DRIED PLUM ENHANCES RECOVERY OF BONE

FOLLOWING HINDLIMB UNLOADING

IN FEMALE RATS

By

ASHLEY LYNN ETHRIEDGE

Bachelor of Science in Dietetics

Oklahoma State University

Stillwater, Oklahoma

2002

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2006

DRIED PLUM ENHANCES RECOVERY OF BONE

FOLLOWING HINDLIMB UNLOADING

IN FEMALE RATS

Thesis Approved:

Dr. Brenda Smith

Thesis Advisor

Dr. Barbara Stoecker

Dr. Edralin Lucas

Dr. Gordon Emslie

Dean of the Graduate College

ACKNOWLEGEMENTS

First and foremost, I would like to thank my advisor, Dr. Brenda Smith. Without her encouragement, support and assistance, I would have never been able to reach this goal. I will always be grateful for her commitment to being my advisor and teacher. I would also like to express appreciation to my graduate committee members, Dr. Barbara Stoecker and Dr. Edralin Lucas for their encouragement and assistance throughout this project.

Furthermore, I would like to express my sincere gratitude to my parents, John and Audrey Ethriedge, for always encouraging me and believing I can do anything. And finally, to my wonderful boyfriend and best friend, Jarrod King, for his love, friendship and daily inspiration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

RESEARCH PROBLEM

Introduction to the Problem

Osteoporosis is a disease of the skeletal system characterized by decreased bone

mass and deterioration of the bone microarchitecture, resulting in increased risk of

fracture (WHO, 2006). Adult bone regularly remodels through a sequence of events

described as bone resorption coupled with new bone formation. During remodeling, bone

tissue is degraded by the actions of bone resorpting osteoclast cells followed by bone

formation by osteoblasts. Once peak bone mass is achieved, which occurs at

approximately 25 to 30 years of age, bone loss continues throughout the life span

(Cosman, 2005). This bone loss is a consequence of the bone resorption rate exceeding

the bone formation rate, causing bones to become thin, more fragile, and therefore

increasing the risk of fracture. Because bone loss can occur for years without an

individual fracturing, osteoporosis has been described as the silent epidemic (NIH, 2006).

Today, 10 million Americans are estimated to suffer from osteoporosis defined as

a bone mineral density (BMD) $\geq$2.5 standard deviations below the average young adult

(WHO, 2006). Another 34 million Americans are estimated to have low BMD (between

-1 to -2.5 standard deviations) or osteopenia, placing them at higher risk for osteoporosis

(NIH, 2006). Furthermore, it is expected that forty percent of white, postmenopausal women will suffer a fracture due to osteoporosis during their lifetime (NIH, 2006). Despite these alarming statistics, drug treatment options may be cost prohibitive (Brixner, 2006) and accompanied with adverse side-effects (Chaiamnuay, 2006; South-Paul, 2001; Muff, 1999).

While white, postmenopausal women are the most prevalent sufferers (Cauley, 2005); there are numerous other factors that enhance the likelihood of developing osteoporosis. These factors include both controllable and uncontrollable risk factors. Controllable factors include chronic low calcium intake, insufficient physical activity, cigarette smoking, and excessive alcohol consumption (NOF, 2006). Uncontrollable risk factors consist of age, gender, immobilization, hormone deficiencies and a family history of osteoporosis (NOF, 2006). Of the factors which individuals can control, the effects of weight-bearing activity on bone health have been the focus of much research (Kannus, 1995; Jacobson, 1984; Nickols-Richardson, 1999; McDonald, 1986).

