EFFECTS OF SOY PROTEIN ON INDICES OF BONE TURNOVER IN MEN AND WOMEN

By

NASRIN SINICHI

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1997

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2000

EFFECTS OF SOY PROTEIN ON INDICES

OF BONE TURNOVER IN

MEN AND WOMEN

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGMENTS

"Anything accomplished has not been done alone." No true words could be applied to this research/Master's endeavor.

Voluminous appreciation goes to my major advisor, Bahram H. Arjmandi, Ph.D., R. D., for providing critical and invaluable ideas, as well as encouragement and support in fulllfilling my research and academic goals. Without his patience and guidance, the completion of this Master's thesis and research would not have been possible.

My sincere gratitude is extended to my thesis committee members: Dr. Gail Gates, Department of Nutritional Sciences and Dr, Joseph Weber, Department of FRCD, and Dr. Mark Payton, Department of Statistics. Their thorough and enthusiastic critique and healthy exchange of ideas and knowledge with me were very important for this final thesis to take place.

Considerable thanks is given to my husband, Massood Saffarian, for being patient, tolerant and helpful in family responsibilities. His love and understanding made this endeavor endurable. Thanks also to my children Neda and Nika who expressed interest, support, and encouragement.

Special acknowledgment is also due to the following staff and students at Oklahoma State University; Dr. Edralin Lucas, Dr. Dania Khalil, Dr. Young Rhee, Lisa Hammond, Brandon Hodges, and Shanil Juma who assisted me in the subject recruiting, data collection, and laboratory analyses. To all unnamed families and friends, who directly or indirectly, supported me, I give my thanks.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

Chapter Page

LIST OF TABLES

List Of Abbreviations

ALP	Alkaline Phosphatase
B-ALP	Bone-Specific Alkaline Phosphatase
BMD	Bone Mineral Density
BMC	Bone Mineral Content
CT	Calcitonin
Dpd	Deoxypyridinoline (pyrilinks-D)
E_2	17β-Estradiol
ERT	Estrogen Replacement Therapy
FDA	Food and Drug Administration
GH	Growth Hormone
IGF-I	Insulin-Like Growth Factor Binding Protein-3
IGFBP-3	Insulin-like Growth Factor-I
IP	Ipriflavone
NTX	N-telopeptide of the Cross-Links of Collagen
PTH	Parathyroid Hormone
SERM	Selective Estrogen Receptor Modulators
SHBG	Sex Hormone Binding Globulin
TRAP	Tartrate-Resistant Acid Phosphatase
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Osteoporosis is the most common skeletal disorder in the United States and is a major health and socioeconomic problem worldwide (Castelo-Branco, 1998). It is characterized by a progressive bone weakness due to gradual loss of bone mineral content and protein matrix leading to increased bone fragility and higher rate of fracture (Castelo-Branco, 1998). According to the recent National Health and Nutrition Examination Survey (NHANES), approximately 26 to 38 million adult in the United States have osteoporosis or are at risk for osteoporosis (Leboff et al., 1999). Osteoporosis is a major cause of morbidity and mortality which currently costs Americans over $13.8 billion dollars each year in addition to the pain, immobility, depression and inability to perform tasks (Finkelstein et al., 1998). Costs are expected to exceed $60 billion by the year 2020 if no drastic changes take place (Tolstoi & Levin, 1992).

As the American population gets older, osteoporosis will become an even greater factor in the health of this society. It is estimated that 50% of women over age 45 and as many as 90% of women over age 75 have osteoporosis (Melton et al., 1989).

Osteoporosis that is associated with ovarian hormone deficiency following menopause (postmenopausal osteoporosis) is by far the most common cause of age-related bone loss. A sharp decrease in ovarian estrogen production is the predominant

cause of rapid hormone-related bone loss. This rapid phase of bone loss occurs within the first 10 years following the cessation of menses or surgical removal of the ovaries, resulting in an increased rate of bone turnover and an imbalance between bone resorption and bone formation, thereby accelerating bone loss (Tolstio & Levin, 1992).

Osteoporosis in men is also a significant problem in clinical medicine (Jackson et al., 1990). Long-term testosterone deficiency and the resulting reduction in bone mineral content are important factors that cause osteoporosis in men (Jackson et al., 1990). The risk for spinal osteoporosis with vertebral fractures increased in men who smoked cigarettes, drank alcoholic beverages, were obese, and had an associated disease known to affect calcium or bone metabolism (Seeman et al., 1983). One out of every eight men over age 50 will experience a bone fracture due to osteoporosis (Lipman, 1999).

Prevention of osteoporosis by interrupting the chain of events that leads to bone fracture is currently the best and most cost-effective approach to osteoporosis (Lindsay, 1993). Appropriate public health strategies during the first, three or four decades of life, to prevent bone fragility, include having adequate calcium intake, maintaining appropriate body weight, obtaining adequate physical activity, and avoiding cigarette and alcohol abuse (United States Department of Health and Human Services, 1988).

Current therapies emphasize the use of anti-resorptive agents, such as estrogen replacement therapy (ERT) (Lobo, 1995), calcitonin (Gennari et al., 1994) and bisphosphonates (Fleisch, 1994). There are also potential bone formation-stimulating agents such as parathyroid hormone (PTH) (Turner, 1991), 1,25 dihydroxy vitamin D_3 ($1,25\ (OH)_2$ vitamin D_3) (Raisz, 1993). While these agents may prevent further bone loss in established osteoporosis, they do not have the capability of restoring bone mass that

already has been lost (Baron, 1993a). Furthermore, the compounds that are reported to have bone forming properties are either associated with certain side effects or they do not produce bone mass with desirable quality (Turner, 1991). It would be most desirable to discover naturally occurring compounds or synthetic substances that could substitute for or reduce the use of these types of drugs and at the same time have fewer associated side effects.

There are some indications that the consumption of a diet rich in fruits, vegetables, grains, and legumes can reduce the incidence of postmenopausal osteoporosis (Anderson et al., 1997). It is reported (Arjmandi et al., 1996; Anderson et al., 1997) that the protective effect of a plant-based diet may be due to the presence of numerous physiologically-active compounds known as "phytoestrogens" (Arjmandi et al., 1996). Phytoestrogens are nonsteroidal plant compounds of diverse structure that may produce estrogenic responses. Most of the known phytoestrogens are, relatively, weak estrogens, but they can have potent biological effects when ingested in large quantities (Arjmandi et al., 1996). Also, the natural food sources with high concentrations of isoflavones, which are naturally occurring phytoestrogens, might be equally affective in modulating bone mass (Gambacciani et al., 1993). Naturally occurring isoflavones have also been found to positively affect bone without toxicity, carcinogenicity or other undesirable side effects (Gambacciani et al., 1993). The purpose of this study was to determine whether soy protein has favorable effects on the indices of bone turnover in both men and women.

Hypothesis

The hypothesis of this study is that inclusion of soy protein in the diet increases bone mineral density by suppressing the rate of bone resorption while maintaining or enhancing the rate of bone formation in both men and women.

Objective

The objective of this study was to determine whether the daily inclusion of 40g soy protein with normal isoflavones (about 90 mg) into the diets of men and women for three months will positively influence bone metabolism as assessed by favorable alterations in the rate of bone turnover.

CHAPTER II

REVIEW OF LITERATURE

Osteoporosis

Osteoporosis is the most common metabolic bone disease. It is characterized by an imbalance between bone formation and bone resorption, resulting in decreased bone mineral density (BMD) or a reduced amount of total bone mass (Eriksen et al., 1994).

The etiology of osteoporosis can be primary (type I and type II) or secondary. Primary osteoporosis in women begins during the perimenopausal period and is due to decreased in concentrations of estrogens and androgens (Johnston et al., 1995). Secondary osteoporosis in men and women is due to various factors such as aging, a chronic low intake of calcium, use of medications, endocrine disorders, gastrointestinal diseases, physical inactivity or immobilization, hyperparathyroidism (Johnston et al., 1995), cigarette smoking, alcohol abuse, medical diseases that affect calcium or bone metabolism (Seeman et al., 1983). The disease types differ in terms of sex and age of those affected, type and rate of bone loss, and fracture sites (Teitelbaum, 1996). Vanderschueren et al. (1990) indicated that aging is associated with decreased in rate of bone formation rather than increased rate of bone resorption. Results from their study of

men and women showed that bone and serum osteocalcin concentrations which reflect elevated osteoblastic activity and increased rate of bone formation were higher in men than in women with average age of (58.3±1.2) years and an age-related decline was observed in both sexes.

Osteoporotic fractures usually involve the hip, spine, and wrist (Teitelbaum, 1996). According to the National Institutes of Health (1999) an estimated 10 million individuals in the United States have osteoporosis and an additional 18 million have low bone mass that places them at increased risk for this disabling disease. Osteoporosis is the underlying factor in more than 1.5 million fractures each year. This includes approximately 300,000 hip fractures, 700,000 vertebral fractures, 250,000 wrist fractures, and more than 300,000 fractures at other sites.

The incidence of fractures increases with age (Castelo-Branco, 1998). It is higher in white than in black individuals and in women than in men (Castelo-Branco, 1998). One out of two women and one in eight men will have an osteoporosis-related fracture at some time in their life (Seeman et al., 1983). Moreover, after the age of 50 years nearly one-half of all white and oriental women will sustain at least one osteoporotic bone fracture (Castelo-Branco, 1998).

Three factors in the pathogenesis of osteoporosis should be considered: (1) changes in the organic matrix which is composed mainly of collagen and several noncollagenous proteins such as osteocalcin and osteonectin, (2) an inorganic component such as hydroxyapatite, and (3) peak bone mass which is achieved in the third decade of life (Leboff et al., 1999). In adults, any factor that maximizes peak bone mass modifies the risk of developing osteoporosis later in life (Finkelstein et al., 1998).

The bone remodeling cycle begins with osteoclastic bone resorption. Osteoblasts then migrate to this newly formed cavity and replace the deficiency with a calcifiable organic matrix. It appears that with each remodeling cycle there is an imperceptible deficit in bone formation. Therefore, the total bone loss is the function of the number of cycles in progress at any one time. Thus, conditions that increase the rate of bone remodeling processes increase the rate of bone loss (Castelo-Branco, 1998). Heredity, race, sex, age and lifestyle have important effects in regulation of the bone remodeling cycle (Meiner, 1999).

Overview of Osteoporosis in Women

Osteoporosis is a public health threat that affects over 25 million Americans 80% of whom are women. Loss of ovarian function increases bone resorption and accelerates bone loss with bone turnover remaining elevated into the late postmenopausal years, which is a determinant of osteoporosis (Garnero et al., 1996b). Osteoporotic risk varies with ethnic origin. For instance, Caucasian women are at greater risk than African and Mexican Americans (Looker et al., 1997) and experience with higher fracture rates (Silverman & Madison, 1998). About 30% of postmenopausal Caucasian women in the U.S. are affected by osteoporosis with 16% experiencing vertebral fractures (Melton, 1997). We have a paucity of data on bone density and osteoporotic risk factors in Asians originating from the Indian subcontinent who now live in the U.S. (Cundy et al., 1995; Alekel et al., 1999) or residing elsewhere (Tobias et al., 1994; Parker et al., 1992; Chin et al., 1997). We do know that osteoporotic fractures usually occur 10 to 20 years earlier in

Indian men and women residing in eastern area than in their western counterparts (Nordin, 1966; Gupta et al., 1967). It is also noteworthy that Chinese and southeast Asians who consume soy products habitually have low osteoporotic fractures and high BMD, whereas Asians from the Indian subcontinent who do not typically consume soy products have been at higher risk of osteoporosis (Kamath et al., 1999). A collective conclusion from above studies could be drawn that some of these differences in osteoporotic risk factors among ethnic groups are inexplicable, but in part may be due to dietary and exercise-related factors specific to each culture.

Overview of Osteoporosis in Men

Osteoporosis in men can be a major health concern. Although less common than in women, it is estimated that one-seventh of all vertebral fractures and one-fifth of all hip fractures occur in men (Jackson, 1993). Men have an increased risk of sustaining fractures of the hip, spine, and forearm with aging. Moreover, a survey conducted in Sweden has indicated that, although the age-adjusted incidence of fractures of the hip may be declining in women, the occurance of such fractures in men is on the rise (Naessen et al., 1989). Projected figures suggest that 17% of men will suffer a hip fracture before they reach the age of 90, leading to significant physical and emotional problems (Avioli, 1991). Osteoporosis develops less commonly in men than in women because men have larger skeletons, bone loss starts later and progresses more slowly, and there is no period of rapid hormonal changes and the accompanying rapid bone loss (Jackson et al., 1990).

There are many causes of osteoporosis in men (Jackson et al., 1990). For example, 40-45% of men with spinal osteoporosis and vertebral compression fractures have disorders or conditions that can produce bone loss and cause osteoporosis. These include hypogonadism, hyperthyroidism, neoplastic diseases, and genetic disorders as well as steroid therapy, immobilization, and gastric surgery (Scane et al., 1993). Currently, the therapy for established osteoporosis in men is inadequate and no single agent has been convincingly capable of preventing osteoporotic fractures in men. However, an animal study conducted by Muhlbauer (1997) showed that natural dietary components such as soy is capable of inhibiting the increased loss of trabecular bone density in rats. Emphasis must be given to prevention of bone loss in men (Jackson et al., 1990).

Food and Drug Admininstration Approved Therapies for Osteoporosis

The approved drugs for the treatment of osteoporosis are estrogen, calcitonin, bisphosphonates , and raloxifene.

Estrogen. Estrogens (E_2) play important hormonal roles among all vertebrates. Estrogen replacement therapy (ERT) prevents bone loss due to ovarian hormone deficiency, and controls bone remodeling (Mundy, 1993; Sue, 1993). Studies show an estimated 25% reduction of hip fractures and 50% reduction of vertebral fractures in subjects receiving ERT compared to untreated subjects (Sue, 1993). Estrogen maintains bone mass by slowing bone turnover which subsequently slows bone resorption and bone formation (Kalu et al., 1991). It may also indirectly inhibit bone loss by its interaction

with parathyroid hormone (PTH), calcitonin (CT) and 1,25 $(OH)_2$ vitamin D_3 and help maintain their balance in circulation (Tolstoi et al., 1992). When ovarian hormone deficiency occurs, estrogen maintains bone integrity by possibly interacting with E_2 receptors on osteoclasts and osteoblasts (Tolstoi et al., 1992; Turner 1991; Raisz, 1993; Mundy, 1993). Zofkova et al. (1996) reported that surgically induced estrogen deficiency increased serum ionized calcium and phosphate levels in blood as well as insulin-like growth factor-I (IGF-I) concentrations, and PTH in perimenopausal women. These parameters return to their preoperative values after short-term transdermal estrogen therapy. Although estrogen is usually considered the most effective therapy in treatment of osteoporosis, unfortunately its use is associated with increase risk of breast and endometrial cancers (Nagata et al., 1998).

