

THE EFFECTS OF PRUNE ON BONE IN
OVARIECTOMIZED RATS

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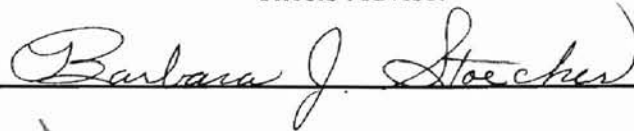
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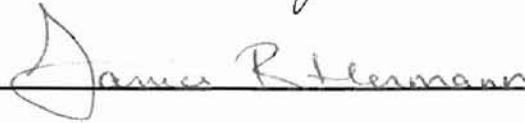
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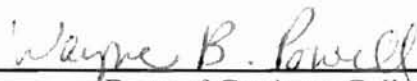
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Dean of Graduate College

Dedicated to the memory of Entisar Draz, My Brother

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TABLE OF CONTENTS

CHAPTER	PAGE
CHAPTER I.....	1
INTRODUCTION.....	1
Hypothesis.....	4
CHAPTER II.....	5
REVIEW OF LITERATURE.....	5
Bone Structure and Biology.....	5
Bone Modeling and Remodeling.....	6
Activation:.....	7
Resorption:.....	7
Reversal:.....	8
Formation:.....	8
Osteoporosis, a Metabolic Bone Disease.....	9
Antiresorptive Agents.....	11
Estrogen Replacement Therapy (ERT).....	11
Bisphosphonates.....	12
Calcitonin.....	13
Bone Formation Stimulating Agents.....	13
Parathyroid Hormone.....	13
Growth Hormone.....	14
Insulin-Like Growth Factors.....	14
Fluoride.....	15
Selective Estrogen Receptor Modulators (SERM).....	16
Raloxifen.....	16
Ipriflavone.....	16
Dietary Factors Influencing Bone Metabolism.....	17
Calcium.....	17
Factors which Affect Calcium Bioavailability.....	19
Vitamin D.....	22
Magnesium.....	23
Potassium.....	25
Boron.....	27
Antioxidants.....	29
The Biology of Oxygen Radicals.....	29

Oxygen Derived Free Radicals (ODFR) in Osteoclasts.....	30
Oxidants as Stimulators of Signal Transduction.....	31
Phytoestrogens / Isoflavonoids.....	35
Prunes.....	36
 CHAPTER III.....	 38
RESEARCH DESIGN AND METHODS.....	38
Appropriateness of the Ovariectomized Rat Model.....	38
Experimental Design.....	39
Feces and Urine Collection.....	42
Necropsy:.....	42
Body and Organ Weights.....	42
Blood Processing.....	43
Serum Alkaline Phosphatase (ALP)and Tartarate-Resistant Acid Phosphatase (TRAP).....	43
Bone Processing.....	44
Bone Chemistry.....	44
Urine Parameters:.....	45
Fecal Parameters:.....	46
Statistical and Data analysis.....	46
 CHAPTER IV.....	 48
RESULTS.....	48
Food Intake, Body Weights, and Organ Weights.....	48
Bone Length and Density.....	49
Bone Mineral Contents.....	49
Bone Alkaline Phosphatase Activity (ALP).....	50
Serum Alkaline Phosphatase (ALP)and Tartarate-Resistant Acid Phosphatase (TRAP).....	51
Urinary Calcium, Hydroxyproline, and Creatinine.....	51
Fecal Mineral Content.....	51
 CHAPTER V.....	 60
 DISCUSSION.....	 60
 CHAPTER VI.....	 64
CONCLUSION, RECOMMENDATIONS, AND SUMMARY.....	64
Conclusion.....	64
Recommendations.....	65
Summary.....	65

REFERENCES.....	67
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LIST OF TABLES

TABLE	PAGE
TABLE I	
Experimental Groups.....	40
TABLE II	
Composition of Diets.....	41
TABLE III	
Effect of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Food Intake, Body Weight and Organ Weight.....	53
TABLE IV	
Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on 4 th Lumbar Vertebral Parameters.....	54
TABLE V	
Effects of Ovariectomy, 5% Prune (LD), 25% Prune on Femoral Parameters.....	55
TABLE VI	
Effects of Ovariectomy, 5% Prune (LD), 25% Prune on Tibial Parameters.....	56
TABLE VII	
Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Serum Alkaline Phosphatase (ALP) and Tartarate-Resistant Acid Phosphatase (TRAP)Activities.....	57
TABLE VIII	
Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Urine Parameters.....	58
TABLE IX	
Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Fecal Parameters.....	59

ABBREVIATIONS

ERT	Estrogen Replacement Therapy
GH	Growth Hormone
IGF-1	Insulin-like Growth Factor-1
IGF- β	Insulin-like Growth Factor- β
PTH	Parathyroid Hormone
BMD	Bone Mineral Density
WHO	World Health Organization
SD	Standard Deviation
E ₂	Estrogen
FDA	Food and Drug Administration
IGFBP	Insulin-like Growth Factor Binding Protein
FGF	Fibroblast Growth Factor
TGF- β ₁	Transforming Growth Factor- β ₁
TGF- β ₂	Transforming Growth Factor- β ₂
ATPase	Adenosine Tri-phosphatase
O ₂ ⁻	Superoxide Anion
H ₂ O ₂	Hydrogen Peroxide
OH [·]	Hydroxyl Radical
OFDR	Oxygen Derived Free Radicals

HCT	Human Calcitonin
SOD	Superoxide Dismutase
TBARS	Thiobarbituric Acid Reactive Substances

CHAPTER I

INTRODUCTION

Osteoporosis is a major concern worldwide as it is in the United States (US). It affects more than 25 million Americans each year, resulting in 1.5 million annual osteoporotic-related fractures (1,2) and costs Americans \$13-18 billion annually (3,4). It is estimated that by the year 2030, 21% of the US population, or 65 million people, will be 64 years and older (2,4). By that time, the cost for osteoporotic related treatment is expected to exceed \$60 billion annually (2,4,5).

Osteoporosis is the second leading cause of hospitalization in individuals 80 years and older, the fastest growing segment of population in the US (3,5). At the age of 70, risk of hip fracture is very probable and thereafter this risk doubles every seven years (1,3). Similarly, the risk for mortality due to fractures increases by 20% (3). Those who survive fractures live with the burden of morbidity, suffering from pain, immobility, dependency and depression (1,6).

Osteoporosis is a metabolic bone disease characterized by reduction in bone mass and microarchitecture of bone tissues (7). Although osteoporosis is a silent disease, its early signs include loss of height and kyphosis that may go unnoticed until fracture occurs (8).

There are two types of osteoporosis: primary and secondary. Primary or involutional osteoporosis is further divided into two types, type I (postmenopausal) and type II (senile). Secondary osteoporosis is a result of endocrinal diseases, gastrointestinal disorders, or long-term use of certain medications (1,9). In postmenopausal osteoporosis, ovarian hormone deficiency gives rise to accelerated bone turnover with bone resorption exceeding bone formation, resulting in less bone mass (1). Currently in the US, one out of three postmenopausal women suffer from osteoporosis (4).

Prevention of osteoporosis can be accomplished through increasing bone mass and mineral content throughout the first three decades of life (6,10). A good strategy for prevention of osteoporosis would include a lifetime adequate intake of calcium and other nutrients, maintenance of a moderate amount of physical activity and avoiding smoking and alcohol abuse (6,11).

Traditional therapy for postmenopausal osteoporosis has emphasized the use of antiresorptive agents. Antiresorptive agents such as estrogen replacement therapy (ERT) (1,4,7), calcium (1,6, 10,11) calcitonin (1,12,13) and bisphosphonates (1,13) have been widely investigated and approved with certain limitations. Antiresorptive agents stabilize, but are relatively ineffective at restoring bone. However, in the case of established osteoporosis, the treatment of choice would be a drug that stimulates bone formation. Parathyroid hormone (PTH) (14,15,16), vitamin D (1,25 (OH)₂ D₃) (17,18,19), and sodium fluoride (1,11,20) are potential bone forming stimulating agents that may also play a role in the treatment of osteoporosis. Local bone factors have been recognized to exert metabolic effects on bone. Examples include growth factors (21),

such as insulin-like growth factor-1 (IGF-I), and transforming growth factor- β (TGF- β) (16,20,22). Even though growth hormone (GH) is currently recognized as bone forming stimulating agent, it has serious side effects (16,21,22). Other bone formation-stimulating agents especially sodium fluoride may neither decrease susceptibility to fracture nor improve bone quality (20). Although estrogen is known to effectively prevent bone loss in postmenopausal women, it is associated with risk factors such as endometrial and breast cancer (7).

Recent reports indicate that naturally occurring compounds such as isoflavones, a subclass of flavonoids and lignans, a group of diphenolic compounds, can inhibit bone resorption while stimulating bone formation (23). In this regard, prunes and prune juice are considered to be good sources of phenols, flavonoids, and antioxidants including vitamin A, vitamin E, and selenium (24). Prune is also a rich source of boron (2.2 mg/100 g) (25). Since antioxidants, phenolic compounds and boron are reported to increase BMD, the reported profile of prune increases its importance in the osteoporosis research field (9,26,27,28). Therefore, it is conceivable to speculate that prune plays an important role in maintaining skeletal health. Hence, the purpose of this study is to investigate the effects of prune on bone and indices of bone turnover in an ovarian hormone deficient rat model of osteoporosis.

Hypothesis

The hypothesis of this study is that prune is effective in preventing ovarian hormone deficiency-associated bone loss in a rat model of osteoporosis. To test this hypothesis, we have two specific aims.

Specific Aim 1: To evaluate the effects of prune in preventing the occurrence of bone loss in a rat model of ovarian hormone deficiency as assessed by bone mineral density measurement.

Specific Aim 2: To examine some of the mechanisms by which prune protects ovarian hormone deficiency-associated bone loss, including analyses of blood and urine indices of bone turnover.

CHAPTER II

REVIEW OF LITERATURE

Bone Structure and Biology

Bone is a mineralized connective tissue, that functions as a mechanical support and movement system, a protector for bone marrow and internal organs, and provides a reservoir for calcium and other minerals (29). Anatomically, the skeleton is made up of two types of bone: 1) flat bones such as the skull, mandible, and vertebrae; and 2) long bones, such as those that make up the tibia, femur, and humerus. In growing bones, the end (epiphysis) is separated from metaphysis (the conical part that connects the epiphysis to the mid shaft) by a cartilage growth plate. The diaphysis (the mid shaft) encloses the medullary cavity where bone marrow is produced (29).

Histologically, there are two kinds of bone: cortical and cancellous. Cortical bone is the thick compact bone that forms the diaphysis of long bones or surrounds trabecular bone. Cancellous or trabecular bone is the bone that fills out the flat bones or long bone epiphysis and metaphysis (29). Trabecular bone is made up of calcified trabeculae intercalating the bone marrow (29). The ratio of cortical bone to cancellous bone is 4:1, but cancellous bone is metabolically more active per unit volume because it is full of

trabeculae, that contain bone marrow. There are two bone surfaces: external (periostium) and internal (endostium). They are in contact with soft tissues and both are lined with osteogenic cells (29).

Bone consists of organic matrix cells and inorganic minerals (30). The extracellular matrix contains collagenous fiber filled with the mineral component. The most important mineral that provides the structural rigidity is calcium (13,30). The organic cells are in two main forms: osteoblasts and osteoclasts. Osteoblasts are found either singularly or in clusters of one or two layers of cuboidal cells along the layer of bone matrix in which they are formed (29). Osteoblasts form collagen and ground substance of bone matrix and its plasma membrane is, characteristically, rich in alkaline phosphatase enzyme (whose concentration in the serum is used as a marker for bone formation) (1,29). Osteoclasts are the giant multinucleated bone lining cells found usually in contact with a calcified bone surface. In each resorptive site, one to four osteoclasts can be found (7,29).

Bone Modeling and Remodeling

Bone is in a dynamic metabolic state throughout life. Bone formation and resorption are parts of the continuous turnover process by which old bone is replaced by new bone (7). Modeling takes place only during skeletal development and ceases when the cartilaginous growth plate closes (13,29). Modeling starts with bone formation which adds new bone and is then followed by resorption which shapes up the whole bone to its

original shape. Both length and volume of the bone expand by the bone modeling mechanism (3).

Bone remodeling occurs later in life after adult bone density is established. With the right stimulus, resorption of a defined quantity of bone is performed by osteoclasts, followed by osteoblast-stimulated bone formation (3,13,29). There are several million active remodeling sites in the skeleton. Four remodeling steps take place in each site: activation, resorption, reversal, and formation (13,16,29).

Activation

The exact mechanisms of activation are yet to be defined. Raisz (16) speculated that the effect of local factors on osteoblasts and their precursors could be the initiators for resorption. Another postulated mechanism includes the production of various proteolytic enzymes from lining cells to prepare the bone surface by removing protein. Removal of protein from the bone surface involves stromal cells and perosteoblasts as well as active and inactive osteoblasts (13,16).

Resorption

Osteoclasts are the main resorptive cells which assemble together or differentiate to excavate an erosion cavity on the bone surface (16). Osteoclasts adhere to the bone surface by the ruffled borders and stimulate the release of proteolytic enzymes which

result in acidification, digestion, and removal of bone cells (13,16). When osteoclastic activity goes array, perforations can occur resulting in removal of the template which makes it impossible to restore bone mass (16).

Reversal

In this phase, resorption stops by osteoclastic inactivation (16,29). Macrophage-like cells are seen in bone surface and in a deposition of the proteoglycan layer. As a result, a cement line is formed between old and new bone to mark the limits of the resorption cavity (16,29). What really stops the resorption phase is yet to be defined, but limited life span of osteoclasts and inactivation of osteoclasts by accumulation of high concentrations of calcium on ruffled borders might be the stopping signals (16). Another possibility is that the release of TGF- β from the matrix attracts the osteoblasts and inactivates the osteoclasts.

