry ice (5 lbs) in the bottom of the shipping container.
4. Place several layers of newspaper to act as a barrier between the dry ice and ziplock bags.
5. Place a layer of crumpled paper towels or other absorbent material on top of the newspaper.
6. Place bagged samples on top of paper towels, and pack additional crumpled paper towels around the remaining sides of the shipping carton so that the samples are cushioned. Please remember that as the dry ice sublimates, your samples may be at greater risk due to increased shifting. It is a good idea to pack as many crumpled paper towels as possible without risking breaking the Styrofoam insulation.
7. Place sample submittal sheet in a ziplock bag on top.
8. Place Styrofoam lid on box. Wrap the seam of the Styrofoam box with packing tape.
9. Attach shipping label and black & white dry ice sticker (if shipping via FedEx) on outside of box.

When packing your samples, **DO NOT:**

1. Ship samples that are not frozen. All samples **MUST** be frozen prior to shipment.
2. Use rubber bands. They will break upon contact with dry ice.
3. Place samples in plastic bags directly on dry ice. If plastic bags contact dry ice, they will split open.
4. Overpack container. Styrofoam will crack when over-packed, causing samples to thaw prior to delivery.

Shipping: We recommend shipping FEDEX Priority Overnight service. You will need to place a black and white dry ice sticker on the outside of the box and complete the FEDEX air bill, stating that there is dry ice inside the container. Call FEDEX (1-800-463-3339) if you need supplies or have any questions. Other carriers are acceptable as well, just be sure to send your samples via overnight delivery.

Please ship samples on Monday, Tuesday, or Wednesday to assure that your samples are not delayed in transit over a weekend. Our lab is closed on weekends.

Please fax (814-234-1608) or email mcurran@salimetrics.com to notify us that the shipment has been made. Please include the carrier and the package air bill/tracking number.

Shipping Address:

Salimetrics, LLC
Attn: Mary Curran
101 Innovation Blvd. Suite #302
State College, PA 16803
800-790-2258

APPENDIX E

Informed Consent Form

The University of Texas Southwestern Medical Center at Dallas
Aston Center

CONSENT TO PARTICIPATE IN RESEARCH

Title of Research: The Relationship Between Endogenous Cortisol, Subjective Stress and Bone Mineral Density in Non-Elderly Women

Sponsor:

Investigator: Sherry Carter, MS, WHCNP	Telephone No. 214-905-2116 (regular office hours)	Telephone No. metro-817-261-5094 (other times)

PURPOSE: The purpose of this research is to: determine the relationship of cortisol to bone mineral ensity in non-elderly women. Cortisol is one of the "stress" hormones your body releases when your are physically or mentally stressed. A secondary purpose will be to determine the relationship between cortisol and a measure of mental stress.

This research is being done because knowledge of all risk factors for low bone mineral density is needed in order to development health education and other interventions for promoting health in women.

PROCEDURES: Informed consent will be obtained within two weeks of obtaining a bone mineral density test. After you have signed informed consent you will place a small cotton pad in your mouth for about 2 minutes to saturate it with saliva. This sample will be tested for cortisol level. Next you will respond to two short questionnaires. One asks questions about personal characteristics (such as age, weight and whether or not you eat dairy products) and one measures the degree of stress that you may experience in life. Completing the questionnaires will take about 20 minutes.

POSSIBLE RISK(S):
Because you already have an appointment for a DXA, the study involves no additional risks other than possible mild psychological unease associated with answering the stress questionnaire.

POSSIBLE BENEFITS
As a result of answering the stress questionnaire, you may become more aware of the effects of stress in your life. With this awareness, you may look for ways to decrease the level of stress in your life. In addition, in the unlikely event that your cortisol level is higher than normal, you will be informed. This information may also indicate levels of stress not previously identified or it may indicate an underlying health disorder.

Benefit to others: Data from this study will add to the knowledge of factors related to low bone mineral density in non-elderly women. The information will contribute to the assessment for health education needs and the development of interventions that promote health for women.

ALTERNATIVES TO PARTICIPATION IN THIS RESEARCH: One alternative is not to participate in this study.

PAYMENT TO TAKE PART IN THIS RESEARCH: You will receive $25. for your time and effort as a participant of the study.

