EFFECT OF BLACK COHOSH ON BIOCHEMICAL MARKERS OF BONE REMODELING IN POSTMENOPAUSAL WOMEN

By

ALICE PETERS CARLISLE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2008
To my husband Andrew Mitchell Carlisle, who supported me without fail. Without his love and support, none of this would have been possible.
ACKNOWLEDGMENTS

I would like to thank my committee chair, Dr. James V. Jessup, for his guidance, patience, and continual encouragement. I appreciate him taking on a nurse midwife, even though he wasn’t always sure what to do with my “midwifery” research ideas.

I thank Dr. Thomas Wronski for taking on a nursing doctoral student, and teaching me almost everything I now know about bones. I appreciate his extensive knowledge of bone metabolism and all the time he spent educating me. I thank Dr. Sharleen Simpson for her extensive knowledge of women’s health and for being with me on yet another nursing research committee. I thank Dr. Saunjoo Yoon for her assistance with my study design and for supporting my dietary supplement research.

I thank Linda Reilly CNM for giving me a work schedule that allowed me to go to all of my classes and for her unwavering support. In addition, I thank all of the midwives, the office manager (Linda Gerds), and all the wonderful staff at Midwives of Ocala for their patience and understanding of my many absences, strange schedule, and nasty moods. Huge thanks go to Hilary Morgan, a true friend and a constant source of encouragement. Even though we started our PhD program at the same time and she graduated a year ahead of me, she never stopped helping and encouraging me. I thank Leslie Thorne and Elaine Kaplan, both good friends and great recruiters for my study. I thank my family (husband Andy, son Chris, and daughter Jennifer) for their unwavering love and support during this long and often stressful PhD program. None of this would have been possible without the three of them behind me. Lastly, I thank my lord God for walking with me every step of the way.
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LIST OF ABBREVIATIONS

BMI      Body mass index
BMD     Bone mineral density
CTX         C-terminal telopeptide
DOH     Department of Health
DEXA Dual energy x-ray absorptiometry
ER     Estrogen receptor
FDA     Food and Drug Administration
FSH    Follicle stimulating hormone
IL     Interleukin
IRB    Institutional Review Board
LH     Luteinizing hormone
LMP    Last menstrual period
NIH    National Institutes of Health
NOF    National Osteoporosis Foundation
NTX    N-terminal telopeptide
OC     Osteocalcin
OPG    Osteoprotegerin
ORS    Office of Research Support
PTH    Parathyroid hormone
RANKL Receptor activator of NF-κB ligand
TGFβ Transforming growth factor beta
TNFα    Tumor necrosis factor alpha
Postmenopausal osteoporosis affects more than eight million American women leading to an imbalance in bone remodeling, resulting in fragile brittle bones which are susceptible to fracture. There are more than 1.5 million fractures annually, many resulting in increased morbidity and mortality. Every year more than $14 billion is spent on health care for osteoporosis-related fractures.

Estrogen replacement has long been known to decrease the bone remodeling that occurs during menopause. Postmenopausal women not taking estrogen replacement are not only at risk for bone loss, but often experience many of the vasomotor symptoms of menopause. The dietary supplement black cohosh is used by many postmenopausal women to decrease or eliminate most of the vasomotor symptoms of menopause. The current mechanism of action of black cohosh is unclear but it is possible that it may have an estrogenic effect on bone. The purpose of this study was to evaluate the effect of black cohosh on bone metabolism, and ultimately to see if black cohosh had an effect on bone that was similar to estrogen.

Forty-eight healthy postmenopausal women were recruited for this study. Forty six women completed the study: 23 women in the black cohosh group and 23 in the placebo group. Participants were randomized into a double-blind, placebo-controlled clinical trial evaluating the
effect of a standardized 40mg dose of black cohosh given orally once daily. All of the women had serum samples drawn at the onset of the study and again after 12 weeks of study medication. Serum samples were analyzed for serum C-terminal telopeptide, a marker of bone resorption; and serum osteocalcin, a marker of bone formation.

Analysis of covariates (ANCOVA) conducted on the results of the bone biochemical markers revealed that there was no statistically significant difference in either bone resorption or formation after 12 weeks of black cohosh therapy. In addition, an ANCOVA was conducted to compare onset and conclusion blood pressures, as hypotension is a documented side effect of black cohosh. Results showed that black cohosh had no statistically significant effect on either systolic or diastolic blood pressure. Since estrogen is well-known to decrease serum markers of bone resorption and formation, these findings suggest that, under the conditions of this clinical study, black cohosh lacks an estrogenic effect on bone remodeling.
CHAPTER I
INTRODUCTION

Statement of the Problem

Osteoporosis is a condition in which bone loss occurs leading to bone that is fragile and at increased risk for life-threatening fractures. Osteoporosis is a major health condition which by current estimates affects over 10 million Americans, 80% of them postmenopausal women (National Osteoporosis Foundation, 2005). In addition, 34 million Americans have osteopenia, or low bone mass, which puts them at increased risk for developing osteoporosis and related fractures in the future. Every year osteoporosis is estimated to be responsible for more than 1.5 million fractures, including 300,000 fractures of the hip, 700,000 fractures of the spinal vertebrae, and 250,000 fractures of the wrist (National Institutes of Health, 2005). Costs associated with osteoporosis and the resulting fractures are staggering, with estimates in the year 2002 at $18 billion (NOF, 2005). In addition, fractures often result in a change in lifestyle and quality of life, with hip fractures resulting in nursing home admissions, impairment in mobility, and death. The National Institutes of Health (NIH, 2005) estimates that 1 out of 5 patients with a hip fracture dies in the year after the fracture.

Loss of bone mass, resulting in weakened bones with a potential for fracture, is thought to be from an imbalance in the bone remodeling process. Bone remodeling is the process of rebuilding new bone and repairing existing bone. Remodeling occurs throughout the skeleton at many different sites, and is ongoing throughout the life span (Jee, 1988). Bone remodeling begins with bone resorption, the removal of old bone, and is completed with bone formation, the laying of new bone matrix. Bone loss, due to an imbalance in remodeling where resorption occurs at a higher rate then formation, occurs approximately 10 years earlier and occurs two times faster in women than in men (Jee). The loss of endogenous estrogen which occurs in the
postmenopausal period results in a change in the remodeling process causing an increase in bone resorption and a smaller increase in bone formation, resulting in an imbalance in the remodeling process.

Menopause is broadly defined as the final menstrual period and the termination of ovarian function, and is made retrospectively after the woman has been without a menstrual period for one year (Dawood, 2000). The postmenopausal period is the time in a woman’s life after the menopause has been completed. Recent estimates are that nearly 46 million women in the United States are experiencing or completing the menopause transition, and that number is expected to increase as the population ages (The North American Menopause Society, 2001).

Menopause is a natural multisystem transition that usually takes 3 to 5 years to complete and is often referred to as the perimenopause or the climacteric. As a woman enters the menopause transition there is a declining level of estradiol, the predominant female estrogen produced by the theca and granulosa cells of the female ovary (Jones & DeCherney, 2005). Vasomotor symptoms such as hot flashes, hot flushes, and night sweats as well as insomnia, forgetfulness and irritability are all well known symptoms of the menopause (Whiteman, Staropoli, Benedict, Borgeest, & Flaws, 2003). In addition to the vasomotor symptoms associated with the menopausal years, the decrease in estrogen also causes an imbalance in the bone remodeling process which results in increased resorption and a smaller increase in formation. Increased bone remodeling causes a loss of bone mineral density and an increased fragility of the bones, specifically cancellous bone, resulting in an increased risk of fractures (Akesson, 2003). As a result of living longer, women will spend approximately one-third of their lives in the postmenopausal period, and therefore increase their risk of osteoporosis and fractures.
Hot flashes, a defining characteristic of the menopause, are the primary reason women in this phase of their life seek medical attention. It is estimated that 40% to 70% of early menopausal women experience hot flashes, nearly 4 to 5 million women in the United States alone, and may continue to experience them for up to 30 years after the menopause transition (Whiteman et al., 2003). Hot flashes are the most common reason that women try estrogen therapy, and research has shown that estrogen replacement therapy alleviates up to 80%-90% of the vasomotor symptoms of menopause (Shanafelt, Barton, Adjei, & Loprinzi, 2002).

Synthetic estrogens were discovered in 1938 and by the 1950’s clinical studies were developed to test their effectiveness and use in postmenopausal women (Ferguson, 2004). In addition to the relief of vasomotor symptoms, estrogen was shown to help prevent bone loss by inhibiting osteoclastic activity, stimulating collagen synthesis, and decreasing osteoporosis-related hip and vertebral fractures by 50% and 90% respectively (Notelovitz, 2003). Estrogens are able to move in and out of cells by simple diffusion and their action is dependent on the presence of an estrogen receptor in the nucleus of the targeted cell (Barrett, 2005). Bord and colleagues (2001) found the presence of estrogen receptors in both cortical and cancellous bone. The degree of the effect of estrogen on the bone varies with the dosage and route of administration of the estrogen used.

Estrogen replacement therapy was widely accepted as a treatment for menopausal symptoms, and as a method of preventing the development of osteoporosis. The Women’s Health Initiative Study, published in 2002, revealed an increased risk of developing breast cancer, stroke, non-fatal myocardial infarction, pulmonary embolus, and deep vein thrombosis. The positive results of the study revealed a decrease in the risk of osteoporosis-related fractures and colorectal cancer, both statistically significant results.
The publication of the Women’s Health Initiative Study, and the resulting media frenzy, spurred postmenopausal women to discontinue their estrogen, and to look into other alternatives with their health care providers (Writing Group for the Women's Health Initiative Investigators, 2002).

Several other treatment options are available for osteoporosis. These include the use of the selective estrogen receptor modulators (SERMs), the bisphosphonates, parathyroid hormone, calcitonin, and the phytoestrogens. These treatments, like estrogen, are aimed at countering the imbalance in the bone remodeling process. All of the above treatments work to decrease bone resorption except parathyroid hormone, which increases bone formation (Akesson, 2003).

Selective estrogen receptor modulators (SERMs) such as Raloxifene and Tamoxifen are commercially available with a prescription and are taken daily. Tamoxifen is primarily used to treat breast cancer, not osteoporosis, though it has been shown to have a beneficial effect of decreasing bone resorption (Notelovitz, 2003). The disadvantage associated with Tamoxifen is that studies have shown that it has a detrimental effect on the endometrium of the uterus. Raloxifene has been shown to prevent postmenopausal bone loss and reduce vertebral fractures by 30%-50%, as well as reducing biochemical markers of bone resorption and formation (Notelovitz).

Bisphosphonates such as Alendronate, Risedronate, and Ibandronate are all commercially available with a prescription, and have the convenience of daily, weekly, and monthly dosing. Bisphosphonates mechanism of action is that they prevent bone resorption by binding to hydroxyapatite, inhibiting osteoclast activity and preventing osteoblast apoptosis (Eqbal, Inzerillo, Moonga, & Zaidi, 2003). The advantage of the bisphosphonates is that studies have shown a decrease in bone resorption through the use of biochemical bone markers and dual
energy X-ray absorptiometry (DEXA) scans and a reduction in the fracture rate. The disadvantages are the side effects such as esophageal irritation, the low rate of intestinal absorption, and the fasting state that is necessary both before and after dosing (Akesson, 2003).

Parathyroid hormone, commercially available by prescription as Teriparatide, is the only approved osteoporosis treatment which increases bone formation. Low intermittent doses of parathyroid hormone can cause growth hormone to increase, stimulate osteoblast activity, and reduce osteoblast apoptosis, thereby increasing bone mineral density (Notelovitz, 2003). Teriparatide has been shown to reduce new vertebral fractures by 65%-69% and non-vertebral fractures by 53% (Akesson, 2003). The disadvantage of Teriparatide is that it must be administered by subcutaneous (SC) injection daily.

Calcitonin is commercially available as both a nasal spray and by SC injection. The most common is Miacalcin, a nasal spray commercially available by prescription. The mechanism of action of calcitonin is that it decreases bone remodeling by interfering with the attachment of the osteoclast to the bone surface, and it interferes with the ability of the osteoclast’s ruffled border to function properly (Mehta, Malootian, & Gilligan, 2003). Calcitonin has also been shown to be beneficial in controlling bone pain that occurs in several diseases in addition to osteoporosis, such as osteoarthritis, bone cancer, and Paget’s disease (Mehta, Malootian, & Gilligan). The disadvantage of calcitonin is that it produces nasal symptoms such as discharge, congestion, bleeding, and sores.

Phytoestrogens are plant compounds available as dietary supplements that have estrogen-like properties and are available commercially without a prescription. Interest in phytoestrogens has increased as women are looking for more natural alternatives without the risk of serious side effects. Numerous herbal products are available commercially without a physician’s
prescription, and the sale of herbal products is a multibillion dollar business in the United States. In the United States alone there was a 380% increase in the use of herbal supplements from 1990 to 1997, with spending in 1997 on herbal supplements estimated at 5.1 billion dollars (Mahady, 2001). In the United States it is estimated that Americans pay out of pocket $10.3 billion annually for a variety of alternative therapies such as acupuncture, massage, chiropractic services and herbal dietary supplements (Eisenberg et al., 1993). It is only within the last twenty years that clinical research has evaluated the effectiveness and potential harmful effects of dietary supplements.

Phytoestrogens are plant derived supplements that have estrogen-like properties. The phytoestrogens usually consists of three main classes: the isoflavones, lignans, and the coumestans (Turner, Rickard, Spelsbery, & Sibonga, 2003). Phytoestrogens are believed to exert their estrogen-like effect by binding to the two subtypes of estrogen receptor, ERα and ERβ, but preferentially to the ERβ, and producing an estrogen like effect in the body similar to, but less potent than, estradiol (Kuiper et al., 1998). Several studies evaluating the effect of phytoestrogens on the primary complaint of the menopause, the hot flash, have shown conflicting results. The difficulty with evaluating the effect of the phytoestrogens is that phytoestrogen products are often unregulated, specifically in the United States, and may contain numerous different ingredients such as two or more phytoestrogens and a variety of fillers.

Several phytoestrogens belonging to the isoflavones category such as genistein and daidzein found in soy products, have been found to have bone protective properties in ovariectomized rats, an animal model for postmenopausal bone loss (Setchell, 1998). A study comparing the soy product genistein with Raloxifene and estradiol in vitro on cultured rat bone marrow cells showed all products decreased the number of osteoclasts present with no
statistically significant difference in osteoclast number between the groups (Sliwinski, Folwarzna, Janiec, Grynkiewicz, & Kuzyk, 2005). Research continues on the many variations of soy products currently available as researchers look for alternative methods for treating and preventing osteoporosis.

Black cohosh is a perennial plant found in the eastern United States and Canada and is formally called *Cimicifuga racemosa* or *Actaea racemosa*. Black cohosh was first mentioned in writing in 1749 by Carl von Linne as a treatment for female problems such as dysmenorrhea and menopausal complaints (Upton, 2002). Black cohosh has been used extensively in Germany for many years, and more recently across the globe for the relief of the hot flashes associated with menopause. It has been suggested that since the herb has the estrogenic activity necessary to relieve hot flashes, it may also have a similar estrogenic activity on bone, a decrease in bone remodeling. Several studies using rat models and biochemical markers of bone resorption and formation have shown promising evidence that black cohosh can reduce bone remodeling (Niblein & Freudenstein, 2003; Seidlova-Wuttke et al., 2003; Wuttke et al, 2003). Wuttke and colleagues (2006) found that the use of black cohosh in postmenopausal women in Germany induced an increase in osteoblast activity and further studies were recommended. A review of the literature shows a need for more randomized double blind, placebo-controlled clinical trials of the herb black cohosh to evaluate the effect it has on bone metabolism and remodeling in humans.

**Rationale for Further Research**

Osteoporosis is a condition which research has shown is treatable and possibly preventable. It does not have to be the end result of the aging process for postmenopausal women. Current pharmacological treatments exist which can prevent the bone loss that occurs with osteoporosis, and the resulting fractures. Estrogen, bisphosphonates, and SERMs are
currently well accepted treatment options. However, women are also looking for more convenient, natural and less risky treatment options such as over the counter dietary supplements.

Americans are using more dietary supplements, however little clinical evidence exists to substantiate claims made by the companies marketing these products. Recently, black cohosh has been shown clinically to relieve the vasomotor symptoms of the menopause. The exact mechanism of action of black cohosh remains unclear. However, it has been shown that phytoestrogens do bind with estrogen receptors in the targeted cell, and produce estrogenic-like activity. It is possible that black cohosh may activate an unknown receptor to produce the positive effect on vasomotor symptoms. Bone has estrogen receptors and may be a site for the estrogen-like effect of black cohosh to exert an influence. Few studies have been done which address the effect black cohosh may have on bone remodeling and most are in animals. Only one published study known to this researcher has addressed the possible effect on bone remodeling in postmenopausal women and further testing was called for. It is clear that well designed studies are needed to address the effect of black cohosh on bone remodeling.

In conclusion, millions of women are using over the counter dietary supplements and black cohosh is one of the most popular supplements in postmenopausal women. Women often look for a more natural option when attempting to treat and deal with the symptoms associated with menopause. It is well known by health care providers that bone remodeling increases in postmenopausal women after estrogen withdrawal. However, few postmenopausal women are concerned with their bone health. Bone health should be an important issue for both postmenopausal women and their health care providers. Postmenopausal women have been shown to have an increased and an imbalanced bone remodeling which causes a loss of bone
mass. This loss of bone mass which results in fragile bones makes women more prone to fractures of the hip, vertebrae, and wrist. The increased morbidity and mortality of osteoporotic fractures is unacceptable if treatment options exist. However, as previously stated the women themselves are looking for alternative options. It is imperative that herbal products undergo more rigorous testing, and the results be made available to women so they may make an informed and educated decision. Future research into black cohosh should investigate the effect on bone health, long term usage, and adverse effects.