Formation

In the formation stage, osteoblasts are taking over. They secrete matrix substances which later will be mineralized (16). At the cement line old and new bone is held together (29). Replication and differentiation of osteoblasts are controlled by local and systemic factors (16). Local factors are derived from osteoclasts, reversal cells, marrow cells, and bone matrix. Systemic factors are hormones such as parathyroid hormone

(PTH), calcitonin, and many other growth factors (16,29).

Osteoporosis, a Metabolic Bone Disease

Bone is a metabolically active tissue; it constantly undergoes modeling and remodeling cycles throughout life (1,3,13). Bone modeling predominates throughout childhood resulting in an increase in bone mineral density (BMD). This remodeling process continues till early adulthood then, the established BMD plateaus between the ages of 30 to 40 years after which remodeling takes over, resulting in a decrease in BMD (4,13,31).

There are several million active remodeling sites in the skeleton which occupy 15-20 % of bone surfaces (32). After menopause, bone loss increases due to hypogonadism which results in prolonged bone turnover time. It would take from five to fifteen years after menopause to reach a new steady state where skeletal mass is no longer decreasing (32). Depending on the BMD present at menopause, the net BMD at a certain time of the postmenopausal resorption process might fall under the osteoporotic threshold. The World Health Organization (WHO) defines osteoporosis as a decrease in BMD by more than 2.5 standard deviations (SD) below the mean value for young women, and by more than 3 SD below the mean value for young men (31). What controls BMD is mainly genetic. Almost 80% of BMD is genetically determined while environmental factors and endogenous sex hormone levels during puberty control the remaining 20% (2,31).

There are two types of osteoporosis: primary and secondary. Primary or involutional

osteoporosis, is further divided into type I (postmenopausal) and type II (senile). Secondary osteoporosis, or type III osteoporosis, is a result of endocrinal diseases, gastrointestinal disorders, or long-term use of certain medications (1,9). Type I osteoporosis occurs in postmenopausal women 5 to 15 years after menopause. Estrogen (E₂) deficiency increases bone turnover and induces an imbalance between resorption and formation in each site. Calcium malabsorption is found in 60-70% of type I osteoporotic patients and calcium loss may exceed the intake of calcium in one-third of the patients (4). Low serum dihydroxy-cholecalciferol [1, 25 (OH)₂ D₃] concentrations may be another cause of bone loss in postmenopausal osteoporosis. Estrogen replacement therapy (ERT) decreases the rate of bone turnover in this group. Also it corrects the imbalance between resorption and formation at each resorptive site (32). The conjugated estrogen dose of 0.625 mg /day is capable of decreasing skeletal loss.

Type II (senile) osteoporosis occurs in much older patients, 80 years and older. Hip fractures are more common in this group. The decrease in BMD for this group is caused by the ultimate decrease in calcium absorption and other minerals in advanced age. Seventy-five percent of the elderly have a documented decrease in calcium absorption (1,4).

Type III (secondary) osteoporosis is the result of non-skeletal conditions that impact bone and mineral metabolism. Partial gastrectomy, malabsorption syndrome, thyrotoxicosis and drug and alcohol abuse can be the cause of type III osteoporosis (1,9).

Antiresorptive Agents

Several antiresorptive agents are currently in use for prevention or treatment of osteoporosis. They include ERT, Bisphosphonates, and calcitonin.

Estrogen Replacement Therapy (ERT)

In the perimenopausal years, the bone-remodeling cycle is taking over. This results in an increase in the rate of bone turnover which leads to an imbalance between resorption and formation (32). Estrogen slows down bone resorption by limiting osteoclastic access to bone and blocks the process of creating new erosion cavities (32). Estrogen is unique in correcting the imbalance between resorption and formation in each erosion site resulting in a net increase in bone mass (32). This increase in bone mass should not be interpreted as an anabolic action for estrogen because it does not continue indefinitely (32). Even though estrogen does not restore the bone mass already lost, it prevents bone loss due to ovarian hormone deficiency (32,33,34). Therefore, E₂ is a preventive rather than a corrective agent (32).

Estrogen's influence on osteoclasts is not yet as clear as its effect on osteoblasts (34). Osteoblasts respond to estrogen by producing local growth factors including insulin-like growth factor-I (IGF-I) (32).

Zofkova and Kancheva (34) studied the effects of estrogen administration on 11 women following hysterectomy and oophorectomy. In their study, bone indices were

measured before ovariectomy, 12-16 weeks after the surgery, and 2 months after transdermal treatment with a reservoir estrogen patch. Estrogen administration to these women increased parathyroid hormone levels after the postoperative drop in their levels, and decreased serum calcium after postoperative serum calcium increases. Estrogen also lowered IGF-I following its increase after the ovariectomy (34). Although ERT plays a central role in controlling bone mass, it is not a risk free option. Estrogen increases the risk of endometrial cancer with a small but real increase in the risk for breast cancer (32,33,23,35).

Bisphosphonates

Bisphosphonates are synthetic analogues of pyrophosphate and are promising agents in the treatment and prevention of osteoporosis (1,36). These drugs have a high affinity for calcium, and attach to it in the hydroxyapatite crystals in bone (1). They are ingested by osteoclasts and impair the resorptive process (13).

Etidronate, a bisphosphonates member, considerably increases the vertebral bone mass and reduces the rate of new vertebral fractures in postmenopausal women (36). It is administered orally in a cyclical dose regimen which is 400 mg daily for two weeks followed by 13-15 weeks of adequate calcium and vitamin D intake to support bone mineralization (1). Although etidronate is effective in preventing bone loss in the first five years after menopause (1,36), its long-term use may inhibit bone mineralization and cause osteomalacia (36).

Alendronate (Fosamax), a new member of the bisphosphonates family, is the first non-hormonal drug to be approved by the FDA for the treatment of osteoporosis in postmenopausal women (1,13). Daily administration of 10 mg of alendronate has been

reported to increase BMD in the spine and hip joint (1,13). Alendronate is poorly absorbed and has low toxicity due to its short half-life (1,13).

Calcitonin

Calcitonin is a small peptide hormone secreted by thyroid “C” cells and is recognized as an effective antiresorptive agent (1,16). In its pharmacological doses, it suppresses osteoclastic activity resulting in reduction of resorption and increase in BMD (13,16). Calcitonin has no effect on calcium homeostasis but is used effectively in the treatment of glucocorticoid-induced osteoporosis (1,16).

Seven published trials evaluating the efficacy of 200 IU nasal salmon calcitonin were analyzed by Nieves et al. (12). Six of the seven studies used a mean calcium dose of 1466 mg/d (which is daily intake from diet and supplement) and one used calcitonin alone, without calcium supplementation (total 627 mg calcium /d). The analysis of the results indicated a 0.2% increase in BMD of lumbar spine per year happened when calcitonin was used alone as compared with a 2.1% increase in BMD of lumbar spine per year when calcium supplement was added (12). Such results indicate that calcitonin can be a reasonable alternative for ERT in at risk cancer cases (12).

Bone Formation Stimulating Agents

Parathyroid Hormone

Parathyroid hormone is a major calcium-regulating hormone in humans (21). When blood calcium concentrations drop below normal levels, PTH draws calcium from bone

to restore normal calcium levels by stimulating bone resorption (14,16). Parathyroid hormone can exert an inhibitory effect on bone formation by inhibiting collagen synthesis as well as stimulating bone formation through intermittent or low dose administration (14). The stimulatory effect of PTH on bone formation is probably mediated through the production of local growth factors such as IGF-I and TGF- β (16).

Parathyroid hormone related peptide (PTHrP) could be a possible factor in activating PTH receptors in bone (14). Injection of small doses of PTH to postmenopausal osteoporotic women can positively shift calcium balance (4). Although PTH stimulates 1,25 (OH)₂ D₃ secretion from the kidneys which in turn enhances calcium uptake (14,17), repeated injection of PTH can result in down regulation of 1,25 (OH)₂ D₃ and consequently a reduction in bone formation (14). Therefore, injection with PTH needs to be accompanied by 1,25 (OH)₂ D₃ to be most effective (4).

Growth hormone

Growth hormone increases bone formation and promotes calcium absorption (15,23). Growth hormone may increase IGF-I synthesis, and decreases renal calcium excretion (16). Since production of both GH and IGF-I decline with advancing age, they may play a role in osteoblastic bone formation (16).

Insulin-like growth factors

A large number of growth factors have been implicated in the regulation of bone formation (16,22). Bone matrix produces IGF-I, IGF-II, bone morphogenetic protein

(BMPs), IGF binding proteins (IGFBPs, except IGFBP-I), and both acidic and basic fibroblast growth factors (FGFs) (16).

Insulin-like growth factor-1 has a strong anabolic effect on bone formation and mediates the anabolic response of PTH (16). It enhances chondrocyte proliferation, differentiation and proliferation of osteoblasts, matrix formation, and type I collagen synthesis (22). Bone morphogenetic proteins including TGF- β_1 and TGF- β_2 (16), have been shown to enhance bone formation in animals (16,22,20). These BMPs stimulate osteoblast differentiation and formation of normal mineralized bone (20). Fibroblast growth factors are potent bone mitogens which decrease collagen synthesis and increase bone resorption (20).

Fluoride

Fluoride enhances new bone formation, mostly trabecular, through the reactivation of osteoblasts (20). This results in an increase in trabecular bone mass, especially in the hip and spine (20). An increase in BMD by approximately 10%, can be achieved by fluoride treatment (20). Unfortunately fluoride treatment is not a risk free drug and it has been associated with gastric distress and pain in the joints and lower extremities (20). Increased rates of non-vertebral fractures due to fluoride treatment, have been reported (20). Currently, the FDA is considering the approval of sodium fluoride as a therapeutic alternative for the treatment of osteoporosis.

Selective Estrogen Receptor Modulators (SERM)

Raloxifen

The challenge in estrogen research is to produce a risk free derivative of E₂. Raloxifen is a new drug on the market that reduces the risk of osteoporosis and heart disease in postmenopausal women (37). It is a synthetic compound and a part of the Selective Estrogen Receptor Modulators (SERMs) class of medication (37). It halts the effects of estrogen by blocking E₂ receptors in the breast and uterus (37). Delmas et al. (37) studied 601 postmenopausal women who took either 60 mg Raloxifen or placebo daily for two years. An increase in bone density of 1.2-1.6% was observed in the Raloxifen-treated group along with a reduction in LDL cholesterol by 10% and no change in HDL cholesterol. By comparison, estrogen generally increases bone mass by 3%-4%, lowers LDL by 15%, and raises HDL by about 7%. Vaginal bleeding and hot flashes were the most common adverse events in the Raloxifen-treated group. Raloxifen is less potent than estrogen and it has a better safety profile regarding breast and uterine cancer compared to ERT (37).

Ipriflavone

Ipriflavone (IP), a synthetic isoflavone derivative, has been reported to act as both an anti-resorptive and bone forming agent. Ipriflavone has been shown to prevent bone loss

in both humans (38,39) and animals (40).

The mechanism by which ipriflavone acts on bone, however, is unknown. Both direct (41) and indirect (42) stimulation of estrogen-induced thyroid calcitonin secretion have been suggested as possible mechanisms.

Nakamura et al. (43) examined the effects of ipriflavone on bone mineral density and calcium regulating hormones and found that bone loss was inhibited possibly through increased serum calcitonin levels (43). By using mouse cells, some investigators (44) have demonstrated that the bone conserving mechanism(s) of ipriflavone involve the inhibition of osteoclast formation and bone resorption (44), the inhibition of mature osteoclast activity and formation (45), or the production of collagen by osteoblasts (45). Others have reported potential effects of IP when used in conjunction with low doses of estrogen (38). However, further studies are needed to elucidate the role of IP in the treatment of osteoporosis.

Dietary Factors Influencing Bone Metabolism

Calcium

Calcium is the most abundant divalent cation in the body. About 1.5% of body weight (1-1.2 kg) is calcium (46). Calcium is an essential nutrient for bone and soft tissues. Ninety-eight percent of calcium is found in bone and the remaining 2% is found in serum and soft tissues. In soft tissues, calcium is involved in regulatory mechanisms

such as cellular secretion, blood clotting, and neurotransmission (46). Due to ultimate calcium loss through urine, feces, and skin, calcium deficiency can be easily induced.

Calcium deficiency in developmental stages results in impaired growth and a weak skeleton. Deficiency in adulthood and the elderly result in a decrease in BMD and ultimately, osteoporosis (47). In a 14 year prospective population study by Holbrook et al. (48), 957 men and women aged 50-79 years were studied in the period from 1973 to 1975 and then followed up to 1987. The baseline distribution of calcium intake (based on a 24-hour intake report) was examined in relation to hip fracture in subjects with no prior history of hip fractures during the 14 year follow-up period. Also, the relations of protein, fat, fiber, caffeine, trace minerals, vitamin D and vitamin C to hip fractures were examined. The only factor consistently and significantly associated with the risk of hip fracture was calcium. Although other nutrients may alter calcium absorption, calcium ingestion was the limiting factor determining calcium availability. A moderate increase in dietary calcium consumption for adults can significantly reduce the risk of hip fracture (48).

Nieves et al. (12) used meta-analysis to evaluate the influence of calcium supplementation on the efficacy of estrogen and calcitonin on bone mass change in osteoporotic patients. Twenty out of 31 published ERT trials were analyzed. Twenty of these trials made diet modifications or used calcium supplement to adjust total consumption to a mean of 1183 mg/day. Eleven trials did not make diet modifications and had a mean intake of 563 mg/day. The analysis showed mean increases in bone mass densities of lumbar spine, femoral neck, and forearm when ERT was used alone of

1.3%/y, 0.9%/y and 0.4%/y, respectively. When ERT was used in addition to calcium, the mean increases to those sites were 3.3%/y, 2.4%/y, and 2.1%/y, respectively.