COSTS TO YOU: Not applicable.

VOLUNTARY PARTICIPATION IN RESEARCH: You have the right to agree or refuse to participate in this research. If you decide to participate and later change your mind, you are free to discontinue participation in the research at any time.

Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. Refusal to participate will not affect your legal rights or the quality of health care that you receive at this center.

RECORDS OF YOUR PARTICIPATION IN THIS RESEARCH

Information kept at UT Southwestern: You have the right to privacy. All information obtained from this research that can be identified with you will remain confidential within the limits of the law.

Information available to other people: An Institutional Review Board (IRB) is a group of people who are responsible for assuring the community that the rights of participants in research are respected. Members and staff of the IRB at this medical center may review the records of your participation in this research. A representative of the Board may contact you for information about your experience with this research. If you wish, you may refuse to answer any questions the representative of the Board may ask.

Publication of the results of the research: The results of this research may appear in scientific publications without identifying you in any way.

YOUR QUESTIONS: Sherry Carter is available to answer your questions about this research at (214-905-2116 or metro 817-261-5094 or by email at sherry.carter@utsouthwestern.edu). The Chairman of the IRB is available to answer questions about your rights as a participant in research. You may telephone the Chairman of the IRB during regular office hours at 214-648-3060.

YOU WILL HAVE A COPY OF THIS CONSENT FORM TO KEEP.
Your signature below certifies the following:

- You have read (or been read) the information provided above.
- You have received answers to all of your questions.
- You have freely decided to participate in this research.
- You understand that you are not giving up any of your legal rights.

_____
Participant's Name (printed)

_____ _____
Participant's Signature Date

_____
Legally responsible representative's name (printed) (if applicable)

_____ _____
Legally responsible representative's Signature Date

_____
Witness' name (printed)

_____ _____
Witness' signature Date

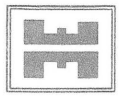
_____
Name (printed) of person obtaining Consent

_____ _____
Signature of person obtaining consent Date

_____
Principle Investigator

APPENDIX F

Index of Clinical Stress



INDEX OF CLINICAL STRESS (ICS)

Name: _____ Today's Date: _____

This questionnaire is designed to measure the way you feel about the amount of personal stress that you experience. It is not a test, so there are no right or wrong answers. Answer each item as carefully and as accurately as you can by placing a number beside each one as follows.

1 = None of the time
2 = Very rarely
3 = A little of the time
4 = Some of the time
5 = A good part of the time
6 = Most of the time
7 = All of the time

---

1. ____ I feel extremely tense.
2. ____ I feel very jittery.
3. ____ I feel like I want to scream.
4. ____ I feel overwhelmed.
5. ____ I feel very relaxed.
6. ____ I feel so anxious I want to cry.
7. ____ I feel so stressed that I'd like to hit something.
8. ____ I feel very calm and peaceful.
9. ____ I feel like I am stretched to the breaking point.
10. ____ It is very hard for me to relax.
11. ____ It is very easy for me to fall asleep at night.
12. ____ I feel an enormous sense of pressure on me.
13. ____ I feel like my life is going very smoothly.
14. ____ I feel very panicked.
15. ____ I feel like I am on the verge of a total collapse.
16. ____ I feel that I am losing control of my life.
17. ____ I feel that I am near a breaking point.
18. ____ I feel wound up like a coiled spring.
19. ____ I feel that I can't keep up with all the demands on me.
20. ____ I feel very much behind in my work.
21. ____ I feel tense and angry with those around me.
22. ____ I feel I must race from one task to the next.
23. ____ I feel that I just can't keep up with everything.
24. ____ I feel as tight as a drum.
25. ____ I feel very much on edge.

5, 8, 11, 13.