Weight-bearing exercise is crucial in the maintenance of bone health (Aloia, 1978; Dalsky, 1989; Krolner, 1983). Previous studies have shown bone to be negatively affected by the reduction or absence of weight-bearing exercise (Kannus, 1995; Nickols-Richardson, 1999). For example, without gravitational or mechanical loading of the skeleton, there is rapid bone loss (Abram, 1988). Bone loss has been reported in individuals who perform limited weight-bearing activity, including those experiencing prolonged bed rest (Donaldson, 1970), astronauts during space flight (Oganov, 1991) and the elderly population as a result of limited use or disuse (Takata, 2001). Because National Aeronautic Space Association (NASA) scientists identified bone loss as one of

the major obstacles to extended space exploration, ground-based models of unloading (i.e. bed-rest studies and hindlimb unloading of rats and mice) were developed (Morey-Holton, 1998; Donaldson, 1970; Arnaud, 1992). These models have resulted in significant advancements in our understanding of the skeletal response to unloading, however, few effective countermeasures have been found to date that can prevent or reverse this process.

Dietary supplements are one possible group of countermeasures that could offer effective, relatively inexpensive treatment options with low risk of side-effects. Dietary supplements that would either prevent bone loss due to unloading or enhance the recovery of bone, once the loss has occurred would be an appealing option. Animal and human studies (Arjmandi, 1999; Arjmandi, 2001; Deyhim, 2005; and Franklin, 2006) conducted in the Nutritional Sciences laboratories at Oklahoma State University have demonstrated that dried plums (*Prunus domestica L*) have osteoprotective (Arjmandi, 2001and Franklin, 2006) and anabolic properties which may reverse previous bone loss (Deyhim, 2005). Dried plums are a rich source of antioxidants as indicated by their high oxygen radical absorbance capacity (ORAC) rating used to evaluate commonly consumed fruits and vegetables. They are also good sources of vitamin K, potassium and the trace element boron (Stacewicz-Sapuntzakis, 2001) which are recognized to play a role in bone metabolism.

The initial dried plum studies were carried out in an animal model of postmenopausal osteoporosis, i.e. the ovariectomized rat. These studies showed that dried plums prevented bone loss as indicated by preserving BMD and bone microarchitecture (Arjmandi, 2001) as well as restoring or reversing the loss of trabecular

bone architectural properties (Deyhim, 2005). Findings from these studies also suggested that dried plum may be a potent stimulator of bone formation (Arjmandi, 2001). More recently, a study in a male animal model of gonadal hormone-induced bone loss (Franklin, 2006) provided evidence that dried plum prevented bone loss by depressing bone resorption while at the same time enhancing insulin-like growth factor (IGF)-I. If as this report suggests that dried plum can depress bone resorption and at the same time enhance bone formation, dietary supplementation with dried plum would provide a very promising treatment option for bone loss.

In light of these very promising findings regarding dried plum's potent effects on bone in gonadal hormone deficiency animal models, and the need for effective interventions that could be used as countermeasures for bone loss resulting from skeletal unloading, the following hypothesis and specific aims have been developed.

Hypothesis

1. Dried plums will dose-dependently enhance the recovery of bone following hindlimb unloading (HLU) and have similar effects to the anabolic agent, parathyroid hormone (PTH).

Specific Aims

1. To establish the most effective dose of dried plum in restoring bone quality following HLU and to compare these effects to the positive control, intermittent PTH. The effectiveness of each dose of dried plum in reversing bone loss will be evaluated based on the improvements of bone quality as indicated by bone density using dual

energy x-ray absorotometry (DXA) and bone strength using 3-point bending of the femur midshaft and compression testing of the vertebral body.

2. To assess the dose-dependent effects of dried plum on trabecular and cortical bone microarchitecture and to compare these effects to PTH, micro-computed x-ray tomography (μCT) will be utilized. μCT analysis will be used to evaluate trabecular bone in the distal femur metaphysis and vertebral bone and the cortical rich region of the femur midshaft.

3. To investigate the dose-dependent effects of dried plum on bone metabolism in rats following HLU and to compare these effects to intermittent PTH. Serum and urinary markers of bone metabolism will be assessed to evaluate indicators of bone resorption (urinary deoxypyridinoline crosslinks) and bone formation (serum alkaline phosphatase, osteocalcin, and IGF-I).