Such results indicate that calcium and ERT have synergetic effects on BMD of all skeletal sites. This synergetic effect of calcium can be explained by a temporarily increased demand for calcium by the pre-existing remodeling sites with the start of anti-resorptive treatment (12). This can be met through increased calcium intake. Although estrogen increases calcium absorption, an additional 550 mg elemental calcium per day is needed for the accomplishment of a BMD increase by 2% per year (12).

Factors which affect calcium bioavailability

Estrogen may be involved in the regulation of intestinal calcium absorption.

Arjmandi et al. (49) investigated estrogen's role in calcium absorption. They detected estrogen receptors (ER) by using immunocytochemical and northern blot analysis.

Immunochemical localization of E₂-receptors in rat duodenum was reported in ninety-five day old female Sprague Dawley rats. Specific nuclear staining of ER was detected in the surface epithelial cells lining the lumen as well as glandular epithelium of the 17β-estradiol treated group (49). 17β-Estradiol (10⁻⁸ mol) caused a significant increase in calcium uptake by the duodenal cells *in vitro* (49).

Arjmandi et al. (50) investigated the *in vivo* effect of 17β-estradiol on intestinal calcium absorption in rats. Arjmandi and colleagues reported that administration of 17β-estradiol caused an increase in intestinal calcium absorption dose-dependently. Both

studies (49,50) support the role of estrogen in intestinal calcium transport indicating that estrogen acts directly on the intestinal cells to promote calcium transport (49,50).

The consumption of protein, fiber, caffeine, alcohol, trace minerals, and vitamin D can affect calcium bioavailability (48). Although protein is needed by bone for growth and integrity, a diet high in protein will increase the acid load resulting from its high content of sulfur-containing amino acids (51). As the body attempts to neutralize urine pH, it pulls more calcium from the serum with a resultant hypercalciuria (11). Consequently, calcium mobilization from bone increases to maintain serum calcium levels (51).

Fiber, the plant polysaccharides and lignin that resist hydrolysis by digestive enzymes of man, is an important food component (52). Calcium absorption may be reduced by increased fiber consumption such as cellulose or other fiber components found in wheat bran. Fiber effect on calcium absorption can be explained by the increase in the bulk of intestinal contents, the decrease in the intestinal transit time and the propensity of fiber to bind cations. Therefore; it can potentially reduce the time available for calcium absorption. Fiber also causes proliferation of intestinal microbes which in turn bind calcium and make it unavailable for absorption (52).

Caffeine consumption has a negative effect on calcium metabolism. In a study conducted by Kynast-Gales and Massey (53), the effects of caffeine consumption on calcium excretion in 17 healthy men and women were evaluated. On the first day they received a diet containing 11.3 mmol calcium and 12.7 mmol magnesium for two days but on the second day 3 mg/kg body weight caffeine was added to the diet. Six hours

after caffeine administration, an increase in the urinary calcium excretion was observed (53). Renal conservation could not compensate for the caffeine effect on calcium.

Cigarette smoking influences BMD especially in postmenopausal women (1). Perimenopausal smokers may experience an earlier onset of menopause. These women also have lower E_2 as a result of an increase in E_2 biotransformation by hepatic enzymes (1). Postmenopausal women who smoke and use ERT also have lower circulating levels of E_2 (1).

Alcohol's impact on bone metabolism depends on the level of its consumption. Moderate alcohol consumption of 70-140 g/week resulted in decreased BMD in perimenopausal women (11). In contrast, there are studies that have shown some beneficial effects of alcohol on BMD when consumed in moderation (11). The mechanism through which alcohol exerts its effects on bone is not clear. However alcohol, in part, may exert positive effects on bone by increasing 17β -estradiol concentrations (11); on the other hand, excessive alcohol consumption may result in reduced calcium absorption and increased renal calcium excretion (11). This results in significant decrease in calcium content and thickness of bone with increased bone fragility (11).

Vitamin D, glucocorticoids, and trace minerals have great impacts on calcium balance. Vitamin D metabolites not only increase calcium absorption in postmenopausal women but also decrease bone resorption and increase bone formation (17). Estrogen replacement therapy enhances calcium absorption in postmenopausal women and improves calcium profile (50).

Glucocorticoids are commonly used to treat conditions such as arthritis, asthma, and allergic skin disorders. Glucocorticoids may decrease both calcium absorption and estrogen production in women and testosterone in men with a resultant bone loss (19). This bone loss can result in osteoporosis. Bone loss is usually most rapid in the first 6 months and can lead to fractures in less than one year. To prevent the bone loss associated with the treatment of these diseases, physicians should start treatment with the lowest possible effective steroid dose with patients suffering from those diseases. Also, the use of topical or inhaled formulations of steroid drugs is recommended with maintaining calcium intake of 1500 mg/ day and of 800 IU/ day for vitamin D (19).

Vitamin D

Vitamin D reaches the liver from the small intestine where it is hydrolyzed into 25 (OH) D₃, the main form of vitamin in the blood. It has a long half-life of three weeks. In the kidney, it is further hydroxylated to 1, 25 (OH)₂ D₃ which is considered the active form of vitamin D with half-life of 4-6 hours. Vitamin D is important in stimulating calcium absorption (17,19). Liang et al. (17) tested calcium uptake in rats at 3, 6, 12, and 24 months. They found that calcium absorption declines in duodenal cells of aged rats with simultaneous decline in 1,25 (OH)₂ D₃ levels (17).

Human studies (18) have also shown that vitamin D levels decrease in postmenopausal women. This decrease in vitamin D levels has been associated with reduced calcium absorption which is corrected by supplemental vitamin D (18).

Magnesium

Magnesium is a vital component of human cells and skeleton (54). Bone is the major storage site for magnesium, holding 53% of body stores for magnesium (55). Serum magnesium is very sensitive to dietary consumption and both bone and serum magnesium are closely linked. Magnesium is involved in the function of more than 200 enzymes including ATPase and in energy production (54). It has been reported that trabecular bone of osteoporotic women has low magnesium levels similar to those of magnesium deficiency (54). Balance studies (54,55) have reported that in osteoporotic women magnesium intake is about 15% lower than in normal women. Magnesium retention increases by 90% in magnesium deficient people after a parenteral magnesium load. A similar increase in magnesium retention also occurs in osteoporotic women which indicates magnesium deficiency in those women (54). The mechanisms by which magnesium status affects osteoporosis are not clear, yet as magnesium is involved in most enzymatic pathways, it would be multifactorial. Magnesium deficiency may destabilize bone minerals by increasing the pH of bone extracellular fluids. Also, the formation of $1,25(\text{OH})_2\text{D}_3$ (calcitriol) needs magnesium. Low calcitriol will reduce both calcium and magnesium absorption from the intestine (54).

Magnesium has been also reported to influence the qualitative changes in bone matrix and hence, it plays an important role in osteoporosis. Calcium is known to increase BMD by a mean of 0.8% per year (12). Even though calcium has been reported to increase BMD, it might result in larger and more brittle bone crystals (56). Magnesium has been shown to enhance bone modeling and remodeling through its enhancement of osteoblastic and osteoclastic activities (55,56).

Magnesium deficiency results in cessation of bone growth, osteopenia and bone fragility through decreasing both osteoclastic and osteoblastic activity (56).

Stendig-Lindberg et al. (57) assessed the effects of magnesium deficiency on trabecular

bone density in postmenopausal osteoporotic women. Bone density loss was documented in a two-year prospective controlled therapeutic trial. Fifty-four women participated in the study and received either treatment or placebo. The treatment group received up to 750 mg magnesium hydroxide daily for six months, followed by 250 mg per day for 18 months. Seventy-one percent of women experienced an increase of 1-8 % in trabecular bone density (57).

Classen et al. (55) investigated the influence of magnesium deficiency in older rats (aged 34 months) and the effects of magnesium preloading before giving the rats magnesium deficient diet. Rats were fed 9000 ppm magnesium as a high diet, 300 ppm magnesium as a marginal diet and 50 ppm magnesium as a low diet. The results showed significant differences in serum magnesium and pelvic bone calcium content between the high magnesium diet and the marginal magnesium diet. The appearance of erythema in 50% of the rats was significantly delayed from the 11th day to the 17th day in the low magnesium diet group which previously was fed a loading diet of 9000 ppm magnesium for 14 days. Body weight decreased continuously and was not affected by magnesium loading prior to administration of low magnesium diet. The delayed appearance of erythema, a symptom of magnesium deficiency, when rats were preloaded by magnesium before they have been given magnesium deficient diet shows the ability of the skeleton to store magnesium (55).

Magnesium deficiency is frequently associated with chronic alcoholism and may contribute to the 47% increased incidence of osteoporosis in the alcoholic population (58). In alcoholics, histologic evaluation of bone biopsies confirmed a reduction in trabecular, and to a lesser extent, cortical bone mass (58). This may be used as explanation for the magnesium reduction in the alcoholic population. Magnesium deficiency may increase bone loss through its effect on mineral homeostasis. Hypocalcemia is considered a major complication of magnesium deficiency (58).

Apparently, impaired PTH which lessens the PTH effects on kidneys and bone, is the principal cause of hypomagnesemia-induced-hypocalcemia. Also, reduction in serum concentrations of vitamin D in hypocalcemic-hypomagnesemic patients can be explained by low PTH secretion. Low PTH enhances 1,25, (OH)₂D₃ production through its effect on 1,25,(OH)₂D₃ 1-α hydroxylase enzyme (58). Hence, alcohol reduces magnesium which causes calcium and vitamin D deficiencies and consequently low BMD with more osteoporosis incidence (58).

Potassium

Potassium is the principal intracellular cation with a concentration of 145 m Eq /liter. It influences muscle contraction, nerve tissue's excitability, and electrolyte and pH balance (46,59). Under normal conditions dietary potassium intake is enough to maintain cellular levels, but deficiency can develop as a result of massive intestinal fluid loss or increased renal excretion (46,59).

Normally, a low level of metabolic acidosis and a positive shift in acid base balance are correlated with metabolism of the consumed diet. An ordinary diet with high protein value causes a shift in acid-base balance to the positive side (60). If this dietary consumption were a life-long process it would present a continuous load on the body's buffering system. As an accommodating mechanism, serum bicarbonate, the known body buffering alkali, falls but stabilizes at a lower level despite the continued acid retention. Bone plays a central role in the acid buffering process. Base mobilization from the skeleton helps stabilize the serum bicarbonate concentration (60). Such a buffering

capacity of bone may contribute to reduced BMD. Sebastian et al. (60) investigated the contribution of bone in buffering a portion of the acid generated from the metabolism of food in postmenopausal women. The researchers used metabolic-balance techniques to assess the effect of alkali on bone metabolism when given in the form of potassium bicarbonate (60 to 120 mmol potassium bicarbonate per day per 60 kg of body weight) to 18 healthy postmenopausal women eating a diet containing 16 mmol calcium and 96 g protein per 60 kg body weight daily. The researchers studied calcium, phosphorus, and acid-base balance. They also measured hydroxyproline excretion and serum osteocalcin concentrations. The results indicated that potassium bicarbonate neutralized endogenous acid production, as reflected by a sharp reduction in net renal acid excretion. In addition urinary excretion of calcium and phosphorus fell without a change in net intestinal absorption. Moreover, bone formation was stimulated, as indicated by a reduction in hydroxyproline excretion (60).

Because potassium bicarbonate improves calcium balance through its effect as a component of the serum buffering system, one would assume that it is the bicarbonate part that is effective in improvement of calcium balance. This was not the case in the study done by Lemann et al. (61). They reported that the administration of sodium bicarbonate rather than potassium bicarbonate to healthy men with a low level of metabolic acidosis did not improve calcium balance. These results suggest an effect of potassium rather than bicarbonate in the bicarbonate salt on calcium balance (61).

Boron

Boron is the first member of metalloids or semiconductors which are intermediate elements between metals and nonmetals. The chemical properties of metalloid elements can not be readily predicted (62). In 1923, boron was accepted as being essential for plants. Recently, however, accumulated evidence indicates that boron is essential for animals and humans (62).

Boron is obtained from a diet rich in fruits, vegetables, nuts and legumes (62). Wine (39.1 ppm), raisins (25 ppm), dried parsley (26.8 ppm) and caviar (5430 ppm) are particularly rich sources of boron (62). The daily intake by humans is estimated to range from 0.3-41 mg per day. Such a wide range is due to variation of the analytical methods used and differences in the soil content of boron (62). Increasing dietary boron results in increases in the tissue concentration of boron. Although large amounts of boron are well tolerated, an oral dose of 3-4 g in adults (63) and 1 g in infants (64) has been suggested to be toxic. Boron deficiency depresses growth and reduces some blood indices, particularly steroid hormone levels (62). Based on animal studies, it can be suggested that 0.5mg / day boron is the minimum daily requirement (62).

Boron is well absorbed from the gastrointestinal tract and excreted through the urine, bile, sweat and breath (62). The retention of boron by tissues is inversely related to its concentration in the diet (62). Boron has been found in all tissues but in small amounts and its concentration in the human body is 0.04 mg/kg body weight (62). Based on tissues obtained from two human cadavers, boron is distributed as follows: skin, 7.8; skeleton, 2.1; liver, 7.8; heart, 0.2; spleen and kidney, 0.1 (mg boron /organ or tissues) (62).

In chemical reactions boron accepts a hydroxyl group and leaves proton. It complexes with organic compounds containing more than two hydroxyl groups with a stronger affinity to hydroxyl groups (62). In humans, boron supplementation enhances the

hydroxylation rate leading to enhanced formation of vitamin D, testosterone and 17β -estradiol. In animals boron supplementation particularly increases testosterone levels in rat (62). At an alkaline pH, borate reacts with hydroxyl groups of estrogen and possibly results in reduction in the hormone methylation (63). Boron competitively inhibits the riboflavin-containing xanthine oxidase, the enzyme that converts xanthine oxidase to uric acid (9). Boron, *in vivo*, seems to be more prominent in cases of magnesium and vitamin D deficiency.