**Research Purpose**

The purpose of this study was to determine if a standardized (2.5% triterpene glycoside) commercial preparation of the herb black cohosh will alter bone remodeling in the postmenopausal female. Bone remodeling is a two step physiologic process which includes bone resorption and bone formation, and can be measured with commercially available biochemical bone markers. A biochemical marker of bone resorption, serum C-terminal telopeptide (CTX), and a biochemical marker of bone formation, serum osteocalcin, were measured at the initiation of the study and again after 12 weeks of therapy with the black cohosh supplement.

**Research Hypotheses**

**Hypothesis 1:** Postmenopausal women taking a standardized 40 mg (2.5% triterpene glycoside) dose of an oral black cohosh supplement daily for 12 weeks will show a decrease from baseline in the level of serum C-terminal telopeptide, a biochemical marker of bone resorption.

**Hypothesis 2:** Postmenopausal women taking a standardized 40 mg (2.5% triterpene glycoside) dose of an oral black cohosh supplement daily for 12 weeks will show a decrease from baseline in serum osteocalcin, a biochemical marker of bone formation.
CHAPTER 2
REVIEW OF THE LITERATURE

Introduction

In the United States it is estimated that over ten million Americans suffer from osteoporosis, with 80% of those Americans being postmenopausal women (National Osteoporosis Foundation, 2005). In addition, approximately 28 million more Americans are estimated to have low bone mass, which puts them at increased risk for developing osteoporosis (National Institutes of Health, 2000). The estimated costs associated with osteoporosis and the resulting fractures in 2002 were $18 billion, and that number is expected to increase as Americans grow older and live longer (NOF, 2005). The risk of a woman developing osteoporosis and experiencing a fracture increases during the postmenopausal years, and with advancing age. Nearly 30 million American women are menopausal, and that number is expected to rise as large numbers of women born during the baby boom generation are entering and completing the menopause transition (Theroux & Taylor, 2003). Osteoporosis is the underlying cause for over 1.5 million fractures a year including fractures of the hip, vertebrae, and wrist (NOF, 2005). Fractures that result from osteoporosis are associated with increased morbidity and mortality, with statistics revealing 20% of Americans with a hip fracture will die within one year, 28% will require long-term care, and 50% will be unable to walk without the assistance of a walker (NIH, 2005).

These alarming figures present the need for future research to thoroughly understand the pathophysiology of osteoporosis, develop better treatments, and finally to better educate health care providers and the public about the risk factors and lifestyle choices which can prevent, or accelerate, the bone loss that occurs with osteoporosis and its precursor osteopenia. The fractures of osteoporosis and the resulting increase in morbidity and mortality can and should be
prevented. Osteoporosis and bone fractures do not necessarily have to be a consequence of aging.

Bone is living dynamic connective tissue which has the ability to constantly repair and rebuild itself. It also provides structure for the body, acts as a reservoir for important minerals such as calcium and phosphorus, and contains specialized tissue in its core for the production of blood cells essential for human life (Ferguson, 2004). Bone tissue responds to a variety of stimuli, both internal and external. Therefore treatments for a bone condition such as osteoporosis are varied. Lifestyle changes such as exercise, appropriate diet, discontinuing smoking and heavy alcohol use, and supplementing the diet with the appropriate vitamins and minerals are all external stimuli proven to affect bone tissue. Internal stimuli such as hormones, cytokines, and mechanical stress also affect bone tissue. To further osteoporosis research an understanding of the physiology of bone, the pathogenesis of postmenopausal osteoporosis and the current and future treatment options is necessary.

**Bone Formation and Structure**

The human body is composed of 206 bones which come in two different architectural forms. Cortical or compact bone forms the outer layer of all bones and accounts for 80% of the human skeleton. Cortical bone is dense and solid, and is the main component of long bones such as the humerus and femur (Barrett & Barrett, 2005). Trabecular or cancellous bone accounts for the remaining 20% and is present in the interior of the long bones and the vertebrae. Trabecular bone is not solid and dense, but is instead composed of a series of lattices and arches (Barrett & Barrett). Though trabecular bone accounts for only 20% of the bone in the human skeleton, the lattice like structure allows for a higher surface to volume ratio, and a bone turnover rate much higher than cortical bone (Barrett & Barrett).
Bone is developed through a process called ossification, and there are two types of ossification: intramembranous and endochondral (Saladin, 2004). The flat bones of the skull, parts of the mandible, and the clavicle or collarbone are formed by intramembranous ossification (Jee, 1999). The bones of the arms, legs, vertebrae, and the pelvic bones, specifically a large majority of cancellous bone, are formed by endochondral ossification. Intramembranous ossification occurs without formation of a cartilage scaffold prior to mineralization, whereas endochondral ossification utilizes a hyaline cartilage model for formation (Jee, 1988).

Intramembranous ossification begins during the embryonic period when mesenchymal cells develop into a sheet of soft tissue with a large supply of blood vessels. Cells from this sheet of mesenchymal connective tissue differentiate into osteogenic cells and form a collection of trabeculae (Saladin, 2004). The osteogenic cells continue to develop near the newly forming trabeculae and become osteoblasts. The osteoblasts deposit osteoid tissue, and the trabeculae begin to connect and develop the primary spongiosa, or cancellous bone (Jee, 1988). The osteoblasts continue to deposit bone within the primary spongiosa, filling in the spaces, and the initial cancellous bone becomes compact or cortical bone. The endosteum is formed when the mesenchymal cells and the inactive osteoblasts on the endosteal inner surface become the endosteum. The periosteum is formed when the connective tissue remaining on the outer surface condenses and becomes fibrous (Saladin). The large supply of blood vessels within the cancellous bone will eventually become the haematopoietic tissue of the bone marrow (Sims & Baron, 2000).

Endochondral ossification begins with the formation of a cartilaginous model or scaffold of the future bone. Mesenchymal cells develop into chondroblasts which secrete a cartilage-like matrix. The chondroblasts become surrounded by newly formed matrix and then become
chondrocytes. The chondrocytes, or cartilage cells, continue to grow and eventually the matrix calcifies (Sims & Baron, 2000). Calcification begins in the center of the cartilage scaffold and progressively moves to the ends of the bone as row after row of layered chondrocytes deposit a matrix which then calcifies. After calcification of the cartilage matrix, blood vessels bring osteoclasts and osteoblasts into the center of the cartilage scaffold which replace the cartilage with woven bone. This woven bone will then be replaced with lamellar bone, the main form of bone in the adult skeleton (Jee, 1999, Sims & Baron).

Eventually two architecturally and functionally different types of bone are formed in the human skeleton, cortical or compact and trabecular or cancellous. Cortical bone accounts for 80% of the bone of the human skeleton and cancellous bone accounts for only 20% of the skeleton (Barrett & Barrett, 2005). Cortical and cancellous bone are functionally different, with cortical bone providing protection of the soft internal organs and mechanical support, and cancellous bone providing the metabolic functions (Marks, Jr. & Hermey, 1996).

Architecturally, cortical bone is solid and dense and cancellous bone is composed of lattices, arches, and plates.

Cortical bone forms the outer surface of bone and is solid and dense. The majority of cortical bone is in the shaft of the long bones. Cortical bone is composed of bone units called osteons, which consist of a central Haversian canal and surrounding lamellae (Jee, 1988). In cortical bone the osteon is 200 μm in diameter and 10-20 mm long with surrounding concentric lamellae 3-7 μm thick (Athanasiou, Zhu, Lanctot, Agrawal, & Wang, 2000). Cortical bone accounts for 33% of bone surface and has a slower bone turnover rate then cancellous bone (Jee, 1999).
Cancellous bone is in the interior portion of bone and consists of a series of connecting trabeculae which form cavities that are then occupied with bone marrow (Jee, 1988). Cancellous bone has a lattice-like appearance, is very porous, and accounts for 67% of bone surface. This lattice-like structure and the larger bone surface accounts for a higher surface to volume ratio, eight times higher than cortical bone (Jee, 1999). Since bone remodeling takes place on the bone surface, cancellous bone has a higher turnover rate. Trabeculae are usually approximately 100-300 μm thick and spaced approximately 300-1,500 μm apart (Athanasiou, Zhu, Lanctot, Agrawal, & Wang, 2000).

Bones do not contain cortical and cancellous bone equally. Prime examples are the long bones such as the femur, humerus, and the ulna compared with the vertebrae of the spine. A long bone consists of three distinct areas: diaphysis, metaphysis, and the epiphysis. The diaphysis is the central shaft of the bone and is composed primarily of cortical bone. The two ends of the bone, the epiphyses, are composed primarily of cancellous bone with a covering of cortical bone. The metaphysis, the portion of the bone which connects the epiphysis to the diaphysis, is also primarily cancellous bone with a cortical bone shell (Jee, 1999). The ulna, a long bone in the forearm, is 92% cortical bone and 8% cancellous bone compared with the spinal vertebrae which is 62% cortical and 38% cancellous bone (Jee).

All bone, whether cortical or cancellous, is composed of multiple layers of lamellae, and a system of connecting channels and canals. Lamellated bone is composed of collagen fibers that are arranged in either a parallel (cancellous) or concentric (cortical) pattern, which assures the highest density of collagen per unit, making lamellated bone much stronger than woven bone (Sims & Baron, 2000). Lamellated bone replaces woven bone at approximately two to three years of age (Jee, 1999).
In cortical bone there are three distinct lamellae patterns: circular, circumferential, and interstitial (Jee, 1988). Circular rings of lamellae surround a central canal called the Haversian canal containing nerves and vessels responsible for carrying nutrients from the bone marrow and periosteum to cortical bone. Connecting one Haversian canal to another are canals called Volkmann’s canals, which are differentiated from Haversian canals by their lack of surrounding lamellae (Jee). Lacunae, small spaces containing an osteocyte, are consistently spaced throughout the lamellated tissue and are connected by channels called canaliculi. This arrangement of lacunae, canals, and channels make it possible for the bone tissue to have access to a constant supply of essential nutrients and fluids. The entire system is referred to as either an osteon or Haversian system. Circumferential lamellae are on the outer and inner surfaces of cortical bone beneath the periosteum and endosteum, respectively, and can extend uninterrupted around the Haversian systems. Interstitial lamellae are remains of circular and circumferential lamellae which fill the spaces between the Haversian systems (Jee).

Cancellous bone consists of trabecular packets and interstitial lamellae. The trabecular packet is comparable to the osteon or Haversian system. The trabecular packets are sheets of parallel lamellae layered one atop the other forming a series of interconnected trabeculae. Interstitial lamellae fill the spaces in the trabeculae packet. The spaces between the trabecular packets are occupied by bone marrow. Osteocytes receive nutrients and fluid via diffusion through canaliculi which extend from the osteocyte to the trabecular surface (Jee, 1988).

Bone is composed of approximately 60% mineral, 30% collagen matrix, and 10% water (Athanasiou et al., 2000). Bone mineral, in the form of small crystals, is situated in and around the collagen fibers and is primarily hydroxyapatite. Hydroxyapatite, an inorganic salt composed primarily of calcium, phosphorus, and a hydroxide, is responsible for the hardness and strength
of the bone (Jee, 1999). Mineralization occurs when hydroxyapatite crystals attach to the collagen matrix and are bound together by specific binding proteins, developing into mature bone (Ferguson, 2004). Mature bone is the main reservoir for calcium in the human body, with 99% of the calcium found in bones (Ferguson). The organic matrix consists of type I collagen and several other non-collagenous proteins. Ninety percent of the organic material of bone is type I collagen and the remaining ten percent are the non-collagenous proteins such as osteocalcin, osteonectin, and osteopontin (Jee).

**Bone Cells**

Four types of bone cells are most recognized as being important to bone metabolism. The four cells are: bone lining cells, osteoclasts, osteoblasts, and osteocytes (Jee, 1988). Bone lining cells are attached to mature inactive bone, and are thought to be derived from osteoblasts that have become inactive. They are flat elongated cells attached directly to the bone surface. Their precise role on the cell surface is unclear, but these cells are thought to play a role in calcium homeostasis (Jee).

Osteoclasts are large, multinucleated bone cells which are responsible for bone resorption (Jee, 1988). Osteoclasts are derived from haematopoietic stem cells which originate from the phagocytic cell line (Ferguson, 2004). Osteoclasts line the surface of bone, though they are not actively resorbing bone all the time. Activation of osteoclasts must occur, and several substances such as parathyroid hormone, vitamin D, and prostaglandins have been proven to activate osteoclasts to begin resorption (Ferguson). The osteoclast forms a seal on the bone surface called the sealing zone, allowing the ruffled border to begin resorption of bone, forming a crater called a Howship’s lacuna (Jee). Resorption of calcified bone and creation of the Howship’s lacuna is possible because the osteoclast is equipped with specialized sodium pumps, ion exchangers, and specialized lysosomal enzymes (Sims & Baron, 2000). After the formation of
the Howship’s lacuna, the osteoblasts move in to begin the bone formation phase of the remodeling process.

Osteoblasts are the cells responsible for bone formation and they secrete a bone matrix which contains collagen and mucopolysaccharides (Ferguson, 2004). Type I collagen accounts for 90% of the bone matrix secreted by the osteoblast. Osteoblasts secrete numerous non-collagen proteins such as osteocalcin, osteonectin, and prostaglandins, with osteocalcin the predominant protein. Osteocalcin is responsible for one percent of the bone matrix and potentially plays a role in calcium binding and mineralization (Sims & Baron, 2000). Serum osteocalcin is currently used as a biochemical marker for osteoblast activity. As with osteoclasts, osteoblasts also contain receptors for substances such as parathyroid hormone, vitamin D, prostaglandins, glucocorticoids, and insulin (Jee, 1988). Osteoblasts are cuboidal in shape, attached to the bone surface in a single layer, and possess microtubules allowing the osteoblasts to communicate with each other and receive nutrients essential for cell life (Ferguson).

Osteocytes are mature bone cells and originate from osteoblasts. A single osteocyte is present in a lacuna surrounded by newly formed bone, and communicates with other osteocytes via gap junctions (Jee, 1988). The osteocyte has a number of channels which allow the flow of nutrients, fluids and wastes among several osteocytes (Ferguson, 2004). In addition, osteocytes are “strain sensors” and when a bone is strained the osteocyte is thought to send a message to the osteoblasts on the surface to begin forming bone (Saladin, 2004).

**Bone Remodeling**

Bones are constantly changing to meet the needs placed on them by humans. The process of building new bone and repairing existing bone is called remodeling. Remodeling occurs throughout the skeleton at many different sites and is ongoing. Bone remodeling is the job of a specialized group of cells called bone remodeling units, which are present on the surface of
trabecular bone and in resorption tunnels in cortical bone (Jee, 1988). The major purpose of the bone remodeling unit is to replace the old bone with new, through resorption of the old bone and formation of new bone. Bone remodeling should be in constant balance with formation equaling resorption. When the balance of bone remodeling is skewed in one direction or the other, disease processes occur, such as osteoporosis when resorption exceeds formation and osteopetrosis where formation exceeds resorption (Key & Ries, 2002).

Bone remodeling occurs differently in cortical bone than trabecular bone. Cortical bone remodeling is done through a resorption tunnel within bone. The osteoclasts form a cone shaped resorption tunnel by removing the existing bone and Haversian system. The resorption tunnel is composed of three zones: cutting zone, reversal zone, and closing zone (Jee, 1988). The cutting zone is composed of a layer of osteoclasts which form a space called a resorption cavity. Immediately behind the cutting zone is the reversal zone. The reversal zone is an area where the osteoclasts have completed the process of resorption, but formation has not yet started (Jee). The resorption tunnel is then closed by a layer of osteoblasts forming a new layer of mineralized bone tissue, thereby closing the resorption tunnel. A new Haversian system with new bone tissue has now replaced the old Haversian system (Jee).

In trabecular bone the resorption process is faster and occurs on the bone surface (Ferguson, 2004). There are two proposed types of trabecular bone remodeling, one where formation does not begin until resorption is completed, and another in which formation begins while resorption is still occurring (Jee, 1988). In trabecular bone remodeling a pit or lacuna is produced on the bone surface by the osteoclasts, then osteoblasts replace the osteoclasts, and finally new bone is formed (Riggs & Melton, 1992).
Remodeling begins when the osteoclasts have been activated and are in place on either the trabecular bone surface or in the interior of cortical bone. The exact mechanism for the activation of osteoclasts and the site selected for the remodeling process remains unclear. It is hypothesized that microfractures in the bone alter the osteocytes around the fracture, and these osteocytes send a signal to the osteoclast precursors (Vaananen, 2005). It has also been suggested to work in the opposite manner with healthy osteocytes sending a signal to inactivate osteoclasts, and when this signaling mechanism stops the osteoclasts begin to move toward that area to begin the remodeling process (Vaananen). The answer may be a combination of factors or one specific factor, but further studies are called for.