Limitations

The HLU rat model has been used extensively to evaluate the response to microgravity and limited weight-bearing activity. This model was designed to mimic alterations in the skeletal loading associated with spaceflight as well as the cephalic fluid shift that occurs. Although only a limited number of individuals experience space travel, this model has provided a valuable system to study the effects of unloading on the skeleton. Despite the advantages provided by this animal model, it is not without some limitations.

1. During the first 3-4 days of HLU, observational data (weight-loss and the development of pyrine around the eyes) suggests that the animals are stressed. These initial changes do not persist past day 7 and studies have shown that, to the contrary, typical physiological alterations in thymus and adrenal gland size and elevated corticosterone levels have not been reported (Morey-Holton, 2002).

2. Another limitation is the fact that we are using an animal model; not humans. While this model does mimic bone response to disuse well, it can not be exactly how the human skeleton would respond. The rat skeleton continues skeletal growth throughout life while the human skeleton reaches peak mass in early adulthood.

3. A further limitation in this study is that the doses of dried plum used in this study are not reasonable or appealing treatment or prevention options for men and women. However, in order to evaluate the effectiveness of this natural alternative treatment, a wide range of doses should be tested.

CHAPTER II

REVIEW OF THE LITERATURE

Osteoporosis

Osteoporosis is a condition of the bone characterized by decreased bone mass and deterioration of the bone microarchitecture, leading to enhanced risk of fracture, typically of the spine, hip and wrist (WHO, 2006). According to the World Health Organization (WHO) osteoporosis is defined as a bone mineral density (BMD) 2.5 or more standard deviations below the average young adult (WHO, 2006). In general, peak bone mass is achieved between 25 to 30 years of age, followed by continuous bone loss throughout the remainder of the lifespan (Cosman, 2005). Because an individual can live with osteoporosis for years without symptoms of fracture, osteoporosis is often described as the silent disease (NIH, 2006). This loss of bone results in thinner, more fragile bones, and consequently increases the risk of fracture.

Today, osteoporosis is the most common bone disease in America and is a health threat for approximately 44 million Americans (NIH, 2006). It is estimated that nearly 10 million of those Americans suffer from osteoporosis, with 68% of those affected being women (NIH, 2006). The remaining 34 million are estimated to have low BMD or osteopenia and are at increased risk for osteoporosis (NIH, 2006). Current estimates indicate that one in two women and one in four men over age 50 will have an

osteoporosis-related fracture in their lifetime (NIH, 2006). In 2006, it was estimated that nearly $14 billion dollars a year are spent on fractures due to osteoporosis, costing approximately $38 million per day (NIH, 2006).

Numerous factors increase the risk of developing osteoporosis, and may be considered as either uncontrollable or controllable risk factors. Examples of uncontrollable risk factors include age, gender, and a family history of osteoporosis (NOF, 2006). Controllable risk factors for osteoporosis on the other hand include insufficient weight-bearing physical activity, estrogen deficiency due to menopause, cigarette smoking, habitually low calcium intake and excessive alcohol consumption (NOF, 2006). Of the risk factors that are considered controllable, weight-bearing activity and its effect on bone has been extensively studied.

Weight-Bearing Exercise and Bone Health

Weight-bearing activity has bee defined by the CDC as any physical activity that imparts a load or impact on the skeleton (CDC, 2006). The effects of weight-bearing exercise on bone health have been the focus of numerous studies and adequate skeletal loading has proven to be essential in the development and maintenance of bone health (Kannus, 1995; Nickols-Richardson, 1999; Dalsky, 1989; Schoutens, 1989). In general, physical activity may reduce the risk of fracture by enhancing bone strength and BMD and by improving balance, leading to decreased risk of falls (Srivastava, 2002). It is estimated that more that 50% of American adults do not get the recommended physical activity of 30 minutes a day despite its known health benefits (CDC, 2006). Furthermore

the amount of physical activity performed tends to decrease with age and is less in females compared to males (CDC, 2006).