Bisphosphonates. Bisphosphonates are drugs with structural analogues of pyrophosphate which combine with hydroxyapatite crystals in bone and inhibits osteoclast or pre-osteoclast activity (Boonecamp et al., 1986). Thus, they easily reach their target organ, bone, for which they have remarkably high affinity. This modification gives the compound a longer biologic half-life in the bone to prevent the osteoclasts cells from resorpting its mineralized matrix.

The original notion about the mechanism of action of bisphosphonates is based on their high-binding affinity for skeletal calcium and their prevention of bone crystal dissolution (Boonecamp et al., 1986). These agents impair the resorptive function of both mature and immature forms of osteoclasts (Boonecamp et al., 1986). In the cyclical therapy, bisphosphonate is administered orally at bedtime for two weeks followed by 13 weeks of a daily supplement of calcium and vitamin D_3 to ensure that adequate calcium

and phosphorus are available for mineralization (Tolstoi et al., 1992). *In vitro*, bisphosphonates stimulate collagen synthesis and fatty acid oxidation (Boonecamp et al., 1986). Variable effects, e. g. increases or decreases depending on the cellular model, are seen in the production of alkaline phosphatase, mitochondrial calcium release, and cell replication (Boonecamp et al., 1986). Bisphosphonates impair the development of the immature osteoclast (Boonecamp et al., 1986) and the function of mature osteoclasts. They depress chemical signaling to adjacent cells and alter the osteoclast life cycle (Satom et al., 1990).

Because the remodeling (repair) process of the skeleton is linked to osteoclastic function, there are concerns that these long-lived agents may actually be detrimental to the skeleton. The major concern is that bisphosphonates might halt the metabolism of the skeleton, stopping the normal repair process (Satom et al., 1990).

Bisphosphonates include etidronate, alendronate sodium, residronate, tiludronate, ibandronate, and zoledronate, most of which are currently under investigation by the Food and Drug Administration (FDA). The only bisphosphonate currently approved by the FDA in the United States (October 1995) for the therapy of established osteoporosis is alendronate (Ragsdale et al., 1998).

Alendronate has been the most intensively studied bisphosphonate so far (Harris et al., 1993; Liberman et al., 1995). In a well-designed 3-year study conducted at multiple sites in the United States and abroad, alendronate increased bone mass of the lumbar spine by approximately 10% and was associated with a reduction in fracture incidence. Further evidence is seen when comparing the loss of height between the placebo and the alendronate treatment groups. Women who did not receive the drug lost an average of 1

inch in height over 3 years, whereas those who received alendronate lost only an average of ¼ inch. Another interesting point about alendronate therapy is that bone mineral density showed incremental increases over the 3 years of treatment period. This suggests that alendronate may have an additional positive effect on bone, besides its anti-resorptive ability (Harris et al., 1993; Liberman et al., 1995).

Alendronate is remarkably well tolerated and gastrointestinal side effects which can occur with bisphosphonates at high doses, are not observed. No other side effects specifically attributable to alendronate have been observed (Harris et al., 1993; Liberman et al., 1995). Less than 10% of an oral dose of the drug is absorbed from the gut which half of it is excreted unchanged by the kidney and the remainder is deposited in the bone (Tolstoi et al., 1992).

Calcitonin. Calcitonin (CT) has been available as an injectable treatment for established osteoporosis since 1984 (Tolstoi et al., 1992). The inconvenience of injection and the resulting side effects have limited the widespread use of injectable calcitonin. The most common side effects of injectable calcitonin are nausea, with or without vomiting, diarrhea, local reaction at the injection site, and flushing of the face and hands (Baron et al., 1993b). In 1995, a new nasal spray formulation, sold under the name, fosamax, by Merck & Co. Inc., was approved by the Food and Drug Administration. Similar to bisphosphonates, calcitonin inhibits bone resorption by directly affecting the osteoclast. Calcitonin reduces osteoclast activity *in vitro* (Tolstoi et al., 1992) and this reduction appears to be its major role *in vivo*. Both *in vitro* and *in vivo* data suggest that calcitonin may have an anabolic osteoblastic effect on bone (Tolstoi et al., 1992) which

could improve bone quality and reduce the incidence of bone fracture (Baron et al., 1993b). Nasal preparations have been licensed for the treatment of osteoporosis in several countries. This drug which cost $2,200 to $3,000 annually looses its efficacy after 18-24 months due to production of CT antibodies or down regulation of its receptor (Tolstoi et al., 1992; Baron et al., 1993b).

Raloxifene. Raloxifene is the most recent FDA approved drug for the treatment of osteoporosis. Its mode of action differs from those of antiresorptive drugs such as estrogen and bisphosphonates. Raloxifene suppresses bone resorption which allows bone formation to continue (Anthony et al., 1997). Hence, individuals who have already lost bone may benefit from this therapy more so than just the antiresorptive drugs (for further discussion of raloxifene refer to the following SERMs section).

Selective Estrogen Receptor Modulators (SERMS) and Bone

Selective estrogen receptor modulators are a growing class of nonsteroidal compounds with estrogen-like actions in bone and lipid metabolism, and antiestrogenic actions in the breast tissue (Weryha et al., 1999). Synthetic compounds such as tamoxifen, raloxifene, and ipriflavone, pharmacologically and structurally similar to estrogen, are effective in preventing or reducing bone loss.

The first SERM, tamoxifen, is a nonsteroidal antiestrogen approved by the US Food and Drug Administration in 1978 for treatment of people with estrogen receptor-positive breast cancers (Weryha et al., 1999). However, similar to estrogen it may prevent bone loss (Wright et al., 1994). The results of an animal study have shown that

tamoxifen acts as an estrogen agonist and prevents bone loss due to ovarian hormone deficiency (Stewart et al., 1986). Also, there is substantial evidence that in postmenopausal women, tamoxifen aids in the preservation of bone density and inhibition of bone resorption without changing bone formation (Robinson et al., 1996). Furthermore, Kalu et al. (1991) reported that the action of tamoxifen on bone is influenced by the level of circulating estrogen. According to this view, at low levels of estradiol, tamoxifen has estrogen agonist action on bone and in the presence of high plasma estrogen levels it has an anti-estrogenic action on bone. The beneficial effects of tamoxifen on bone have been found in other studies but its major drawback is its endometrial stimulatory effects (Fornander et al., 1989).

Another SERM, raloxifene, is the latest FDA approved drug for osteoporosis. It mimics estrogen action in some tissues, while preventing the risk of cancer. Raloxifene exerts positive effect on both bone and cholesterol without affecting the uterine and breast tissues (Delmas et al., 1997). Raloxifene is a major alternative to hormone therapy for bone and unlike tamoxifen does not stimulate the endometrium (Delmas et al., 1997; Heaney & Draper 1997). However, a drawback of raloxifene is that it may increase hot flushes in some women (Draper et al., 1996; Walsh et al., 1998), thus limiting its use to those who are well beyond the menopausal transition. The proposed mechanism of action for SERMs on bone is to decrease bone resorption. Although these potential beneficial effects of SERMs make them very attractive in preventing and treating osteoporosis, naturally occurring SERMs such as soy isoflavones may be more acceptable to many postmenopausal women than the synthetic analogues because they can be obtained via dietary means.

Ipriflavone (IP), an isoflavone derivative of plant origin (Havsteen, 1983), has been used in preventing and treating postmenopausal osteoporosis (Fujita et al., 1986; Agnusdei, et al. 1989; Agnusdei et al., 1992) and in several models of experimental osteoporosis (Yamazaki et al., 1986a; Yamazaki, 1986b). The effect of ipriflavone on bone resorption seems to be directed to inhibition of preosteoclast recruitment and differentiation of osteoclasts (Morita et al., 1992; Bonucci et al., 1992). It acts as both anti-resorptive and bone-forming agent without producing the adverse side effects associated with estrogen (Melis et al., 1992; Agnusdei et al., 1992). A recent study by Arjmandi et al. (2000), compared the effect of IP to those of E_2 on bone in rats with ovarian hormone deficiency. The investigators of the study concluded that IP as well as E_2 is capable of preventing loss of bone density due to ovariectomy. Ipriflavone is a promising agent and represents an attractive alternative for the prevention of osteoporosis in postmenopausal women particularly in those who present contraindications to estrogen replacement therapy.

Soy Protein

Soy beans are excellent source of protein, fiber, and minerals (Messina, 1999) and are popular in the diets of Asian countries (Omi et al., 1994). There are fewer cases of bone fractures due to osteoporosis and lower incidence of breast cancer in Asian women compared to women in Western countries where a limited amount of soy products are included in their diets (Zava, 1994; Omi et al., 1994). Asians consume 20-50 times more soy-based food per capita than do Americans (Kim et al., 1998).

Soybeans are unique among the legumes because they are a concentrated source of isoflavones (Messina, 1999). Isoflavones are a subclass of flavonoids (Messina, 1999) containing flavone nucleus which is composed of two benzene rings linked through a heterocyclic pyrane ring (Messina, 1999). There are about 500 varieties of flavonoids each possessing many different pharmacological and biochemical properties (Brandi, 1993). As drug applications, some flavonoids produce anti-microbial, anti-asthmatic or estrogenic activities (Brandi, 1993). The soy isoflavones have estrogenic effects of approximately 1×10^{-5} the activity of the E_2, the natural female sex hormone (Brandi, 1993; Barnes et al., 1993; Zava, 1994).

The primary isoflavones present in soybeans are genistein (4,5,7-trihydroxyisoflavone) and daidzein (4,7-dihydroxyisoflavone) (Messina, 1999).

The similarity in structure between the isoflavones and estrogen and the weak estrogenic properties of isoflavones provided the initial basis for speculation that they may promote bone health. Another speculation about the benefits of isoflavones was the similarity in chemical structure between the soybean isoflavones and the synthetic isoflavone, 7-isopropoxy-3-phenyl-4H-1-benzopyran-4-one (ipriflavone) which was shown to increase bone mass in postmenopausal women (Messina, 1999) and act as both an anti-resorptive and a bone formating agent without producing estrogenic side effects (Brandi, 1993; Gambacciani et al., 1993). Ipriflavone also can be used as an alternative treatment at the level of 600-1200 mg per day for prevention of osteoporosis in postmenopausal women who exhibit contraindications to ERT (Gambacciani et al., 1993; Head, 1999).

Studies have shown the potential effects of soy protein on both osteoblasts and osteoclasts. The findings of *In vitro* and *in vivo* studies by Blair et al. (1996) indicated that when overectomized rats were given genistein (44 μmol/d) for four weeks they had 12% higher ($p<0.005$) dry femural mass than the control group. The *in vitro* findings of their studies showed that genistein suppressed osteoclastic activity with a low potential for toxicity. In a study by Fanti et al. (1998), tibial bone mineral loss was reduced significantly after 21 days after subcutaneous injection of 5 and 25 μg genistein/g body weight to ovariectomized rats. Ishimi et al. (1999) looked at the effects of genistein and E_2 on bone in ovariectomized mice. Both genistein and E_2 restored the marked bone loss occurred in the distal metaphysis of the femoral cancellous bone after ovariectomy. However, only E_2 restored the loss in uterine weight following ovariectomy indicating that genistein exhibits estrogenic activity in bone but not in uterus (Ishimi et al., 1999).

A study of soy milk peptides conducted by Omi et al. (1994), showed that regular soy milk protein, high-molecular soybean milk peptides and low-molecular soybean milk peptides all increased BMD and mechanical bone strenth in comparison with casein deit (control). The suggested mechnism may be increasing the intestinal calcium absorption, by some peptides presented in bovine bone. However, additional research is needed to identify the components of soybeans which influence calcium absorption and bone metabolism.

Estrogenic Effect of Isoflavones

Phytoestrogens or plant estrogens including isoflavones, coumestans, and lignans have been identified in whole grains, fruits, and vegetables. Soy isoflavones including

genistein, daidzein, and glycitein are structurally similar to E_2, are reported to protect against chronic diseases such as osteoporosis, breast cancer, and cardiovascular disease (Kurzer & Xu 1997). Isoflavones exert an estrogenic effect on the central nervous system, stimulate growth of the genital tract in female mice, and bind to estrogen receptors (Liberman, 1996). Shutt and Cox (1972) determined that phytoestrogens bind to estrogen receptors but, in comparison with E_2, have much lower binding affinity for estrogen receptors.

In postmenopausal women consuming 130 mg isoflavones/day, Kurzer (unpublished observations) found a significant decrease in estrone sulfate (E_1SO_4; p=0.002), increase in sex hormone binding globulin (SHBG) (p=0.04), and trends toward decreased 17β-estradiol (E_2) and estrone (E_1) with increased isoflavone intake. Initially, soy isoflavones were thought to act purely as hormones. However, the actions of isoflavones are quite diverse with no single action explaining all their *in vivo* and *in vitro* effects (Setchell & Adlercreutz, 1988; Setchell, 1995; Adlercreutz, 1995). Isoflavones are thought to inhibit protein tyrosine kinase, have antioxidant effects (Kurzer & Xu, 1997), and arrest cell growth by interfering with signal transduction (Higashi & Ogawara, 1994). There is some evidence that isoflavones *in vitro* may also inhibit aromatase (Adlercreutz et al., 1993), the rate-limiting enzyme in estrogen synthesis. Thus, these data suggest that one underlying mechanism by which isoflavones act is hormonal, but most likely this is not the sole mechanism.

Isoflavones, Bone, and Biochemical Markers of Bone

Recent animal work and one published human study indicate that soy and/or soy isoflavones increase bone density. The latter study (Potter et al., 1998) was designed to examine the lipid-related effects of soy protein and isoflavones, but also indicated that isoflavone-rich soy increased lumbar spine BMD and BMC in postmenopausal women. However, there are no published long-term studies in early postmenopausal women documenting their effect on BMD or BMC. Alekel and colleagues (1999) conducted a 24-week study in perimenopausal women. Their results indicated that percentage change in lumbar spine BMD or BMC did not decline in the isoflavone-rich soy group; however, significant loss occurred in the control group who were consuming whey protein and loss approached significance in the isoflavone-deficient group for BMD.