APPENDIX G

Demographic and Practices Questionnaire

THE RELATIONSHIP BETWEEN ENDOGENOUS CORTISOL, SUBJECTIVE STRESS, AND BONE MINERAL DENSITY IN NON-ELDERLY WOMEN
Demographic and Practices Questionnaire

The following questionnaire is designed to provide information essential for examining variables that might be related to bone mineral density.
Your responses are confidential. Only a case number will be linked to other data collected for the study.
Date: Time:

The Researcher will help you complete the information in this box.
Case number: _____ Cortisol level: _____ BMD:_____ Stress Score_____

Age: ____ Blood Pressure: _____ Weight: _____Height: _____BMI: _____

Gravidity:____ Para: ____Menses: [ ] Regular [ ] Irregular [ ] Menopausal LMP: _____

Ovaries: [ ] Yes [ ] No

Name _____

Phone number:_____Email address: _____

If needed, may I contact you? [ ] No, do not contact me

[ ] Yes Indicate your preference: [ ] Phone [ ] Email

Do you want a copy of the study results? [ ] No [ ] Yes

Preferred address to send results:

_____

Race or Ethnic Identification: Please designate the group you belong in, identify with or are regarded in the community as belonging to:

_____White: (a person having origins in any of the original peoples of Europe, North Africa, or the Middle East, not of Hispanic origin)

_____African American or Black: (a person having origins in any of the Black racial groups of Africa, not of Hispanic origin)

_____Hispanic/Latino: (a person of Mexican, Puerto Rican, Cuban, Central or South American, or other Spanish culture or origin, regardless of race)

_____Asian or Pacific Islander: (a person having origins in any of the original peoples of the Far East, Southeast Asia, the Indian subcontinent, or the Pacific Islands.
This area includes, for example, China, Japan, Korea, the Philippine Islands and Samoa)

_____American Indian or Alaska Native: (a person having origins in any of the original peoples of North and South America and who maintains cultural identification through tribal affiliation or community recognition)

_____Multi-Ethnic/Other: (a person having origins in more than one of the groups listed above).
Please specify: _____

1. Has any member of your family had osteoporosis (brittle bones)?

[ ] Yes **[ ] No** **[ ] Don't know**

If "yes," please indicate the family member:

[ ]Mother

[ ]Maternal grandmother

[ ]**Maternal grandfather**

[ ]**Father**

[ ]Paternal grandmother

[ ]Paternal grandfather

[ ]**Brother**

[ ]**Sister**

2. Do you currently take hormone replacement therapy?

[ ] No

[ ] Yes, estrogen and progestin How long have you taken it? _____

[ ] Yes, estrogen only How long have you taken it? _____

[ ] Yes, but don't know if I take estrogen alone or estrogen plus progestin

How long have you taken it? _____

3. Are you currently being treated for any health condition?

[ ] Yes What is the condition? _____

[ ] No

4. Are you currently taking any prescribed medication other than hormone replacement therapy?

[ ] Yes Name of the medication/medications: _____

[ ] No

5. Are you taking any non-prescription medication/medications?

[ ] Yes

[ ] No

6. Do you take a calcium supplement daily?

[ ] Yes What dosage?_____ [ ] Don't know

What brand? _____

[ ] No, I do not take a calcium supplement daily.

Dietary Calcium Intake

Estimate the amount in cups (1 cup, ½ cup, etc.) per day OR per week OR per month.	Never	Monthly	Weekly	Daily	Duration of use (years)
7. How often do you drink milk?	_____	_____	_____	_____	_____
8. How often do you eat cheese/cottage cheese?	_____	_____	_____	_____	_____
9. How often do you eat fresh or frozen yogurt?	_____	_____	_____	_____	_____
10. How often do you eat dark green leafy vegetables?	_____	_____	_____	_____	_____
11. How often do you eat pinto beans?	_____	_____	_____	_____	_____

Tobacco, Alcoholic Beverage & Caffeine Usage

Estimate the number per day OR per week OR per month.	Never	Monthly	Weekly	Daily	Duration of use (years)
12. How often do you smoke cigarettes?	_____	_____	_____	_____	_____
13. How often do you drink beer or wine?	_____	_____	_____	_____	_____
14. How often do you drink a mixed drink?	_____	_____	_____	_____	_____
15. How often do you drink caffeinated coffee, tea, or cola?	_____	_____	_____	_____	_____

Exercise

Estimate the number of times per day OR per week OR per month.	Never	Monthly	Weekly	Daily	Duration of activity (years)
16. How often do you walk, run, or skate for at least 20 minutes?	_____	_____	_____	_____	_____
17. How often do you use a weight lifting machine or lift free weights?	_____	_____	_____	_____	_____
18. How often do you lift heavy objects?	_____	_____	_____	_____	_____

Thank you for your time and effort!