Once a signal has been received, osteoclasts move toward the area for remodeling and a resorption zone is established. Three separate domains are recognized in osteoclasts: the ruffled border, the sealing zone, and a functional secretory domain. The ruffled border is the actual area where the breakdown of the hydroxyapatite surface and the collagen occurs, and its formation is the first step in the resorption process (Vaananen, 2005). The sealing zone is responsible for anchoring the osteoclast to bone. The functional secretory domain is responsible for ridding the cell of the byproducts of bone degradation. The byproducts of degradation are moved through the cell by endocytosis and transported by a transcytotic pathway, where they are then secreted into the extra-cellular fluid (Wang, Miller, Kopeckova, & Kopecek, 2005).

Once a sealing zone and a ruffled border are established HCL acid is released onto the surface of the bone and resorption begins. In order for the osteoclasts to have a steady supply of HCL, proton pumps and chloride channels are present in the cell membrane. The hydrogen ion is provided via the carbonic anhydrase reaction converting carbonic acid to H+ and bicarbonate. The bicarbonate is exchanged for chloride through a bicarbonate/chloride exchanger located in
the membrane of the osteoclast. Mitochondrial-provided ATP pumps the H⁺ ion toward the ruffled border where it combines with the chloride ion forming HCL. The HCL then begins the process of breaking down the hydroxyapatite surface and collagen fibers (Vaananen, 2005).

The byproducts of bone resorption must be removed from the cell in order for resorption to continue. Cathepsin K, a protease, is responsible for digesting the calcium, phosphate and collagen fibers produced by the resorption process. These byproducts are then removed from the cell and secreted into the extra-cellular fluid (Wang et al., 2005).

Once resorption has been completed the osteoclasts disappear leaving a reversal zone. The edges of the resorption pit are then smoothed over and a cement line is formed (Jee, 1988). The next step is new bone formation and that is the function of the osteoblasts. Bone formation is a multi-step process and the first step is the formation of the bone matrix, the osteoid. The osteoblasts secrete a number of substances in the production of the bone matrix. These substances include osteocalcin, type-I pro-collagen peptides, and bone specific alkaline phosphatase (Srivastava et al., 2005). After the bone matrix is deposited, mineralization begins and is completed in two steps: primary and secondary mineralization. Primary mineralization occurs approximately 5-10 days after hydroxyapatite crystals are deposited between the organic matrixes. Secondary mineralization is a much slower, gradual process and increases the bone mineral density by increasing the number of hydroxyapatite crystals and the crystal size, but not the volume of the new bone (Boivin & Meunier, 2002).

Bone formation and bone resorption are usually well balanced, or coupled in the adult providing they are healthy, have good nutrition, are active, and exercise. Bone remodeling is a continuous process and necessary for normal bone growth and repair. Bone mass in males and females, peaks at approximately 30 years of age. Normal healthy adults will usually lose a small
amount of bone throughout their lives, about 0.4% per year after age 30. However, postmenopausal women have increased bone loss secondary to an imbalance in bone remodeling, and lose approximately 1% to 2% of bone a year for the first 5-8 years if there is no intervention to prevent bone loss (Srivastava et al., 2005). Bone loss occurs when bone resorption exceeds bone formation. This is exactly what occurs in the condition known as postmenopausal osteoporosis.

**The Role of Cytokines in Bone Remodeling**

Cytokines are small size proteins secreted by several different types of cells such as macrophages, leukocytes and mast cells (Saladin, 2004). Cytokines have many roles in the human body, with the regulation of bone metabolism being only a small part of their function. Interleukins, tumor necrosis factor, and transforming growth factor β are the cytokines most often involved with bone metabolism.

Receptor activator of NF-κB (RANK) and receptor activator of NF-κB ligand (RANKL) are members of the tumor necrosis factor (TNF) family, and play a part in osteoclast differentiation and osteoclast resorption respectively (Eqbal, Inzerillo, Moonga, & Zaidi, 2003). RANKL is produced by osteoblasts and stromal cells and is necessary for osteoclast development. RANKL stimulates bone resorption by interacting with the RANK receptor on the surface of the preosteoclast. RANKL can also bind to the decoy receptor osteoprotegerin, which inhibits osteoclast formation and bone resorption (Khosla, 2001; Reddy & Roodman, 2004).

Osteoprotegerin, a glycoprotein produced by osteoblasts, blocks the differentiation of the osteoclast by antagonizing RANKL and interfering with the signaling pathway necessary for cell differentiation (Blair, Robinson, & Zaidi, 2005). A fine balance between the effects of RANKL and osteoprotegerin (OPG) is necessary to control the bone resorption process. Several substances have been identified as having either a stimulatory or inhibitory effect on RANKL
and OPG. TNFα, IL-1, and vitamin D increase the expression of RANKL. Parathyroid hormone (PTH) and glucocorticoids increase the expression of RANKL and decrease the production of OPG, which promotes osteoclastogenesis and bone resorption (Khosla, 2001; Rubin et al., 2002). TGF-β increases production of OPG and stimulates RANK, and estrogen increases production of OPG and interferes with RANKL signaling, thereby inhibiting osteoclastogenesis (Khosla; Rubin et al.).

TNF α and β are both potent stimulators of bone resorption, are produced by osteoblast-like cells, and their release appears to be influenced by the presence of estrogen (Reddy & Roodman, 2004). Turner and colleagues (1994) found that estrogen inhibited the release of TNFα in cultured bone cells in vitro. When estrogen was reduced, osteoclast numbers were increased, and when estrogen was replaced the osteoclast number decreased.

Interleukin 1 (IL-1) and Interleukin 6 (IL-6) are potent stimulators of bone resorption, produced by a variety of cells such as monocytes, macrophages, and stromal cells, and their effects appear to be mediated by estrogen (Reddy & Roodman, 2004). Experiments done on transgenic mice in which the IL-6 gene was eliminated showed that mice without the IL-6 gene showed no bone loss after removal of the ovaries, as opposed to the normal mice which showed considerable bone loss after ovariectomy (Turner, Riggs, & Spelsberg, 1994). IL-6 stimulates osteoclast differentiation, and its expression may be stimulated by IL-1, parathyroid hormone (PTH), TNFα, and vitamin D, while estrogen inhibits its expression (Eqbal et al., 2003). IL-1 is produced by a number of cells including osteoclasts, and it stimulates an increase in osteoclast precursors as well as stimulating mature osteoclasts to begin bone resorption (Reddy & Roodman). IL-4 inhibits osteoclast activity thereby decreasing bone resorption; however some studies show that high levels of IL-4 may also inhibit bone formation (Reddy & Roodman). IL-7
is a potent cytokine responsible for increasing the osteoclast population in the absence of estrogen. Weitzmann and colleagues (2002) found that in the absence of estrogen, IL-7 had an increased presence in the bone marrow resulting in an increase in osteoclastogenesis.

Transforming growth factor β (TGF-β) is secreted by both osteoblasts and osteoclasts, and is an osteoblastic stimulator and an osteoclastic inhibitor (Reddy & Roodman, 2004). TGF-β works in an autocrine manner to increase osteoblast cell differentiation and proliferation, inhibit osteoclast proliferation, and induce osteoclast apoptosis through the protein osteoprotegerin (Eqbal et al., 2003). Turner and colleagues (1994) reported that the presence of estrogen increased the levels of TGF-β in cultured bone cells. Reddy and Roodman reported that very low levels of TGF-β increased osteoclast formation and bone resorption in cultured bone cells.

**Role of Estrogen in Bone Remodeling**

Three forms of estrogen are present in the female human body: estradiol, estrone, and estriol. The most potent form of endogenous estrogen in the female is estradiol, which is carried by the blood and produced mainly by the theca and granulosa cells of the dominant follicle in the female ovary through steroidogenesis of cholesterol (Jones & DeCherney, 2005). As a woman ages the pool of potential follicles diminishes, and the primary source of estradiol is lost. The endocrine changes that occur as a woman ages include decreasing ovarian estrogen production and increased pituitary follicle stimulating hormone. This endocrine transition is referred to as the menopause or the climacteric. Menopause is defined as the cessation or the absence of the menstrual period for one year; however menopause is actually a multisystem transition that occurs in the lives of all middle aged women (Greendale, Lee, & Arriola, 1999). The average age of menopause is 48 to 55 years of age. Current estimates for the year 2000 are that there are 46 million postmenopausal women in the United States, and that number is expected to reach 50 million in the year 2020. In 1998 it was estimated that there are more than 477 million
postmenopausal women worldwide and that number is expected to reach over one billion by the year 2025 (The North American Menopause Society, 2001). As the life expectancy of women increases, a large majority of women will spend one quarter to one third of their lives in menopause. During the menopause years the potent estrogen estradiol is lost, and the primary estrogen becomes estrone, a much weaker estrogen with no discernable estrogenic effects (Jones & DeCherney). It has long been understood that estradiol has protective effects on bone, and the loss of these protective effects in the menopausal years leads to an increase in bone loss and potentially osteoporosis.

Estrogen plays a vital role in the preservation of bone by maintaining a balance between bone resorption and formation, and by protecting cancellous bone from excessive remodeling (Turner et al., 1994). Estrogen also regulates many cytokines that are responsible for osteoclast formation and activation such as IL-1, IL-6, IL-7, TNF, and RANKL and exogenous estrogen replacement has been shown to decrease the production of these cytokines (Uemura et al., 2005).

To understand the effect of estrogen on bone it is necessary to understand how estrogen enters and affects a target organ. Estrogens have the ability to move in and out of cells by simple diffusion, but the action of estrogen is dependent on the presence and activation of an estrogen receptor in the nucleus of the targeted cell (Barrett, 2005). There are two estrogen receptor subtypes, ER\textsubscript{α} and ER\textsubscript{β}, which are distinctive from each other and located on different chromosomes (Bord, Horner, Beavan, & Compston, 2001). Bord and colleagues found that both ER\textsubscript{α} and ER\textsubscript{β} were present in human bone, with ER\textsubscript{α} present in higher concentrations in cortical than cancellous bone, and ER\textsubscript{β} present in higher concentrations in cancellous bone than cortical bone. The presence of distinctive and entirely different ERs suggests that each receptor may have a different function in the estrogen modulation of bone remodeling. Once the hormone has
bound to a specific estrogen receptor in the nucleus, the receptor then becomes a specific transcription factor which will bind with a specific hormone response element (HRE) resulting in gene transcription (Igarashi, 2005). Estrogen receptors, both ERα and ERβ, have been identified in the three most important bone cells: osteoclasts, osteoblasts, and osteocytes (Vaananen & Harkonen, 1996).

Estrogen is necessary to maintain bone mass. The presence of estrogen maintains a balance between bone resorption and formation by suppressing bone remodeling. Estrogen has been demonstrated to decrease osteoclast formation, activation, and lifespan and has been suggested to increase osteoblast formation (Riggs, Khosla, & Melton, 2002). The loss of circulating estradiol, the most potent estrogen, causes an estrogen deficiency which then causes an imbalance in bone remodeling. Without estrogen a rapid loss of bone occurs when bone resorption is markedly increased and bone formation is only slightly increased. Riggs and colleagues found that when estrogen levels decreased in menopause there was an increase in bone biochemical markers, however the increase was not equal. Biochemical markers of bone resorption increased by 90% at menopause; however biochemical markers of bone formation only increased by 45%, half as much. This inequality leads to a remodeling imbalance which in turn leads to bone loss, structural weakness, and perforation of the bone, specifically the cancellous bone (Vaananen & Harkonen, 1996).

The substantial decrease in circulating estradiol decreases the regulatory effect that estrogen exerts over cytokines. Cytokines have been shown to increase osteoclast activity and promote bone resorption. The most potent cytokines in bone resorption IL-1, IL-6, and TNF-α have been shown to be modulated by endogenous estrogen (Pacifici, 1998). The removal of the regulating effect of estrogen on cytokines increases their expression, therefore increasing
osteoclast differentiation and activation. This in turn increases a skewed form of bone remodeling with resorption exceeding formation. This imbalanced bone remodeling leads to a loss of bone resulting in more fragile bones which are prone to fracture. This condition is commonly called postmenopausal osteoporosis.

**Effect of Calcium on Bone Remodeling**

Calcium is the most abundant mineral in the human body with 99% of the body’s calcium stored in the bones (Ferguson, 2004). Calcium is necessary for bone strength, nerve and muscle conduction, and the maintenance of a regular heartbeat (Lyon & Sutton, 1993). Calcium is the primary mineral of hydroxyapatite, an inorganic salt which is responsible for the hardness and strength of bone (Jee, 1999). Calcium is released from the bone during resorption, and then replaced in the form of hydroxyapatite by the osteoblasts during formation (Parfitt, 1993). Serum levels of calcium are regulated by two hormones: parathyroid hormone and calcitonin. Parathyroid hormone is secreted when serum levels of calcium are low and calcitonin is secreted when serum levels of calcium are high (Barrett & Barrett, 2005).

Vitamin D is a fat soluble vitamin that is primarily stored in the body fat. The primary form of vitamin D is the dihydroxylated metabolite of vitamin D₃, Calcitrol (1,25(OH)₂D₃). Vitamin D is synthesized in the skin when the skin is exposed to ultraviolet light, mainly in the form of sunlight. Vitamin D acts mainly on the kidney, bone and intestine to regulate serum calcium under the influence of parathyroid hormone (Barrett & Barrett, 2005). Vitamin D promotes absorption of calcium from the intestine, increases calcium reabsorption in the kidney, and has both an indirect and direct effect on bones (Barrett & Barrett). The immediate direct effect of vitamin D is to obtain calcium from the bones by promoting the development of mature osteoclasts, thereby increasing bone resorption. The indirect effect of vitamin D is the enhanced absorption from the intestine and reabsorption from the kidney, creating an overall surplus of
serum calcium, promoting storage of calcium in the bone, and bone mineralization (Barrett & Barrett).

Aging decreases the intestinal absorption of both dietary calcium and vitamin D, resulting in decreased available stores and the necessity for mobilizing calcium from other stores, mainly the bones (Lyon & Sutton, 1993). In addition, advanced age limits or prevents women from being outdoors, resulting in decreased vitamin D stores. The deficiency of both calcium and vitamin D increases net bone loss, resulting in a loss of bone mineral density and increased risk of fractures.

Dawson-Hughes and colleagues (1990) studied the effect of calcium supplementation in postmenopausal women in a double-blind, placebo-controlled trial. It was noted that calcium supplementation showed no difference in bone loss from the spine in women who were less than five years postmenopausal. Women who were six or more years postmenopausal showed less bone loss than women on placebo. In 1997 Dawson-Hughes and colleagues looked at the effect of calcium and vitamin D supplementation in men and women over age 65 and found reduced bone loss in the spine and femoral neck, as well as a reduced incidence of nonvertebral fractures.

Riggs and colleagues (1998) investigated the effect of long term calcium supplementation in postmenopausal women and found that supplementation decreased the age-related increase in serum parathyroid hormone and bone resorption, and decreased bone loss. A meta-analysis done by Kanis (1999) revealed that calcium supplementation slows bone loss but is not effective at rebuilding bone, and the effect is more pronounced in older women than women at the beginning of menopause. It is generally accepted that supplementation with calcium and vitamin D will not prevent postmenopausal bone loss in trabecular bone but can slow age related bone loss in cortical bone (Dawson-Hughes, 2000). Flynn (2003) concluded in a meta-analysis that calcium
and vitamin D supplementation reduced the incidence of nonvertebral fractures and reduced morbidity in elderly men and women. Prince and colleagues (2006) found that calcium supplementation with calcium carbonate reduced the risk of an osteoporosis-related fracture in women who were at least 80% compliant with the medication regimen. However, this large study (n=1460) showed that the majority of the women in the study were not compliant with the recommended dosage of calcium carbonate due to the side effects. Therefore this is not suggested as a form of single therapy for the prevention of osteoporosis-related fractures. It is currently recommended that women not on estrogen replacement take calcium 1500 mg/day and Vitamin D 400 IU/day (Goldfeder, 2005; Luckey, 1999; Lyon & Sutton, 1993).

**Postmenopausal Osteoporosis**

Menopause is a natural midlife transition that all older women will experience in their lifetime. Currently in the United States there are an estimated 46 million women who are postmenopausal (North American Menopause Society, 2001). It is well known that menopause is due to a loss of ovarian function causing a decrease in circulating levels of estradiol, the most potent female estrogen. This loss of estrogen causes an imbalance in the bone remodeling process which favors resorption over formation. This imbalance causes a loss of bone which increases the fragility of the remaining bone. The increasingly fragile bone becomes more susceptible to fractures, specifically in cancellous bone of the hip and vertebrae. This disease in which bones are susceptible to fracture due to a decrease in bone mass and strength secondary to loss of endogenous estrogen is known as postmenopausal osteoporosis.

Adult human bone is constantly being remodeled. In adulthood, 25 percent of cancellous bone is remodeled every year while only 3 percent of cortical bone is remodeled yearly (Manolagas & Jilka, 1995). The imbalance in bone remodeling that occurs in menopause therefore affects cancellous bone more than cortical bone. Beginning in the fourth or fifth
decade of life, with the average age of menopause 48-55 years, bone loss accelerates, sometimes as much as ten times the amount of loss that occurs in premenopausal women (Manolagas & Jilka). Bone loss in cancellous bone weakens the plates and promotes the alteration of plates to the less substantial rods (Raisz, 1988). The weakened more fragile bone is then susceptible to the fractures that characterize postmenopausal osteoporosis.