Isoflavones such as genistein and daidzein aside from their bone protective effects also have additional health benefits including reducing the risk of breast cancer (Ingram et al., 1997) and improving lipid profiles, hence reducing the risk of cardiovascular disease (Ingram et al., 1997). These phytoestrogens normally compete with estradiol for binding to estrogen receptors and may, therefore, interfere with estrogen-induced cellular proliferation (Nagata et al., 1998). A study on female mice showed that the estrogenic activity in three isoflavones: daidzein, genistein, and glycitein was 1000-5000 times lower than that of E_2 (Song et al., 1999). However, with increased levels of dietary intake, these isoflavones can play an important role in reducing the incidence of estrogen deficiency diseases and symptoms and have cell growth-inhibitory action (Zava et al., 1997).

In a study by Arjmandi et al. (1996), markers of bone formation (alkaline phosphatase) and resorption (tartrate-resistant acid phosphatase) were greater in ovariectomized rats consuming soy protein compared to sham-operated control rats. However, vertebral and femoral bone densities were also significantly higher in the soy fed compared with ovariectomized control rats with vertebral bone density similar to that in the estrogen-treated group. In support of these observations calcium and phosphorus levels of the vertebrae were higher in soy-fed rats compared to sham-operated controls. Results from this research and another recent study (Harrison et al., 1998) suggest that bone formation stimulated by soy protein and/or its isoflavones exceeds the resorption induced by ovariectomy. Other studies have found the effects of coumestrol *in vitro* that have been shown to inhibit bone resorption and stimulate mineralization (Tsutsumi et al., 1995). Yet, the findings of the study by Alekel et al. (1999) in perimenopausal women on soy isoflavones appear to confirm the animal findings by Arjmandi et al. (1996) in the rat model. In the study by Alekel et al. (1999), the analyses of the biochemical markers of bone indicated that bone formation appeared to be maintained and bone resorption was unaltered in the isoflavone-treated group throughout the 24 weeks of treatment. Whereas, these results did not observed in the isoflavone-deficient soy protein or control groups. Based on these findings from the biochemical markers of bone turnover, soy isoflavones do not appear to decrease bone resorption as does estrogen therapy but it may prevent a decrease in bone formation which otherwise typically occurs during the menopausal transition (song et al., 1999). Thus, the mechanism by which isoflavones appear to attenuate bone loss may involve osteoblast stimulation and thus maintenance of bone formation, not inhibition of resorption as does estrogen. Animal studies by Arjmandi et

al. (1996, 1998b) suggest a role for IGF-I in modulating these effects. This question of whether isoflavones inhibit bone resorption or stimulate bone formation needs to be further studied.

Isoflavones, Protein Intake, Mineral Metabolism, and Calcium Absorption

It has also been suggested that a soy protein diet may protect against bone loss by mechanisms independent of its estrogenic effects. For example, soy foods are a good source of calcium. Also, isoflavones may enhance intestinal calcium absorption, similar to the direct estrogen-enhancing effects on calcium uptake *in vitro* (Arjmandi et al., 1993). Further, a high soy protein diet may result in less urinary calcium loss compared to diets high in animal protein. Breslau et al. (1988) compared urinary losses of calcium in high soy versus high animal protein diets. Urinary calcium loss with the soy protein was about 50% of that of the animal protein diet. This may be due to the sulfur-containing amino acids in animal protein. A related question posed in light of this study is whether the calcium recommendations (age 50+y, 1200 mg/d) for Caucasians should apply to Asians who typically consume ~500 mg/d (Pun et al., 1990) and whose body and skeletal sizes are smaller. A study (Kung et al., 1998) in non-osteoporotic postmenopausal Chinese women found that calcium absorption with a 600 mg supplement was 58%, during the unmodified period was 60%, but rose to 71% during calcium deprivation (<300 mg/d). These absorption values are two-fold higher than what has been reported in Caucasians (Heaney et al., 1989) or African Americans (Dawson-Hughes et al., 1993). The difference in calcium absorption may be related to the high vegetable and soy intakes of Chinese (Weaver, 1998),

which provide 41% of their calcium compared with the U.S. vegetable and soy intake which provide <10% of their calcium (Lau, 1995). The issue of plant- versus animal-based diets, calcium absorption, ethnicity, and bone is not fully understood, let alone that of isoflavones and calcium balance.

Soy Protein or Its Isoflavones, IGF-I and Its Binding Proteins

The effects of growth hormone on bone are likely mediated locally through IGFs (Jones & Clemmons, 1995). Bone cells synthesize both IGF-I and IGF-II, while IGF-I is more potent in stimulating osteoblasts (Zhang et al., 1991), increasing collagen synthesis, and matrix apposition (Hock et al., 1988). Serum IGF-I declines with age in both sexes (Yamamoto et al., 1991) but also has been shown to decline immediately after menopause (Romagnoli et al., 1993). Administration of IGF-I stimulates bone turnover (Bianda et al., 1997), similar to growth hormone (Brixen et al., 1995), and is thought to play a role in regulating bone remodeling (Boonen et al., 1996). In addition, IGF-I concentrations are correlated positively with bone mass in pre- (Romagnoli et al., 1993), peri- (Nasu et al., 1997), and post-menopausal women (Boonen et al., 1996).

This is complicated by the finding that IGF-I, but not growth hormone (GH) (Brixen et al., 1995), stimulates production of $1,25(OH)_2$ vitamin D *in vivo* (Bianda et al., 1997), likely through enhancing renal 1α-hydroxylase activity. Furthermore, GH may be the influence of binding proteins on IGF action. The major serum binding protein for IGF-I, IGFBP-3 (Baxter, 1987) decreases with age but these changes are not necessarily associated with decreases in estrogen. In perimenopausal women, serum IGFBP-2 was

inversely related and IGFBP-3 was directly related to IGF-I, despite the lack of direct relation of IGFBP-2 to bone density (Nasu et al., 1997). In contrast, serum IGFBP-3 concentrations were reduced in young osteoporotic men (Johansson et al., 1997), suggesting a role for IGF binding proteins in addition to IGF-I in bone metabolism. Circulating IGFBPs not only may influence the effects of IGF-I on bone, but other hormones can alter the concentration of these binding proteins or modulate IGF receptors (Johansson et al., 1997). A recent study by Arjmandi et al. 1998a examined the effects of feeding soy protein with isoflavones versus reduced isoflavones to ovariectomized rats with established bone loss. Results indicated that bone IGF-I mRNA levels was increased ($p \leq 0.001$) by both diets but more significantly ($p < 0.05$) in the soy with isoflavones group suggesting an effect of soy on protein synthesis. However, little is known about the effect of soy isoflavones on serum IGF-I and its binding proteins in humans which may provide insight into its purported skeletal effects.

Safety Aspects of Soy Isoflavone Intake

The potential of soy isoflavones to cause toxicity must be considered, despite epidemiologic observations in many Asian women who consume high isoflavone-containing diets, but have low rates of breast (Wu et al., 1998) and endometrial (Wynder et al., 1991) cancer. A short-term study of dietary soy intake in premenopausal women with benign and malignant breast conditions, soy intake was shown to stimulate breast tissue proliferation (McMichael-Phillips et al., 1998). However, estrogenic effects in postmenopausal women are less impressive with only slight increases in vaginal cell

maturation (Baird et al., 1995; Wilcox et al., 1990). Whereas in another study, 130 mg consumption of isoflavones daily had no effects on either vaginal cytology or endometrial biopsy (Kurzer, 1997). Unlike estrogens, soy isoflavones (Arjmandi et al., 1998a; 1998b) or genistein alone (Murrill et al., 1996) in animal studies have shown no uterotropic activity. Furthermore, surgically-induced postmenopausal female macaques who consumed isoflavone-rich soy did not show proliferation in endometrial or mammary tissue (Foth & Cline, 1998). Doses of 80 and 120 mg daily used in this study are within the range of what might be consumed from moderate intakes of soy in humans (Wang & Murphy, 1994).

Significance of the Research

Inclusion of soy protein in diets is believed to delay the onset on bone loss which occurs during aging. (Kalu et al., 1988). Age-related increase in serum PTH and senile bone loss are not inevitable consequences of aging and may be prevented by food restriction or by maintaining a soy-protein rich diet (Kalu et al., 1988). Soy is a unique source of the isoflavones which structurally are similar to the synthetic phytoestrogens (tamoxifen and IP) that have shown positive bone conserving properties (Arjmandi et al., 1996). However, It is not known what component of soy affects bone metabolism or by what mechanism it works (Omi et al., 1994).

The postmenopausal period typically occupies one-third of a woman's life (Barrett-Connor, 1993), with more than 40 million in the U.S. now in the postmenopausal phase (Hargrove & Eisenberg, 1995). Menopause is accompanied not only by symptoms, but

by greater risks of osteoporosis (Lindsay, 1993). A study conducted by Agnusdei & Bufalino (1997) showed that isoflavone increased bone density and prevent fractures in elderly women with established osteoporosis.

In animals, Arjmandi et al. (1998a), showed that by giving isolated soy protein, either with or without isoflavones, to ovariectomized rats after bone loss was already established, both diets were somewhat effective in reversing femoral but neither fourth lumbar bone-density loss.

The effect of soy isoflavones on male bone whether animal or human, has received little attention. This study provides the first insight as to whether soy protein with normal isoflavone content is effective in improving markers of bone formation and bone resorption in males.

Although animal studies have provided us with valuable information on potential mechanisms of action, controlled clinical trials ultimately must be conducted to confirm the long-term effects of soy isoflavones in humans.

CHAPTER III

RESEARCH DESIGN AND METHODS

Subject Characteristics

Men and women with diverse ethnic backgrounds, who lived in Stillwater, Oklahoma or adjacent communities were recruited for this study. A total of 135 mobile individuals (65 men and 70 women), with self reported joint pain or diagnosed osteoarthritis ranging in age from 27 to 87 years were included in the study. Subjects were pre-screened via a phone interview which included a short medical history questionnaire to identify qualified potential participants. Subjects were excluded if they had rheumatoid arthritis, joint pain due to injury, cancer or a history of cancer, type 1 diabetes mellitus, kidney disease, gastrointestinal or chronic digestive disorders, or allergy to milk, eggs, or soy. A total of 90 subjects (44 men and 46 women) completed the study.

Study Overview

In a double-blind study, eligible men and women were recruited and randomly assigned to either a soy or casein regimen. Neither researchers nor subjects knew in advance if the subjects are taking soy or casein regimen. Each subject was asked to consume 40 g soy or casein (placebo) supplement on a daily basis for a three-month period. One hundred fifty men and women were initially recruited and randomly assigned to one of the two treatment groups but only 135 subjects showed up for the second visit where they gave their baseline blood and 24-hr urine specimens. Sixty-four of the subjects were on the soy regimen and 71 on the casein regimen. It was made clear to the study participants that they had an equal chance of being placed on either of these regimens. The supplements were provided to the subjects on a monthly-basis for daily consumption of two packets containing a powdered drink mix prepared and donated by Protein Technologies International (St. Louis, MO). Both regimens supplied equal amounts of protein (40g) and carbohydrate (18g). The rationale for choosing the soy dose (40g/day) was based on the amount previously used in clinical studies conducted by Alekel et al. (1999) and Potter et al. (1998). Both studies showed positive effect of soy on bone mineral density in women.

Compliance with the study protocol was monitored via the following means: 1) subjects were provided with a monthly calendar for recording consumption of the contents of the provided packets on a daily basis; 2) subjects returned any unconsumed packets to the investigators on their monthly visits, unused supplies were counted and recorded; and 3) body weight was monitored. Monthly visits were made and if needed subjects were advised how to best incorporate the supplement into their diets by reducing

extra protein intake such as meats, eggs and legumes in order to avoid excess calories. A total of 90 subjects (44 men and 46 women) completed the study with 45 subjects in each of the two treatments.

Subjects met with the investigators for a total of five visits. Visit 1 included a verbal and a written explanation of the project, signing a consent form, a detailed medical history questionnaire to confirm prescreening findings and to insure that subjects do not have any of the conditions violating the inclusion/exclusion criteria. Subjects were scheduled to come back to the study site and instructed regarding blood and urine collection for their next visit. Visit 2 occurred between the hours of 8-10 a.m. for the collection of overnight fasted blood samples (20 ml) and a 24-hour urine specimen. Anthropometric measurements included: height, weight, waist to hip ratio, and percent body fat were also obtained in this visit. Participants were given their food supplies at this visit. Visits 3 and 4 were monthly visits for the purposes of replenishing the subject's food supply, monitoring intake of the supplies as well as body weight. Visit 5, which was the final visit, occurred three months from the start of the study and included all measures and assessments which were performed on visit 2. Visiting subjects started on the beginning of April and ended at the end of October of 1999.

Subject Confidentiality

Upon entrance into the study, each subject was assigned an identification number. This number was used for tracking the subject's records throughout the study. The subjects' data such as medical history were kept in a secured cabinet with restricted

access. A number was assigned to each subject and thereafter, the samples from each study participant carried a number with no personal information available to the laboratory or data entry personnel.

Blood and Urine Collection

Blood Collection. Fasting venous blood was collected at a designated time from each subject in vacutainer tubes with appropriate anticoagulants or without anticoagulants at baseline and at the end of the study. Serum and plasma were separated using 1500x g for 20 minutes within 2 hours of collection and immediately aliquoted into small volumes and stored at -80°C until required for analyses. In this study, serum bone specific alkaline phosphatase (B-ALP) activity, total alkaline phosphatase (ALP) activity, tartrate resistant acid phosphatase (TRAP) activity, serum insuline-like growth factor-I (IGF-I), and 17β-estradiol (E_2) were measured. All the measurements are reported in SI Units.

Urine Collection. Each subject was asked to collect 24-hours of urine in acid-washed polyethylene containers prior to the day of the blood draws. The subjects provided urine twice during the study (baseline and at the end of the study). Subjects were instructed on proper urine collection method and the exclusion of the first void of the day. Total volume of the urine sample was measured to an accuracy of 0.1 ml and recorded. Urine was aliquoted from each collection and kept at -20°C for analyses. In this study, we measured pyrilinks-D, phosphorous, calcium, and magnesium. All the measurements are reported in SI Units.

Anthropometric Measurements

Height, weight, and waist:hip ratio were obtained at baseline. Waist:hip ratio was also measured at the final visit. Weight was monitored during each monthly follow-up visit. If weight gain was apparent, counseling was made available to make adjustments in the diet to prevent further gain. The Lee and Nieman (1996) method were used for assessing anthropometric measurements.

Weight and Height. Body weight was measured on a Health-O-Meter, Continental Scale Corp. scale (Chicago, IL) and subjects dressed in light clothing without shoes or jewelry. The height was taken on a Acustat Genetech Stadiometer scale (San Francisco, CA). Subjects were asked to take a deep breath, stand with their feet at a comfortable distance apart, and their shoulders and head touching the back of the stadiometer. Heights were measured to the nearest 0.1 inch.