Boron has been implicated with a number of diseases because of its effect on plasma steroid hormone concentrations and their diverse roles in metabolism. In a study conducted by Nielsen et al. (26), 12 women aged between 48 and 82 years were housed in a metabolic unit. A boron supplementation of 3 mg/day for 24 days after 119 days of consuming a conventional diet with 0.25 mg/day boron reduced the urinary excretion of calcium and magnesium in all the postmenopausal women. Calcium excretion was reduced by 22 mg/day in the five women who consumed adequate magnesium and by 52 mg/day in the seven women who consumed a magnesium deficient diet. Boron supplementation markedly elevated 17β -estradiol and testosterone (26). This effect was more marked in the low-magnesium consuming women (26).

However in another study (65), supplementation of boron at 3 mg/day to postmenopausal women for 3 weeks after a low boron diet (0.33 mg/day) did not significantly change mineral metabolism, steroids or urinary pyridinium cross-links.

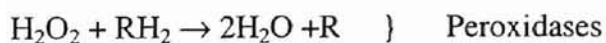
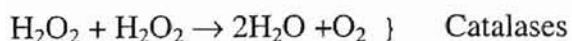
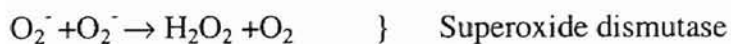
Fourteen subjects, five men aged over 45 years, four perimenopausal women and five postmenopausal women on estrogen therapy were fed a low boron containing diet (0.23 mg/2000 kcal) for 63 days, then fed the same diet supplemented with 3 mg boron/day for 49 days. The diet was low in magnesium (115 mg/2000 kcal) and marginally adequate in copper (1.6 mg/2000 kcal). A higher erythrocyte superoxide dismutase, serum enzymatic ceruloplasmin, and plasma copper were reported during boron repletion than boron

depletion (66). Boron is also capable of enhancing and mimicking some of the effects of estrogen on bone (66).

Antioxidants

The biology of oxygen radicals:

The complete reduction of an oxygen molecule to water is a univalent pathway which requires 4 electrons (67). Such a reduction results in several intermediates: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical, (OH^\cdot). Because of their high activity, these intermediates must be contained. Superoxide dismutase (SOD) eliminates O_2^- . Catalases and peroxidases eliminate H_2O_2 . The release of OH^\cdot is decreased to a great extent by elimination of O_2^- and H_2O_2 and by the action of glutathione peroxidase (GSH-Px) (67). This pathway for oxygen is not a major route of O_2 consumption by biological system, but is important for some biological reactions, such as autoxidation of catecholamines, leukoflavins, thiols and reduced ferredoxins (67). Superoxide anion, O_2^- is produced by red blood cells (RBCs) during the conversion of hemoglobin and myoglobin to methemoglobin and metmyoglobin. Methemoglobin reductase, xanthine oxidase, and aldehyde oxidase are enzymes that are involved in the conversion. O_2^- and H_2O_2 can form OH^\cdot which in turn forms ethylene, a potent oxidant. SOD and catalases inhibit ethylene production by eliminating O_2^- and H_2O_2 (67).



Oxygen derived free radicals (ODFR) in osteoclasts

Key and colleagues (28) were able to detect ODFR in the media surrounding osteoclasts. The reduction of nitroblue tetazolium (NBT) by cellular oxidants to formazan (insoluble precipitate) was used as an indicator for ODFR, particularly superoxide, production. Three media preparations of NBT, NBT+SOD, NBT+ human calcitonin (hCT) were added to three groups of isolated osteoclasts from the tibiae and clavaria of Sprague Dawley rats. The optical density of NBT in the media contained NBT+SOD and NBT+ hCT (human calcitonin) was compared to the optical density of NBT alone in its media by using microspectrophotometric densitometry. The media of NBT alone had the highest density indicating that SOD and hCT have reduced the production of ODFR (28). Under the transmission electron microscopy (TEM) calvarium tissue sections showed much more accumulation of formazan granules in the area where ruffled borders of osteoclasts face bone surface than the surrounding tissues (28). This shows ODFR production by osteoclasts. Superoxide dismutase is a large molecule which can not cross the cell membrane. Its inhibition to formazan accumulation in the media preparations (which represent osteoclastic extracellular fluid) and inside the osteoclastic cells under the transmission electron microscopy indicates that the reduction of NBT to formazan involved O_2^- . Superoxide is suggested to be produced by osteoclasts because formazan was detected in the media surrounding the isolated osteoclasts. These results indicated that ODFR may contribute to bone resorption (28).

Oxidants as stimulators of signal transduction

Suzuki et al. (68) defined signal transduction as a process that transmits the extracellular message to the intracellular environment. Oxidative-reduction reaction (redox) is a chemical regulator of signal transduction. Redox signaling is mediated by reactive oxygen species such as O_2^- , H_2O_2 , HO^\cdot and lipid peroxidases. These oxidants induce biological processes such as cell growth, apoptosis, and cell adhesion. In bone, vitamin D₃, PTH and interleukin-1 use reactive oxygen species as second messengers to signal for osteoclastic bone resorption (68).

Superoxide anion role in osteoclasts is suggested to be due to the reduction of formazan in the media surrounding osteoclasts. Superoxide anion blocks both O_2^- production and bone resorption by interleukin-1 and PTH. The detection of the role of catalases in osteoclastic bone resorption suggested a role for H_2O_2 in osteoclasts. Thus oxidants have the ability to mimic stimulus-mediated signal transduction in the physiological system.

Oxidants have been shown to stimulate Ca^{2+} signaling by an increase or a decrease in cytosolic Ca^{2+} concentration. Some reports (68) show a direct interaction between Ca^{2+} channels and oxidants which results in Ca^{2+} release from sarcoplasmic reticulum of cardiac and skeletal muscles.

The destructive role of oxidants to biological components has been revised and a new role as a regulator of physiological systems is considered. If so, then antioxidants may

alter biological function by blocking oxidant-mediated signal transduction events.

Xu and Watkins (69) evaluated the effect of two doses of dietary vitamin E on tissue lipid peroxidation, trabecular bone formation and epiphyseal growth plate cartilage development. Two levels of vitamin E (30 and 90 IU/ kg diet) were used. After 14 days, in the group that was fed the high dose of vitamin E, thiobarbituric acid reactive substances (TBARS) were lower than in the group fed the low vitamin E diet. These results indicate that the higher doses of vitamin E can suppress lipid peroxidation in animal tissue (69). In the high vitamin E group, lower hypertrophic chondrocyte zone (LHCZ) thickness was significantly increased which may be due to decreased cartilage resorption at the metaphyseal site. This data agrees with current reports that vitamin E protects against cellular lipid peroxidation in cartilage which helps in maintaining normal bone growth and modeling (69).

Umegaki et al. (70) investigated the influence of dietary vitamin E on DNA damage in rat bone marrow. Low-vitamin E supplemented diet (30 mg /kg diet) or a high-vitamin E supplemented diet (1000 mg/kg diet) were fed for 50 weeks. DNA damage was evaluated using a micronucleus assay and sister chromatid exchange in bone marrow cells at 6 weeks and 50 weeks periods. Results showed that lipid peroxidation in bone marrow cells was higher in the low vitamin E diet group, while it was not lowered by increasing vitamin E in the diet of the high vitamin E group (70). Neither at six weeks nor at 50 weeks did the change in vitamin E level in the diet affect bone marrow. There was no increase in DNA oxidative damage rate due to diet change. This indicates that DNA is well protected against oxidative damage in vivo (70).

Cohen and Meyer (71) examined the effects of dietary vitamin E supplementation on alveolar bone loss in the mandibular bone of Rice (*Oryzomys palustris*) rats. Two levels of vitamin E were introduced for 35 days to low animal groups, then rats were assigned to normal or high-stress environments. The stressed group was subjected to 1-min. period of a 30 rev / min cage rotation every 4 hours. As a result of stress, stressed animals practiced an increase in catecholamines and glucocorticoids due to the release of cytotoxic free radicals. Due to high variability, no significant effect of vitamin E was seen. But in the non-stressed group, a significant protective effect of vitamin E against alveolar bone loss was detected (71).

Vitamin A toxicity and deficiency have profound effects on bone. Zile et al. (72) designed an experiment to investigate vitamin A metabolism in rats. Male Holtzman rats were fed a retinol-deficient diet or a retinol adequate diet with all other nutrients in normal level concentrations. Animals were fed low calcium, retinol-deficient diet after they had the assigned retinol-deficient diet for 14, 21, or 28 days. Serum calcium was determined every 2 to 3 days until the animals became severely deficient and died. Animals fed retinol adequate diet were subjected to similar treatment. Calcium mobilization from bone and maintenance of normal levels of plasma calcium were not affected or only slightly altered by vitamin A deficiency indicating that PTH secretion and function were not affected (72). Major abnormalities in collagen metabolism and mineralization were seen in vitamin A deficient group. In the deficiency group, bone maturation was not complete with development of hypertrophied cartilage and improperly calcified bone. Vitamin A deficiency decreased alkaline phosphatase (ALP) activity in

bone, which supports the postulate that vitamin A enhances bone formation (72).

In a study by Metz et al. (73), diets with different levels of vitamin A and vitamin D were introduced to large male white turkey poults to test the interaction between these two vitamins and skeletal development. There was a marked reduction in growth rate in poults after both the high and low vitamin A treatments compared to other groups. All birds developed lameness, and many were unable to walk. At necropsy, bones from the deficient birds were soft and flexible; whereas cortical bone was thin and bone was fractured easily in the vitamin A deficient group. Vitamin A was effective in ameliorating the acute toxicity associated with hypervitaminosis D. Higher dietary levels of vitamin A were effective in preventing the tubular mineralization and growth depression associated with hypervitaminosis D (73).

Seleno-proteins are involved in mechanisms of cell differentiation and defense. Using ⁷⁵-selenium metabolic labeling of viable fetal human osteoblasts (hFOB-cells), the expression of seleno-protein like glutathione peroxidase (GSH-Px) has been identified by Derher et al. (74). Basal cellular glutathione peroxidase was further increased by the addition of sodium selenate to the culture medium for three days indicating that selenium can act as an antioxidant as well (74). Cellular and plasma GSH-Px expression in osteoblasts indicates that antioxidants are needed by growing osteoblasts since GSH-Px protects against H₂O₂ produced by osteoclasts during bone remodeling (74).

Phytoestrogens / Isoflavonoids

Phytoestrogens are naturally occurring compounds found in many foods.

Phytoestrogens are structurally or functionally similar to estradiol (27). Phytoestrogens consist of a number of classes with lignans and isoflavones being the most common. Lignans are found mainly in flaxseed, cereal bran, whole cereal, fruits and vegetables while isoflavones occur at high concentrations in soybean, other legumes and clovers (27). The lignanic compounds: enterolactone, enterodiol, and mataricesinol all have been isolated in human serum, urine, feces, bile, and semen (27). They also have been isolated in cow's milk.

The major isoflavones in soy are genistein and daidzein. Daidzein is formed from formononetin and metabolized to equol and 0-desmethyl-angolensin (0-DMA) and genistein is formed from biochanin. Genistein, daidzein, and equol have been detected in human plasma and equol in cow's milk (27).

Lignans and isoflavones estrogenic activities vary from weak estrogenic to anti-estrogenic activities (tamoxifen-like activity). Relative potencies of isoflavones compared with E₂ (considered 100) were coumestrol (0.202), genistein (0.084), equol (0.061) daidzein (0.013). The therapeutic effects of lignans and flavonoids are antiviral, bactericidal, antifungal, anti-inflammatory, and antiproliferative. Biochemically, flavonoids are inhibitors of tyrosine kinases which play a key role in tumorigenesis, topoisomerases I and II, microsomal lipid peroxidation, superoxide anion formation, and sodium, potassium, calcium dependent ATPase pump.

isoflavone-derived agent structurally similar to naturally occurring phytoestrogens, exerts a direct inhibitory effect on osteoclastic activity and a stimulatory effect on osteoblastic activity (75).

Arjmandi et al. (76) reported lower liver lipid and liver cholesterol in ovariectomized rats receiving ipriflavone or soy protein, compared to other groups. Also, the ovariectomy-induced increase in abdominal fat was completely reversed by the soy treatment. Arjmandi et al. (76) suggested that their results, together with the reported effects of ipriflavone in preventing bone loss associated with ovarian hormone deficiency (44,45), constitute a good foundation for a potential alternative therapy in treatment of ovarian hormone deficiency associated heart disease and osteoporosis since it demonstrates effects similar to those associated with estrogen (76).

Prunes

Prunes and prune juice are reported to be excellent sources of fiber, compounds with antioxidant properties such as phenols and flavonoids, vitamin A and vitamin E, potassium, calcium, and boron. Prunes are considered a good source of protein (77). Processed prune juice which is a water extract of dried prunes, is unique in its predominance of free amino acids: α amino butyric acid, citrullin, taurine, o-phosphoethanolamine, and quinic acid (77). Boron concentration in prune is about 2.2 mg/100g (25).

The health benefits of prunes including its hypocholesterolemic effects have hardly

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been studied. The human study conducted by Tinker and colleagues (78) has shown that consumption of 100 g prunes on a daily basis by hypercholesterolemic men lowers serum total- and LDL-cholesterol concentrations. The cholesterol lowering effects of prunes have also been studied in rats. In another study (79), when diet-induced hyperlipidemic rats were fed prune fiber, they had significantly lower plasma LDL- and liver total-cholesterol concentrations in comparison with control animals.