In the first few years of menopause circulating estradiol levels have been shown to drop to 10-15 percent of the premenopausal level (Riggs, Khosla, & Melton, 2002). This decrease in circulating estrogen causes a loss of the regulatory effect that estrogen has on bone remodeling. Bone remodeling increases, however it is not the balanced remodeling that occurs prior to menopause. This postmenopausal remodeling favors bone resorption (Doran & Khosla, 2000). Without estrogen suppressing osteoclast formation and activation, as well as inducing osteoclast apoptosis, more osteoclasts are active on the bone surface. The suggested osteoblast formation that is promoted by estrogen is also lost (Riggs, Khosla, & Melton). Bone resorption exceeds formation in the hypoestrogenic state of menopause. In addition, the suppressive effect of estrogen on potent cytokines involved in bone resorption such as IL-1, IL-6, and TNF-α is lost and bone resorption is further increased (Vaananan & Harkonen, 1996).

The role of RANK, RANKL and OPG has recently been shown to play a role in postmenopausal osteoporosis. RANKL is produced by osteoblasts and is a membrane bound factor of the tumor necrosis factor family shown to stimulate osteoclast differentiation (Troen, 2003). RANK is a membrane bound receptor and also a member of the tumor necrosis factor family. OPG is a decoy receptor which can bind to RANKL in the place of RANK and inhibit osteoclastogenesis (Troen). Aubin and Bonnelye (2000) found that estrogen inhibits the production of RANKL and subsequently RANKL-induced osteoclastogenesis. Hofbauer and
colleagues (1999) found that estrogen increased production of the decoy receptor OPG in cell cultures of human osteoblast cells. Eghbali-Fatourechi and colleagues (2003) found that an estrogen deficit state induced the expression of RANKL promoting increased bone resorption, however they were unable to determine if it was a direct effect of the estrogen deficiency, or the increased expression of cytokines such as IL-1 and TNF-α in menopause which are also known to increase expression of RANKL.

The decreased estrogentic state of menopause also increases the sensitivity of the bone to parathyroid hormone (PTH), which is responsible for calcium homeostasis in the body. The increased sensitivity to PTH causes even higher bone resorption which leads to an increasing level of serum calcium. The body compensates by increasing the excretion of calcium through the urine and decreasing calcium absorption in the intestinal system preventing hypercalcemia (Barrett & Barrett, 2005; Riggs, Khosla, & Melton, 1998).

As women age a second cause of bone loss is identified. Approximately 10-15 years after the menopause serum levels of PTH begin to rise. Research has shown that aging impairs the intestinal absorption of calcium and the renal reabsorption of filtered calcium, causing a decrease in circulating calcium. PTH secretion increases and calcium is mobilized from the bones, where 99% of calcium is stored (Riggs, Khosla, & Melton, 1998, 2002). This increased resorption of calcium from the bones causes bone loss which results in fragile bones and increased risk of osteoporotic fractures.

The diagnosis of osteoporosis is currently done with either the presence of an osteoporotic fracture or the use of bone mineral density measurements. Biochemical markers of bone turnover are also being used to assess bone remodeling, but have not proven useful in predicting the fracture risks associated with osteoporosis, and are currently being used more in research,
specifically for testing antiresorptive drugs (Kenny & Prestwood, 2000). Bone mineral density (BMD) measurement is the current acceptable form of diagnosing osteoporosis and osteopenia. BMD can be measured in a variety of ways including single and dual energy X-ray absorptiometry (DEXA) scan, ultrasound measurement, computed tomography and radiography (Kanis, 2002). Bone mineral density has been proven to correlate well with the load bearing ability of bone and the potential risks of fracture (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001).

Dual energy X-ray absorptiometry (DEXA) scans are used to measure the mineral content of the skeleton, specifically the sites prone to fractures such as the vertebrae and the hip. The results are given in standard deviation (SD) measurements as compared to a young healthy population and the measurement obtained is called the T-score (Kanis, 2002). Osteoporosis is diagnosed when the T-score of the hip is at or below 2.5 SD of the young adult female. Osteopenia, or low bone mass, is diagnosed when the T score of the hip is between 1 SD and 2.5 SD below the young adult female. Severe osteoporosis is diagnosed when the T score of the hip is more than 2.5 SD below the young adult female or the diagnosis of at least one fracture due to bone fragility (Kanis). The major use of DEXA scans is the assessment of the mineral content of the bones and the risks of fracture. The information obtained from DEXA scans can aid the health care practitioner in treating osteoporosis prior to a fracture with antiresorptive drugs such as the commercially available bisphosphonates, hormone replacement therapy, or calcitonin.

**Biochemical Markers of Bone Remodeling**

Biochemical markers of bone remodeling are divided into two groups, markers measuring bone resorption and markers measuring bone formation. Markers commonly used to measure bone resorption are urine pyridinolines and deoxypyridolines, commonly referred to as cross-links. In addition, newer type I collagen C- telopeptides (CTx) and N- telopeptides (NTx) are
markers of resorption measured by both serum and urine sampling. Markers commonly used to measure bone formation are bone specific alkaline phosphatase (BSAP), osteocalcin, and propeptides of type I collagen, all serum markers (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001).

The collagen of mature bone is held in place by non-breakable cross-links formed primarily by amino acids. The primary cross-links in bone are hydroxylysyl-pyridinoline and lysyl-pyridinoline commonly known as pyridinoline (Pyr) and deoxypyridinoline (D-Pyr, DPD) (Ziambaras & Civitelli, 1998). When bone is broken down during the process of resorption, the collagen cross-links are liberated, released into the systemic circulation and then excreted in the urine intact, as they are not metabolized or reused in the body again (Eyre, 1994). D-Pyr is present in high concentrations in the collagen of bone and the dentin of the teeth, and since bone is the primary source of collagen in the body, it is an excellent marker for the collagen breakdown that occurs during bone resorption (Ziambaras & Civitelli). Immunoassays are available which measure the amount of free Pyr or D-Pyr concentrations in the urine and are the basis for the biochemical markers of bone resorption. In addition, immunoassays have been developed to identify the amount of the terminal fragments of the type I collagen peptide chain known as the N-terminal telopeptide (NTx) and the C-terminal telopeptide (CTx). The N-terminal of the type I collagen molecule is thought to be responsible for approximately 60% of the D-Pyr in human bone collagen and the C-terminal responsible for the remaining 40% (Ziambaras & Civitelli) making them a useful source for measuring bone resorption. These markers are not affected by a person’s diet and are specific for type I collagen; however since bone remodeling follows a diurnal pattern, they are best collected consistently and in the early morning hours (Hanley, 2000).
Studies using the C-terminal telopeptide (CTX) have shown that CTX is an excellent marker of bone resorption in osteoporosis. Chailurkit and colleagues (2001) compared numerous bone biochemical markers in evaluating the effect of hormone replacement and calcium supplementation alone and in combination and concluded that serum CTX is an excellent indicator for determining response to treatment. The effect of estrogen withdrawal has been well documented. Sornay-Rendu and colleagues (2003) used serum CTX to evaluate the effect on bone remodeling in women who were on estrogen replacement and then discontinued treatment. A rapid increase in bone remodeling was documented using biochemical markers of both bone resorption and formation, specifically serum CTX. Serum CTX was significantly lower in a study which compared the response of several bone biochemical markers to alendronate therapy, and serum CTX was declared to be the most effective bone biochemical marker used in the study (Fink et al., 2000). Recently serum CTX was utilized in evaluating the effectiveness of a once a month dose of Ibandronate (Boniva). Serum CTX was significantly decreased after three months of treatment with once-monthly Boniva (Reid, 2006). Serum CTX is unaffected by diet, but fasting has been shown to reduce the effect of the diurnal pattern of high levels in the early morning hours and low levels in the afternoon (Delmas et al., 2000). It is recommended that to reduce the effect of the diurnal pattern of serum CTX that all specimens be collected after the subject has fasted and the timing be tightly controlled, collecting specimens at the same time of the day both before and after treatment.

Mature active osteoblasts secrete several non-collagenous proteins such as osteocalcin, bone specific alkaline phosphatase and type I pro-collagen peptides, such as N-terminal PINP and C-terminal PICP, during the formation phase of bone remodeling (Srivastava et al., 2005). Osteoblasts secrete these non-collagen proteins, in addition to type I collagen, and then the newly
formed bone is mineralized in two phases when hydroxyapatite is deposited. Since formation involves the laying of both type I collagen and the non-collagenous proteins, it is possible to measure the rate of bone formation by measuring the amount of these non-collagenous proteins in the blood.

Alkaline phosphatase is an enzyme that originates from a variety of tissues in the human body, specifically the liver, intestines, spleen, bone, kidney, and placenta (Delmas et al., 2000). In the healthy adult the alkaline phosphatase in serum comes in equal parts from the liver and bone. Total alkaline phosphatase is not specific in determining the tissue of origin, but the bone specific alkaline phosphatase (BSAP) is specific to the bone tissue, and is considered a highly specific test (Delmas et al.). Bone specific alkaline phosphatase uses an antibody that recognizes and binds to a glycoprotein on the surface of the osteoblast cell known as an immunoassay. There is some cross reactivity between bone and liver alkaline phosphatase, approximately 10-20%, but it is still considered highly specific (Delmas et al.).

In the osteoblast, prior to secretion, the type I collagen is held in a triple helix formation by disulfide bonds between the C-terminal propeptides and stored in the golgi complex until it is time for secretion. After the type I collagen is secreted by the osteoblast, the N-terminal and the C-terminal peptides are removed and released into the systemic circulation, allowing them to be measured through serum assays (Hanley, 2000). These peptides are specific for newly formed type I collagen and are termed PINP for the amino N-terminus and PICP for the carboxy C-terminus of the peptide extension (Delmas et al., 2000).

Osteocalcin is a non-collagenous protein with an affinity for binding to hydroxyapatite secreted by the osteoblast. Osteocalcin is often called Gla-protein as it contains three gamma-carboxyglutamic acid residues which are believed to be responsible for the calcium binding
properties of the osteocalcin protein (Hanley, 2000). Though the exact function of osteocalcin is unclear, it is considered to be a reliable marker of osteoblast function (Delmas et al., 2000). Osteocalcin, like deoxypyridinoline, has a diurnal pattern and peaks in the early morning and is dependent on adequate kidney function for excretion (Hanley). Therefore it should not be relied on in someone with kidney disease or inadequate kidney function.

Biochemical markers are used extensively in research involved with osteoporosis. The markers used vary widely, and sometimes combinations of markers are used in the same study. Biochemical markers are used to study bone loss, risk of fracture, and the efficacy of medications such as estrogen, bisphosphonates, calcium, calcitonin, parathyroid hormone, and the phytoestrogens. Currently, in addition to dual energy X-ray absorptiometry (DEXA) scans, biochemical bone markers are a staple in osteoporosis research, and are beginning to be used in clinical practice.

It is known that bone mineral density (BMD) is different in men and women as well as different ethnic groups. African American women have been shown to have higher BMD at every site tested in DXA scans and a slower rate of bone loss from the femur and spine than Caucasian women (Aloia, Vaswani, Yeh, & Flaster, 1996). In addition to African Americans having a larger bone mass, Asians have a lower bone mass than Caucasian women, with Hispanic women similar to Caucasian women. Barrett-Connor and colleagues (2005) found that African American women had the highest BMD followed by Hispanic women, Native American women, Caucasian women, and finally Asian women. Though Asian women have the lowest BMD they have lower fracture risks then Hispanic and Caucasian women (Barrett-Conner et al.).

There is also an ethnic difference noted in biochemical bone markers. Aloia and colleagues (1996) found differences in biochemical bone markers between whites and blacks,
with black women having lower levels of bone turnover markers, and higher levels of parathyroid hormone and serum calcitriol, a metabolite of vitamin D. Han and colleagues (1997) found that postmenopausal African-American women had statistically significant lower levels of serum osteocalcin than Caucasian women, but there was not a statistically significant difference in bone specific alkaline phosphatase. Aloia and colleagues (1998) found that several indices of bone turnover were lower in postmenopausal African-American women than postmenopausal Caucasian women such as bone specific alkaline phosphatase, osteocalcin, urine hydroxyproline, urine pyridinoline cross-links, and urine N-terminal telopeptide. Gundberg and associates (2002) found that osteocalcin was lower in African-American women compared to Caucasian and Hispanic women, and bone specific alkaline phosphatase was lower in Caucasian women than African-American and Hispanic women. These studies show differences in individual biochemical bone markers dependent on race/ethnicity, which would make comparing groups with different ethnic origins more difficult. It would be more beneficial to look at an intervention tested on one specific racial/ethnic group, and then compare each racial/ethnic group to another to see how each group individually responded to the intervention.

Biochemical markers of bone remodeling have long been used in drug intervention trials. Estrogen replacement in the postmenopausal years has been shown to have statistically significant changes in biochemical markers of both bone resorption and formation. Studies have shown that with estrogen therapy both markers, serum osteocalcin and urine deoxypyridinoline, have significantly decreased, evidence that bone remodeling has decreased, a goal for the prevention of the bone loss that accompanies menopause (Castracane, 2005; Lindsey, 2002; Riggs, 2003; Watts, 2000; Zhan, 1999). Research studies on antiresorptive agents, the bisphosphonates alendronate and risedronate, have used biochemical bone markers. Serum
osteocalcin, bone specific alkaline phosphatase (BSAP), urine D-Pyr, and urine NTx all decreased in women taking alendronate (Delmas, 2000; Greenspan, 2005).

Studies involving many phytoestrogens have also used bone biochemical markers for assessing the effect of their product on bone remodeling. Yamori and colleagues (2002) found a statistically significant decrease in urine deoxypyridinoline after ten weeks of soy supplementation in postmenopausal women compared with placebo. Nikander and colleagues (2004) reported a statistically significant decrease in urine D-Pyr in the phytoestrogen soy group compared with placebo after three months of therapy, but no change in bone specific alkaline phosphatase. Changes were noted in the N-terminal and C-terminal telopeptides, markers of bone resorption, in the soy group, but they were not statistically significant.

Black cohosh, Cimicifuga racemosa, has long been grouped in the phytoestrogen category due to the fact that its exact mechanism of action was unclear. It remains to be proven if black cohosh works on estrogen receptors, dopamine receptors, or a combination of both receptor types (Viereck, Emons, & Wuttke, 2005). In ovariectomized rats, Niblein and Freudenstein (2003) found that black cohosh had significantly reduced urine Pyr and D-Pyr after nine weeks of therapy. In Germany, Wuttke and colleagues (2003) found that black cohosh has a positive influence on the bone turnover index (BSAP/Crosslaps ratio) of 62 postmenopausal women and may have implications in the prevention of osteoporosis.

**Effect of Calcium and Vitamin D Supplementation on Biochemical Bone Markers in Postmenopausal Women**

It is currently accepted as a standard of care to recommend both calcium and vitamin D supplementation to postmenopausal elderly women. Current recommendations for women not taking estrogen replacement are calcium 1500mg/day and vitamin D 400 IU/day (Goldfeder,
Biochemical bone markers have been used in the past to evaluate the effect of calcium on bone turnover, both resorption and formation.

In 1994 Blumsohn and colleagues noted that supplementation with calcium citrate in the evening hours showed a decrease in morning measurements of urine DPD and NTX, whereas supplementation of calcium in the morning hours had no effect on either urine DPD or NTX excretion the following morning. Kenny and colleagues (2004) found calcium supplementation with calcium citrate for 12 weeks decreased urinary DPD, CTX, and NTX while supplementation with calcium carbonate showed no significant change in the same biochemical markers. Ulrich and colleagues (2004) found that calcium carbonate supplementation for 18 weeks in early postmenopausal women showed no change in serum OC, BSAP, and CTX or urinary NTX. Supplementation with an effervescent Sandocal 400 mg tablets one daily in healthy young adults showed a decrease in serum CTX, demonstrating in this study that calcium can decrease bone remodeling in young adults (Sidideen & Swaminathan, 2004).

**Role of Black Cohosh (Cimicifuga Racemosa)**

Black cohosh is a native plant in North America and Canada. Black cohosh was originally used by the Native Americans for a variety of female complaints including: dysmenorrhea, childbirth, snakebite and pain relief (Hardy, 2000). Black cohosh has been known by many names including snakeroot, squawroot, rattle root, rattle weed, rattle top, and bug-bane. The names involving the word rattle were believed to be due to the distinctive rattle appearance to the flowering part of the black cohosh plant (Skidmore-Roth, 2004). It is important not to confuse black cohosh with blue or yellow cohosh. Black cohosh is best known by the name *Cimicifuga racemosa* and is still often referred to by that name. It has since been renamed *Actaea racemosa* due to a reclassification into a different genus; however many researchers interchange the names, and use both *Cimicifuga* and *Actaea* (Upton, 2002). The word cohosh is an Algonquin Indian
word meaning “it is rough”. Black cohosh was used by many Native American tribes including the Cherokee, Delaware, Iroquois, Mikmaq, and the Penobscot (Upton). The tribes used the plant as a treatment for a variety of complaints including: hives, rheumatic conditions, sedative and gynecological.

The first published reference to black cohosh occurred in Carl von Linne’s work in 1749 and its uses were listed as treatments for swelling and female problems (Upton, 2002). In 1831 the use of black cohosh was introduced in the United States through a paper written by Young and published in the American Journal of Medical Science. Black cohosh was used as a treatment for smallpox in 1832, a belief that was supported for forty years. In 1820 black cohosh was listed in the United States Pharmacopoeia as an anti-inflammatory and antispasmodic.

Black cohosh appeared in the United States Dispensatory in 1833 and remained there for 122 years as a stimulant for the kidneys, skin and pulmonary mucous membranes. In 2001 it was proposed to include black cohosh in the United States Pharmacopoeia- National Formulary, and it is currently approved in Germany for relief of dysmenorrhea and menopausal symptoms.