Waist-to-Hip Ratio. Circumferences were measured with measuring tape while subjects were wearing light clothing, relaxed, standing erect, and had their arms at their sides and feet together. Waist circumference was measured midway between the lower rib and iliac crest whereas hip circumference was measured at the outermost points of the greater trochanters (WHO, 1995). Wrist to hip ratio was obtained by dividing waist circumference to hip circumference.

Percent Body Fat. Percent body fat was measured using Body Composition Analyzer (Biodynamics, Modle 310e). The analyzer was based on the bioimpedance method of testing and was designed to give a level of accuracy. According to the manufacturer, test results were based upon regression equations which correlate the bioresistance method to the hydrostatic weighing method. Test result are accurate to within ±1.5 percentage points of body fat with a correlation coefficient of 0.97. For a test, the individual's gender, age, height and weight were entered into the Analyzer

Body Mass Index. Quetelet's Index were used for indicating body mass index (BMI). Quetelet's Index were obtained by dividing weight in kilograms by height in meters squared (Lee & Nieman 1996).

Analytical Assays

Biochemical markers of bone turnover were determined two times during the study period: at baseline and at the end of the study (three months). Two markers of bone formation in serum: bone-specific alkaline phosphatase and total alkaline phosphatase activities and two markers of bone resorption: urinary deoxypyridinoline (DPD) and serum tartrate resistant acid phosphatase (TRAP) activity were measured at baseline and three months later. Serum insulin-like growth factor (IGF-1), urinary calcium, phosphorus, magnesium, and creatinine were measured at the same time points. Serum levels of 17β-estradiol (E_2) were assessed at the beginning and at the end of the study to

evaluate the estrogenicity of soy protein. Serum and urinary markers of bone turnover were considered primary outcome variables.

Markers of Bone Formation. The most responsive marker of formation, serum bone specific alkaline phosphatase (B-ALP) activity was measured using an ELISA kit (Alkphase, Metra Biosystems, Mountain View, CA) (Gomez et al., 1995). This is an immunoassay in a microtiter strip format utilizing a monoclonal antibody against bone ALP which is coated on the strip to capture bone-ALP in the sample. Anti-bone-ALP antibody at 5 mg/L was coated onto the microtiter plates and dried at 37°C overnight. To each well of the antibody-coated plate, 125 μL of buffer (100 mmol/L sodium phosphate and 150 mmol/L sodium chloride, PH 7.0) was added followed by 20 μL of bone-ALP calibrator or serum sample. After 3 h of incubation at room temperature, the plate was washed with a buffer containing 10 mmol/L sodium phosphate, 0.15 mol/L sodium chloride, and 0.5 g/L Tween-20. Then 150 μL of 2 g/L p-nitrophenyl phosphate substrate in 0.4 mol/L zinc sulfate, and 2 mmol/L magnesium acetate, pH 10.4, was added to each well and incubated for 30 min at room temperature. The reaction was stopped with 100 μL of 1 mol/L NaOH, and the absorbance was read at 405 nm with a microplate reader. The results were a measure of the activity of captured bone-ALP expressed in units/L.

Serum alkaline phosphatase (ALP) was measured using a commercially available kit from Roche Diagnostics (Branchburg, NJ). Serum ALP was determined using 4-nitrophenylphosphate and 4-nitrophenoxide ion. The rate of increased absorbance at 405 nm is proportional to the enzyme activity. The test was measured by Cobas Fara II clinical analyzer (Montclair, NJ). We did not deem it necessary to assess other markers

of formation, e.g., carboxy-terminal extension peptide of type-I procollagen, because of the high cost of the measurements and the redundancy of the information that is obtained. The ALP activity was reported in units/L, where one unit of activity is defined as that amount of enzyme which will produce one μmol of 4-nitrophenoxide ion per minute under the conditions of the assay.

Marker of Bone Resorption. The best urinary marker of bone resorption (Hanson et al., 1992), deoxypyridinoline (Dpd) was assessed at baseline and at the end of the study. Deoxypyridinoline has been shown to be one of the most consistent and reliable bone resorption assays (Hsin-Shan et al., 1997). Deoxypyridinoline was measured by an ELISA kit (Pyrilinks-D, Metra Biosystems, Mountain View, CA) that uses a monoclonal antibody which has less than 1% cross-reactivity with free pyridinoline and no significant interaction with cross-linked peptide (Garnero et al., 1996a).

Serum tartrate resistant acid phosphatase (TRAP) activity was measured using a commercially available kit from Roche Diagnostics (Branchburg, NJ). The test is based on the method by Fabiny-Byrd et al. (1972), hydrolyzing α-naphthylphosphate by acid phosphatase to produce a dye which absorbs at 405 nm. Tartrate resistance acid phosphatase values are expressed in units/L activity, where one unit of activity is defined as that amount of enzyme which will produce one μmol of α-naphthol per minute under conditions of the assay.

Other Nutritionally-Related Parameters. Urinary creatinine was measured using a commercially available kit from Roche Diagnostic (Branchburg, NJ). Urinary creatinine

concentration was determined colorimetrically using a kinetic modification of the Jaffe reaction (Jaffe, 1886). The rate of formation of the red colored complex was measured at 520 nm using the Cobas Fara II clinical analyzer (Montclair, NJ).

Urine samples for calcium and magnesium were prepared by diluting 100 μL of urine sample with 6.00 mL of 0.5% nitric acid and 0.5% lanthanum chloride. Calcium and magnesium concentrations were determined using Perkin Elmer model 5100 (Norwalk, Connecticut) atomic absorption spectrophotometer.

Urinary phosphorus was measured using a commercially available kit from Roche Diagnostic (Branchburg, NJ). Inorganic phosphorus reacts with ammonium molybdate in dilute sulfuric acid to form the unreduced phosphomolybdate complex which is measured at 340 nm (Daly et al., 1972). The test was performed using the Cobas Fara II clinical analyzer (Montclair, NJ). All of these indices were determined at baseline, and at the end of the study.

Serum IGF-I. Serum IGF-I was extracted and measured using a radioimmunoassay kit from Nichols Institute Diagnostics (San Juan Capistrano, CA) (Kalu et al., 1994). First, IGF-1 was extracted from serum using the acid-ethanol procedure. For the acid-ethanol procedure, 100 μL of serum was placed in polypropylene tubes, acidified by adding 900 μL of acid-ethanol solution to each tube. The tubes were parafilmed, vortexed and centrifuged at 1300-1500 RCF for 30 min at 4°C. Next, the supernatant was diluted with RIA buffer consisting of 1.4 mL sodium phosphate (monobasic) to give a final serum dilution of 1:225. The immunoreactive IGF-I was measured using the manufacturer's protocol. Fifty μL of extracted, standards serum and controls, 350 μL of phosphate buffer

and 100 μL of IGF-I antiserum were added to polypropylene tubes. The tubes were vortexed and incubated at room temperature for 1 h. Following incubation, 100 μL of IGF-I (I^{125}-labeled) was added to each tube, vortexed and the tubes incubated for 16-18 h at 4 (C. Unlabeled goat anti-rabbit immunoglobulin in polyethylene glycol (500 μL) and normal rabbit serum (50 μL) were added to each tube, vortexed, incubated at room temperature for 20 min and the tubes centrifuged at 1500 g for 30 min at 4 (C. The supernatant was discarded immediately and the pellet counted for 4 min using the Cobra II Series gamma counter. The IGF-I concentrations of the test samples were calculated from a standard curve generated from recombinant IGF-I standards that were treated in a similar manner as the serum extracts (Kalu et al., 1994).

Estradiol. Serum levels of E_2 were assessed using double antibody assay for estradiol from Diagnostic Products Corporation (Los Angeles, CA). This procedure is a sequential radioimmunoassay in which the sample is preincubated with anti-estradiol antiserum. Radiolabeled ^{125}I-Estradiol was then added which competes with estradiol in the serum for antibody sites. After incubation for a fixed time, separation of bound is achieved by polyethylene glycol (PEG)-accelerated double-antibody method. Finally, the antibody-bound fraction was precipitated by centrifuging for 15 minutes at 3000 xg and the supernatant was discarded immediately by decanting. The pellet was counted for 1 min using Cobra II series gamma counter (Packard Instrument Company, Meriden, CT).

Data Management and Statistical Analyses

Data Management. The data from subjects were compiled on a weekly basis into the central database filing system by a trained graduate student for permanent storage. The laboratory-generated raw data and printouts were recorded/kept in a secured storage area. All of the original data were stored in a locked cabinet with restricted access.

Data Analyses. Descriptive statistics were calculated for all variables and included means and standard deviations. The data was analyzed using PC SAS version 6.12 (SAS Inst., Carry, NC). The primary outcome variables were biochemical markers of bone formation and bone resorption. Independent sample T-tests were used to assess treatments (soy vs. control) effect and compare baseline and final values within groups. Significance level set was at $p< 0.05$.

CHAPTER IV

RESULTS

Subject participation

Subjects participating in this study included 135 healthy men and women with a mean (SD) age of 57.6 ±1.1 years. Subject dropouts included a total of 45 over the entire course of the study. Dropouts excluded from statistical analyses. Reasons for discontinuing included taste aversion, gastrointestinal disturbance, inconvenience of powdered-protein consumption, or starting a new drug therapy that could affect the outcome of the study.

A. Anthropometric measurements

A.1. All subjects. Consumption of 40 g soy protein or casein daily for a three-month period did not significantly increase body weight. However, both dietary protein regimens: soy protein ($p<0.01$) and casein ($p<0.02$) decreased percent body fat. In the casein group, there was a significant increase in both waist ($p<0.04$) and waist-to-hip ratio ($p<0.03$). In the soy group, there were no significant differences in these two measurements, however this group also tended ($p<0.06$) to increase their waist-to-hip ratio **(Table I).**

A.2. Women only. Women receiving soy protein experienced a significant (p<0.02) decrease in body fat. There were also a tendency for decreasing percent body fat in casein (p<0.09). There were no other observed significant alterations in anthropometric measurements between baseline and final values in the two treatment groups **(Table II)**.

A.3. Men only. Men receiving soy protein regimen had lower final body weight, albeit not significantly ($p < 0.09$), in comparison with initial body weight. Percent body fat tended to increase ($p < 0.08$) in subjects who consumed casein in comparison with their baseline values. Men in casein group also experienced increases in both waist ($p < 0.04$) and waist-to-hip ratio ($p < 0.01$) compared with their corresponding baseline values. There were no other observed significant differences in anthropometric measurements within treatment groups among men **(Table III)**.

B. Serum Parameters: Serum total alkaline phosphate (ALP), bone specific alkaline phosphatase (B-ALP), tartrate resistant acid phosphatase (TRAP) activities, estradiol, and IGF-I concentration

B.1. All subjects. The effects of dietary regimens on serum total ALP, B-ALP, TRAP activities, estradiol, and IGF-I concentrations for the entire pool of subjects are presented in **Table IV**. Casein regimen increased total ALP (p<0.05) activity compared to baseline activity. Soy consumption reduced (p<0.03) bone-specific ALP activity in comparison with the corresponding values. However, the soy regimen also tended to decrease (p<0.08) TRAP activity, a marker of bone resorption. Both soy (p<0.0001) and casein (p<0.04) consumption increased

serum insulin-like growth factor-I (IGF-I) concentrations **(Table IV)** in comparison with their corresponding baseline values.

B.2. Women only. Soy consumption suppressed ($p<0.005$) serum TRAP activity, in comparison with baseline values, while had no effect on serum total ALP and B-ALP activities. In comparison with baseline, casein regimen significantly ($p<0.03$) increased total ALP activity but tended to decrease ($p<0.09$) B-ALP activity. Soy and casein regimens had no estrogenic effects as assessed by serum E_2 levels. Soy consumption increased ($p<0.0001$) serum IGF-I concentrations in comparison with corresponding baseline **(Table V)**.

B.3. Men only. Soy and casein consumptions had no significant effects on serum ALP, B-ALP, TRAP activities, and E_2. Similar to the findings in women, soy consumption in men also elevated ($p<0.0001$) serum IGF-I concentrations in comparison with baseline. Casein consumption also elevated ($p<0.05$) serum IGF-I concentrations in men in comparison with baseline **(Table VI)**.

C. Urinary Parameters: Deoxypridinoline (Dpd), Phosphorous (P), Magnesium (Mg), and Calcium (Ca)

C.1. All subjects. The effects of dietary regimens on urinary Dpd, P, Mg, and Ca per 24-hr of urine for all subjects are presented in **Table VII**. Casein regimen

significantly ($p<0.04$) increased the urinary excretion of P and tended ($p<0.06$) to increase the urinary excretion of magnesium in comparison with baseline values.

C.2. Women only. Casein regimen significantly increased urinary excretions of Dpd ($p<0.05$), P ($p<0.005$), Mg ($p<0.002$), and Ca ($p<0.04$), while soy protein regimen had no such increasing effects **(Table VIII)**.

C.3. Men only. There were no significant changes in 24-hr urinary excretions of Dpd, P, Mg, and Ca in either soy or casein regimens **(Table IX)**.

D. Differences between final and baseline values on treatment.

There was no significant differences in serum (total ALP, B-ALP, TRAP) or urinary parameters (P, Dpd, Mg, Ca) of interests between the two regimens, except serum IGF-I. Soy regimen significantly increased ($p<0.01$) serum IGF-I concentrations in comparison with casein regimen **(Table X)**.

E. Differences between final and baseline values on gender

There was no significant differences in serum (total ALP, B-ALP, IGF-I, TRAP) or urinary (P, Dpd, Mg, Ca) parameters of interests between the two regimens in men and women **(Table XI)**.