Although the beneficial effects of prunes on skeletal health have not been studied, it is conceivable to propose such a role for prunes. Prunes are considered good sources of vitamin A (199 RE compare to 3094 RE in carrot) (24) which is reported to stimulate bone formation (73). Vitamin A activity of prune is due to the presence of various provitamin A carotenoid compounds. In the prune the primary carotenoid is β -carotene (1.192 μg) (80). β -carotene can prevent bone resorption as a potent antioxidant. Prune contains 2.5 mg vitamin E per 100 g (24) which stimulates bone formation and reduces bone resorption (70,71,72).

Prune has a good content of magnesium (24). Magnesium increases bone formation and reduces bone loss (54,56,57). Prune also contains potassium which decreases bone resorption (60,61). Selenium and boron are trace minerals found in prune and both are reported to play roles in preserving BMD (9,66,74). Based on the functional properties and nutritional composition of prune, it would be reasonable to assume a positive role for prune in osteoporosis.

CHAPTER III

RESEARCH DESIGN AND METHODS

Appropriateness of the ovariectomized rat model

Ovariectomized rats were selected as the model used in this study because postmenopausal bone loss is characterized in this experimental model and is similar to the bone loss that occurs in postmenopausal women (81). The same mechanisms control bone resorption and bone formation in rats and humans (81). The osteopenia associated with estrogen deficiency in women and with disuse follows the same patterns of bone loss and bone tissue changes occurring in the rat (81). Therefore, the rat skeleton provides a good model of human osteoporosis. In this study, 90 day-old female Sprague-Dawley rats were used because these rats are considered mature and their bones have nearly ceased to grow longitudinally (81). Additionally, these rats show marked skeletal sensitivity to ovx-induced bone loss in a relatively short period of time (81).

Experimental design

This study was designed to represent preventive intervention for postmenopausal

bone loss due to ovarian hormone deficiency. Forty-eight (Table I) female Sprague-Dawley rats, aged 90 days, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). On arrival at our institution, rats were individually housed in wire bottom cages and kept in an environmentally controlled animal room. The rats were fed a semi-purified casein-based diet, AIN-93M, (Table II) and acclimated for 4 days. Rats had free access to deionized water throughout the study (50). When the animals were 95 days old they were divided into four weight-matched groups (12 animals/group) using a complete block design (Table I) (50). Under halothane anesthesia, bilateral ovariectomy or sham operations were performed (82). Treatment intervention (Table I) began after surgery and continued for 45 days. The sham and ovx control groups continued to receive casein-based diet (AIN-93M) whereas the prune treatment groups received similar powdered diets in which 5% or 25% of diets were replaced with dried-powdered prune with known composition. The prune was provided by the California Prune Board (Pleasanton, CA). Guidelines for the ethical care and treatment of animals from the Animal Care and Use Committee of the Oklahoma State University were strictly followed.

TABLE I
EXPERIMENTAL GROUPS

Experimental Groups				
	Surgery Day 1	Treatment Started Day 2		Sacrifice Day 45
SHAM	◆	..	_____ Casein _____	◆
OVX ¹	◆	..	_____ Casein _____	◆
OVX +LD ²	◆	..	_____ LD _____	◆
OVX + HD ³	◆	..	_____ HD _____	◆

¹OVX=ovariectomized animal

²LD=5% prune diet

³HD=25% prune diet

TABLE II
COMPOSITION OF DIETS

Ingredients	Control	OVX-LD ¹	OVX-HD ²
Casein (g)	140.0	138.5	132.5
Cornstarch (g)	465.7	465.7	465.7
L-cysteine (g)	1.8	1.8	1.8
Maltodextrin (g)	155.0	155.0	155.0
Sucrose (g)	100.0	100.0	100.0
Oil (g)	40.0	39.75	38.75
Cellulose (g)	50.0	45.5	27.5
Vitamin Mix ³ (g)	10.0	10.0	10.0
Choline bitartrate (g)	2.5	2.5	2.5
Prune ⁴ (g)	0	50.0	250
Mineral mix (Ca, P deficient) ⁵ (g)	13.4	13.4	13.4
Calcium carbonate (g)	9.88	9.79	9.43
Potassium phosphate, monobasic (g)	5.6	5.48	5.0
Sodium phosphate, monobasic (g)	3.44	3.32	2.84
Potassium citrate, monobasic (g)	0.9	0.9	0.9
Sucrose (g)	1.78	2.02	2.98

¹LD=5% prune

²HD=25% prune

³Vitamin Mixture; From Harlan Teklad, Madison, WI. (TD94047)

⁴Prune in dry powdered form provided by the California Prune Board (Pleasanton, CA).

⁵Mineral Mixture: from Harlan Teklad, Madison, WI. This mineral mix is a modification of AIN 76 formulation.

Feces and urine collection

Seven days prior to termination of the study, animals were housed individually in metabolic cages, fed known amounts of food daily for 4 days and feces were collected for four days. Carmine red (25 mg/100 g food) was added to their food the first and last days to mark the beginning and end of the fecal collection. However this was not effective since the red color was masked by pigments present in prunes. Two days prior to the termination of the study, rats were placed in metabolic cages and urine was collected from 8:00 p.m. to 8:00 a.m. During this 12-hour urine collection period, rats had free access to deionized water but no food. Urine was collected in acid-washed tubes and acidified using 6 mol/L HCl. Samples were frozen at -20°C until analyzed (50).

Necropsy

At the termination of the study, animals received intraperitoneal injection of a combination of xylazine (5 mg/kg body weight) and ketamine (100 mg/kg body weight) anesthesia and were bled from their abdominal aortas.

Body and organ weights

Animals initial and final body weights were recorded. Weights were recorded for uterine tissue, liver, abdominal fat, spleen, heart, right and left kidney, and right and left

adrenal gland for individual animals.

Blood processing

Blood samples were collected and centrifuged (4 °C) at 1500 x g for 15 minutes and serum was immediately aliquoted into small volumes (83) and stored at -20°C until required for analysis. Serum parameters, including 17β-estradiol, alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activities were determined.

Serum alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) Serum

ALP and TRAP activities were determined colorimetrically using commercial kits from Sigma Diagnostics, Inc. (St. Louis, MO) (84). Based on optimized methods, the rate of increase in absorbance due to the formation of p-nitrophenol which is directly proportional to ALP activity was measured at 405 nm. The rate of formation of TRAP which is proportional to acid phosphatase activity, was measured at 405 nm by the diazot dye formation rate.

These tests were performed on a Cobas-Fara II Clinical Analyzer (Montclair, NJ) following the manufacturer's instructions and using commercially available calibrator and quality control samples.

Bone processing

Right femurs, right tibiae and fourth lumbar vertebra were dissected, cleaned of soft tissues and stored in stoppered glass vials at -20°C . For determination of bone density, each femur, tibia and vertebra was placed in an unstopped glass vial filled with deionized water. The vial was placed into a dessicator connected to a vacuum for 90 minutes so that all trapped air diffused out of the bone (83). The bone was weighed in a boat completely immersed and suspended in deionized water previously equilibrated to room temperature (83). The bone was returned to the deionized water in the vial. Next, the bone weight in air was determined by blotting the bone with a tissue and re-weighing it in air. The bone density was calculated by Archimedes' principle (83):

$$\text{bone density} = \frac{\text{bone air weight}}{\text{bone air weight} - \text{bone under water weight}}$$

Bone mineral density and bone mineral content of 4th lumbar vertebrae were also measured by dual x-ray absorptiometry (DXA)

The lengths of the femurs were measured to the nearest 0.1 mm using a vernier caliper. Bones were dried overnight ($90-95^{\circ}\text{C}$), weighed, then ashed in covered crucibles at 600°C for 16 hours, cooled and weighed to determine the percent mineral content.

Bone chemistry

Femur, tibia, and 4th lumbar vertebrae were extracted after measuring BMD. Bones obtained from each animal were rinsed with phosphate buffered saline and extracted in a

solution of Triton X-100 (containing 0.02% sodium azide) for 72 hours at 4 °C and then centrifuged. Bone ALP and TRAP activities and total bone protein were measured from aliquots of bone extract using the Cobas-Fara II Clinical Analyzer. Detection of bone ALP and TRAP activities were based on colorimetric reactions using commercially available kits from Sigma Diagnostics, Inc. (St. Louis MO). These tests were performed on a Cobas-Fara II Clinical Analyzer (Montclair, NJ) following the manufacturer's instructions and using commercially available calibrators and quality control samples.

Inorganic constituents of the right femur, right tibia, and the fourth lumbar vertebra were determined from the ashed bone. After measuring bone enzymes, bone was ashed in porcelain crucibles overnight at 600°C. Ashed samples were diluted with 0.5% nitric acid and 0.5% lanthanum chloride solution. Bone calcium and magnesium were analyzed using atomic absorption spectrophotometry (Model 5100 PC, Perkin-Elmer, Norwalk, CT), and levels were expressed as milligram of calcium or magnesium per gram bone.

Urine Parameters

Urine was collected over a 12-hour period, preserved with 6 mol/L HCL and stored at -20°C until analyses were performed. Urine hydroxyproline was measured as an index of bone collagen content following the methods of Bergman and Loxley and reported in µg per mg creatinine. Creatinine was measured colorimetrically using a commercially available kit from Roche Diagnostics (Branchburg, NJ) based on the 12-hour night urine volume using Cobas-Fara II Clinical Analyzer (Montclair, NJ). Urine calcium and magnesium were analyzed by diluting urine samples with 0.1% nitric acid and 0.1% lanthanum chloride solution. Bone calcium and magnesium were analyzed using atomic

absorption spectrophotometry (Model 5001 PC, Perkin-Elmer, Norwalk, CT), and concentrations were expressed as milligrams of calcium or magnesium per gram bone.

Fecal parameters:

The collected feces were stored in polyethylene tubes and kept frozen at -20°C . Dried feces were ground and one gram was weighed and ashed in porcelain crucibles overnight at 600°C . The ashed samples were diluted with 0.5% nitric acid and 0.5% lanthanum chloride solution. Fecal calcium and magnesium were analyzed using atomic absorption spectrophotometry (Model 5001 PC, Perkin-Elmer, Norwalk, CT) and concentrations were expressed as milligrams of calcium or magnesium per gram bone.

Statistical and data analysis

Data analysis (Graph Pad InStat Software version 2.00, 1993, San Diego, CA) involved estimation of means and SD for each of the groups (85). Analysis of variance (ANOVA) was performed to determine whether there were significant ($p < 0.05$) differences among the groups. When ANOVA indicated a significant difference among the means, the Tukey-Kramer multiple comparison test was used to determine which means were significantly different.

The primary statistical analysis was conducted to determine the effect of the independent variable treatments ovariectomy, 5% prune diet and 25% prune diet on the

dependent variable, BMD, measured for the right femurs, right tibiae and the fourth lumbar vertebra. An ANOVA was performed for each outcome variable(85). Statistical significance was set at $p < 0.05$ for all analyses.

A secondary analysis was done to determine whether the effect of treatment on BMD was reflected by biochemical markers of bone metabolism. An ANOVA was performed for each dependent variable: the % mineral content of the femur, tibia, and vertebra; femur calcium and magnesium; serum ALP activity; serum TRAP activity; urinary hydroxyproline; and creatinine concentrations. If the ANOVA was significant, the means for each treatment group were compared to the sham and ovx control groups. For each ANOVA, the Tukey-Kramer multiple comparison procedure was used to control for Type I error (85).

CHAPTER IV

RESULTS

Food intake, body weights, and organ weights

Sham animals consumed significantly ($p<0.05$) less food than ovx control group animals (Table III). There were no significant differences in food intake among the ovx groups (Table III). Although all animals started with similar body weights, at the end of the study all the ovx groups regardless of dietary treatments had a significantly higher mean body weight than the sham group (Table III). Among the ovx groups, the ovx LD mean weight gain was significantly ($p<0.05$) higher than ovx control group but not significantly different from ovx HD group (Table III). As expected, the mean uterine weight (g) of the animals in the ovx control group was significantly lower than the sham group (Table III) indicating the success of the ovariectomy. Furthermore, prune diets had no uterotrophic effects on ovx groups, indicating the lack of estrogenicity in prunes. The mean weights of heart, kidneys, and adrenal glands, when expressed per 100 g body weight, were significantly ($p<0.05$) lower in ovx animals than the sham (Table III). There were no differences among the groups in mean liver, spleen, and abdominal fat weights when expressed as g per 100 g body weight.

Bone length and density

The mean right femoral (Table V) and tibial (Table VI) lengths in ovx-control animals were significantly ($p<0.05$) greater than the sham animals. Prune treatments did not significantly affect the ovx-induced increase in femoral and tibial length.

The ovx-control animals had significantly ($p<0.05$) lower 4th lumbar vertebral bone mineral densities than the sham animals (Table IV). The 4th lumbar bone mineral density was not significantly changed by 5% prune diet, but the loss was completely prevented by 25% prune diet as indicated by the significantly higher BMD for ovx-HD than that for ovx-control group. The 4th lumbar vertebral bone density data were also confirmed using dual-energy x-ray absorptiometry (DXA) which gave similar results (Table IV).

The mean femoral density for ovx-control was significantly ($p<0.05$) lower than for sham animals (Table V). Similarly, the mean tibial density of ovx-control group was significantly ($p<0.05$) lower than the sham animals (Table VI). High dose prune, but not LD prevented this ovx-induced tibial bone loss.

The ovx-induced bone mineral density loss was not affected by 5% prune in the diet but was completely prevented by 25% prune in the diet for tibia and 4th lumbar vertebra (Table IV and VI).