Black cohosh is an herbaceous perennial. It consists of a stem, leaves, flowers, fruit and root system. The stem of the plant is approximately eight feet tall with leaves approximately 6-15 cm long and 6-16 cm wide and green in color (Upton, 2002). The flowers are cream colored and the fruit is a 5-10 cm oval brown pod with multiple seeds. The rhizome is 2-15 cm long with many tightly packed curving branches with a brownish-black exterior. The roots are 3-16 cm long and dark brown in color. The plant is found only in the United States and Canada. Currently the entire supply of black cohosh is from the United States, specifically Kentucky, Tennessee, Georgia, Ohio, North Carolina, Michigan, South Carolina, Virginia, West Virginia and Wisconsin. The plant is found in forests, meadows, creek margins and mountains. The
rhizome and root are harvested when the plants are at least two years in age and the plant is in the dormant cycle (Upton, 2002).

The rhizome portion of the black cohosh plant is used in commercial preparations of black cohosh extract. The exact chemical composition of any black cohosh supplement is varied according to the manufacturer, and may contain many different compounds. The main active ingredients are the triterpene glycosides, and a black cohosh supplement may contain as many as 20 different types of triterpene glycosides (Upton, 2002). Commercial preparations of black cohosh contain approximately 2.5% triterpene glycosides and include the following: actein, 23-epi-26-deoxyactein, and cimiracemoside A. In addition, commercial preparations contain varying amounts of aromatic acids, flavonoids, tannins, resins, fatty acids, starch, and sugars (Upton). The flavonoids are phytoestrogenic in nature but their presence in black cohosh preparations are currently under debate. Three flavonoids have been reported to be present in black cohosh preparations: biochanin A, formononectin, and kaempferol (Upton). The presence of formononectin was identified in early studies, but recent studies have failed to prove its presence in current commercial preparations of black cohosh (Kennelly et al., 2002). The presence or absence of biochanin A and kaempferol has been debated and cannot be proven; further studies are recommended (Upton). The exact composition of black cohosh can be measured by mass spectroscopy and is recommended for ensuring the consistency of the product. The exact metabolism and excretion (Johnson & vanBreemen, 2003), and the exact mechanism of action of black cohosh in humans remains unknown (Skidmore-Roth, 2004).

It has long been proposed that black cohosh contained substances were estrogenic in nature, or were similar to the selective estrogen receptor modulators (SERMs). Due to the multitude of chemical components in a black cohosh preparation, it has been difficult to
determine the exact mechanism of action. Black cohosh has been classified as a phytoestrogen because it was believed to behave similarly to estrogen and was plant derived. However, all phytoestrogens have different mechanisms of action, and most are believed to exert their effects through the estrogen receptors, ERα and ERβ (Wuttke, Jarry, Westphalen, Christoffel, & Seidlova-Wuttke, 2003). Studies on black cohosh have produced conflicting results because, although believed to work by exerting an influence on target organs via estrogen receptors, several studies have shown that the compounds in black cohosh do not directly bind with estrogen receptors. Initially, black cohosh was thought to be estrogenic in nature due to the fact that preparations of black cohosh, specifically Remifemin, decreased circulating levels of luteinizing hormone (LH), but not follicle stimulating hormone (FSH) in postmenopausal women (Duker, Kopanski, Jarry, & Wuttke, 1991). However, several studies have shown that black cohosh does not have estrogenic effects, a fact that has become important to women seeking options for relief of postmenopausal vasomotor symptoms who have been treated for breast cancer. Liu and colleagues (2001) found that using four different assays to measure estrogen receptor binding in vitro, black cohosh did not bind to estrogen receptors α and β, and did not exert an estrogenic activity through the estrogen receptors. Lupu and colleagues (2003) found that black cohosh did not demonstrate any estrogenic activity in assays measuring the activation of the estrogen-response-element (ERE) required for estrogen receptor function. Black cohosh has also demonstrated no effect on the MCF-7 cell line, obtained from human breast adenocarcinoma which contains estrogen receptors, and is therefore a model for estrogen-responsive cells (Amato et al., 2002; Einbond et al., 2004; Stromeier et al., 2005).

The possibility that black cohosh is a new SERM has been proposed. A SERM is a substance which has estrogen-like effects in some target organs and anti-estrogenic effects or no
effect at all on other target organs. Seidlova-Wuttke and associates (2003) found that black cohosh exerted estrogenic activity by significantly reducing serum levels of LH and decreasing the amount of bone loss in ovariectomized rats compared to placebo. It was proposed that black cohosh contained an unidentified component which exerted selective estrogenic activity. Jarry and colleagues (2003) also suggest the presence of an unidentified component in black cohosh preparations which they termed ER$\gamma$. They found that black cohosh competed with radioactive-labeled estradiol in a cytosolic ER preparation from pork uteri, demonstrating phytoestrogen-like activity in vitro. Viereck and colleagues (2005) also suggest that black cohosh is not a classic phytoestrogen, and due to the fact that it has ER binding and an estrogen agonistic effect on bone tissue, and no estrogenic activity on breast or endometrial tissue, it should be classified as a SERM. In addition, it is postulated that the mechanism of action of black cohosh may be through serotonin receptors or dopamine receptors.

The possibility that black cohosh may have an effect on cytokines, which themselves have an effect bone remodeling, has been proposed. It is known that an increased expression of cytokines such as: tumor necrosis factor-$\alpha$ (TNF-$\alpha$), Interleukins (IL) 1, 4, 6 and 7, receptor activator of NF-$\kappa$B ligand (RANKL), and osteoprotegerin (OPG) can either stimulate or inhibit bone resorption (Khosla, 2001; Reddy & Roodman, 2004; Turner, Riggs, & Spelsberg, 1994; Turner, Rickard, Spelsbery, & Sibonga, 2003). Black cohosh was shown to inhibit the production of IL-4, and TNF-$\alpha$ in human mast cells (Kim et al., 2004) and increase the expression of OPG mRNA in cultured human osteoblast cells (Viereck et al., 2005). Qiu and colleagues (2007) found that one of the triterpene glycosides found in black cohosh (25-acetylcimigenol xylopyranoside [ACCX]) blocked in vitro osteoclastogenesis induced by RANKL or TNF alpha.
The exact mechanism of action of black cohosh remains unclear, but it is accepted that black cohosh does not act through the common estrogen receptors ERα and ERβ. It has been suggested that black cohosh might act through an unknown receptor, or possibly the serotonin receptors. It has also been suggested that black cohosh may have an effect on the regulation of cytokines which regulate bone remodeling. However, the exact mechanism of action in postmenopausal women remains unknown.

The use of black cohosh by postmenopausal women has increased in the last few years, possibly from fear of synthetic estrogens, from a desire to use something more natural, and/or a desire to be more in control of the decision for taking medication or supplement. Recently studies have shown that black cohosh is effective in relieving the vasomotor symptoms of the postmenopausal woman. The most common and disrupting vasomotor symptom in the postmenopausal woman is the hot flash. Black cohosh has shown promising results in reducing the number of hot flashes in postmenopausal women (Nappi et al., 2005; Osmers et al, 2005; Pockaj et al., 2004; Uebelhack et al., 2006; Verhoeven et al., 2005).

Recently it has been suggested that in addition to relief of vasomotor symptoms, black cohosh may have a preventive effect on the bone loss that occurs in the postmenopausal period. Studies done in ovariectomized rats, a rat model of osteoporosis, show changes in bone biochemical markers. Niblein and Freudenstein (2003) found that biochemical markers of bone resorption (PYR, DPY) decreased, and BMD loss was significantly less, in ovariectomized Sprague-Dawley rats when supplemented with black cohosh. Seidlova-Wuttke and colleagues (2003) found a significant bone sparing effect in the tibia of ovariectomized rats with black cohosh supplementation over the control group, but less than the estradiol group. Serum osteocalcin was lower in both the estradiol and the black cohosh groups, but serum crosslaps
were reduced only in the estradiol group. A study done in Germany by Wuttke and colleagues (2003) in postmenopausal women found an improvement in climacteric complaints, a decrease in the biochemical marker of bone resorption Crosslaps, and an increase in the biochemical marker of bone formation, bone-specific alkaline phosphatase. They concluded that black cohosh had a positive effect on osteoblast activity and decreased osteoclast activity. A repeat study done by Wuttke and colleagues (2006) in postmenopausal women found a statistically significant increase in osteoblast activity, but no significant change in osteoclast activity after 12 weeks of black cohosh. These studies show that there is potential for black cohosh as an acceptable supplement to decrease the bone loss that accompanies menopause. Further studies are needed to understand the mechanism of action of black cohosh and its effect on bone and the bone remodeling that accompanies menopause.

**Adverse Effects of Black Cohosh**

Few adverse effects of standardized black cohosh at the recommended dosage are mentioned. Skidmore-Roth (2004) lists nausea, vomiting, hypotension and slow heart rate. Upton (2002) reported headache, vertigo, weight gain, nausea, vomiting, and a stimulant effect. The National Center for Complementary and Alternative Medicine (NCCAM) list in their official material on black cohosh the following side effects: headaches, stomach discomfort, heaviness in the legs and weight problems (2005). Though adverse events are rare in standardized doses many preparations of black cohosh are not standardized and prepared from different parts of the plant. Recently several cases of liver failure have been linked to black cohosh and several have been reported in the United States. A 54 year old postmenopausal woman taking 1,000 mg of black cohosh daily presented to the clinic with symptoms of fatigue, forgetfulness, and a 10 pound weight loss. Liver failure was diagnosed and a liver transplant was required (Lynch, Folkers, & Hutson, 2006). In addition, a 50 year old postmenopausal woman
taking 500 mg of black cohosh daily presented to a hospital with jaundice, dark urine and light-colored stools. Liver failure was diagnosed and a successful liver transplant was performed (Levitsky, Alli, Wisecarver, & Sorrell, 2005). Several cases of hepatitis and liver failure requiring transplant have been documented outside the United States. Lüde and colleagues (2007) found that the liver in rats fed varying doses of black cohosh showed liver cell death by apoptosis both in vitro and in vivo, suggesting that liver damage is a possible adverse event with black cohosh administration and should be looked for in all subjects who take the supplement.

**Standardization of Black Cohosh**

The use of herbal medicine dates back thousands of years originating in China and India and is still used quite extensively in Asia. In the United States herbal medicine began in colonial times and was greatly influenced by the Native American culture. Many herbal medications were folk remedies passed on through the generations (Bedi & Shenefelt, 2002). Currently herbal products are sold in the United States as dietary supplements and are therefore not subject to stringent regulations with regard to standardization, safety, or quality. In order to be classified as a dietary supplement, a herb may not claim to prevent, treat, or cure a disease (Bent & Ko, 2004). Many herbs are sold as extracts of the original plant that have been obtained by boiling or “percolating” the herb in water or alcohol, which removes the active ingredient (Bent & Ko). Many herbal products contain a number of active ingredients, some as yet unidentified, as well as contaminants such as pesticides, prescription drugs, and heavy metals (Barnes, 2003).

In 1994 congress passed the Dietary Supplement Health and Education Act which set forth the definition for a dietary supplement as well as how they may be promoted, guidelines for labeling the product, and placed the burden of proving the product unsafe on the Food and Drug Administration (U.S. Food and Drug Administration, 1994). This law does not require the manufacturers of dietary supplements to conform to current good manufacturing practices.
On March 13, 2003 the Food and Drug Administration (FDA) published a proposed rule which would require that manufacturers ensure that their dietary supplement did not contain contaminants or impurities, and that the supplement be labeled accurately with regard to the active ingredients, as well as other ingredients contained in the supplement (USFDA, 2004). Under this new proposal dietary supplement manufacturers would be required to evaluate the purity, quality, strength and composition of their products as well as report all adverse effects to the FDA (USFDA, 2004). Currently there are several manufacturers that produce standardized products that contain a specific quantity of the active herbal ingredient, and have removed the harmful or toxic ingredients (Barnes, 2003). However, there are no current legally imposed regulations for standardization of dietary herbal supplement, and the term standardization may vary from manufacturer to manufacturer (National Institute of Health Office of Dietary Supplements, 2004).

Standardization of black cohosh refers to the amount of the active ingredient obtained from the root of the plant known as triterpene glycosides, or sometimes referred to as triterpene saponins, which is expressed as 26-deoxyactein (National Institutes of Health Office of Dietary Supplements, 2005). Each standardized tablet of 20 mg or 40 mg (dependent on manufacturer) of black cohosh extract contains 1 mg of triterpene glycosides. Chen and colleagues (2002) isolated a triterpene glycoside known as 26-deoxyactein from the root of the cimicifuga racemosa plant as well as 23-epi-26 deoxyactein formerly identified as 27-deoxyactein, a triterpene glycoside used for calculating the total triterpene content of black cohosh preparations using chromatography on silica gel. As the triterpene glycosides are the active ingredient in the black cohosh plant, Panossian and colleagues (2004) recommends standardizing dosages to the triterpene glycosides and the one most commonly used is 23-epi-deoxyactein, occasionally still
known as 27-deoxyactein. Most commercial black cohosh root products standardize their product to contain 2.5% triterpene glycosides, which is equal to 1mg of 26-deoxyactein per tablet (National Institute of Health Office of Dietary Supplements, 2005).

**Future Research on Black Cohosh**

The use of dietary supplements has continued to rise in the past decade due to a number of reasons. Many Americans believe that dietary supplements are more natural, and therefore better for them. In addition, dietary supplements are available without a costly visit to the health care provider, and are often less expensive than prescription medication. However, dietary supplements have not undergone the rigorous research that is required in the United States by the Food and Drug Administration and therefore lack information on quality, efficacy, and safety. In addition, dietary supplements are not required by law to report adverse effects and drug interactions, further compromising patient safety.

Black cohosh has gained popularity recently as a dietary supplement for the relief of menopausal vasomotor symptoms specifically, the hot flash. Recently more studies are being done utilizing randomized, double-blind, placebo-controlled clinical trials evaluating the effect of black cohosh on vasomotor symptoms and the quality of life of postmenopausal women. Literature review revealed one study conducted by Wuttke and colleagues (2006) that investigated the effect of black cohosh on bone metabolism. Wuttke found that black cohosh stimulated osteoblast activity, but had no statistically significant effect on osteoclast activity. The sample size was small (N=62 randomized into 3 study groups) and further studies replicating these findings have not been located.

This study utilized a randomized, double-blind, placebo controlled trial investigating the effect of a daily dose of black cohosh, standardized to 1 mg of triterpene glycosides in the form of a 40mg tablet once daily for 12 weeks. In addition, subjects in the control and experimental
group were given calcium carbonate and vitamin D supplementation. Bone biochemical markers of resorption and formation were evaluated at the onset of the study and after 12 weeks of therapy. To minimize the effects of confounding variables such as exercise, smoking, and excessive alcohol intake subjects with those behaviors were excluded from the study. To minimize the effects of ethnicity on bone mineral density and bone biochemical markers only Caucasian women were included in this initial study. As bone remodeling occurs at different rates depending on age and the amount of postmenopausal years only women greater than one year post menopause and less than 6 years were included. This study attempted to evaluate the effect of black cohosh on the most homogenous sample possible. Results from this study will be used to educate postmenopausal women on the possibility of a viable alternative to estrogen replacement to prevent postmenopausal bone loss.
Figure 2-1. Human femur

Figure 2-2. Haversian system (Barrett & Barrett, 2005).
Figure 2-3. Osteoclast (Barrett & Barrett, 2005).
Declining levels of circulating estradiol

Loss of inhibition of cytokine production

Increased production of cytokines TNFα, IL-1, IL-6

↑ production of RANKL
↓ production of osteoprotegerin

Loss of regulatory effect on bone remodeling

? Role in osteoblast formation

↑ osteoclast formation
And activation osteoclast apoptosis

↑↑ bone resorption
↑ bone formation

Imbalance in bone remodeling
Results in BMD loss

Postmenopausal Osteoporosis

Figure 2-4. Postmenopausal osteoporosis theoretical model.
Figure 2-5. Black cohosh theoretical model on mechanism of action. Broken arrows indicate proposed, but unproven theories of the mechanism of action of black cohosh.
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<th>Cytokines</th>
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<td>Stimulates</td>
</tr>
<tr>
<td>OPG</td>
<td>Decoy receptor for RANKL, interferes with RANKL signaling</td>
<td>Stimulates production of OPG</td>
<td>Inhibits</td>
</tr>
<tr>
<td>TNFα</td>
<td>Increases RANKL expression</td>
<td>Inhibits production of TNFα</td>
<td>Stimulates</td>
</tr>
<tr>
<td>IL-1</td>
<td>Increases RANKL expression</td>
<td>Mediates effects</td>
<td>Stimulates</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td>Inhibits</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td>Inhibits effects</td>
<td>Stimulates</td>
</tr>
<tr>
<td>IL-7</td>
<td></td>
<td>Mediates effects</td>
<td>Stimulates</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Increases OPG expression and stimulates RANK</td>
<td>Increases production of TGFβ</td>
<td>Inhibits</td>
</tr>
</tbody>
</table>
CHAPTER 3
METHODS

This study was designed to determine the effect of black cohosh (*Cimicifuga Racemosa*) on the biochemical bone markers of resorption and formation in the postmenopausal woman. In the postmenopausal woman it is known that due to estrogen deficiency, bone remodeling increases resulting in fragile brittle bones, often leading to bone fracture. The dual process of bone remodeling, resorption and formation can be assessed with the use of commercially available bone biochemical markers. This chapter includes the following sections: research design, subjects, measures, procedure, statistical analysis, and ethical considerations.