Table I. Comparison between baseline and final anthropometric data for all subjects in soy and casein treatment

	Soy Treatment			Casein Treatment		
Measurements	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Age (y)	57.3±1.6 (n=45)	-	-	57.8±1.5 (n=45)	-	-
Bodyweight (lb)	211.7±6.5 (n=45)	206.3 ± 8.7 (n=45)	$P<0.2$	195.7±6.1 (n=45)	199.1 ± 8.7 (n=45)	$P<0.6$
Height (in)	67.9±0.4 (n=45)	-	-	67.8±0.4 (n=45)	-	-
Bodyfat %	36.0±1.0 (n=45)	35.1±1.2 (n=45)	$P<0.01$	35.3±0.9 (n=45)	35.0±1.1 (n=45)	$P<0.02$
BMI (kg/m^2)	32.4±0.9 (n=45)	31.1±1.2 (n=45)	$P<0.3$	30.2±0.9 (n=45)	30.6±1.2 (n=45)	$P<0.6$
Waist (in)	40.1±0.8 (n=45)	40.9±1.0 (n=45)	$P<0.2$	38.8±0.8 (n=45)	39.9±1.0 (n=45)	$P<0.04$
Hip (in)	45.3±0.8 (n=45)	44.9±1.0 (n=45)	$P<0.9$	43.5±0.8 (n=45)	43.9±1.0 (n=45)	$P<0.9$
Waist/Hip Ratio	0.89±0.01 (n=45)	0.91±0.01 (n=45)	$P<0.06$	0.89±0.01 (n=45)	0.91±0.01 (n=45)	$P<0.03$

Values represent mean±SD

Table II. Comparison between baseline and final anthropometric data for female in soy and casein treatment

	Soy Treatment			Casein Treatment		
Measurements	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Age (y)	59.2 ± 2.2 (n=23)	-	-	58.7 ± 2.2 (n=23)	-	-
Bodyweight (lb)	189.7 ± 8.9 (n=23)	187.6 ± 12.4 (n=23)	*P*<0.9	191.7 ± 8.6 (n=23)	195.9 ± 11.9 (n=23)	*P*<0.7
Height (in)	64.9 ± 0.6 (n=23)	-		65.9 ± 0.6 (n=23)	-	-
Bodyfat %	38.8 ± 1.4 (n=23)	38.2 ± 1.6 (n=23)	*P*<0.02	39.7 ± 1.3 (n=23)	39.2 ± 1.5 (n=23)	*P*<0.09
BMI (kg/m^2)	31.9 ± 1.4 (n=23)	31.5 ± 1.7 (n=23)	*P*<0.9	31.2 ± 1.3 (n=23)	31.9 ± 1.6 (n=23)	*P*<0.7
Waist (in)	38.2 ± 1.1 (n=23)	38.9 ± 1.4 (n=23)	*P*<0.1	38.3 ± 1.2 (n=23)	39.2 ± 1.4 (n=23)	*P*<0.4
Hip (in)	44.8 ± 1.1 (n=23)	44.7 ± 1.5 (n=23)	P<0.8	44.2 ± 1.2 (n=23)	44.8 ± 1.4 (n=23)	*P*<0.7
Waist/Hip Ratio	0.86 ± 0.01 (n=23)	0.87 ± 0.01 (n=23)	*P*<0.2	0.87 ± 0.01 (n=23)	0.87 ± 0.01 (n=23)	*P*<0.6

Values represent mean±SD

Table III. Comparison between baseline and final anthropometric data for male in soy and casein treatment

	Soy Treatment			Casein Treatment		
Measurements	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Age (y)	55.4 ± 2.4 (n=22)	-	-	56.9 ± 2.2 (n=22)	-	-
Bodyweight (lb)	233.7 ± 9.6 (n=22)	224.9 ± 12.2 (n=22)	$P<0.09$	199.8 ± 8.7 (n=22)	202.4 ± 12.7 (n=22)	$P<0.7$
Height (in)	71.0 ± 0.7 (n=44)	-	-	69.7 ± 0.6 (n=22)	-	-
Bodyfat %	33.2 ± 1.5 (n=22)	31.9 ± 1.6 (n=22)	$P<0.2$	30.0 ± 1.4 (n=22)	30.8 ± 1.6 (n=22)	$P<0.08$
BMI (kg/m^2)	32.8 ± 1.5 (n=22)	30.7 ± 1.7 (n=22)	$P<0.1$	29.2 ± 1.4 (n=22)	29.3 ± 1.8 (n=22)	$P<0.7$
Waist (in)	41.9 ± 1.2 (n=22)	42.9 ± 1.4 (n=22)	$P<0.6$	39.2 ± 1.1 (n=22)	40.7 ± 1.5 (n=22)	$P<0.04$
Hip (in)	45.9 ± 1.2 (n=22)	45.3 ± 1.5 (n=22)	$P<0.9$	42.8 ± 1.1 (n=22)	42.9 ± 1.5 (n=22)	$P<0.6$
Waist/Hip Ratio	0.92 ± 0.01 (n=22)	0.96 ± 0.01 (n=22)	$P<0.2$	0.92 ± 0.01 (n=22)	0.94 ± 0.02 (n=22)	$P<0.01$

Values represent mean±SD

Table IV. Effect of treatments on serum parameters for all subjects

	Soy Treatment			Casein Treatment		
Serum Parameters	Baseline	Final	*P*- value	Baseline	Final	*P*- value
Total ALP (μkat/L)	1.39±0.40 (n=45)	1.42±0.35 (n=45)	*P*<0.5	1.25±0.35 (n=45)	1.34±0.41 (n=45)	*P*<0.05
Bone-Specific ALP(μkat/L)	0.43±0.15 (n=45)	0.40±0.14 (n=45)	*P*<0.03	0.40±0.14 (n=45)	0.39 ±0.15 (n=45)	*P*<0.3
TRAP (U/L)	3.9±2.9 (n=45)	3.1±0.8 (n=45)	*P*<0.08	3.1±0.6 (n=45)	3.4±0.7 (n=45)	*P*<0.6
Estradiol (pg/mL)	52.5±12.8 (n=45)	65.8±16.2 (n=45)	*P*<0.5	77.9±12.6 (n=45)	96.7±14.9 (n=45)	*P*<0.3
IGF-I (ng/ml)	114.5±54.8 (n=45)	212.9±150.7 (n=45)	*P*<0.0001	136.4±69.4 (n=45)	171.4±77.3 (n=45)	*P*<0.04

Values represent mean±SD

Table V. Effect of treatments on serum parameters for female

	Soy Treatment			Casein Treatment		
Serum Parameters	Baseline	Final	*P* value	Baseline	Final	*P* value
Total ALP (μkat/L)	1.46±0.40 (n=23)	1.51±0.37 (n=23)	*P*<0.9	1.26±0.41 (n=23)	1.34±0.46 (n=23)	*P*<0.03
Bone-Specific ALP(μkat/L)	0.43±0.14 (n=23)	0.41±0.15 (n=23)	*P*<0.1	0.39±0.13 (n=23)	0.36 ±0.10 (n=23)	*P*<0.09
TRAP (U/L)	4.5±3.6 (n=23)	3.3±0.8 (n=23)	*P*<0.005	2.9±0.7 (n=23)	3.6±0.7 (n=23)	*P*<0.7
Estradiol (pg/mL)	52.5±12.8 (n=23)	65.8±16.2 (n=23)	*P*<0.5	77.9±12.6 (n=23)	96.7±14.9 (n=23)	*P*<0.3
IGF-I (ng/ml)	102.3±55.9 (n=23)	204.3±184.7 (n=23)	*P*<0.0001	113.6±65.5 (n=23)	139.8±69.1 (n=23)	*P*<0.3

Values represent mean±SD

TABLE VI. Effect of treatments on serum parameters for male

	Soy Treatment			Casein Treatment		
Serum Parameters	Baseline	Final	*P*- value	Baseline	Final	*P*- value
Total ALP (μkat/L)	1.3±0.3 (n=22)	1.3±0.3 (n=22)	*P*<0.4	1.2±0.3 (n=22)	1.3±0.4 (n=22)	*P*<0.5
Bone-Specific ALP(μkat/L)	0.42±0.13 (n=22)	0.39±0.13 (n=22)	*P*<0.1	0.41±0.15 (n=22)	0.42±0.19 (n=22)	*P*<0.9
TRAP (U/L)	3.1±1.8 (n=22)	2.9±0.8 (n=22)	*P*<0.7	2.9±0.9 (n=22)	3.5±0.7 (n=22)	*P*<0.7
Estradiol (pg/mL)	19.1±13.1 (n=22)	17.9±14.2 (n=22)	*P*<0.9	31.5±11.4 (n=22)	17.9±15.3 (n=22)	*P*<0.5
IGF-I (ng/ml)	134.2±54.2 (n=22)	220.5±117.4 (n=22)	*P*<0.0001	154.3±65.5 (n=22)	209.3±70.6 (n=22)	*P*<0.05

Values represent mean±SD

Table VII. Effect of treatments on urinary parameters per 24-hr urine for all

	Soy Treatment			Casein Treatment		
Urinary parameters	Baseline	Final	*P*- value	Baseline	Final	*P*- value
Phosphorous (mole/24hr)	25.9±13.3 (n=45)	28.4±15.1 (n=45)	*P*<0.7	25.6±11.7 (n=45)	37.8±34.6 (n=45)	*P*<0.04
Pyrilinks-D (umol/24hr)	78.9±50.9 (n=45)	61.7±49.0 (n=45)	*P*<0.2	63.7±30.8 (n=45)	77.0±115 (n=45)	*P*<0.4
Magnesium (mole/24hr)	5.3±3.9 (n=45)	4.7±2.8 (n=45)	*P*<0.4	4.4±0.2 (n=45)	5.8±5.5 (n=45)	*P*<0.06
Calcium (mole/24 hr)	5.61±5.5 (n=45)	4.5±2.9 (n=45)	*P*<0.4	3.9±2.4 (n=45)	6.0±5.4 (n=45)	*P*<0.2

Values represent mean±SD

Table VIII. Effect of treatments on urinary parameters in 24-hr urine in women

	Soy Treatment			Casein Treatment		
Urinary parameters	Baseline	Final	*P*- value	Baseline	Final	*P*- value
Phosphorous (mole/24 hr)	20.7±10.4 (n=23)	22.0±12.1 (n=23)	*P*<0.8	23.7±13.5 (n=23)	45.4±36.8 (n=23)	*P*<0.005
Pyrilinks-D (umol/24 hr)	69.6±46.7 (n=23)	53.5±32.2 (n=23)	*P*<0.5	64.1±33.3 (n=23)	97.1±15.6 (n=23)	*P*<0.05
Magnesium (mole/24 hr)	3.8±1.9 (n=23)	3.7±1.7 (n=23)	*P*<0.9	3.4±1.3 (n=23)	6.5±7.3 (n=23)	*P*<0.002
Calcium (mole/24 hr)	3.9±2.7 (n=23)	4.2±3.5 (n=23)	*P*<0.8	3.3±1.8 (n=23)	7.3±5.6 (n=23)	*P*<0.04

Values represent mean±SD

Table IX. Effect of treatments on urinary parameters in 24-hr urine in men

	Soy Treatment			Casein Treatment		
Urinary parameters	Baseline	Final	*P*- value	Baseline	Final	*P*- value
Phosphorous (mole/24 hr)	31.1±14.2 (n=22)	34.8±15.3 (n=22)	*P*<0.8	27.8±9.6 (n=22)	29.5±11.6 (n=22)	*P*<0.9
Pyrilinks-D (umol/24 hr)	87.9±54.2 (n=22)	69.0±60.2 (n=22)	*P*<0.2	63.3±28.7 (n=22)	54.9±26.3 (n=22)	*P*<0.6
Magnesium (mole/24 hr)	6.7±4.7 (n=22)	5.7±3.3 (n=22)	*P*<0.3	5.5±2.6 (n=22)	5.1±1.9 (n=22)	*P*<0.6
Calcium (mole/24 hr)	7.3±6.9 (n=22)	4.8±2.3 (n=22)	*P*<0.2	4.7±2.7 (n=22)	4.7±2.9 (n=22)	*P*<0.9

Values represent mean±SD

Table X. Differences* by treatment between final and baseline values

Urinary parameters	**Soy Treatment**	**Casein Treatment**	**P- value**
Phosphorous (mg)	14.8±303.0 (n=45)	-7.8±261.2 (n=45)	P<0.8
Pyrilinks-D (nmol)	-15.9±37.1 (n=45)	8.4±21.8 (n=45)	P<0.2
Magnesium (mg)	-5.5±40.5 (n=45)	4.5±25.5 (n=45)	P<0.2
Calcium (mg)	-8.8±116.3 (n=45)	9.1±71.9 (n=45)	P<0.4
Serum parameters			
Total ALP (μkat/L)	0.03±0.2 (n=45)	0.09±0.3 (n=45)	P<0.4
Bone-Specific ALP(μkat/L)	-0.03±0.07 (n=45)	-0.01±0.08 (n=45)	P<0.4
TRAP (U/L)	-0.8±4.9 (n=45)	0.2±0.8 (n=45)	P<0.1
IGF-I (ng/ml)	98.5±144.9 (n=45)	57.2±21.3 (n=45)	P<0.01

* Differences calculated by subtracting baseline values from final values

Table XI. Differences* by gender between final and baseline values

Urinary parameters	**Female**	**Male**	**P- value**
Phosphorous (mg)	-34.6±229.7 (n=46)	45.9±327.3 (n=44)	P<0.1
Pyrilinks-D (nmol)	-12.4±25.8 (n=46)	-12.1±35.5 (n=44)	P<0.8
Magnesium (mg)	5.1±19.3 (n=46)	-6.7±44.4 (n=44)	P<0.2
Calcium (mg)	9.1±54.0 (n=46)	-10.1±128.9 (n=44)	P<0.4
Serum parameters			
Total ALP (μkat/L)	0.07±0.4 (n=46)	0.04±0.2 (n=44)	P<0.8
Bone-Specific ALP(μkat/L)	-0.03±0.08 (n=46)	-0.01±0.1 (n=44)	P<0.3
TRAP (U/L)	-0.8±4.8 (n=46)	0.2±0.7 (n=44)	P<0.1
IGF-I (ng/ml)	60.2±130.5 (n=46)	68.7±97.0 (n=44)	P<0.9

*Differences calculated by subtracting baseline values from final values

CHAPTER V

DISCUSSION

Although the effect of soy protein on bone has been examined in animals (Arjmandi et al. 1996; 1998a, 1998b) and two short-term human studies, limited to post- (Potter, et al. 1998) or peri-menopausal women (Alekel et al., 1999), its effect on bone metabolism in men and women at large has not been studied. Postmenopausal women, in particular, can benefit from soy consumption since soy products, including its protein isolate contain considerable amounts of estrogen-like compounds (phytoestrogens) including isoflavones such as genistein and daidzein (Kanazawa et al., 1993). Soy isoflavones are structurally similar to raloxifene and tamoxifen (Agnusdei et al., 1989) that positively affect bone and cholesterol metabolisms (Delmas et al., 1997; Wright et al., 1994). Both of these compounds exhibit weak estrogenic (0.2% that of estradiol) (Shutt and Braden, 1963) and anti-estrogenic effects (Martin et al., 1978). The estrogenic action of raloxifen, tamoxifen, and other synthetic SERMs seem to be tissue specific. In tissues such as bone and prostate, they act as estrogen agonists, whereas in tissues such as uterus and breast they act as estrogen antagonists (Love et al., 1992).