Numerous studies have evaluated the osteoprotective effects of various physical activity regimens on the skeleton. A recent study (Srivastava, 2002) showed that weight-bearing exercise has an effect on both lumbar spine and femoral neck bone density while non-weight bearing exercise only positively affects lumbar spine bone density. Findings by Nelson and others (1994), demonstrated the effectiveness of high-intensity resistance training in maintaining femoral neck BMD as well as improving muscle mass, strength and balance in postmenopausal women. In contrast, Bemben and others (2004) showed that in postmenopausal women neither high-load nor high-repetition (HR) resistance training for 6 months combined with 1500 mg of supplemental calcium produced a significant increase in spine or hip BMD. An additional study evaluated the effect of resistance training and calcium supplementation (600 mg/day) on BMD (Kerr, 2001). A significant increase was reported at the hip site following 2 years of resistance training in postmenopausal women suggesting that long-term resistance training combined with calcium supplementation is beneficial. Going and colleagues (2002), assessed the effects of exercise, including both aerobic weight-bearing activity and weightlifting 3 times a week and hormone replacement therapy (HRT) with exercise on BMD in postmenopausal women given calcium supplementation (800 mg/day). They showed that improvements in BMD at the femoral neck, lumbar spine, and total body occurred with exercise, but not to the level of HRT with exercise. These findings related to resistance training alone and in combination with calcium supplementation demonstrate the importance of weight-bearing and bone health.

Skeletal Unloading and the Human Skeleton

Bone loss occurs rapidly in individuals such as astronauts, individuals who are immobilized, and the elderly, who perform limited weight-bearing activity. In adults, the absence of weight-bearing activity reduces bone mass (Martin, 1990), produces hypercalcemia (Zerath, 1998), decreases osteoblast number (Vico, 1987) and increases osteoclast numbers (Zerwekh, 1998). These alterations in osteoblasts and osteoclasts result in a depressed rate of bone formation and increased bone resorption. Decreased bone mass results from the microgravity environment of space. Smith and others (1999) reported that during space-flight, male astronauts experience a 50% decrease in calcium absorption and greater than a 50% increase in bone resorption. During spaceflight, astronauts have lost as much bone mass in 1 month as a postmenopausal women would in 1 year (Cavanaugh, 2005). These alterations in bone metabolism induced during spaceflight appear to persist for an extended period, as a significant elevation in bone resorption markers has been shown 4-6 months following space-flight (Smith, 2005).

Bone loss attributable to space flight is an enormous concern that could hinder extended stays at the International Space Station and long-term space exploration, such as expeditions to Mars (Bikle, 1997). Upon return to earth, the effects of weightlessness have been shown to render the musculoskeletal system incapable of enduring the stress of normal gravity (Bikle, 1997). Because of this concern, the National Aeronautic Space Association (NASA) developed ground-based research models such as bed-rest studies with humans (Arnaud, 1992) and hindlimb unloading of rodents to simulate the effects of weightlessness on the skeleton (Morey-Holton, 1998). This model has shown similar

10

results to spaceflight by creating a negative calcium balance, decreasing bone mass and producing the coinciding cephalic fluid shifts associated with microgravity (Zerwekh, 1998).