**Research Design**

This study was a randomized, placebo-controlled, double-blind clinical trial. After obtaining informed consent, qualified subjects were randomized into two separate groups, an experimental group and a control group. Both groups had an initial set of bone biochemical markers measured. Serum C-terminal telopeptide (CTX) was utilized as the marker for bone resorption. Serum osteocalcin (OC) was utilized as the marker of bone formation. After the biochemical markers of bone remodeling were measured each group received the study medication. All participants received calcium carbonate and vitamin D (OsCal +D) supplementation in tablet form. The experimental group took a standardized dose of black cohosh 40 mg (triterpene glycosides 2.5%) once daily for 12 weeks. The placebo group took an identical appearing placebo capsule containing lactose once daily for 12 weeks. After 12 weeks, measurements of the two biochemical markers for bone resorption and formation were repeated.

**Sample**

Subjects were recruited using flyers and advertisement in local hospitals, doctor’s offices, women’s clubs, and word of mouth in North Central Florida. In an effort to encourage subject
participation a $20.00 honorarium was provided to all subjects who completed the study. A prorated amount was given to subjects who completed at least 3-6 weeks of the study. Inclusion criteria were:

- Women who were naturally or surgically menopausal for at least one year but not more than six years.
- Women able to give voluntary consent
- Women not taking any hormone replacement therapy or selective estrogen receptor modulators (SERMs) for the past three months
- Women who had not been diagnosed with osteoporosis or an osteoporosis-related bone fracture
- Women who were sedentary and not involved in a regular exercise program, defined as at least 30 minutes at a time three times a week
- Women who were Caucasian females between the ages of 35 and 60
- Women who had not taken black cohosh for the past three months

Women were excluded from the study if they:

- Were current smokers
- Failed to take the medication as directed per study protocol
- Had a patient-reported history of kidney or liver disease, diabetes, parathyroid disease or documented osteoporosis with DEXA scan.
- Were lactose intolerant
- Became ill or were diagnosed with osteoporosis or fracture during the study time period
- Changed their mind about being included in the study
- Had a history of taking bisphosphonates at any time in their life

The above inclusion and exclusion criteria were altered slightly during the trial due to low subject recruitment and an impending expiration date on all of the study medication. All changes in the inclusion and exclusion criteria were approved by the Doctoral Supervisory Committee, the University of Florida Institutional Review Board, and the State of Florida Department of Health Institutional Review Board. The following changes were made. Women who were surgically menopausal and women with thyroid disease under control with medication were included in the study. The age range was changed to reflect the inclusion of surgically menopausal women and was changed from 45-60 to 35-60. The length of time from surgical and naturally occurring menopause remained unchanged. Subjects were provided with free calcium
carbonate (Os-cal + D) and vitamin D supplementation during the time period of the study, and
given education on postmenopausal osteoporosis.

To achieve a power of 80% and an alpha level of 0.05 using a one-tailed test and a
medium effect size 0.6 the study required 23 subjects per group. To allow for the accepted
attrition rate of 20%, the goal for recruitment was 25 subjects per group. The effect size was
computed by averaging the effect size of the two bone biochemical markers. Fifty subjects
consented to the study. Forty eight subjects met the inclusion criteria and were randomized into
the study. Forty six subjects completed the study, 23 per study group. Two subjects withdrew
from the study prior to the second blood sampling. One subject (study drug B- placebo)
withdrew due to a subject-reported weight gain. The subject declined to be reweighed by the
principal investigator. The second subject (study drug A-black cohosh) to withdraw from the
study did so because the principal investigator was unable to obtain the second blood sample
after two attempts on two separate occasions.

Measures

Biochemical markers of bone remodeling have been used for over 20 years in bone and
osteoporosis research, and are more specific to bone tissue (Delmas et al., 2000). As bone
remodeling is divided into two phases, resorption and formation, two bone biochemical markers
were used.

Serum CTX was used to measure the C-terminal peptide fragments that are released when
type I collagen is broken down during the resorption process. Serum CTX testing was done on
serum samples using a Metra serum crosslaps CTX Elisa kit (Quidel Corporation, San Diego,
CA). The serum crosslaps CTX Elisa is an enzyme immunological test for the products of the C-
terminal telopeptides released during bone resorption.
Serum osteocalcin (OC) was used to measure the activity of osteoblasts during bone formation. Serum osteocalcin testing was done on a serum sample using a Metra Osteocalcin EIA kit (Quidel Corporation, San Diego, CA). The Metra osteocalcin assay is a competitive immunoassay which uses osteocalcin coated strips, a mouse anti-osteocalcin antibody, an anti-mouse IgG-alkaline phosphatase conjugate, and a pNPP substrate to detect and measure the osteocalcin in the sample.

Demographic variables were assessed using a questionnaire which included age, weight, height, body mass index (BMI), last menstrual period (LMP), postmenopausal or surgical menopause, and current medications. The demographic questionnaire (Appendix A) was administered at the beginning of the study after the participant had signed the informed consent and prior to the serum sample collection. The demographic questionnaire is a one page fill in the blank questionnaire. The participant was assigned a number at the onset of the study and the questionnaire was coded with the participant’s number. The medical history questionnaire (Appendix B) is a two page form that the participant filled out after completing the demographic questionnaire. The medical history questionnaire provided the researcher with important facts on the participant’s health history and allowed the researcher to determine if the participant was eligible for the study.

**Operationalization of the Variables**

The independent variable is the administration of a standardized dose of black cohosh, 40mg once daily and is a dichotomous variable with the participant either taking the black cohosh or not. Each standardized capsule of black cohosh contained 40 mg of black cohosh root, or the equivalent of 1mg of triterpene glycosides. The placebo medication was packaged in an identical appearing capsule and was taken once daily. The identical-appearing placebo was
compounded and packaged in clear gelatin capsules by Francks Compounding Pharmacy in Ocala.

The dependent variables are the two biochemical markers of bone remodeling and are continuous variables. Serum C-terminal telopeptide (CTX) was used to assess bone resorption. The CTX results are expressed as ng/ml.

Serum osteocalcin (OC) is the marker that was used to assess bone formation. Serum osteocalcin is measured in ng/ml and does not need to be corrected unless the sample was diluted for any reason, but in this study, the samples did not need to be diluted. The monoclonal anti-osteocalcin antibody has a high specificity showing 100% reactivity when compared to bovine osteocalcin, which shares a significant homology with human osteocalcin. The antibody has a high specificity for recognizing intact osteocalcin and not fragments of bone resorption such as the N- and C- terminal propeptides. The Metra Osteocalcin kit requires 25µL of sample for analysis. Demographic variables such as age, weight, height, BMI, and years postmenopause are continuous variables. Demographic statistics are discussed in Chapter four.

Procedure

Participants that met the inclusion criteria and agreed to participate in the study were advised of the benefits and risks of the study, and then asked to sign an informed consent which had been approved by the Institutional Review Board (IRB) of both the University of Florida and the State of Florida Department of Health. After entry into the study the participant was given a participant number, and then randomized into either the experimental or the control group using a computer generated randomization table.
Laboratory Tests Protocol

After randomization, the subject was asked to fill out the demographic questionnaire and the medical history questionnaire. If the inclusion criteria were met and none of the exclusion criteria were met, the subject was included in the study. All serum samples were obtained during the morning hours and the subject did not need to be fasting.

Blood was drawn by venipuncture using aseptic technique from an antecubital or dorsal hand vein using a 21-gauge needle at the beginning of the study, and again after 12 weeks on the study medication. Two 10ml red top serum separator tubes were collected at each blood draw. The tubes were centrifuged at 5000g for 10 minutes to separate the serum component. After the serum was separated, it was pipetted into three or four 1.5 ml aliquots and then packed in dry ice for transport according to OSHA standards. The principal investigator completed a four hour training program required by the Department of Transportation (DOT) titled “Shipping and Transport of Biological Materials”. This training is required to transport blood in a vehicle from one site to another. The frozen serum was transported to the University of Florida for storage in the College of Nursing Office for Research Support (ORS) physiology wet lab. It was frozen and stored at $\leq -80^{\circ}\text{C}$ in the College of Nursing wet laboratory freezer until all specimens were collected. The longest time period a specimen was stored in the freezer was 350 days.

At the completion of the study both the samples from the beginning and the end of the study for each participant were analyzed in the wet lab by the Principal Investigator under the supervision of the laboratory supervisor and the guidance of the Doctoral Supervisory Chair. All the samples were analyzed in the College of Nursing wet lab by the principal researcher over the course of several days.

Samples were thawed immediately prior to use. All samples used in the bone assays were freshly thawed samples, refrozen samples were not utilized for this study. After the samples
were thawed they were thoroughly mixed using a vortex spinner. After mixing the serum was pipetted into the assay plates provided in the assay kits. The assays were performed by the principal investigator over the course of two weeks in the College of Nursing physiology wet lab. Each assay kit tested 40 samples in duplicate plus the standards and controls. Each assay kit required 4-6 hours to complete. All assays were done in duplicate, and the controls and standards of both assays were within the range provided by the manufacturer of the assay kits. Assays were performed following specific directions provided by the manufacturer.

**Medication Protocol**

After completing the initial laboratory tests, the medication, which was prepared by Francks pharmacy, was dispensed to the participant. The subject received either the black cohosh or an identical appearing placebo. The black cohosh was obtained from a local GNC (General Nutrition Corporation) retail store in Ocala. All the bottles of black cohosh bore the same lot number. The black cohosh was manufactured for GNC by the Nutra Manufacturing Company. A certificate of analysis from the Nutra Manufacturing Company is included in this dissertation (Appendix C). The bottles of black cohosh were then taken to the compounding laboratory of Francks Pharmacy to be repackaged as study medication. The study medications were packaged by the pharmacy and were in identical appearing plastic brown containers. The bottles were now labeled either study drug A or study drug B. The medication was then given to the participant by the blinded researcher. The participant was given 28 capsules of either study medication A or study medication B depending on group assignment, and instructed to take a capsule once daily for four weeks. After four weeks if there were no adverse events, the participant received 28 more capsules. After eight weeks, if there were no adverse events, the participant received the final 28 capsules. At the end of twelve weeks, the second serum sample was drawn. In addition to receiving the black cohosh or placebo, all participants were provided
with calcium carbonate and vitamin D supplementation in the form of Oscal caplets + D which they were instructed to take by mouth twice daily. During the study the participants were contacted by phone weekly to address any concerns or questions and to detect any adverse events. In addition, the participants were given the cell phone number of the principal researcher to call in the event of an emergency.

**Statistical Analysis**

Data was analyzed using SPSS (SPSS Inc., Chicago, IL). Descriptive statistics including means, standard deviation, and frequency distributions were analyzed. Levene’s test for equality of variances and the student’s t-test for equality of means were conducted to analyze the group differences. Repeated measures Analysis of Covariates (ANCOVA) was used to determine the difference in the bone biochemical markers within groups, between groups, and within-by-between group interaction after controlling for age and body mass index (BMI). A p value of $\leq 0.05$ was required for statistical significance.

**Ethical Considerations**

All participants who met the inclusion criteria for the study were included. The research proposal was reviewed and approved by the Institutional Review Board (IRB) at the Health Sciences Center at the University of Florida and the State of Florida Department of Health. In addition, an investigational new drug number (IND) was obtained from the Food and Drug Administration (FDA). The IND letter from the FDA is included as Appendix D. Participants were asked to sign an informed consent which outlined the risks, benefits, and goals of the study. All participants were assured that they were free to leave the study at any time for any reason if they were uncomfortable with the study protocol. All participants were provided with calcium and vitamin D supplementation which is currently a standard of care for postmenopausal women. Subjects were randomized using a computer generated randomization table to ensure that all
subjects had an equal opportunity to receive the study medication. Confidentiality was protected by using assigned subject numbers and no names, and current HIPAA laws were adhered to. All material related to the study subject was kept in a numbered file. All confidential subject files were kept inside the principal investigator’s secure home in a locked filing cabinet.
Table 3-1. Research Design for a two group study including an experimental group and a placebo group. R= randomization, O₁= First laboratory values of CTX and OC, X= introduction of the black cohosh intervention, O₂= Second laboratory values of CTX and OC. Duration of the study from O₁ to O₂ was 12 weeks.

<table>
<thead>
<tr>
<th>R- Group 1</th>
<th>O₁</th>
<th>X</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>R- Group 2</td>
<td>O₁</td>
<td>O₂</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-2. Timeline for study participants.

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2-4</th>
<th>Week 5-8</th>
<th>Week 9-12</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1: met the subject, inform subject about study, complete informed consent, complete demographic questionnaire and medical history form and answer questions.</td>
<td>Each week subjects were called by phone to determine if there were any problems or questions.</td>
<td>Weekly calls to subject to determine if there were any problems or questions.</td>
<td>Weekly calls to subject to determine if there were any problems or questions.</td>
<td>Visit 5: met with the subject to draw morning blood samples. Answer any questions the subject had and make a final determination if any adverse events occurred.</td>
</tr>
<tr>
<td>Visit 2: draw morning blood. Provide subject with 28 days worth of study medication and give subject instructions for taking the medication correctly, answer questions.</td>
<td>Visit 3: met with the subject, determine if there are any adverse events, answer any questions, and provide the subject with the next 28 days worth of study medication.</td>
<td>Visit 4: met with the subject, determine if there are any adverse events, answer any questions, and provide the subject the final 28 days of study medication.</td>
<td>Subject completed the final 28 days of medication.</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3-1. Conceptual framework and relationship of the research variable
CHAPTER 4
ANALYSIS AND RESULTS

Data analysis for this study was conducted using SPSS statistical software version 11.5 (SPSS Inc., Chicago, IL). Descriptive statistics were obtained to provide summary measures for the data. Bivariate statistics were analyzed to compare the two groups, the black cohosh and the placebo group. Analysis of covariates (ANCOVA) was performed to test the research hypotheses and to answer the research questions.

Forty-eight subjects were recruited into the study to determine the effect of the dietary supplement black cohosh (Cimicifuga racemosa) on bone biochemical markers of resorption and formation in postmenopausal women. All subjects were recruited from the North Central Florida area. All subjects were females between the ages of 35 and 60 that had been estrogen depleted for at least one year and less than six years. A total of 48 subjects were recruited into the study, 46 subjects completed the study. Two subjects voluntarily dropped from the study, one due to weight gain and the other due to the inability to obtain the second blood sample by the principal investigator. All subjects were recruited through word of mouth, flyers, or discussion of the study in a public forum.

Demographic Statistics

All of the subjects who participated in the study were female and between the ages of 35 and 60. The overall mean age of the women was 53.44 (SD=4.70) with the minimum age of 36 and a maximum age of 60. The mean age of the subjects taking drug A was 54.08 (SD= 4.95) and the mean age of the subjects taking drug B was 52.79 (SD= 4.44). Age difference was not significant (p=.552) for Levene’s test and the difference was not statistically significant (t-test=.951, p=.346). The largest portion of women in the study were in their fifties (83%), followed by women in their forties (6.3%), sixty year old women -(6.3%), and women in their thirties.

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The overall mean body mass index (BMI) was 29.43 (SD= 5.71) with the minimum BMI of 20.14 and a maximum of 44.48. The mean BMI of subjects taking drug A was 29.02 (SD=5.38) and the mean BMI of those taking drug B was 29.84 (SD=6.09). Body Mass Index (BMI) was not significant (p= .502) for Levene’s test and the difference between treatment groups was not significant (t-test= -.494, p= .623). The educational status of the subjects is as follows: 15 had a high school diploma (31.3%), 10 had an Associate degree (20.8%), 10 had a Masters Degree (20.8%), 8 had a Bachelors degrees (16.7%), 3 had completed a GED certificate (6.3%), and 2 had completed a doctoral degree (4.2%). The years postmenopausal were five years (35%), four years (13%), three years (17%), two years (10%) and one year (25%). The overall mean for years postmenopausal was 3.23 (SD= 1.63). The mean for years postmenopausal in the black cohosh group was 3.71 (SD=1.46) and the placebo group was 2.75 (SD=1.67). Years postmenopausal was not significant (p=.225) for Levene’s test, however the difference in mean years postmenopausal between groups was significant (t-test= 2.114, p=0.04).

Thirty (62.5) of the subjects were married, 13 (27.1%) were divorced, and 5 (10.4%) of the subjects were single. Thirty-five (72.9%) of the subjects were employed full time, 7 (14.6%) of the subjects were employed part time, 4 (8.3%) of the subjects were unemployed, and 2 (4.2%) of the subjects were retired.

Blood pressure was assessed two times in the study, at the onset and at the conclusion. The mean group onset systolic blood pressure was 122.17mm/Hg (SD=16.01) and the mean group onset diastolic was 78mm/Hg (SD=8.31). The mean group conclusion systolic blood pressure was 123.09mm/Hg (SD=16.37) and the mean group conclusion diastolic blood pressure was 77.28mm/Hg (SD=8.43). The mean onset systolic blood pressure of subjects taking drug A was 121.21mm/Hg (SD=16.17) and subjects taking drug B was 122.13mm/Hg (SD= 16.14). The
The mean group onset systolic blood pressure was 78.0mm/Hg (SD=8.31) and the mean group conclusion systolic blood pressure was 123.67mm/Hg (SD=18.50) and those taking drug B was 122.48mm/Hg (SD= 14.20). Systolic blood pressure at the onset (p= .680) and conclusion (p= .300) of the study were not significant for Levene’s test and the difference between treatment groups was not significant at the onset (t-test= -.411, p= .683) or at the conclusion (t-test= .246, p= .807).