Our laboratory previously reported that a diet containing soy protein isolate that is rich in isoflavones, a class of naturally occurring phytoestrogens, prevented ovariectomy-induced bone loss in rats (Arjmandi et al., 1996, 1998a, 1998b). The question left unanswered by that study was whether the protective effect of the diet can also be observed in humans. The present study demonstrates that soy protein isolate with its normal isoflavone content given as a daily supplement has positive effects on some indices of bone metabolism in both men and women. The findings of a recent clinical trial by Potter et al. (1998) have also indicated the importance of soy protein with normal levels of isoflavones in the diets of postmenopausal women for ameliorating bone mass. We speculate that the beneficial effects of soy on bone, in part, is due to its isoflavone content. In support of this view, genistein, one of the isoflavones present in soybeans, has been shown to effectively prevent ovarian hormone deficiency-associated bone loss (Anderson et al., 1997; Fanti et al., 1998). Two weeks of genistein treatment (1.0 mg/d) in lactating ovariectomized rats was effective in maintaining trabecular bone tissue in comparison with ovariectomized control animals (Anderson et al., 1997). Furthermore, in the same report, genistein stimulated ALP activity *in vitro* using an osteoblast-like cell line, suggesting a positive effect on bone formation. Fanti et al. (1998), also reported that genistein (5 mg/kg body weight) maintained both cortical and trabecular bones in ovariectomized rats. However, the aforementioned studies also suggest that the bone-sparing effect of genistein is biphasic.

Although the soy regimen significantly suppressed the serum index of bone resorption, TRAP activity, in this study suppressive effect was not as effective as that of

estrogen (Kalu et al., 1991). Hence, it can be concluded that the bone protective effects of soy with normal isoflavone content may not be ideal and only partially prevent bone loss. Thus, the amount of isoflavones consumed by these subjects may have provided a suboptimal or excessive dose, resulting in only partial protection of bone. However, future studies are necessary to explore the bone protective effects of soy protein or its isoflavones using more defined study populations, e.g. postmenopausal women not on hormone replacement therapy, and elderly men and women (older than 70 y). Furthermore, efficacious doses of isoflavones need to be established on the basis of the model of osteoporosis, e.g., age or ovarian hormone deficiency-associated bone loss both in men and women and the type of isoflavone being used.

Although the mechanism by which soy isoflavones exert their protective effects on bone is unclear, in women, soy's bone protective effects may be additive to that of estrogen. This view is supported, in part, by the fact that 35 out of 46 women who participated in this study were on hormone replacement therapy and yet soy regimen significantly reduced serum marker of bone resorption (TRAP activity) and tended to reduce marker of bone resorption (B-ALP activity). These observations imply that soy isoflavones may exert further estrogenic effects on bone while not having such effects on other tissues such as uterus. We cannot offer an explanation as to why soy reduced serum B-ALP activity but not total ALP activity. However, it should be noted that serum total ALP activity is a nonspecific marker of bone formation which can be influenced by many other factors.

In this study, casein was used as a comparative control regimen. Casein's effects on indices of bone turnover especially IGF-I have not been studied and it is of particular interest to document its effect on modulating the markers of bone turnover.

Because the soy treatment did not appear to ameliorate the indices of bone formation in men or women, and yet tended to decreased the indices of bone resorption, the data suggest that soy protein or its isoflavones may decrease bone resorption resulting in a net increase in bone formation (formation exceeds resorption).

Both men and women on soy had significantly greater serum IGF-I levels at the end of the study in comparison with their baseline valuesand casein. It is well recognized that insulin-like growth factor-I concentrations enhance osteoblastic activity in both humans (Rosenfeld et al., 1994; Zhang et al., 1991) and animals (Tanaka et al., 1994). Sugimoto et al. (1997) indicated that insulin-like growth factor-I could be an important modulator of the rate of bone formation in humans. IGF-I increases collagen synthesis, and matrix apposition rate (Hock et al., 1988) both of which are indicative of enhanced bone formation rate. These findings confirm earlier observation (Arjmandi et al., 1998a) in which soy protein with isoflavones significantly enhanced serum IGF-I levels. Therefore, the elevated serum concentrations of IGF-I may indicate that consumption of soy protein by humans increases the rate of bone formation and hence, the bone protective effects of soy, in part, may be due to enhanced rate of bone formation.

The findings of this study demonstrated that soy protein improves body composition in comparion with casein. In the soy protein group, a decrease in body fat percentage on average was observed in both men (-1.26%) and women (-0.58%), but the

decrease only reached a significant level among women. It has been shown in animal (Barton-Davis et al., 1999) and human studies (Welle, 1998; Carter, 1995) that IGF-I has a positive effect on lean body mass, specifically by increasing muscle mass, and decreasing fat mass (Skaggs et al., 1991). Therefore, the decreases in percent body fat observed in the soy group can most likely be attributed to the aforementioned increases in IGF-I levels among men and women. These observations further suggest that soy protein or its isoflavones potentially influence bone metabolism, because increased lean body mass has been reported to correlate with higher bone mineral density (Fox et al., 2000).

Although calcium absorption was not assessed in this study, soy also may positively affect bone through enhanced intestinal absorption of calcium. Arjmandi, et al. have demonstrated that soy protein enhances the *in vitro* uptake of calcium (unpublished data) and their results indicated that soy, regardless of its isoflavone content, stimulated duodenal calcium transport. Their observations are supported by those of Omi et al., (1994), who reported a beneficial effect of a soybean milk-based diet on bone mineral density of 6-wk-old female rats. The authors suggested that this positive effect may have been due to the enhanced intestinal calcium absorption. However, the calcium-absorption-promoting effects of soy do not appear to be related to vitamin D (Barengolts et al., 1995). Therefore, the effects of soy protein or its isoflavones on calcium absorption may not require modulation of serum vitamin D concentrations.

From the findings of this study and our previously published work, it appears that the mechanism for soy isoflavone action is distinct from that of estrogen. Despite reports of soy isoflavones, particularly genistein, having mild estrogenic activity, there were

pronounced differences in blood and urinary markers of bone formation and bone resoprtion from what is expected of estrogen. For instance, estrogen decreases serum IGF-I concentrations (Kalu et al. 1994) whereas, soy increases its levels. Estrogen increases urinary excretions of calcium and phosphorus (Arjmandi et al., 1993), while soy has no such increasing effects. Estrogen suppresses both indices of bone formation and bone resorption (Mundy, 1993), whereas in this study soy protein regimen tended to suppressed the indices of bone resorption. Additionally, the differences between estrogen and soy protein or its isoflavones become more evident when serum IGF-I is brought into this equation. In this study, both men and women who consumed soy protein regimen experienced significant increases in their serum levels of IGF-I, whereas estrogen treatment has been reported to reduce serum concentrations of IGF-I (Span et al., 2000; Kalu et al., 1994).

Animal studies also support the notion that soy or its isoflavones may be devoid of any true estrogenic properties. Arjmandi et al. have repeatedly reported (1996, 1998a, 1998b) that in contrast to the uterine-stimulating effect of estradiol, neither soy nor its isoflavones had demonstrated uterotrophic activity. This suggests that isoflavones have a different effect on the uterus than does estrogen. In this regard, the isoflavones present in soy protein may act analogously to tamoxifen, which is structurally similar to isoflavones, has an antiestrogenic effect on breast tissue (Kalu et al., 1991b), and has a proestrogenic effect on bone (Zava et al., 1997). However, one study showed an additive effect of tamoxifen and estrogen on cancellous bone (Zava et al., 1997) whereas another did not find any such effect (Messina et al., 1994). Our findings in this study that soy

consumption did not modulate serum estrogen levels in both men and women are supported by the recent observations by Baird et al. (1995), who showed that 4 wk of soy supplementation did not produce any of the expected estrogenic effects. Furthermore, women with greater urinary isoflavone concentrations did not show a greater estrogenic responses than did women with modest urinary isoflavone concentrations.

Isoflavones in soy, particularly genistein, have a mild estrogenic effect, approximately $1x10^{-3}$ to $1x10^{-5}$ that of estradiol (Farmakalidis et al., 1985). Although the estrogenic effects of isoflavones may be weak, they may still interfere with the actions of the more powerful endogenously occurring estrogens in several important ways (Farnsworth et al., 1975; Feldman et al., 1989). When isoflavones are provided along with estrogen, the effects of estradiol are suppressed (Feldman et al., 1989). This is in line with the reported beneficial effect of soy protein of lowering the risk of breast cancer and colon cancer, because isoflavones are thought to prevent tumor growth by binding to estrogen receptors on the tumor and hence competitively inhibiting estrogen uptake (Miksicek, 1993; Shutt, 1967).

In summary, soy protein may have a modest beneficial effect on bone. However, the bone protective effects of soy or its isoflavones in human require longer-term studies in which bone density would also be evaluated. In postmenopausal women, research is needed to determine whether the estrogen dose can be lowered if given in conjunction with soy-containing products. Moreover, the intake of isoflavones from soy over an extended period may have a more positive effect on bone than if consumed only during a short period of time.

CHAPTER VI

CONCLUSIONS

The results of this study suggest that soy regimen has a favorable effect on bone metabolism in both women and men by tended to suppress. Elevation of serum insulin-like growth factor-I concentrations by soy consumption indicate the enhanced osteoblastic activity and altering bone formation in both women and men.

Longer study duration, measurement of bone density, and more specifically designed markers of bone resorption such as urinary collagen cross-link-associated N-telopeptide of the cross-links of collagen (NTX) are necessary to confirm the findings of this study.

If the findings of this study are confirmed by other investigators, further studies will be needed to clarify whether the beneficial effects of soy protein on bone metabolism is due to soy consumption as such or to its specific constituents such as the isoflavones.

Furthermore, the use of *in vivo* and *in vitro* models of osteoporosis are necessary to elucidate the mechanisms by which soy protein or its constituents protect skeletal mass in human.

CITED LITERATURE

Adlercreutz H, Bannwart C, Wähälä K, Mäkelä T, Brunow G, Hase T, Arosemena PJ, Kellis JT Jr, Vickery LE. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Molec Biol. 1*988; 44: 147-53.

Adlercreutz H. Phytoestrogens: Epidemiology and a possible role in cancer prevention. *Environ Health Perspect.* 1995; 103: 103-112.

Agnusdei D, Camporeale A, Zacchei F, Gennari C, Baroni MC, Costi D, Biondi M, Passeri M, Ciacca A, Sbrenna C, Falsettini E, Ventura A. Effects of ipriflavone on bone mass and bone remodeling in patients with established postmenopausal osteoporosis. *Curr Ther Res.* 1992; 52: 81-92.

Agnusdei D, Zacchei F, Bigazzi S, Cepollaro C, Nardi P, Montagnani M, Gennari C. Metabolic and clinical effects of ipriflavone in established postmenopausal osteoporosis. *Drugs Exp Clin Res.* 1989; 15: 87-104.

Agnusdei D, Bufalino L. Efficacy of isoflavones in established osteoporosis and long-term safety. *Calcif Tissue Int.* 1997; 61 (suppl 1): S23-7.

Alekel DL, Mortillaro E, Hussain EA, West B, Ahmed N, Peterson CT, Werner RK, Arjmandi BH, Kukreja SC Lifestyle and biologic contributors to proximal femur bone mineral density and hip axis length in two distinct ethnic groups of premenopausal women. *Osteoporos Int.* 1999; 9: 327-338.

Alekel DL, Peterson C, St. Germain A, Hanson K. Isoflavone-rich soy isolate exerts significant bone-sparing in the lumbar spine of perimenopausal women. *Am Soc Bone Miner Res.* 21st annual meeting. 1999; 14 (suppl): S208.

Anderson JJ, Ambrose WW, Garner SC. Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. *Proc Soc Exp Biol Med.* 1997; 12: 67-73.

Anderson JJB, Ambrose WW, Garner SC. Orally dosed genistein from soy and prevention of cancellous bone loss in two ovariectomized rat models. *J Nutr.* 1995; 125: 799S.

Anthony M, Christianses C. Effects of Raloxifene on Bone Mineral Density, Serum Cholesterol Concentrations, and Uterine Endometrium in Postmenopausal Women. *N Engl J Med.* 1997; 337: 1641-1648.

Arjmandi BH, Alekel L, Hollis BW, Amin D, Staceeewicz-Sapuntzakis M, Guo P, Kukreja SC. Dietary soybean protein prevents bone loss in an ovariectomized rats modle of osteoporosis. *J Nutr.* 1996; 126: 161-167.

Arjmandi BH, Birnbaum R, Goyal NV, Getlinger MJ, Juma S, Alekel L, Hasler CM, Drum ML, Hollis BW, Kukreja SC. The bone-sparing effect of soy protein in ovarian hormone deficient rats is related to its isoflavone content. *Am J Clin Nutr.* 1998b; 68 (Suppl): 1364S-1368S.

Arjmandi BH, Birnbaum RS, Juma S, Barengolts E, Kukreja SC. The synthetic phytoestrogen, ipriflavone, and estrogen prevent bone loss by different mechanisms. *Calcif Tissue Int.* 2000; 66: 61-65.

Arjmandi BH, Getlinger MJ, Goyal NV, Alekel L, Hasler CM, Juma S, Drum ML, Hollis BW, Kukreja SC. The role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. *Am J Clin Nutr.* 1998a; 68 (Suppl): 1358S-1363S.

Arjmandi BH, Salih MA, Herbert DC, Sims SH, Kalu DN. Evidence for estrogen receptor-linked calcium transport in the intestine. *Bone Miner.* 1993; 21: 63-74.

Avioli LV. Significance of osteoporosis: a growing international health care problem. *Calcif Tissue Int.* 1991; 49 (suppl): S11-S14.

Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KDR, Weinberg CR, Haney AF, Wilcox AJ, Mclachlan JA. Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. *J Clin Endocrinol Metab.* 1995; 80: 1685-1690.

Barengolts EI, Lathon PV, Lindh FG. Progesterone antagonist RU 486 has bone sparing effects in ovariectomized rats. *Bone.* 1995; 17: 21-5.

Barnes S, Messina MJ. Soy and its role in cancer prevention. *The Soy Connection.* 1993; 1: 1-4.

Baron R. Prevention of osteoporosis. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Second Edition. Raven Press, New York. 1993a: 240-245.

Baron R. Anatomy and ultrastructure of bone. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Second Edition. Raven Press. New York, 1993b; 3-9.

Barrett-Connor E. Epidemiology and the menopause: A global overview. *Int J Fertil.* 1993; 38: 6-14.

Barton-Davis ER, Shoturma DI, Sweeney HL. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiol Scand.* 1999; 167: 301-5.

Baxter R. Circulating binding proteins for the insulin-like growth factors. *Tr Endocrin Metab.* 1987; 64: 884-888.