Bone mineral contents

Percent mineral content of fourth lumbar vertebrae was significantly ($p<0.05$) lower for the ovx-control animals than the sham animals (Table IV). Ovx-HD group had

significantly ($p<0.05$) greater fourth lumbar percent mineral content than both the ovx-control and the ovx-LD groups (Table IV). Fourth lumbar magnesium (g/whole bone) was significantly greater in ovx-HD animals than the ovx-control group (Table IV). Fourth lumbar magnesium (g/whole bone) was not significantly different in ovx-control than in sham group or in ovx-LD group (Table IV).

Right femoral percent mineral contents of the ovx animals were not significantly affected by prune treatments (Table V). Ovx-control group had significantly ($p<0.05$) lower femoral magnesium concentration (g/g bone) than the sham group. The 25% prune in the diet had significantly ($p<0.05$) greater magnesium concentrations than both ovx-control and ovx-LD groups (Table V).

There was no significant difference in magnesium (mg/ whole bone) content of ovx-control group of tibia than of the sham. Ovx-HD group had significantly ($p<0.05$) higher magnesium concentrations than the sham group in tibia (Table VI).

Bone alkaline phosphatase activity (ALP)

Fourth lumbar vertebral ALP activity (U/g bone) was not significantly different in ovx-control group in comparison with the sham group. However, its activity was significantly ($p<0.05$) lower in ovx-LD group in comparison with ovx-control group (Table IV). There was no significant difference among all groups in femur ALP activity (Table V). Tibial ALP activity (U/ whole bone) was significantly ($p<0.05$) greater in the ovx-control animals in comparison with the sham animals (Table VI). Both ovx-LD and ovx-HD groups had significantly ($p<0.05$) lower tibial ALP activities than the ovx-control group but not significantly different than the sham group (Table VI).

Serum alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activity

Mean serum ALP activity did not differ among the treatment groups (Table VII). The ovx-control group had significantly ($p<0.05$) greater serum TRAP activity than the sham group. There was a trend in mean serum TRAP activity in the ovx-HD group showing a lowering effect of 25% prune diet on serum TRAP activity in comparison with ovx control group (Table VII).

Urinary calcium, hydroxyproline, and creatinine

Mean calcium, expressed as either mg calcium per 12-hour urine or mg calcium per mg creatinine, was not significantly altered by ovariectomy, however, both LD and HD prune diets elevated urinary calcium excretion. However, mean urinary calcium excretion was significantly ($p<0.05$) greater only in HD compared to ovx-control group (Table VIII). Mean urinary hydroxyproline concentrations, when expressed in terms of μg per mg creatinine, were not affected by ovariectomy or prune treatments (Table VIII).

Fecal mineral content

Fecal mineral concentration was not significantly different in the ovx-control group compared with sham group. There was a significant ($p<0.05$) decrease in mean mineral concentration of feces in HD group compared to the LD group (Table IX). However, among the ovx group, LD and the HD prune diets dose dependently lowered fecal mineral content ($p<0.05$) for both groups in comparison with ovx-control group.

Fecal magnesium concentration did not differ in ovx-control group from the sham group. Fecal magnesium concentration was significantly ($p<0.05$) lower in ovx-HD than in ovx-control (Table IX).

TABLE III

Effects of Ovariectomy, 5% Prune Diet (LD), 25% Prune Diet (HD) on Food Intake, Body and Organ Weights

Parameter	Sham-Control	Ovx-Control	Ovx- LD	Ovx- HD	P value
¹ Food Intake (g/rat/day)	11.9 ± 0.7 ^b	16.4 ± 1.0 ^a	16.2 ± 1.0 ^a	14.7 ± 0.9 ^{a,b}	<0.0044
Body Weights (g)					
Initial	227.8 ± 2.2	228.3 ± 2.3	226.2 ± 1.7	231.5 ± 1.8	>0.3169
Final	265.6 ± 4.0 ^b	313.9 ± 4.8 ^a	328.5 ± 4.8 ^a	327.8 ± 3.5 ^a	< 0.0001
Weight gain	41.5 ± 3.2 ^c	86.4 ± 4.7 ^b	102.3 ± 4.1 ^a	96.3 ± 4.3 ^{a,b}	< 0.0001
Organ Weights (wt/100g body wt)					
Liver	2.756 ± 0.082	2.649 ± 0.077	2.492 ± 0.066	2.696 ± 0.063	>0.0763
Spleen	0.233 ± 0.010	0.231 ± 0.006	0.236 ± 0.005	0.232 ± 0.008	>0.9755
Uterus	0.265 ± 0.019 ^a	0.045 ± 0.003 ^b	0.044 ± 0.004 ^b	0.044 ± 0.003 ^b	< 0.0001
Kidney	0.674 ± 0.018 ^a	0.575 ± 0.017 ^b	0.548 ± 0.015 ^b	0.554 ± 0.016 ^b	< 0.0001
Heart	0.368 ± 0.010 ^a	0.328 ± 0.010 ^b	0.322 ± 0.007 ^b	0.320 ± 0.008 ^b	<0.0012
Adrenal	0.038 ± 0.002 ^a	0.028 ± 0.002 ^b	0.029 ± 0.001 ^b	0.029 ± 0.001 ^b	< 0.0001
Abdominal fat	2.016 ± 0.154	1.324 ± 0.183	2.061 ± 0.260	1.335 ± 0.194	<0.0114

Values are means ± S

^{a,b,c} Within a row, values that do not share the same superscript letters are significantly (p<0.05) different from each other.

¹Mean food intake from a six days collection

TABLE IV

Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on 4th Lumbar Parameters

Parameter: 4 TH Lumbar:	Sham	Ovx Control	Ovx-LD	Ovx-HD	<i>p</i> values:				
					Sham vs. ovx-cont	Ovx vs. Ovx-LD	Ovx vs. Ovx-HD	Ovx-LD vs. Ovx-HD	Overall
¹ BMD (DXA)	0.238 ± 0.009 ^a	0.216 ± 0.010 ^b	0.223 ± 0.011 ^b	0.235 ± 0.010 ^a	<0.001	ns	<0.001	<0.05	<0.0001
BMD (<i>displacement</i>)	1.275 ± 0.020 ^{ao}	1.240 ± 0.020 ^b	1.247 ± 0.020 ^{bo}	1.302 ± 0.040 ^a	<0.01	ns	<0.001	<0.001	<0.0001
² BMC	0.117 ± 0.007	0.111 ± 0.010	0.115 ± 0.012	0.119 ± 0.010	ns	ns	ns	ns	>0.2978
%Mineral Content	60.10 ± 0.74 ^a	57.42 ± 0.91 ^b	57.15 ± 1.45 ^b	59.05 ± 1.04 ^a	<0.001	ns	<0.01	<0.001	<0.0001
Calcium (<i>mg/g bone</i>)	206.00 ± 7.30	202.00 ± 8.87	198.00 ± 18.24	204.00 ± 10.45	ns	ns	ns	ns	>0.3936
Calcium (<i>mg/ whole bone</i>)	41.32 ± 3.02	39.14 ± 3.73	40.73 ± 4.42	43.24 ± 3.01	ns	ns	ns	ns	0.0578
Magnesium (<i>mg/g bone</i>)	3.97 ± 1.26	3.85 ± 0.42	4.44 ± 0.65	4.57 ± 0.61	ns	ns	ns	ns	0.0937
Magnesium (<i>mg/ whole bone</i>)	0.79 ± 0.24 ^{ab}	0.75 ± 0.12 ^b	0.91 ± 0.13 ^{ab}	0.97 ± 0.15 ^a	ns	ns	<0.05	ns	<0.0079
Protein (<i>mg/g bone</i>)	0.68 ± 0.02 ^a	0.82 ± 1.00 ^{ab}	0.87 ± 0.2 ^b	0.89 ± 0.19 ^b	ns	ns	ns	ns	<0.0226
Protein (<i>mg/ whole bone</i>)	0.137 ± 0.038	0.156 ± 0.022 ^c	0.178 ± 0.040 ^{bo}	0.189 ± 0.043 ^{ab}	ns	ns	ns	ns	>0.6163
³ ALP activity (<i>U/g bone</i>)	6.06 ± 1.72 ^a	5.78 ± 2.03 ^{ac}	3.62 ± 1.70 ^b	3.83 ± 2.08 ^{bc}	ns	<0.05	ns	ns	<0.0023
ALP activity (<i>U/whole bone</i>)	1.223 ± 0.353 ^a	1.102 ± 0.349 ^{ab}	0.744 ± 0.357 ^b	0.808 ± 0.416 ^b	ns	ns	ns	ns	<0.0055

^{a,b,c}Within a row, values that do not share the same superscript letters are significantly ($p < 0.05$) different from each other.Values are means ± SD; n=12; ns= not significant ($p > 0.05$)¹BMD: Bone Mineral Density, ²BMC: Bone Mineral Content, ³ALP: Alkaline Phosphatase

TABLE V

Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Femur Parameters

Parameter Femur:	Sham	Ovx Cont	Ovx-LD	Ovx-HD	<i>p</i> values:				
					Sham vs. ovx-cont	Ovx vs. Ovx-LD	Ovx vs. Ovx-HD	Ovx-HD vs. Ovx-LD	Overall
Length (cm)	3.669 ± 0.059 ^b	3.690 ± 0.062 ^a	3.685 ± 0.059 ^a	3.658 ± 0.070 ^a	<0.001	ns	ns	ns	<0.0001
¹ BMD (displacement)	1.517 ± 0.023 ^a	1.475 ± 0.054 ^b	1.469 ± 0.022 ^b	1.506 ± 0.057 ^b	<0.05	ns	ns	ns	0.0163
%Mineral Content	63.54 ± 1.15 ^a	61.85 ± 1.23 ^b	60.95 ± 1.03 ^b	62.10 ± 1.2 ^b	<0.01	ns	ns	ns	<0.0001
Calcium (mg/g bone)	234.0 ± 10.7	232.0 ± 13.7	224.0 ± 20.6	237.0 ± 26.6	ns	ns	ns	ns	0.3872
Calcium (mg/whole bone)	136.0 ± 8.6	137.0 ± 9.0	136.0 ± 13.0	142.0 ± 9.0	ns	ns	ns	ns	0.4044
Magnesium (mg/g bone)	4.963 ± 0.507 ^a	4.412 ± 0.413 ^c	4.410 ± 0.397 ^c	4.784 ± 0.523 ^b	<0.05	ns	<0.001	<0.001	<0.0001
Magnesium (mg/whole bone)	2.880 ± 0.322	2.614 ± 0.275	2.680 ± 0.258	2.858 ± 0.202	ns	ns	ns	ns	0.0439
Protein (mg/g bone)	1.41 ± 0.46	1.19 ± 0.46	1.39 ± 0.39	1.13 ± 0.32	ns	ns	ns	ns	0.2460
Protein (mg/whole bone)	0.812 ± 0.250	0.703 ± 0.270	0.849 ± 0.260	0.697 ± 0.210	ns	ns	ns	ns	0.3357
² ALP activity (U/g bone)	1.50 ± 0.54	1.38 ± 0.46	1.56 ± 0.41	1.51 ± 0.32	ns	ns	ns	ns	0.7839
ALP activity (U/whole bone)	0.870 ± 0.300	0.820 ± 0.260	0.957 ± 0.280	0.929 ± 0.210	ns	ns	ns	ns	0.5926

^{a,b,c} Within a row, values that do not share the same superscript letters are significantly ($p < 0.05$) different from each other.Values are means ± SD; ns = not significant ($p > 0.05$)¹ BMD: Bone Mineral Density, ²ALP: Alkaline Phosphatase

TABLE VI

Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Tibia Parameters

Parameter Tibia:	Sham	Ovx Control	Ovx-LD	Ovx-HD	p values:				
					Sham vs ovx-cont	Ovx vs Ovx-LD	Ovx vs. Ovx-HD	Ovx-LD vs. Ovx-HD	Overall
Length (cm)	3.919 ± 0.067 ^a	4.023 ± 0.044 ^a	4.052 ± 0.058 ^a	4.012 ± 0.050 ^a	<0.001	ns	ns	ns	<0.0001
¹ BMD (displacement)	1.557 ± 0.024 ^a	1.510 ± 0.029 ^{b,c}	1.523 ± 0.042 ^{a,c}	1.556 ± 0.040 ^a	<0.01	ns	<0.05	ns	<0.0025
%Mineral Content	63.85 ± 2.08	63.53 ± 0.60	63.48 ± 0.79	63.29 ± 0.65	ns	ns	ns	ns	0.7207
Calcium (mg/g bone)	253.0 ± 30.9	244.0 ± 23.0	243.0 ± 19.0	238.0 ± 12.0	ns	ns	ns	ns	0.4157
Calcium (mg/ whole bone)	111.0 ± 14.2	111.0 ± 14.0	111.0 ± 10.0	110.0 ± 7.2	ns	ns	ns	ns	0.9956
Magnesium (mg/g bone)	4.924 ± 0.418	4.884 ± 0.677	5.051 ± 0.696	5.180 ± 0.304	ns	ns	ns	ns	0.5465
Magnesium (mg/whole bone)	2.160 ± 0.189 ^b	2.205 ± 0.273 ^{ab}	2.306 ± 0.240 ^{ab}	2.392 ± 0.084 ^a	ns	ns	ns	ns	0.0368
Protein (mg/g bone)	1.68 ± 0.55	1.49 ± 0.57	1.60 ± 0.58	1.27 ± 0.33	ns	ns	ns	ns	0.2400
Protein (mg/whole bone)	0.734 ± 0.240	0.675 ± 0.270	0.736 ± 0.270	0.587 ± 0.150	ns	ns	ns	ns	0.3751
² ALP activity (U/g bone)	1.84 ± 0.48 ^b	1.82 ± 0.64 ^b	1.89 ± 0.58 ^b	1.89 ± 0.52 ^a	ns	ns	<0.01	<0.001	<0.0001
ALP activity (U/whole bone)	0.805 ± 0.210 ^b	1.32 ± 0.300 ^a	0.874 ± 0.290 ^b	0.871 ± 0.230 ^b	<0.001	<0.001	<0.001	ns	<0.0001

^{a,b,c} Within a row, values that do not share the same superscript letters are significantly ($p < 0.05$) different from each other.