The mean onset diastolic blood pressure for those subjects taking drug A was 75.54mm/Hg (SD= 7.70) and subjects taking drug B was 80.46mm/Hg (SD= 8.31). The mean conclusion diastolic blood pressure for subjects taking drug A was 75.88mm/Hg (SD=10.06) and those taking drug B was 78.74mm/Hg (SD= 6.21). Diastolic blood pressure at the onset (p=.798) and at the conclusion (p=.099) of the study were not significant for Levene’s test and there was no significant (p=.249) difference in means in the conclusion diastolic blood pressure between the two groups. The onset mean diastolic blood pressure was significantly (t-test=-2.125, p=0.039) different between the two treatment groups. The means and standard deviations are summarized in Table 4-1. Frequency and percentages for categorical data are summarized in Table 4-2.

Bivariate statistics were performed between the experimental and placebo group to evaluate any differences between the two groups. Age and body mass index (BMI) were analyzed using the independent samples t-test. Levene's test for equality of variances was not significant for either age (p=.552) or BMI (p=.502) demonstrating that both the black cohosh and the control group did not violate the assumption of homogeneity of variances. The t-test for equality of means was not significant for age (p=.346) or BMI (p=.623).
Bivariate statistics were performed between the experimental and the placebo groups to evaluate differences in blood pressure. Levene’s test for equality of variances was not significant for onset systolic blood pressure (p=.680), conclusion systolic blood pressure (p=.300), onset diastolic blood pressure (p=.798) and conclusion blood pressure (p=.099) demonstrating that both the black cohosh and the placebo group did not violate the assumption of homogeneity of variances. The t-test for equality of means was significant for differences in onset diastolic blood pressure (t-test= -2.125, p=.039) but was not significant for conclusion diastolic blood pressure (t-test= -1.169, p=.249), onset systolic blood pressure (t-test= -.411, p=.683), and conclusion systolic blood pressure (t-test=.246, p=.807).

Cross tabs analysis was conducted on education and marital status as these were not continuous variables. The cross tabs analysis using Pearson Chi-Square between the two groups (black cohosh or placebo) for education (p=.216) and marital status (p=.158) were not statistically significant.

Hypotension is a documented side effect of black cohosh. Two separate analyses of covariates (ANCOVA) controlling for age and BMI were conducted to evaluate the effect of black cohosh on blood pressure, one on systolic blood pressure and one on diastolic blood pressure. The assumption for homogeneity of variance and normality were met in both analyzes. The results of the ANCOVA revealed no significant difference on systolic blood pressure levels within administration (pretreatment vs. posttreatment), F (1,43) = 3.655, p = .063; no significant group, (drug A vs. drug B) by administration (pretreatment vs. posttreatment) interaction on systolic blood pressure levels, F (1,43) = .314, p = .578; and no significant difference on systolic blood pressure levels between group (experimental vs. control), F (1,43) = .032, p = .859. There
was also no significant difference in BMI between the two groups. Results are summarized in table 4-3.

A second ANCOVA was conducted on diastolic blood pressure. The results of the ANCOVA revealed no significant difference on diastolic blood pressure levels with groups (pretreatment vs. posttreatment), $F(1,43) = 2.306, p = .136$; no significant group (drug A vs. drug B) by administration (pretreatment vs. posttreatment) interaction on diastolic pressure levels, $F(1,43) = .469, p = .497$; and no significant difference on diastolic blood pressure between groups (experimental vs. control), $F(1,43) = 3.909, p = .054$. The results are summarized in Table 4-3.

**Research Question**

The purpose of this study was to determine if a standardized (2.5% triterpene glycoside) commercial preparation of the dietary supplement black cohosh would alter bone remodeling in the postmenopausal female. Bone remodeling is a coupled process including both resorption and formation. Usually in postmenopausal women both resorption and formation increase, but not in equal amounts, which results in a net bone loss. It was hypothesized that both resorption and formation would decrease with the administration of black cohosh. Bone resorption and formation were measured with two commercially available bone biochemical assays. Serum C-terminal telopeptide (CTX) was used to measure bone resorption and serum osteocalcin (OC) was used to measure bone formation. In order to determine if there was a statistically significant effect on C-terminal telopeptide (CTX) from pretreatment to posttreatment, and between groups (black cohosh versus placebo), an analysis of covariates (ANCOVA), controlling for age and BMI, was conducted. The assumptions for homogeneity of variance and normality were met. The results of the ANCOVA revealed no significant difference in CTX levels within groups upon administration of black cohosh (pretreatment vs. posttreatment), $F(1,42) = .332, P = .568$.
no significant group (drug A vs. drug B) by administration of black cohosh (pretreatment vs. posttreatment) interaction on CTX levels, $F (1,42) = .071, p = .791$; and no significant difference on CTX levels between groups (experimental vs. control), $F (1,42) = .095, p = .759$. The results are summarized in Table 4-4.

To determine if there was a statistically significant effect on serum osteocalcin (OC) levels from pretreatment to posttreatment and between groups who took a standardized dose of black cohosh and those that did not, while controlling for both age and BMI, an ANCOVA was conducted. The assumptions of homogeneity of variance and normality were met. The results of the ANCOVA revealed no significant difference on OC levels within groups administration of black cohosh (pretreatment vs. posttreatment), $F (1,42) = .184, p = .670$; no significant group (drug A vs. drug B) by administration of black cohosh (pretreatment vs. posttreatment) interaction, $F (1,42) = .255, p = .616$; and no significant difference on OC levels between groups (experimental vs. control), $F (1,42) = .172, p = .680$. The results are summarized in table 4-4.
Table 4-1. Means and Standard Deviations for Age, BMI, Years Postmenopause and Blood Pressure.

<table>
<thead>
<tr>
<th></th>
<th>Overall N= 46</th>
<th>Overall Minimum</th>
<th>Overall Maximum</th>
<th>Black Cohosh n=23</th>
<th>Placebo n=23</th>
<th>Significance</th>
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<tr>
<td>Age (years)</td>
<td>53.44 (SD=4.70)</td>
<td>36</td>
<td>60</td>
<td>54.08 (SD=4.95)</td>
<td>52.79 (SD=4.44)</td>
<td>p= 0.346</td>
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<tr>
<td>Body Mass Index (BMI)</td>
<td>29.43 (SD=5.71)</td>
<td>20.14</td>
<td>44.48</td>
<td>29.02 (SD=5.38)</td>
<td>29.84 (SD=6.09)</td>
<td>P=0.623</td>
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<tr>
<td>Years Postmenopausal</td>
<td>3.23 (SD=1.63)</td>
<td>1</td>
<td>5</td>
<td>3.71 (SD=1.46)</td>
<td>2.75 (SD=1.67)</td>
<td>P=0.040</td>
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<td>Systolic Blood Pressure (mm/Hg)</td>
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<td>Onset</td>
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<td>148</td>
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<td>123.13 (SD=16.14)</td>
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<tr>
<td>Conclusion</td>
<td>123.09 (SD=16.37)</td>
<td>95</td>
<td>165</td>
<td>123.67 (SD=18.50)</td>
<td>122.48 (SD=14.20)</td>
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<td>Diastolic Blood Pressure (mm/Hg)</td>
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<td>Onset</td>
<td>78.00 (SD=8.31)</td>
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<td>96</td>
<td>75.54 (SD=7.70)</td>
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<tr>
<td>Conclusion</td>
<td>77.28 (SD=8.43)</td>
<td>57</td>
<td>101</td>
<td>75.88 (SD=10.06)</td>
<td>78.74 (SD=6.21)</td>
<td>p=0.249</td>
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<tr>
<td>Education</td>
<td>Frequency</td>
<td>Percentage</td>
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Table 4-3. Analysis of covariates on Systolic and Diastolic Blood Pressure after Controlling for Age and BMI.

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<tr>
<td>Systolic Blood Pressure Levels*Drug</td>
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<tr>
<td>Error</td>
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</tr>
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<td><strong>Within Groups</strong></td>
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Table 4-5. Means and Standard Deviation of Serum Osteocalcin and C-terminal Telopeptide at the Onset and Conclusion of the Study

<table>
<thead>
<tr>
<th></th>
<th>Black Cohosh</th>
<th>Placebo</th>
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<tr>
<td><strong>Osteocalcin</strong></td>
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<tr>
<td>(ng/mL)</td>
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<tr>
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<td>N=23</td>
<td>SD=2.40</td>
<td>SD=3.65</td>
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<tr>
<td><strong>C-terminal</strong></td>
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</tr>
<tr>
<td><strong>Telopeptide</strong></td>
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<tr>
<td><strong>CTX</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Onset</strong></td>
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</tr>
<tr>
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<td><strong>Conclusion</strong></td>
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<td>0.575</td>
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<td>N=23</td>
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Menopause is a natural midlife transition that all women will experience as they age. Menopause is broadly defined as the final menstrual period and the termination of ovarian function resulting in a significant decrease in circulating estrogen. This decrease in circulating estrogen produces a skewed form of bone remodeling, resulting in a net bone loss. This condition is termed postmenopausal osteoporosis. Postmenopausal osteoporosis currently affects more than eight million American women, and that number is expected to increase significantly in the next ten years. This net bone loss results in a more fragile brittle bone, predisposing women to fractures. Every year more than 1.5 million fractures occur due to osteoporosis, resulting in an increased morbidity and mortality. Estrogen replacement therapy has been shown to prevent bone loss and therefore decrease the incidence of postmenopausal osteoporosis. The publication of the Women’s Health Initiative (WHI) study in 2002 produced a fear of estrogen therapy in both women and their healthcare providers. Many health care providers will not prescribe estrogen therapy to healthy women, let alone women with chronic diseases such as diabetes, hyperlipidemia and hypertension.

To find some relief for the vasomotor symptoms and to exert some autonomy over their lives, many postmenopausal women have turned to dietary supplements, resulting in a multibillion dollar industry. Dietary supplements such as black cohosh, evening primrose oil, chasteberry tree extract, and many soy products are marketed to the postmenopausal woman looking for symptom relief. Many of these supplements are poorly regulated and have little to no research documenting their effects.

This study was done in an effort to determine if the dietary supplement black cohosh had an effect on bone remodeling, and may therefore be an alternative option for retarding the bone
loss that occurs during the menopausal years. This study was a randomized double-blind, placebo-controlled clinical trial to evaluate the effect of a standardized dose of black cohosh on the two processes of bone remodeling, resorption and formation. Healthy postmenopausal women who currently chose not to take estrogen supplements were invited to participate.

**Discussion of the Findings**

Forty-eight healthy postmenopausal Caucasian women were recruited into the study. Forty-six women completed the study. After consent the women were randomized into either an experimental group or a control group. After randomization serum blood samples were obtained and the women were provided with either study drug A or study drug B and instructed to take one capsule orally every day for 12 weeks. All women were provided with a bottle of Oscal + D and instructed to take one calcium tablet by mouth twice daily. Serum samples were obtained after 12 weeks of drug therapy. Bone biochemical assays were performed on both the pretreatment and posttreatment serum samples and the results were analyzed using Analysis of Covariates (ANCOVA) after controlling for age and BMI.

Descriptive and bivariate statistics revealed both groups had equal number of subjects at 23 per group. All women were Caucasian females between the ages of 36 and 60. There was no statistically significant difference in ages between the black cohosh (52.79) and the placebo group (52.79). All of the subjects had been without a menstrual period for at least one year but less than six years. Women with hysterectomy were included in the study if they had also had removal of the ovaries as well as the uterus. This was done in an effort to increase recruitment. Of the 48 subjects who entered the study 39 (81.3%) were naturally menopausal and 9 (18.7%) were surgically menopausal. Thirty-one (64.6%) subjects reported experiencing hot flashes entering the study and 17 (35.4%) reported no hot flashes prior to entering the study. Women entering the study were generally healthy and were included in the study if they had a health
problem unrelated to bone metabolism, or their health problem was under control with medication. Women were excluded from the study if they had any medical problem which could interfere with bone metabolism such as diabetes, parathyroid disease or osteoporosis. Women were also excluded if they had a health problem which would prevent them from taking black cohosh such as liver or kidney disease or breast or endometrial cancer. Smokers were also excluded from the study. Women who were taking any form of estrogen supplement (pill, patch, injection, or ring) or were on a SERM (Evista) were excluded from the study.

Women with medical conditions under control, unaffected by black cohosh, or with no effect on bone remodeling were recruited into the study. Women with thyroid disease were recruited into the study if they were controlled with medication and were currently euthyroid. The thyroid status was self reported and no thyroid labs were drawn and the patient medical files were not assessed. Forty (83.8%) subjects reported no thyroid disease and 8 (16.7) reported controlled thyroid disease. Ten (21%) of the women had hypertension controlled with medication. Four (8%) of the subjects reported having mitral valve prolapse (MVP) with no current symptoms.

Bone biochemical assays were performed using two commercially available kits. Bone resorption was assessed utilizing an assay which measured the C-terminal telopeptide (CTX). There was no difference in mean values for CTX between the black cohosh group (.484ng/mL) and the placebo group (.563ng/mL) on the first serum sample done prior to instituting treatment. After 12 weeks of treatment serum samples were collected again. There was no difference in the mean values for CTX between the black cohosh group (.556ng/mL) and the placebo group (.575ng/mL). After 12 weeks of treatment with black cohosh there was no statistically significant difference in the mean values for serum CTX within each treatment group.
(pretreatment vs. posttreatment) or between treatment groups (black cohosh vs. placebo). The results of this study reveal that a standardized dose (2.5% triterpene glycosides) of black cohosh 40mg taken once daily by mouth has no effect on bone resorption.

Bone formation was assessed utilizing an assay which measured serum osteocalcin (OC). There was no difference in mean values for OC between the black cohosh group (10.65ng/mL) and the placebo group (11.19ng/mL) on the first serum sample done prior to instituting treatment. After 12 weeks of treatment serum samples were performed again. There was no statistically significant difference in the mean values for OC between the black cohosh group (11.35ng/mL) and the placebo group (11.51ng/mL). After 12 weeks of treatment with black cohosh there was no difference in the mean values for serum OC within each treatment group (pretreatment vs. posttreatment) or between treatment groups (black cohosh vs. placebo). The results of this study reveal that a standardized dose (2.5% triterpene glycosides) of black cohosh 40mg taken once daily by mouth has no effect on bone formation.

Blood pressure was assessed at the beginning and at the end of the study in all subjects. All blood pressure measurements were obtained by the principal investigator and were performed using an electronic blood pressure cuff. Several subjects (21%) had essential hypertension prior to entering the study. Hypotension is a documented side effect of black cohosh. There was no difference in the mean systolic blood pressure between the black cohosh group (121.20mm/Hg) and the placebo group (123.12mm/Hg) at the onset of the study. There was no difference in the mean systolic blood pressure between the black cohosh group (123.67mm/Hg) and the placebo group (122.48) at the conclusion of the study. There was a statistically significant difference in the mean diastolic blood pressure between the black cohosh group (75.54mm/Hg) and the placebo group (80.46mm/Hg) at the onset of the study. However, at the conclusion of the study
there was no difference in mean diastolic blood pressure between the black cohosh group (75.88mm/Hg) and the placebo group (78.74). The results of this study show that black cohosh had no effect on blood pressure measurement.

This study shows that even though black cohosh may help women with the vasomotor symptoms of menopause it does not have an effect, either positive or negative on bone metabolism. This is an important fact for health care providers to understand. As women age during menopause, bone loss is occurring. Women who choose not to take estrogen replacement have few options available to them to protect or rebuild bone. Many women are choosing supplements because advertising has led them to believe that supplements are better for them, and they are easily accessible without a prescription. This study demonstrates that a standardized 40mg dose of black cohosh once daily has no effect on bone metabolism in women who are recently postmenopausal.

**Research Hypotheses**

The research hypotheses presented in this study were not supported. Two hypotheses were postulated and each will be discussed individually.

- **Hypothesis 1:** Postmenopausal women taking a standardized 40mg (2.5% triterpene glycoside) dose of an oral black cohosh supplement daily for twelve weeks will show a decrease from baseline in the level of serum C-terminal telopeptide, a biochemical marker of bone resorption.

  This hypothesis was not supported by the results of this study. The serum C-terminal telopeptide drawn at the conclusion of the study did not significantly change from the baseline levels drawn at the onset of the study in either the black cohosh group or the placebo group. However, it is possible that a low sample size could have led to a Type II error. This will be discussed later in this chapter.
• Hypothesis 2: Postmenopausal women taking a standardized 40 mg (2.5% triterpene glycoside) dose of an oral black cohosh supplement daily for twelve weeks will show a decrease from baseline in serum osteocalcin, a biochemical marker of bone formation. This hypothesis was not supported by the results of this study. The serum osteocalcin drawn at the conclusion of the study did not significantly change from the baseline levels drawn at the onset of the study in either the black cohosh group or the placebo group. Again, the possibility of a Type II error is present due to a low sample size.

**Strengths of the Study**

One of the strengths of the study is the study design. There are few randomized placebo-controlled, double-blind clinical trials on the dietary supplements used by postmenopausal women. Many of the studies done on black cohosh have been done without a placebo, without blinding or randomization, and many were measuring only severity and frequency of hot flashes. Several studies have been conducted in Germany measuring bone metabolism, but those have study flaws as well. The strength of this study design is that it was randomized, double-blinded, and placebo-controlled.