Beresford SAA, Weiss NS, McKnight B. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. *Lancet.* 1997; 349: 458-461.

Bianda T, Hussain MA, Glatz Y, Bouillon R, Froesch ER, Schmid C. Effects of short-term insulin-like growth factor-I or growth hormone treatment on bone turnover, renal phosphate reabsorption, and 1,25 dihydroxyvitamin D_3 production in healthy man. *J Intern Med.* 1997; 241: 143-150.

Blair HC, Jordan SE, Peterson TG, Barnes S. Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. *Cell Biochem.* 1996; 61: 629-637.

Bonucci E, Ballanti P, Martelli A, Mereto E, Brambilla G, Bianco P, Bufalino L. Ipriflavone inhibits osteoclast differentiation in parathyroid transplanted parietal bone of rats. *Calcif Tissue Int.* 1992; 50: 314-319.

Boonecamp PM, van der Wee-Pals LJA, van Wijk-van Lennep MML, Thesing C, Bijvoet OLM. Two modes of action of bisphosphonates on osteoclastic resorption of mineral ized matrix. *Bone Mineral.* 1986; 1: 27-39.

Boonen S, Lesaffre E, Dequeker J, Aerssens J, Nijs J, Pelemans W, Bouillon R. Relationship between baseline IGF-I and femoral bone density in women aged over 70 years: Potential implications for prevention of age-related bone loss. *J Am Geriatr Soc.* 1996; 44: 1301-1306.

Brandi Ml, Gennari C, Bonucci E. Ipriflavone. New insights into its mechanisms of action on bone remodeling. *Calcif Tissue Int.* 1993; 52: 151-152.

Brandi ML. Ipriflavone influences the osteoblastic phenotype in vitro. *Osteoporosis Int.* 1993; 1 (suppl): S226-S229.

Breslau NA, Brinkley L, Hill KD, Pak CY. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J Clin Endrocinol Metab. 1988;* 66: 140-146.

Brixen K, Kassem M, Nielsen HK, Loft AG, Flyvbjerg A, Mosekilde L. Short-term treatment with growth hormone stimulates osteoblastic and osteoclastic activity in osteopenic postmenopausal women: A dose response study. *J Bone Miner Res.* 1995; 10: 1865-1874.

Bryant HU, Dere WH. Selective estrogen receptor modulators: An alternative to hormone replacement therapy. *Proc Soc Exp Biol Med.* 1998; 217: 45-52.

Castelo-Branco C. Management of Osteoporosis. *Drug & Aging.* 1998; 12 (suppl): 25-32.

Carter WJ. Effect of anabolic hormones and insulin-like growth factor-I on muscle mass and strength in elderly persons. *Clin Geriatr Med.* 1995; 11: 735-48.

Chin K, Evans MC, Cornish J, Cundy T, Reid IR. Differences in hip axis and femoral neck length in premenopausal women of Polynesian, Asian, and European origin. *Osteoporos Int* 1997; 7: 344-347.

Collaborative Group on Hormonal Factors in Breast Cancer (CGHFBC). Breast cancer and hormone replacement therapy: Collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 without breast cancer. *Lancet.* 1997; 350: 1047-1059.

Cundy T, Cornish J, Evans MC, Gamble G, Stapleton J, Reid IR. Sources of interracial variation in bone mineral density. *J Bone Miner Res.* 1995; 10:368-373.

Daly JS, Ertingshausen G. Direct method for determining inorganic phosphate in serum with the "CentrifiChem". *Clin Chem.* 1972; 18: 263-5.

Dawson-Hughes B, Harrison S, Kramich C, Dallal G, Rasmussen HM. Calcium retention and hormone levels in black and white women on high- and low-calcium diets. *J Bone Miner Res. 1993;* 8: 779-787.

Delmas PD, Bjarnason NH, Mitlak BH, Ravoux A-C, Shah AS, Huster WJ, Draper M, Christiansen C. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *N Engl J Med.* 1997; 337: 1641-1647.

Draper MW, Flowers DE, Huster WJ, Neild JA, Harper KD, Arnaud C. A controlled clinical trial of raloxifene (LY139481) HCl: Impact on bone turnover and serum lipid profile in healthy postmenopausal women. *J Bone Mineral Res.* 1996; 11: 835-842.

Eriksen EF, Axellrod DW, Melsen F. Bone histomorphometry. New York: Raven Press, 1994.

Fabiny-Byrd DL, Ertingshausen G. Kinetic method for determining acid phosphatase activity in serum with use of the "CentrifiChem". *Clin. Chem.* 1972; 13: 841-4.

Fanti O, Faugere MC, Gang Z, Schmidt J, Cohen D, Malluche HH. Systematic administration of genistein partially prevents bone loss in ovariectomized rates in a nonestrogen-like mechanism. *Am J clin Nutr.* 1998; 68 (suppl): 1517S-8S.

Farmakalidis E, Hathcock JN, Murphy B. Oestrogenic potency of genistein and daidzein in mice. *Food Chem Toxicol.* 1985; 23: 741-5.

Farnsworth NR, Bingel AS, Cordell GA, Grane FA, Fong HHS. Potential value of plants as sources of antifertility agents. *J Pharm Sci.* 1975; 64: 535-98.

Feldman S, Minne HW, Parvizi S, Pfeifer M, Lempert UG, Bauss Zeigler R. Antiestrogen and antiandrogen administration reduce bone mass in the rat. *Bone Miner.* 1989; 7: 245-54.

Finkelstein JS, Klibanski A, Arnold AL, Toth TL. Prevention of estrogen deficiency-related bone loss with human parathyroid hormone: a randomized controlled trail. *JAMA.* 1998; 280: 1067-1073.

Fleisch H. The use of biosphosphonates in osteoporosis. *Br Clin Pract.* 1994; 48: 323-6.

Fornander T, Rutquist LE, Cedermark B, Glas U, Mattsson A, Silfversward C, Skoog L, Somell A, Theve T, Wilking N, Askergren J, Hjalmar M-L. Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. *Lancet.* 1989; 1: 117-120.

Foth D, Cline JM. Effects of mammalian and plant estrogens on mammary glands and uteri of macaques. *Am J Clin Nutr.* 1998; 68 (Suppl): 1413S-1417S.

Fox KM, Magaziner J, Hawkes WG, Yu-Yahiro J, Hebel JR, Zimmerman SI, Holder L, Michael R. Loss of bone density and lean body mass after hip fracture . *Osteoporosis Int.* 2000; 11: 31-5.

Fujita T, Yoshikawa S, Ono K, Inoue T, Orimo H. Usefulness of TC-80 (ipriflavone) tablets in osteoporosis: Multi-center, double-blind, placebo-controlled study. *Igaku Noayumi.* 1986; 138: 113-141.

Gambacciani M, Spinetti A, Cappagli B, Taponeoco F, Felipetto R, Parrini D, Cappelli N, Fioretti P. Effects of ipriflavone administration on bone mass and metabolism in ovariectomized women. *J Encrinol Invest.* 1993; 16: 333-337.

Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Breart G, Meunier PJ, Delmas PD. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS prospective study. *J Bone Miner Res.* 1996a; 11: 1531-1538.

Garnero P, Sornay-Rendu E, Chapuy MC, Delmas PD. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J Bone Miner Res.* 1996b; 11: 337-349.

Gennari C, Agnusdei D. Calcitonins and osteoporosis. *Br Clin Pract.* 1994; 48: 196-200.

Gomez B, Ardakani S, Ju J, Jenkins D, Cerelli MJ, Daniloff GY, Kung VT. Monoclonal antibody assay for measuring bone-specific alkaline phosphatase activity in serum. *Clin Chem.* 1995; 41: 1560-1566.

Gupta A. Osteoporosis in India – the nutritional hypothesis. *Nat Med J Ind* . 1996; 9: 268-274.

Gupta AK, Samuel KC, Kurian PM, Rallan RC Preliminary study of the incidence and aetiology of femoral neck fractures in Indians. *Indian J Med Res.* 1967; 55: 1341-1348.

Hanson DA, Weis MAE, Bollen AM, Maslan SH, Singer FR, Eyre DR. A specific immunoassay for monitoring human bone resorption: Quantitation of type-I collagen cross-linked N-Telopeptides in urine. *J Bone Miner Res.* 1992; 7: 1251-1258.

Hargrove JT, Eisenberg E. Menopause. *Office Gynecology.* 1995; 98: 1337-1356.

Harris ST, Gertz BJ, Genant HK. The effect of short-term treatment with alendronate upon vertebral density and biochemical markers of bone remodeling in early postmenopausal women. *J Clin Endocrinol Metab.* 1993; 76: 1399-1406.

Harrison E, Adjei A, Ameho C, Yamamoto S, Kono S. The effect of soybean protein on bone loss in a rat model of postmenopausal osteoporosis. *J Nutr Sci Vitaminol Tokyo.* 1998; 44: 257-268.

Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol.* 1983; 32: 1141-1148.

Head KA. Ipriflavone: an important bone-building isoflavone. *Altern Med Rev.* 1999; 4: 10-22.

Heaney RP, Draper MW. Raloxifene and estrogen: Comparative bone-remodeling kinetics. *J Clin Endocrinol Metab.* 1997; 82: 3425-3429.

Heaney RP, Recker RR, Stegman MR, May AJ. Calcium absorption in women: Relationships to calcium intake, estrogen status, age. *J Bone Miner Res.* 1989; 4: 469-475.

Higashi K, Ogawara H. Daidzein inhibits insulin- or insulin-like growth factor-1-mediated signaling in cell cycle progression of Swiss 3T3 cells. *Biochim Biophys Acta.* 1994; 1220: 29-35.

Ho SC, Wong E, Chan SG, Lau J, Chan C, Leung PC. Determinants of peak bone mass in Chinese women aged 21 - 40 years. III. Physical activity and bone mineral density. *J Bone Miner Res.* 1997; 12: 1262-1271.

Hock JM, Centrella M, Canalis E. Insulin-like growth factor-1 has independent effects on bone matric formation and cell replication. *Endocrinology.* 1988; 122: 254-260.

Hsin-Shan Ju, Leung S, Brown B, Stringer MA, Leigh S, Scherrer C, Shepard K, Jenkins D, Knudsen J, Cannon R. Comparison of analytical performance and biological variability of three bone resorption assays. *Clin Chem.* 1997; 43: 1570-1576.

Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-estrogens and breast cancer. *Lancet.* 1997; 350: 990-994.

Ishimi Y, Miyaura C, Ohmura M, Onoe Y, Sato T, Uchiyama Y, Ito M, Wang X Suda T, Ikegami S. Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. *Endocrinology.* 1999; 140: 1893-1900.

Jackson JA, Kleerekoper M. Osteoporosis in men: diagnosis, pathophysiology, and prevention. *Medicine.* 1990; 69: 137-151.

Jackson JA. Osteoporosis in men. *In: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Second Edition, Favus MJ. (eds) Raven Press, New York. 1993; 255-257.

Jaffe MZ. Fundamentals of clinical chemistry. *Physio Chem.* 1886; 10: 391.

Johansson AG, Eriksen EF, Lindh E, Langdahl B, Blum WF, Lindahl A, Ljunggren O, Ljunghall S. Reduced serum levels of the growth hormone-dependent insulin-like growth factor binding protein and a negative bone balance at the level of individual remodeling units in idiopathic osteoporosis in men. *J Clin Endocrinol Metab.* 1997; 82: 2795-2798.

Johnston CC, Slemenda CW. Pathogenesis of osteoporosis. *Bone.* 1995; 17: 19S-22S.

Jones JI, Clemmons DR. Insulin-like growth factors and the binding proteins: Biological actions. *Endocr Rev.* 1995; 16: 3-34.

Kalu DN, Arjmandi BH, Liu CC, Salih MA, Birnbaum RS. Effects of ovarictomy and estrogen on the serum levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3. *Bone Miner.* 1994; 24: 135-148.

Kalu DN, Liu CC, Salerno E, Hollis B, Echon R, Ray M. Skeletal response of ovariectomized rates to low and high doses of 17 β-estradiol. *Bone Miner.* 1991a; 14: 175-187.

Kalu DN, Masora EJ, Yu BP, Hardin RR, Hollis BW. Modulation of age-related hyperparathyroidism and senile bone loss in Fischer rats by soy protein and food restriction. *Endocrinol.* 1988; 122: 1847-54.

Kalu DN, Salerno E, Liu CC, Echon R, Ray M, Garza-Zapata M, Hollis BW. A comparative study of the action of tamoxifen, estrogen and progesterone in ovariectomized rat. *Bone Miner.* 1991b; 15: 109-124.

Kamath SK, Murillo G, Chatterton RT Jr, Hussain EA, Amin D, Mortillaro E, Peterson CT, Alekel DL. Breast cancer risk factors in two distinct ethnic groups: Indian and Pakistani versus American premenopausal females. *Nutr Cancer.* 1999; 35: 16-26.

Kanazawa T, Tanaka M, Uemura T, Osanal T, Onodera K, Okubo K, Metoki H, Oike Y. Anti-atherogenicity of soybean protein. *Ann NY Acad Sci.* 1993; 672: 202-214.

Kim H, Peterson TG, Barnes S. Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor β signaling pathways. *Am J Clin Nutr.* 1998; 68 (suppl): 1418S-1425S.

Kung AWC, Luk KDK, Chu LW, Chiu PKY. Age-related osteoporosis in Chinese: An evaluation of the response of intestinal calcium absorption and calcitropic hormones to dietary calcium deprivation. *Am J Clin Nutr.* 1998; 68: 1291-1297.

Kurzer MS, Xu X. Dietary phytoestrogens. *Ann Rev Nutr.* 1997; 17: 353-81.

Lau EM, Kwok T, Woo J, Ho SC. Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians, and omnivores. *Eur J Clin Nutr.* 1998; 52: 60-64.

Lau EMC. Osteoporosis in Asians: the role of calcium & other nutrients. *Challenge Mod Med.* 1995; 7: 45-54.

Lee RD, Nieman DC. Nutritional assessment. 1996; 223-88.

Leboff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *JAMA*. 1999; 281: 1505-1510.

Liberman S. Are the differences between estradiol and other estrogens, naturally occurring or synthetic, merely semantical? *J Clin Endrocinol Metab.* 1996; 81: 850.

Liberman VA, Weiss SR, Broll J. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. *N Engl J Med.* 1995; 33: 1437-1443.

Lindsay R. Prevention and treatment of osteoporosis. *Lancet.* 1993; 341: 801-805.

Lipman MM. Office visit: osteoporosis: not for women only. *Consumer Reports on Health.* 1999; 11: 11.