Values are means ± SD, ns = not significant ($p > 0.05$)

¹BMD: Bone Mineral Density

²ALP= Alkaline Phosphatase

TABLE VII

Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Serum

Alkaline Phosphatase (ALP) and Tartarate-Resistant Acid Phosphstase (TRAP).

Parameter: Serum:	Sham	Ovx Cont	Ovx-LD	Ovx-HD	<i>p values:</i>				
					Sham vs. Ovx-cont	Ovx vs Ovx-LD	Ovx vs Ovx-HD	Ovx-LD vs. Ovx-HD	Overall
ALP activity (U/L)	82.0 ± 15.5	95.7 ± 31.7	90.5 ± 23.8	97.4 ± 14.9	ns	ns	ns	ns	0.3206
TRAP activity (U/L)	1.70 ± 0.71 ^c	2.90 ± 1.00 ^{ab}	3.50 ± 0.67 ^a	2.60 ± 0.48 ^b	<0.01	ns	ns	<0.05	<0.0001

^{a,b,c} Within a row, values that do not share the same superscript letters are significantly ($p < 0.05$) different from each other.

Values are means ± SD; n=12

ns=not significant ($p > 0.05$)

TABLE VIII

Effects of Ovariectomy, 5% Prune (LD), 25% Prune (HD) on Urine Parameters

Parameter: Urine:	Sham	Ovx Cont	Ovx-LD	Ovx-HD	<i>p</i> values:				
					Sham vs. Ovx-cont	Ovx vs. Ovx-LD	Ovx vs. Ovx-HD	Ovx-LD vs. Ovx-HD	Overall
Calcium (<i>mg/12 hrs urine</i>)	0.483 ± 0.260 ^b	0.569 ± 0.440 ^b	1.389 ± 1.790 ^b	2.211 ± 1.390 ^a	ns	ns	<0.05	ns	<0.0123
Calcium (<i>mg /mg creatinine</i>)	0.216 ± 0.242 ^b	0.162 ± 0.122 ^b	0.291 ± 0.269 ^b	0.508 ± 0.316 ^a	ns	ns	<0.05	ns	<0.0170
Magnesium (<i>mg/ 12 hrs urine</i>)	0.584 ± 0.611	0.877 ± 1.270	0.467 ± 0.240	1.205 ± 1.670	ns	ns	ns	ns	0.4074
Magnesium (<i>mg /mg creatinine</i>)	0.325 ± 0.574	0.239 ± 0.314	0.120 ± 0.040	0.256 ± 0.306	ns	ns	ns	ns	0.5526
Hydroxyproline (<i>µg/mg creat</i>)	17.38 ± 11.15	17.38 ± 6.21	18.02 ± 6.78	15.47 ± 3.52	ns	ns	ns	ns	0.8280

^{a,b,c} Within a row, values that do not share the same superscript letters are significantly ($p < 0.05$) different from each other.

Values are means ± SD; n=12

ns= not significant ($p > 0.05$)

TABLE IX

Effects of ovariectomy, 5% Prune (LD), 25% Prune (HD) on Fecal Parameters

<u>Parameter</u> <u>Feces :</u>	Sham	Ovx Cont	Ovx-LD	Ovx-HD	<u>p values:</u>				
					Sham vs. Ovx-cont	Ovx vs. Ovx-LD	Ovx vs. Ovx-HD	Ovx-LD vs. Ovx-HD	Overall
% Mineral Content	14.08 ± 1.24 ^{ab}	14.93 ± 1.80 ^a	13.25 ± 1.18 ^b	10.34 ± 0.40 ^c	ns	<0.05	<0.001	<0.001	<0.0001
Calcium (mg/g feces)	47.45 ± 5.80 ^a	44.37 ± 5.80 ^{ab}	39.96 ± 2.80 ^{bc}	34.82 ± 3.50 ^{cd}	ns	ns	ns	ns	<0.0002
Magnesium (mg/g feces)	3.09 ± 0.52 ^a	3.11 ± 0.56 ^a	2.95 ± 0.56 ^a	1.91 ± 0.23 ^b	ns	ns	<0.001	<0.001	<0.0001

^{a,b,c} Within a row, values that do not share the same superscript letters are significantly ($p < 0.05$) different from each other.

Values are means ± SD; n=12

ns= not significant ($p > 0.05$)

CHAPTER V

DISCUSSION

The purpose of this study was to compare the effect of high dose (25%) and low dose (5%) prune on bone, bone mineral densities, and bone parameters in ovariectomized rats. Because, we fed the prune diets immediately after the surgery, any effects on bone we observed in 5% prune or 25% prune can be attributed to a preventive property of prune in ovarian hormone deficiency. The significantly higher food intake of ovx-control animals compared to sham animals was a result of ovariectomy. This agrees with the findings of Arjmandi et al. (76) and Kalu et al. (83) who reported significant increases in food intake of ovariectomized animals in comparison with sham animals. Arjmandi et al. (76) reported that administration of estrogen to ovariectomized rats decreased their food intake. The significantly higher final mean body weights and weight gains of ovariectomized animals compared to the sham animals suggest a shift in energy metabolism due to ovarian hormone deficiency (76). Human (83) and animal (76,83) studies that investigated estrogen effects on ovx-induced increased body weight have concluded that there is a regulatory role for E₂ on the rate of energy influx. Uterine weight was significantly lower in the ovx-control compared to the sham group. This is an expected effect of ovx on uterus due to the lack of estrogen (49).

The fact that prune did not increase the uterine weight of the ovx animals indicates a lack of estrogenicity of prune at least as far as the uterus is concerned. This suggests that prune may exert its action on bone tissue in a way different than that of estrogen.

Since abdominal fat did not significantly increase in the ovx-control group and organ weights tended to be similar with the sham, the increase in body weight may be due to the increase in muscle weight rather than organ weights.

Femur and tibial lengths were significantly higher in the ovx-control group than in the sham group. This may be due to a transitory increase in GH synthesis and secretion in ovariectomized rats as suggested by Kalu et al. (83).

Bone densities of 4th lumbar vertebra, femur, and tibia were significantly lower in ovx control groups than in the sham group. These findings are in agreement with those of Arjmandi and colleagues (50). This can be explained by the lack of estrogen in ovx rats which accelerates the rate of bone turnover (32). High-dose prune diet may prevent BMD loss by different mechanism(s) than that of estrogen.

Percent mineral content of the ovx-HD group was significantly higher than that for the ovx-control group in the 4th lumbar vertebrae but not significant in femur or tibia. Although prune is a good source of fiber which might have increased fecal mineral loss, percent mineral content of feces was significantly lower in both 5% and 25% prune diets. We speculate that prune diet may alter the pH of the digestive tract and that might increase mineral absorption. Together, these findings suggest that the increase in BMD, in part, may be associated with enhanced calcium absorption, an important factor in bone mineralization. The reason that percent mineral content is significantly higher with 25%

prune in the 4th lumbar vertebrae but not in femur or tibia may be explained, in part, by bone biology. Lumbar vertebrae are predominately made of trabecular bone which is more sensitive to changes in bone turnover than cortical bone from which femur and tibia are made (32). Prune is a good source of antioxidants such as phenolic compounds, vitamin E, β -carotene and selenium (24). Oxidants have a deteriorating effect on BMD as they accelerate bone resorption by osteoclasts (28,70). Vitamin E protects against cellular peroxidation in cartilage which helps in maintaining normal bone growth and remodeling (71). Vitamin A enhances bone growth and development and its deficiency results in a decrease in serum ALP activity and an increase in hydroxyproline in urine indicating that it stimulates bone formation and suppresses bone resorption (73). Selenium can act as an antioxidant through its effect on GSH-Px (74).

Incorporation of 25% prune into the diet tended to decrease TRAP activity. Hence, antioxidant activity of prune together with suppression of bone resorption and enhanced mineral absorption may be important in protecting BMD loss.

Another reason for a protective effect of prune may be due to enhanced magnesium absorption and its incorporation into bone as was the case in this study. In this study, the magnesium concentrations of 4th lumbar vertebra and femur of rats receiving the 25% prune diet were significantly higher than the ovx controls.

Additionally, magnesium is involved in most enzymatic pathways including vitamin D₃ synthesis which regulates calcium and magnesium absorption (54). Therefore, this mechanism could justify a postulated role for magnesium in a better bone mineralization. Magnesium is also involved in osteoclastic and osteoblastic activities and hence

influences bone metabolism in general (55). Magnesium stabilizes bone minerals by decreasing the pH of extracellular fluids of bone cells (55,56).

Fourth lumbar vertebral protein content also increased significantly in 25% prune fed animals in comparison to ovx-controls. Since the bone matrix is mainly collagen protein, the increased bone content of protein may enhance the vital processes performed by the matrix such as bone cells formation.

CHAPTER VI

CONCLUSION, RECOMMENDATIONS, AND SUMMARY

CONCLUSION

In the first few years following menopause or surgical ovariectomy, both osteoblastic and osteoclastic activities are increased in women (32). Serum and urinary biochemical indices of bone turnover such as urinary hydroxyproline, serum alkaline phosphatase, and serum tartrate-resistant acid phosphatase activities are used for their reflection of bone metabolism activities. Local bone factors such as IGF-I and IGFBP-3 may play a role in increasing osteoblastic activity following ovarian hormone deficiency.

The results of this study suggest that prune is capable of preventing bone loss in ovarian hormone deficiency. Bone protective mechanisms of prune may differ from those of estrogen as it is indicated by uterine weight. Prune may enhance bone formation and suppress bone resorption as observed by serum indices of bone turnover.

Beneficial effects of prune may be due to a number of factors including: its contents of minerals, vitamins, and diphenolic compounds that are known to positively influence bone metabolism. Prune may enhance intestinal absorption of minerals including calcium which is known to play an important role in skeletal health.

RECOMMENDATIONS

The findings of the present study suggest that prune can effectively prevent the ovarian hormone deficiency-induced bone loss in a rat model of osteopenia. Since prune is also a good source of soluble dietary fiber, pectin, it may also prevent the ovarian hormone-associated rise in serum total cholesterol. Therefore, prune might be considered a desirable food for dealing with postmenopausal women's increased risks of coronary heart disease and osteoporosis. However, future human studies are needed to confirm the findings of this animal study. Additionally, dose-response studies can help to identify optimal amounts of prune to be incorporated into humans' diets.

Animal studies are also warranted to investigate the bone protective mechanisms of action of prune using histomorphometric and molecular techniques.

SUMMARY

The purpose of this study was to evaluate the effects of prune in preventing the occurrence of bone loss in a rat model of ovarian hormone deficiency, with exploration of some of the mechanisms by which prune exerts its action on bone, blood, and urine indices. The study examined the effects of incorporation of 5 and 25% prune into the rat diet. Forty-eight Sprague-Dawley 90-day old female rats were used in the study. Rats were randomly divided into four treatment groups as follows: Sham operated (sham),

ovx, ovx+5% prune (LD), and ovx+25% prune (HD). Treatments were started after surgery and continued for 45 days. After the end of the treatment period, rats were sacrificed and bone, blood, and liver were collected and stored properly for various analyses. Statistical analysis involved estimation of means and standard deviations (SD) for each of the groups. Analysis of variance (ANOVA) and Tukey Kramer multiple comparison tests were used to determine the significant differences between the groups. The findings of this study indicate that ovariectomy significantly decreased BMD of 4th lumbar vertebrae, tibia, and femur in comparison with sham. Prune incorporation to the diet at the 25% level successfully prevented the bone loss observed in the ovx group. When BMD was evaluated by dual-energy x-ray absorptiometry, the results confirmed the displacement BMD values. From our findings it can be concluded that the ovx-induced bone loss is completely preventable by the 25% prune diet in ovariectomized rat model. Prune at the 25% level tended to decrease serum tartrate-resistant acid phosphatase, an index of bone resorption, compared with the ovx-control group. Hence it may be logical to postulate that prune suppresses bone resorption while allowing bone formation to continue. Furthermore, incorporation of prune in diet enhanced percent mineral content of 4th lumbar vertebrae, tibia, and femur which may be explained by increased mineral absorption including calcium. However, the protective mechanisms of prune need further investigation.

REFERENCES

1. Kujdych N, Pitman JR. Osteoporosis--New Prespective. *Drug Topics*. 1996;140:108-117.
2. Lysen VC, Walker R. Osteoporosis Risk Factors in Eighth Grade Students. *J Sch Health*. 1997;67:317-321.
3. Anderson JB. Symposium: Nutritional Advances in Human Bone Metabolism. *J Nutr*. 1996;126:1150S-1152S.
4. Gallagher JC. The Pathogenesis of Osteoporosis. *Bone Miner*. 1990;9:215-227.
5. Barefold E. Osteoporosis-Related Hip Fractures Cost \$ 13-18 Billion Yearly. *Food Rev*. 1996;19: 31-36.
6. United State Department of Health and Human Services. *The Surgeon General's Report on Nutrition and Health*. DHHS Publication No. 88-50211, 1988.
7. Kanis JA. Osteoporosis: A View Into the Next Century. *Nether J Med*. 1997;50:198-203.
8. Black DM. Why Elderly Women Should be Screened and Treated to Prevent Osteoporosis. *Am J Med*. 1995;98:2A65S-2A75S.
9. Volpe SL, Taper JL, Meacham S. The Relationship Between Boron and Magnesium Status and Bone Mineral Density in the Human: A review. *Magnesium Res*. 1993;6:291-296.
10. Thomas ML, Simmons DJ, KidderL, Ibarra MJ. Calcium Metabolism and Bone Mineralization in Female Rats Fed Diets Marginally Sufficient in Calcium: Effect of Increased Dietary Calcium Intake. *Bone Miner*. 1991;12:1-14.
11. Bunker VW. The Role of Nutrition in Osteoporosis. *Br J Biomed Sci*. 1994;51:228-240.
12. Nieves JW, Komar L, Cosman F, Lindsay R. Calcium Potentates The Effect of Estrogen and Calcitonin on Bone Mass: Review and Analysis. *Am J Clin Nutr*. 1998;67:18-24.
13. Arnaud CD. Osteoporosis: Using 'Bone Markers' for Diagnosis and Monitoring. *Geriatrics*. 1996;51:24-30.
14. Bonn D. Parathyroid Hormone for Osteoporosis. *The Lancet*. 1996;347:50.