The study drug used in this study was obtained from a reputable source and was accompanied by a certificate of analysis which stated that there were no contaminants present. The entire supply of study drug came from the same lot number, which helped to assure that subjects were getting the same consistency and strength of study medication. Efforts to encourage treatment fidelity to the study protocol were as follows: 1) subjects were called weekly to remind them to take the study drug, as well as determine if any adverse events had occurred; 2) subjects met with the principal investigator every four weeks and were told to return the previous bottle of study medication, which was assessed for pill count; 3) subjects were given
the phone number of the principal investigator in case there was any problems with adhering to
the study protocol.

A strength of the study is that all of the bone biochemical assays were performed by the
same person, the principal researcher. The principal researcher performed all of the assays
required for the study on freshly thawed serum samples. No refrozen serum samples were
utilized. The researcher was a novice lab technician, but all samples were assayed in exactly the
same manner increasing intrarater reliability. All serum samples were collected, centrifuged, and
stored in the same manner by the same researcher guaranteeing that the samples were handled
with consistency.

Limitations of the Study

Design Limitations

Every study no matter how well planned will have limitations. This study had several
study design limitations. One limitation dealt with the subjects’ medical history. The medical
history questionnaire was filled out by the subject prior to beginning the study. The researcher
counted on the subject honestly filling out the form. No medical records or lab reports were
obtained by the researcher prior to or during the study. If the subject had lab work documenting
menopause or a stable thyroid condition the researcher did not obtain any medical records,
relying on the subject for the correct value and interpretation. One question on the medical
history questionnaire asked the question “Do you currently participate in a regular exercise
program?” Subjects had a difficult time determining what exercise constituted a regular exercise
program. If they walked 20 minutes once a week they considered this regular exercise. This
question is often on health histories and subjects will often exaggerate how much they exercise.
Some subjects considered exercise caring for their grandchildren for the day. It was left up to the
researcher to determine what constituted regular exercise and the definition of regular exercise
was at least 30 minutes of exercise at least three times a week. In addition, subjects were advised not to start a regular exercise program as regular exercise can have an effect on bone metabolism. This encouragement not to exercise for the duration of the twelve week study could constitute a break in the standard of care for postmenopausal women. As health care providers we encourage all women to participate in a regular exercise program for bone, cardiac, and generalized good health.

As with all studies, subject recruitment proved to be more difficult than expected. Inclusion and exclusion criteria were strict and many women who were interested in the study were excluded for any number of reasons. The incentive for women to take black cohosh is that they consider it a natural alternative to estrogen and it is available without a prescription. Therefore women who had contraindications to estrogen replacement therapy such as breast cancer, older age, heart attack, or stroke felt that they should be able to take black cohosh. They however were excluded from the study. Two subjects with a history of breast cancer told me their physician said they should take black cohosh and did not understand why they were excluded from the study. Many women who had completed menopause many years before, but had been taken off estrogen therapy were also interested in the study. It was very frustrating for the researcher to be turning down more people than were accepted into the study.

In an effort to boost subject recruitment several changes in the inclusion/exclusion criteria were made halfway through data collection. It was decided to accept women who had a hysterectomy as long as they had their ovaries removed as well. The number of years postmenopause remained at less than six years, but the age range was changed from 45-60 to 35-60. This enabled the researcher to accept nine more subjects. Women with thyroid disease
under control with medication were also accepted into the study. This change allowed 8 more subjects into the study.

A recalculation of sample size was done approximately halfway through the study due to low recruitment. The power remained at 80% and the alpha level remained at 0.05 but the effect size was altered. Initially, the effects size was based on the lower effect size of the bone biochemical marker serum osteocalcin. The serum C-terminal telopeptide had a higher effect size. It was decided by the researcher to lower the effect size by averaging the effect size of the two bone markers. This new effect size required only 23 subjects per group instead of 49 subjects per group. This smaller sample size increases the risk for a Type II error.

To increase recruitment into the study the researcher also used a convenience, non-random sampling technique. The researcher recruited heavily at her place of employment, a local hospital in Marion County, by visiting many of the departments and talking with the female employees. All departments of the hospital were visited and the researcher discussed the study with the staff for approximately 5-10 minutes. Nineteen (40%) subjects were recruited from the hospital environment. The other subjects were recruited by word of mouth (37%) and flyers (23%) posted at health food stores, groceries stores, the local health department, churches, and the University of Florida parking garage. Using this form of sampling can pose a threat to the external validity of the study.

Another limitation of the study was that the investigator did not devise a method for determining if the subjects took their calcium supplement correctly or at all. The investigator called the subjects weekly to inquire if the subject had experienced any adverse effects, and to remind them to take the study medication. The investigator did not follow up on compliance to the calcium regimen unless the subject provided that information without prompt. Calcium can
have an effect on bone remodeling and this may have had an effect on the possibility of a Type II error.

Another limitation of the study is that the bone biochemical assays were performed by the principal investigator. The principal investigator is a novice lab technician and mistakes in performing the assays might have occurred. One subject in the study had very high levels of serum osteocalcin, markedly different from the other subjects in the study. The assay was performed again on a fresh sample of the subject’s serum and the results were not significantly different. This resulted in the subject being classified as an outlier, and therefore dropped from the statistical analysis. The investigator performed the assays after the minimal training required by the laboratory. The assays were performed by the researcher, but overseen by the laboratory manager at the College of Nursing physiology wet lab and under the guidance of the Doctoral Supervisory Chair.

**Statistical Analysis Limitations**

All studies are at risk for Type II errors. Type II errors can occur when the researcher incorrectly fails to reject the null hypothesis. The data may not support rejecting the null hypothesis when in truth the null hypothesis is not true. The data from this study do not reject the null hypothesis, and it was determined that there was no change in bone biochemical markers from baseline after 12 weeks of black cohosh therapy. If in the population black cohosh does significantly lower bone remodeling, then a Type II error occurred. A Type II error may occur with small sample sizes and an incorrect sample size, both of which are possible in this study.

**Conclusions**

The conclusion drawn from this study is that the black cohosh did not demonstrate any effect on bone remodeling in the postmenopausal female subjects. The study revealed no statistically significant differences in either the bone resorption or bone formation marker after
taking a standardized 40mg dose of black cohosh for 12 weeks. However, it continues to be
difficult to rule out the possibility that black cohosh has an effect on bone remodeling. It is
possible for this researcher to conclude that the results of the study occurred due to a small
sample size and the utilization of the larger effect size.

Another conclusion drawn from this study is that black cohosh had no effect on either
systolic or diastolic blood pressure. Blood pressures were measured at the onset and the
conclusion of the study period and no significant change in blood pressure was demonstrated. It
can be concluded that black cohosh is a safe alternative for postmenopausal women who are
normotensive or have controlled hypertension.

This study revealed different findings from those documented in the study by Wuttke and
colleagues (2006) who described an increase in osteoblast activity with a standardized dose of
black cohosh. Wuttke’s study had three groups: black cohosh, conjugated estrogens, and placebo
with a total N= 62 (approximately 20 subjects per group). The dose of black cohosh and the
duration of treatment were the same as the current study. The inclusion criteria for the Wuttke
study were not as strict. All women between the ages of 40 and 60 were included in the study.
The number of years postmenopause was not addressed and women who had been amenorrheic
for 6 months were included. Women with a Body Mass Index (BMI) of greater than or equal to
30 were excluded from the study. The difference in the results of the current study and the study
done by Wuttke et al. could be the more stringent inclusion and exclusion criteria in the current
study. Most importantly, the study by Wuttke et al. included both pre- and postmenopausal
women with highly variable estrogen status whereas the current study was restricted to women
who were estrogen deplete for 1-6 years.
Recommendations for Future Research

Although this study did not find any statistically significant effect on bone remodeling with black cohosh, it is possible for future studies to observe a positive effect. Prior studies conducted in Germany measuring the effect of black cohosh on bone metabolism have shown positive results. Currently there are other studies in progress evaluating the effect of black cohosh on bone in both Germany and the United States. In addition, studies measuring the effect of black cohosh on hot flashes continue, as the studies previously conducted have demonstrated conflicting results.

The sale of black cohosh is a multimillion dollar industry and numerous brands and variations of it exist in the retail market. It is important that research continue on black cohosh as women will continue to take this supplement as it is easy to obtain, and has a good word of mouth reputation. The future direction in black cohosh research is to: 1) conduct more randomized, double-blind, placebo-controlled clinical trials on the effect of black cohosh on bone remodeling; 2) conduct studies to identify the mechanism of action of black cohosh; 3) continue research on its effect on the severity and frequency of hot flashes; 4) conduct studies to identify the mechanism of action for liver damage and failure in women taking black cohosh.

Implications for Clinical Practice

It is imperative for the women’s healthcare provider to be knowledgeable about the common dietary supplements postmenopausal women take. It is often not acknowledged that women take herbal supplements in the healthcare providers’ office. It is important for the healthcare provider to understand that most dietary supplements have little to no clinical research done on them, and what is done is often poorly designed and sponsored by the supplement company. The current goal of the nursing healthcare provider is to document the use of dietary
supplements and to educate the client on the current research, contraindications, and adverse
effects of the most commonly used dietary supplements.

It is also in the realm of nursing research to continue with well designed clinical trials on
dietary supplements. Although this study did not demonstrate that black cohosh had any effect
on bone remodeling, future nursing studies may. It is vitally important that nurse researchers
perform well-designed clinical trials on dietary supplements. The American public is spending
billions of dollars on dietary supplements that have little to no clinical research (Bent & Ko,
2002). Postmenopausal women aged 45-60 are the top spenders on dietary supplements, many
purchasing products to alleviate the vasomotor symptoms of menopause such as hot flashes and
insomnia (Kang, Ansbacher, & Hammoud, 2002). The future direction of dietary research
should include studies to evaluate effectiveness, safety, and the interaction of dietary
supplements with other drugs. Consumers and health care providers need to be educated about
dietary supplements. Nurse researchers are in a great position to participate fully in supplement
research and then educate the consumer with the most up to date information.
APPENDIX A
DEMOGRAPHIC INFORMATION QUESTIONNAIRE

Name ________________________________________
   Last                           first                             m.i.

Address _____________________________________
   __________________________________________

Telephone   home ( ) work ( ) _________________

Age ___________    Date of Birth _______________________

Marital Status ______________________

Highest educational degree
   ___ Middle School
   ___ GED Certificate
   ___ High School Diploma
   ___ Associate Degree
   ___ Bachelor’s degree
   ___ Master’s degree
   ___ Doctoral degree
   ___ Other degrees        Please list ________________________

Present work status
   ___ Not employed
   ___ Disabled
   ___ Working Part time
   ___ Working Full time
   ___ Retired

Last menstrual period _________________________

Date form completed _________________________
APPENDIX B
MEDICAL HISTORY QUESTIONNAIRE

Name _____________________________

Id number __________________

General Health History
Yes    No
___   ___   1. Do you consider yourself to be generally healthy?
___   ___   2. Have you ever been diagnosed with a heart condition?
___   ___   3. Have you ever been diagnosed with high blood pressure?
___   ___   4. Have you ever had a heart attack?
___   ___   5. Have you ever had a stroke?
___   ___   6. Have you ever been diagnosed with diabetes?
___   ___   7. Have you ever been diagnosed with kidney disease?
___   ___   8. Have you ever been diagnosed with a thyroid condition?
___   ___   9. Have you ever been diagnosed with a parathyroid problem?
___   ___  10. Have you ever been diagnosed with osteoporosis?
___   ___  11. Have you ever been diagnosed with breast cancer?
___   ___  12. Have you ever been diagnosed with a blood disorder?

Medication History
Yes    No
___   ___   1. Have you ever taken bisphosphonates (Actonel, Fosamax, Boniva)?
___   ___   2. Have you ever taken parathyroid hormone (Forteo, Teriparatide)?
___   ___   3. Are you currently taking estrogen replacement?
___   ___   4. Are you currently taking Evista?
___   ___   5. Are you currently taking calcium supplements?
___   ___   6. Are you currently taking oral contraceptives (birth control pills)?
___   ___  7. Please list all medications and supplements you are taking below:

Social History
Yes    No
___   ___   1. Do you currently smoke or use tobacco products?
___   ___   2. Do you currently drink alcohol? If yes how much and how often?
___   ___   4. Do you currently participate in a regular exercise program?
   If yes please list __________________________________________
Reproductive History

Yes   No

___ ___ 1. Are you currently menopausal (having no menstrual period)?
   Date of last menstrual period _______________________

___ ___ 2. Do you still have your uterus?

___ ___ 3. Do you still have your ovaries?

___ ___ 4. Are you currently experiencing hot flashes?

___ ___ 5. Have you ever experienced hot flashes?

___ ___ 6. Have you been told by a health care provider that you are menopausal or postmenopausal?

___ ___ 7. Have you had lab work done by a health care provider that confirms you are menopausal?

__________________________
Signature of Participant

__________________________
Signature of Investigator

__________________________
Date Form Completed

_____ Recommended for study

_____ Not recommended for study
CERTIFICATE OF ANALYSIS

PRODUCT: HP BLACK CCHCNSH 40 mg CAPSULES

<table>
<thead>
<tr>
<th>APPEARANCE:</th>
<th>Clear Hard Gelatin Capsule, Tan Powder Fill</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIZE/SHAPE:</td>
<td>#2 Vegetable</td>
</tr>
<tr>
<td>AVG. FILL WEIGHT:</td>
<td>188 - 194 mg</td>
</tr>
<tr>
<td>FILL WEIGHT:</td>
<td>304.2 mg</td>
</tr>
<tr>
<td>CODE:</td>
<td>1970</td>
</tr>
<tr>
<td>LOT:</td>
<td>4957KF1970</td>
</tr>
</tbody>
</table>

IDENTIFICATION: Compares to type

DATE OF ANALYSIS: 11/21/05

DATE OF MANUFACTURE: 11/10/2005

When packaged in a 100 cc HDPE bottle with plastic cap and screw cap seal, Nutra Manufacturing normally assigns a 2.5 year expiration date to the product.

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>LABEL CLAIM (per unit)</th>
<th>RELEASE RANGE (per unit)</th>
<th>ASSAY RESULTS (per unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Cohosh PE</td>
<td>40 mg</td>
<td>(38 - 48 mg)</td>
<td>40 mg INPUT</td>
</tr>
<tr>
<td>Triterpene Glycosides</td>
<td>1 mg</td>
<td>(0.85 - 1.2 mg)</td>
<td>1 mg INPUT</td>
</tr>
</tbody>
</table>

DATE: 12-5-06

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APPENDIX D
INVESTIGATIONAL NEW DRUG NUMBER (IND)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 75,692

Alice P. Carlisle, Ph.D.
2850 SE 35th Street
Ocala, FL 34471

Dear Dr. Carlisle:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned: 75,692
Sponsor: Alice P. Carlisle, Ph.D.
Name of Drug: Black Cohosh Root Extract 40 mg
Date of Submission: July 25, 2006
Date of Receipt: August 1, 2006

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before August 31, 2006, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations).
Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)], (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)], and (3) submitting annual progress reports [21 CFR 312.33].

Please forward all future communications concerning this IND in triplicate, identified by the above IND number, to the following address:

**U.S. Postal Service/Courier/Overnight Mail:**

Central Document Room
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Metabolism and Endocrinology Products, HFD-510
Attention: Document and Records Section
5601-B Ammendale Road
Beltville, MD 20705-1266

If you have any questions, contact me at (301) 796-1224.

Sincerely,

(See appended electronic signature page)

Randy Hedin, R Ph.
Senior Regulatory Management Officer
Division of Metabolism and Endocrinology Products
Office of Drug Evaluation II
Center for Drug Evaluation and Research
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Alice Peters Carlisle graduated from Palm Beach Community College with an Associate of Science degree in nursing in 1979. She began her nursing career working as a staff nurse on a medical-surgical floor at Good Samaritan Hospital in West Palm Beach Florida. In 1981 she went to work in the labor and delivery unit, and that was the beginning of a lifelong love of women’s health care and the care of pregnant women. She worked as a labor and delivery nurse in West Palm Beach, Vero Beach, and Gainesville Florida until 1995, when she began her career as a nurse midwife.

Alice completed a Bachelor of Science degree in nursing in 1992 and a Master of Nursing with a certificate in nurse midwifery in 1995 at the University of Florida. She began working as a nurse midwife in private practice in Ocala Florida with Rasik Nagda MD in January 1996 and stayed at that practice until June 2004.

In 2003 Alice began her doctoral studies at the University of Florida with an interest in postmenopausal women’s health. In 2004 she left private practice and began working part time for Midwives of Ocala, a hospital owned midwifery service. She continues to work for them providing prenatal care and hospital birth to the women of Marion County. Since 2004 Alice has also worked part time as a family planning nurse practitioner at the Marion County Health Department Belleview clinic.

Alice was also an American Society of Psychoprophylaxis in Obstetrics (ASPO) certified childbirth educator and taught childbirth classes in Palm Beach County from 1981 until 1989. She continues to use that knowledge to educate pregnant women in her current position. Alice was also a part time adjunct clinical professor at the University of Florida College Of Nursing from 2005 to 2006.
Alice is an active member of the American College of Nurse Midwives (ACNM), and served as the secretary of the Gainesville-Ocala chapter for four years. Alice is also a member of the American Nurses Association (ANA), Florida Nurses Association, and Sigma Theta Tau (Alpha Theta chapter).