Lobo R. Benefits and risks of starting estrogen replacement treatment. *Am J Obstet Gynecol.* 1995; 173: 982-9.

Looker AC, Orwoll ES, Johnston CC Jr, Lindsay RL, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP. Prevalence of low femoral bone density in older U.S. adults from NHANES III. *J Bone Miner Res.* 1997; 12: 1761-1768.

Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *N Engl J Med.*. 1992; 326: 852-856.

Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinol.* 1978; 103: 1860-1867.

McMichael-Phillips DF, Harding C, Morton M, Roberts SA, Howell A, Potten CS, Bundred NJ. Effects of soy protein supplementation on epithelial proliferation in the histologically normal human breast. *Am J Clin Nutr*. 1998; 68 (Suppl): 1431S-1436S.

Meiner SE. Osteoporosis: treatment options today. *ADVANCE for Nurse Practitioners.* 1999; 27-31.

Melis GB, Paoletti AM, Cagnacci A, Bufalino L, Spinetti A, Gambacciani M, Fioretti P. Lack of any estrogenic effect of ipriflavone in postmenopausal women. *J Endocrinol Invest.* 1992; 15: 755-761.

Melton LJ III, Kan SH, Frye MA, Wahner HW, O Fallon WM, Rigges LB. Epidemiology of vertebral fractures in women. *Am J Epidemiol.* 1989; 129: 1000-1011.

Melton LJ III. Epidemiology of spinal osteoporosis. *Spine.* 1997; 24 (Suppl): 2S-11S.

Messina MJ, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer.* 1994; 21: 113-131.

Messina MJ. Legumes and soybeans. overview of their nutritional profiles and health effects. *Am J Clin Nutr.* 1999; 70 (suppl): 439S-50S.

Miksicek RJ. Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol.* 1993; 44: 37-43.

Morita I, Sakaguchi K, Kurachi T, Murota SI. Ipriflavone inhibits murine osteoclast formation in vitro. *Calcif Tissue Int.* 1993; 51 (Suppl): S15-S25.

Muhlbauer RC, Li F. Frequency of food intake and natural dietary components are potent modulators of bone resorption. *Biomed Pharmacother.* 1997; 51: 360-3.

Mundy GR. Bone resorbing cells. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Raven Press, New York, 1993; 25-32.

Mundy GR. Visions for the future in osteoporosis research. *Osteoporosis Int.* 1993; 2 (suppl): S29-S34.

Murrill WB, Brown NM, Manzolillo PA, Zhang J-X, Barnes S, Lamartiniere CA. Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. *Carcinogenesis.* 1996; 17: 1451-1457.

Naessen T, Parker P, Persson I, Zack M, Adami HO. Time trends incident rates of first hip fracture in the Uppsala health care region, Sweden 1965-1983. *Am J Epid.* 1989; 130: 288-299.

Nagata C, Takatsuka N, Inaba S, Kawakami N, Shimizu H. Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. *Nat Cancer Inst.* 1998; 90: 1830-1835.

Nasu M, Sugimoto T, Chihara M, Hiraumi M, Kurimoto F, Chihara K. Effect of natural menopause on serum levels of IGF-I and IGF-binding proteins. Relationship with bone mineral density and lipid metabolism in perimenopausal women. *Euro J Endocrinol.* 1997; 136: 608-616.

National Institutes of Health. Osteoporosis: progress and promise. www.nih.gov/niams/healthinfo/opbkgr.htm [accessed 6/13/99].

Nordin BEC. International patterns of osteoporosis. *Clin Orthop.* 1966; 45: 17-30.

Omi N, Aoi S, Murata K, Ezawa I. Evaluation of the effect of soy bean milk and soybean milk peptide on bone metabolism in the rat model with ovariectomized osteoporosis. *J Nutr Sci Vitaminol.* 1994; 40: 201-211.

Parker M, Anand Jk, Myles JW, Lodwick R. Proximal femoral fractures: Prevalence in different racial groups. *Euro J Epidemiol.* 1992; 8: 730-732.

Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman Jr JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr.* 1998; 68 (suppl): 1375S-9S.

Pun KK, Chan LWL, Chung V, Wong FHW. Calcium and other dietary constituents in Hong Kong Chinese in relation to age and osteoporosis. *J Appl Nutr.* 1990; 43: 12-17.

Ragsdale AB, Barringer TA, Anastasio GD. Alendronate treatment to prevent osteoporotic fractures. *Arch Fam Med.* 1998; 7: 583-6.

Raisz LG. Bone cell biology. new approaches and unanswered questions. *J Bone Miner Res.* 1993; 8 (suppl): S457-S465.

Robinson E, Kimmick GG, Muss HB. Tamoxifen in postmenopausal women. *Drugs & Aging.* 1996; 8: 329-337.

Romagnoli E, Minisola S, Carnevale V, Scarda A, Rosso R, Scarnecchia L, Pacitti MT, Mazzuoli G. Effect of estrogen deficiency on IGF-I plasma levels: Relationship with bone mineral density in perimenopausal women. *Calcif Tissue Int.* 1993; 53: 1-6.

Rosenfeld RG, Pham H, Cohen P, Fielder P, Gargosky SE, Muller H, Nonoshita L, Oh Y. Insulin-like growth factor binding proteins and their regulation. *Acta Paediatr.* 1994; 399: 154-158.

Russell-Aulet M, Wang J, Thornton JC, Colt EWD, Pierson RN Jr. Bone mineral density and mass in a cross-sectional study of white and Asian women. *J Bone Miner Res.* 1993; 8: 575-582.

Satom M, Grasser W. Effects of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. *J Bone Miner Res.* 1990; 5: 31-40.

Scane AC, Sutcliffe AM, Francis RM. Osteoporosis in men. *Baillieres Clin Rheumatol.* 1993; 7: 589-601.

Scharbo-DeHaan M. Hormone replacement therapy. *Nurse Pract.* 1996; 21: 1-13.

Seeman E, Melton III LJ, O'Fallon WM, Riggs BL. Risk factors for spinal osteoporosis in men. *Am Med.* 1983; 75: 977-982.

Setchell KDR, Adlercreutz H. Mammalian ligands and phyto-oestrogens. Recent studies on their formation, metabolism and biological role in health and disease. In: Rowland, I.A. (Ed.) *The Role of Gut Microflora in Toxicity and Cancer*. New York: Academic Press. 1988. pp. 315-345.

Setchell KDR. Non-steroidal estrogen of dietary origin: Possible role in health and disease, metabolism and physiological effects. *Proc Nutr Soc N Z.* 1995; 20: 1-21.

Shutt DA, Cox RI. Steroid and phytoestrogen binding in sheep uterine receptors in vitro. *Endocrinol.* 1972; 52: 299-310.

Shutt DA, Braden AWH. The significance of equol in relation to the oestrogenic responses in sheep ingesting clover with high formononetin content. *Austr J Agr Res.* 1963; 19: 545-553.

Shutt DA. Interaction of genistein with oestradiol in the reproductive tract of the ovariectomized mouse. *J Endocrinol.* 1967; 37: 231-2.

Silverman SL, Madison RE. Decreased incidence of hip fracture in Hispanics, Asians, and Blacks. California hospital discharge data. *Am J Public Health.* 1998; 78: 1482-1483.

Skaggs SR, Crist DM. Exogenous human growth hormone reduces body fat in obese women. *Horm Res.* 1991; 35: 19-24.

Song TT, Hendrich S, Murphy PA. Estrogenic activity of glycitein, a soy isoflavone. *J Agric Food Chem.* 1999; 47: 1607-1610.

Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG. Gender defference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adult: role of sex hormone replacement. *J Clin Endocrinol Metab.* 2000; 85: 1121-5.

Stewart PJ, Stern PH. effects on the antiestrogens tamoxifen and clomiphene on bone resorption in vitro. *Endocrinology.* 1986; 118: 125-131.

Sue CA. Estrogen replacement therapy in postmenopausal women. *Pharmacy Times*. 1993; (suppl): 21-27.

Sugimoto T, Nishiyama K, Kuribayashi F, Chihara K. Serum levels of Insulin-like growth factor (IGF) I, IGF-binding protein (IGFBP)-2, and IGFBP-3 in osteoporotic patients with and without spinal fractures. *J Bone Miner Res*. 1997; 12: 1272-1279.

Tanaka H, Quarto R, Williams S, Barnes J, Liang CT. In vivo and in vitro effects of insulin-like growth factor-I on femoral mRNA expression in old rats. *Bone*. 1994; 15: 647-53.

Teitelbaum SL. The osteoclast and osteoporosis. *Mount Sinai Med.* 1996; 63: 399-402.

Tobias JH, Cook DG, Chambers TJ, Dalzell N. A comparison of bone mineral density between Caucasian, Asian, and Afro-Caribbean women. *Clin Sci.* 1994; 87: 587-591.

Tolstoi LG, Levin RM. Osteoporosis—The treatment controversy. *Nutr Today.* 1992; 27: 6-12.

Tsai KS. Osteoporotic fracture rate, bone mineral density, and bone metabolism in Taiwan. *J Formos Med Assoc.* 1997; 96: 802-805.

Tsutsumi N. Effect of coumestrol on bone metabolism in organ culture. *Biol Pharm Bull* 1995; 18: 1012-1015.

Turner CH. Toward a cure for osteoporosis. Reversal of excessive bone fragility. *Osteoporosis Int.* 1991; 2: 12-19.

United States Department of Health and Human Services. *The Surgeon General's Report on Nutrition and Health.* 1988. DHHS. Publication No. 88-50211.

Vanderschueren D, Gevers G, Raymaekers G, Devos P, Dequker J. Sex and age-related changes in bone and serum osteocalcin. *Calcif Tissue Int.* 1990; 461: 179-182.

Walsh BW, Kuller LH, Wild RA, Paul S, Farmer M, Lawrence JB, Shah AS, Anderson PW. Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. *JAMA*. 1998; 279: 1445-1451.

Wang H-J, Murphy PA. Isoflavone content in commercial soybean foods. *J Agric Food Chem*. 1994; 42: 1666-1673.

Weaver CM. Calcium requirements: The need to understand racial differences. Editorial. *Am J Clin Nutr*. 1998; 68: 1153-1154.

Welle S. Growth hormone and insulin-like growth factor-I as anabolic agents. *Curr Opin Clin Nutr Metab Care.* 1998; 1: 257-62.

Wenger NK, Speroff L, Packard B. Cardiovascular health and disease in women. *N Engl J Med.* 1993; 329: 247-256.

Weryha G, Pascal-Vigneron V, Klein M, Leclere J. Selective estrogen receptor modulators. *Curr Opin Rheumatol.* 1999; 11: 301-306.

WHO Expert Committee on Physical Status. The use and interperetation of anthropometry. *Report of a WHO Expert Committee.* Geneva: World Health Organization, 1995.

Wilcox G, Wahlqvist ML, Burger HG, Medley G. Oestrogenic effects of plant foods in postmenopausal women. *Br Med J.* 1990; 310: 905-906.

Wright CDP, Garrahan NJ, Stanton M, Gazet J-C, Mansell RE, Compston JE. Effect of long-term tamoxifen therapy on cancellous bone remodeling and structure in women with breast cancer. *J Bone Miner Res.* 1994; 9: 153-159.

Wu AH, Ziegler RG, Nomura AMY, West DW, Kolonel LN, Horn-Ross PL, Hoover RN, Pike MC. Soy intake and risk of breast cancer in Asians and Asian Americans. *Am J Clin Nutr.* 1998; 68 (Suppl): 1437S-1443S.

Wynder EL, Fujita Y, Harris RE, Hirayama T, Hiyama T. Comparative epidemiology of cancer between the United States and Japan: A second look. *Cancer.* 1991; 67: 746-763.

Xu L, Lu A, Zhao X, Chen X, Cummings SR. Very low rates of hip fracture in Beijing, People's Republic of China, the Beijing Osteoporosis Project. *Am J Epidemiol.* 1996; 144: 901-907.

Yamamoto H, Sohmiya M, Oka N, Kato Y. Effects of aging and sex on plasma IGF-I levels in normal adults. *Acta Endocrinol* (Copenh). 1991; 124: 497-500.

Yamazaki I, Shino A, Shimizu Y, Tsukuda R, Shirakawa Y, Kinoshita M. Effect of ipriflavone on glucocorticoid-induced osteoporosis in rats. *Life Sci.* 1986a; 38: 951-958.

Yamazaki I, Shino A, Tsukuda R. Effect of ipriflavone on osteoporosis induced by ovariectomy in rats. *J Bone Min Metab.* 1986b; 3: 205-210.

Zava DT, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. *Nutr Cancer.* 1997; 27: 31-40.

Zava DT. The phytoestrogen paradox. *The Soy Connection.* 1994; 3: 2-4.

Zava T, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. *Nut Cancer*. 1997; 27: 31-40.

Zhang RW, Simmons DJ, Crowther RS, Mohan S, Baylink DJ. Contribution of marrow stromal cells to the regulation of osteoblast proliferation in rats: Evidence for the involvement of IGFs. *Bone Miner.* 1991; 13: 201-215.

Zofkova P, kancheva RL. Effect of estrogen status on bone regulating hormones. *Bone*. 1996; 19: 227-232.

APPENDIX

INSTITUTIONAL REVIEW BOARD APPROVAL FORM

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

DATE: 10-28-98 **IRB #: HE-99-032**

Proposal Title: THE EFFECT OF SOY OR ITS ISOFLAVONES ON OSTEOARTHRITIS

Principal Investigator(s): Bahram H. Arjmandi, Mark E. Munson

Reviewed and Processed as: Modification

Approval Status Recommended by Reviewer(s): Approved

Signature: Carol Olson Date: January 27, 1999

Carol Olson, Director of University Research Compliance

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modification to the research project approved by the IRB must be submitted for approval. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board.

VITA

Nasrin Sinichi

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF SOY PROTEIN ON INDICES OF BONE TURNOVER IN MEN AND WOMEN

Major Field: Nutritional Sciences

Education: Received Bachelor of Science in Dietetics, Department of Nutritional Sciences, College of Human Environmental Sciences, Oklahoma State University, Stillwater, Oklahoma, in May 1997. Completed the requirements for the Master of Science degree with a major in Nutrition Sciences at , Oklahoma State University, Stillwater, Oklahoma in May 2000.

Experience: Graduate Research assistant, Department of Nutritional Sciences, College of Human Environmental Sciences, Oklahoma State University, Stillwater, OK , 1997 to present. Computer Lab monitor, College of Business Administration, Oklahoma State University, Stillwater, Oklahoma, 1997. Persian Language instructor, Farsi School, Oklahoma City and Stillwater, Oklahoma, 1996 to present.