15. Kiebzak G, Smith R, Gunberg CC, Howe JC, Sactor B. Bone Status of Senescent Male Rats: Chemical, Morphometric, and Mechanical Analysis. *J Bone Miner Res.* 1988;3:37-45.
16. Raisz LG. Bone Cell Biology: New Approaches and Unanswered Questions. *J Bone Mine Res.* 1993;8:S457-S465.
17. Liang CT, Barnes J, Takamoto S, Sactor B. Effect of Age on Calcium Uptake in Isolated Duodenum cells: Role of 1,25-Dihydroxyvitamin D₃. *Endocrinology.* 1989;124:2830-2836.
18. Thomas MK, Lloyd-Jones DM, Thadhani RI. Hypovitaminosis D in the medical Inpatients. *N Engl J Med.* 1998; 338:777-783.
19. Anonymous. Corticosteroid-Induced Osteoporosis: Guidelines Spell Out Preventative Steps. *Geriatrics.* 1997;52:11, 15.
20. Mundg GR. Vision for the Future in Osteoporosis Research. *Osteoporosis Int.* 1993;2:S29-S34.
21. Rudman D, Feller AG, Nagraj HS. Effects of Human Growth Hormone in Men 60 Years Old. *N Engl J Med.* 1990;323:1-6.
22. Cadogan J, Eastell R, Jones N, Barker M. Milk Intake and Bone Mineral Acquisition in Adolescent Girls: Randomised, Controlled Intervention Trial. *Br Med J.* 1997;315:1255-1261.
23. Arjmandi BH, Alekel L, Hollis BW, Amin D. Dietary Soybean Protein Prevents Bone Loss in an Ovariectomized Rat Model of Osteoporosis. *J Nutr.* 1996;126:161-167.
24. Whitney EN and Rolfes SR (ed). *Understanding Nutrition.* 7th edition West Publishing Company, St. Paul. MN. 1996.
25. Anderson, Cuningham, Lindstrom. Concentration and Intakes of B, Se, K, Na and Cl. *J Food Comp Analysis.* 1994;7:59-82
26. Nielsen FH, Hunt CD, Mullen LM, Hunt JR. Effect of Dietary Boron on Mineral, Estrogen, and Testosterone Metabolism in Postmenopausal Women. *FASEB J.* 1987; 1:394-397.
27. Knight DC, Eden JA. A Review of the Clinical Effects of Phytoestrogens. *Obestet Gynecol.* 1996;87:897-904.

28. Key LL, Riez WL, Taylor RG, Hays BD, Pitzer BL. Oxygen Derived Free Radicals in Osteoclasts: The Specificity and Location of Nitroblue Tetrazolium Reaction. *Bone*. 1990;11:115-119.
29. Baron RE. Anatomy and Ultrastructure of Bone. In: *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Second Edition. New York: Raven press, 1993.
30. Anonymous. *Dorland Illustrated Medical Dictionary*, 28th edition, Philadelphia:WB Saunders Company, 1994.
31. Eriksen EF, Langdahl BL. The Pathogenesis of Osteoporosis. *Hormone Res*. 1997;48:78-82.
32. Kanis JA. Estrogens, the Menopause, and Osteoporosis. *Bone*. 1996;19:185S-190S.
33. Abe T, Chow JWM, Lean JM, Chambers TJ. Estrogen Does Not Restore Bone Lost After Ovariectomy in the Rat. *J Bone Miner Res*. 1993;8:831-838.
34. Zofkova I, Kancheva RL. Effects of Estrogen Status on Bone Regulating Hormones. *Bone*. 1996;19:227-232.
35. Gibaldi M. Prevention and Treatment of Osteoporosis: Does the Future Belong to Hormone Replacement Therapy?. *J Clin Pharmacol*. 1997;37:1087-1099.
36. Meunier PJ, Confavreux E, Tupinon I, Hardouin C, Delmas PD, Balena R. Prevention of Early Postmenopausal Bone Loss with Cyclical Etidronate Therapy (A Double-Blind, Placebo-Controlled Study and 1-Year Follow Up). *J Clin Endocrin Metab*. 1997;82:2784-2791.
37. Delmas PD, Bjarnason NH, Mitlak BH, Ravoux A, 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;337:1641-1648.
38. Agnusdei D, Zachei 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;15:97-104.
39. Reginster JL. Ipriflavone: Pharmacological Properties and Usefulness in Postmenopausal Osteoporosis. *Bone Miner*. 1993; 23:223-232.
40. Yamazaki I, Shino A, Tsukuda R. Effect of Ipriflavone on Osteoporosis Induced by

Ovariectomy in Rats. *J Bone Miner Metab.* 1986;3:205-210

41. Kakai Y, Kawase T, Mikuni-Takagaki Y, Saito S. Effect of Ipriflavone and Estrogen on The Differentiation and Proliferation of Osteogenic Cells. *Calcif Tissue Int.* 1992;51:S11:S15.
42. Yamazaki I, Kinoshita M. Calcitonin-Secreting Property of Ipriflavone in the Presence of Estrogen. *Life Sci.* 1986;38:1535-1541.
43. Nakamura S, Takamoto S, Onishi T, Fukuo K, Koh E, Kitano S, Miyshita Y, Yasuda O, Tamatani M, Nakahashi T, Ogihara T. Effect of Ipriflavone on Mineral Density and Calcium-Related Factors in Elderly Females. *Calcif Tissue Int.* 1992;51:S30-S34.
44. Suda A. Effect of Parathyroid Hormone on Parietal Bones of Rats, and Effect of Ipriflavone. *J Bone Miner Metab.* 1987;5:51-56.
45. Notoya K, Tsukuda K, Taketomi S. Stimulatory Effect of Ipriflavone Formation of Bone-Like Tissue in Rat Bone Marrow Stromal Cell Culture. *Calcif Tissue Int.* 1992;51:S16-S20.
46. Flynn A. Mineral and Trace Elements in Milk. *Adv Food Nutr Res.* 36:209-252.
47. Nordin B. Calcium and Osteoporosis. *Nutr.* 1997;13:664-686.
48. Holbrook TL, Barrett-Connor E, Wingard D. Dietary Calcium and Risk of Hip Fracture: 14-Year Prospective Population Study. *Lancet.* 1988;7:1046-1051.
49. Arjmandi BH, Salih MA, Herbert DC, Sims SH, Kalu DN. Evidence for Estrogen Receptor-Linked Calcium Transport Into the Intestine. *Bone Miner.* 1993; 21:63-74.
50. Arjmandi BH, Hollis BW, Kalu DN. In Vivo Effect of 17 β -Estradiol on Intestinal Calcium Absorption in Rats. *Bone Miner.* 1994;26:181-189.
51. Licata AA, Bou E, Bartter FC, West Fredetts. Acute Effects of Dietary Protein on Calcium Metabolism in Patients With Osteoporosis. *J Geront.* 1976. 36:14-19.
52. Harland bf, Oberleas D. Phytate in foods. *Wld Rev Nutr Diet.* 1987;52:235-259.
53. Kynast-Gales SA, Massey GL. Effect of Caffeine on Circadian Excretion of Urinary Calcium and Magnesium. *J Am Coll Nutr.* 1984;13:467-472.
54. Dreosti IE. Magnesium Status and Health. *Nutr Rev.* 1995;53:S23- S27.

55. Classen UG, Seitz G, Grimm P and Classen HG. Influence of High and Low Dietary Magnesium Levels on Functional, Chemical and Morphological Parameters of Old Rats. *Magnes Res.* 1994;7:233-243.
56. Sojeka JE, Weaver CM. Magnesium Supplementation and Osteoporosis. *Nutr Rev.* 1995;53:71-74.
57. Stendig-Lindberg G, Tepper R, Leichter I. Trabecular Bone Density in A Two Year Controlled Trial of Peroral Magnesium in Osteoporosis. *Magnes Res.* 1993;6:155-163.
58. Abbott L, Nadier J, Rude RK. Magnesium Deficiency in Alcoholism: Contribution to Osteoporosis and Cardiovascular Disease in Alcoholics. *Alcohol Clin Exp Res.* 1994;18:1076-1082.
59. Eckerstrom S. Clinical Aspects of Metabolism in Elderly. *Geriatrics.* 1966;21:161-165.
60. Sebastian A, Harris S, Ottaway JH, Todd KM, and Morris CR. Improved Mineral Balance and Skeletal Metabolism in Postmenopausal Women Treated With Potassium Bicarbonate. *N Engl J Med.* 1994;330:1776-1781.
61. Lemann J, Gary RW, Pleuss JA. Potassium Bicarbonate, But Not Sodium Bicarbonate, Reduces Urinary Calcium Excretion and Improve Calcium Balance in Healthy Men *Kidney Int.* 1989;35:688-695.
62. Naghii MR, Samman S. The Role of Boron in Nutrition and Metabolism. *Prog Food Nutr Sci.* 1993;17:331-349.
63. Dixon RL, Lee IP, and Sherines RJ. Methods to Assess reproductive Effects of Environmental Chemicals Studies of Cadmium and Boron Administrated Orally *Environ health Prspect.* 1976,13:59-67
64. Renolds EF. Martindale. *The Extra Pharmacopia*, London. 1982.
65. Beattie JH. The Influence of a Low –Boron Diet supplementation on Bone Metabolism, Major Mineral and Sex Steroid Hormone in Postmenopausal Women. *Nutr.* 1992;3:69-83.
66. Neilsen FH. Biochemical and Physiological Consequences of Boron Deprivation in Humans. *FASEB J.* 1994;7:59-63
67. Duke JB. The Biology of Oxygen Radicals. *Science.* 1978;201:875-880.

68. Suzuki YJ, Forman HJ, Sevanian A. Oxidants as Stimulators of Signal Transduction. *Free Rad Biol Med.* 1997;22:269-285.
69. Xu H, Watkins BA, Seifert MF. Vitamin E Stimulates Trabecular Bone Formation and Alters Epiphyseal Cartilage Morphometry. *Calcif Tissue Int.* 1995;57:293-300.
70. Umegaki K, Uratoto H, Esashi T. Lack of Influence of a Long-Term High or Low Vitamin E Diet on the Oxidative DNA Damage in the Bone Marrow of Mice. *Internat J Vit Nutr Res.* 1997;67:159-154.
71. Cohen M, Meyer DM. Effect of Dietary Vitamin E Supplementation and Rational Stress on Alveolar Bone Loss In Rice Rats. *Br J Biol.* 1993;38:601-606.
64. Zile M, Ahren H, Deluca HF. Vitamin A and Bone Metabolism in Rat. *J Nutr.* 1973;103:308-313.
73. Metz AL, Walser MM, Olson W. The Interaction of Dietary Vitamin A and Vitamin D Related to Skeletal Development in the Turkey Poultry. *J Nutr.* 1985;115:929-935.
74. Derher I, Schutze N, Baur A, Hesse K, Schneider D, Kohrle J and Jakob F. Selenoproteins are Expressed in Fetal Human Osteoblast-Like Cells. *Biochem Biophys Res Commun.* 1998;245:101-107.
75. Gambacciani M, Cappagli B, Piaggese L, Ciaponi M, Genazzani AR. Ipriflavone Induced by GnRH-Agonists. *Calcif Tissue Int.* 1997;61:S15-S18.
76. Arjmandi BH, Khan DA, and Juma SS. The Ovarian Hormone Deficiency-Induced Hypercholesterolemia is Reversed by Soy Protein and the Synthetic Isoflavone, Ipriflavone. *Nutr Res.* 1997;17:885-894.
77. Gorsel HV, Edurado L, Chingying L, Smith M, Kader AA. Compositional Characterization of Prune Juice. *J Agric Food Chem.* 1992;40:784-789.
78. Tinker LF, Schneeman BO, Davis PA, Gallaher DD. Consumption of Prune as a Source of Dietary Fiber in Men with Mild Hypercholesterolemia. *Am J Clin Nutr.* 1991;53:1259-1265.
79. Tinker LF, Davis PA, Schneeman BO. Prune Fiber or Pectin Compared With Cellulose Lowers Plasma and Liver Lipids in Rats With Induced Hyperlipidemia. *J Nutr.* 1994;124:31-40.
80. Somogyi LP. Prune A Fiber Rich Ingredient. *Cereal Food World.* 1987;32:541-544.
81. Frost HM, Jee WS. On the Rat Model of Human Osteopenias and Osteoporosis.

Bone Miner. 1992;18:227-236.

82. Waynforth HB. *Experimental and Surgical technique in the rat.* Academic Press, New York, NY. 1980.
83. Kalu DN, Arjmandi BH, Liu CC, Salih MA, Bimbaum RS. Effects of Ovariectomy and Estrogen on the Serum Levels of Insulin-Like Growth Factor Binding Protein-3. *Bone Minr.* 1994;25:135-148.
84. Kalu DN, Salerno E, LiuCC, Ferarro F, Arjmandi BH, Salih MA. Ovariectomy-Induced Bone Loss and the Hematopoietic System. *Bone Minr.* 1993;23:145-161.
85. Daniel WE. *Biostatistics: A Foundation for Analysis in the Health Sciences.* John Wiley & Sons, New York, NY. 1991.

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