To better understand the potential early responses of bone to spaceflight, Arnaud et al. (1992) carried out a one-week study on 8 healthy men restricted to bed rest with head down tilt of 6° to mimic the acute response unloading of the skeletal system. By the sixth and seventh days of bed rest, serum parathyroid hormone and 1,25-dihydroxyvitamin D were decreased significantly. Donaldson et al. (1970) showed that restricting healthy males to complete bed rest for 30-36 weeks, increased urinary calcium and phosphorous excretion which started to normalize within 3 weeks of re-ambulation. A more recent study of male volunteers showed that following 14 days of 6 degrees head-down bed rest, bone resorption was increased and bone formation remained either normal or was reduced (Kim, 2003). Collet and others (1997) collected data on 2 men before and after spaceflight. After 6 months of spaceflight, they reported a significant loss of both trabecular and cortical bone in the tibia and following a 6 month recovery period, there was still a significant decrease in trabecular bone while cortical bone had recovered. An additional study focusing on recovery reported an incomplete recovery of BMD 1 year after a 4-6 month spaceflight (Lang, 2006).

Studies evaluating countermeasures to prevent bone loss due to spaceflight have been conducted using a variety of modalities. For example, a lower body negative pressure chamber (LBNP) was used to study the effects of exercise in identical twins (Smith, 2003). Following 30 days, markers for bone resorption were increased in the sedentary group, leading to the conclusion that weight-bearing exercise may counteract

11

the negative effects of weightlessness. Schneider et al. (2003) compared the effects of an interim elastomer-based resistive exercise device (iRED) to free weight training in adult males experiencing a weightless environment. They reported that although iRED training produced similar muscle responses to the free weight group, it was unable prevent bone loss.

While preventing bone loss is one approach to counter the deleterious effects of the skeletal unloading, enhancing the recovery of bone would also prove to be beneficial. To look at enhancing recovery, Carvalho and others (2006), evaluated male quadriplegics (n=21) and the effect of treadmill gait training. Following the 6 month treatment period, 81.8% of subjects showed a significant increase in bone formation and 66.7% presented a significantly decreased rate of bone resorption. These studies have allowed us to further understand the effects of skeletal unloading and recovery.

Skeletal Unloading and the Rat Skeleton

In addition to bed-rest studies, researchers at NASA also developed an animal model of unloading the hindlimbs of rats and mice to mimic the alterations in loading that occur during space flight. This model of skeletal unloading rats has proven to closely resemble the skeletal response observed in humans in terms of bone loss in the hindlimbs, changes in calcium metabolism and biochemical markers (Giangregorio, 2002).

The decrease in bone mass resulting from skeletal unloading in the HLU model results from bone resorption becoming uncoupled with bone formation (Bikle, 2003). Initial studies related to hindlimb unloading were performed using young growing male animals and showed that bone loss resulted primarily from decreased periosteal bone

formation (Morey, 1978 and Wronski, 1983). However, later studies using older male rats (i.e. 6-months in age) indicated that mature animal may more closely mimic the uncoupling of bone remodeling that occurs with humans during unloading (Dehority, 1999 and Shackelford, 2004). For example, evidence from some studies with older animals indicates that based on bone histomorphometry and biochemical maker data, increased bone resorption may be the primary culprit inducing bone loss (Hefferan, 2003 and Smith, 2002). Dehority and others (1999) showed that up to 5 weeks of HLU in the mature male rat resulted in diminished serum 1,25-dihydroxyvitamin D and decreased vertebral bone mass. An additional study by Bloomfield (2002) demonstrated that mature male rats HLU for 28 days experienced a 20-21% decrease in cancellous BMD at the proximal tibia and femoral neck while no significant differences were found in cortical.

To compare the gender differences in the response to HLU, Hefferan and colleagues (2003) exposed 6-month-old male and female rats to 2 weeks of unloading. They reported that aside from the gender differences observed at baseline, the alterations in bone induced by HLU were similar in both males and females. A longer term study by Allen and colleagues (2003) focused on mature female rats indicated that 28 days of HLU resulted in a decrease in bone mineral content, cortical bone area and cortical bone formation rate, but no loss of trabecular bone. The fact that bone loss occurred in the cortical bone compartment as compared to the trabecular bone compartment may have resulted from the use of retired breeders that had lost considerable trabecular bone as a result of multi-gravida and multiple lactation cycles. More recently Lecog and colleagues (2006) conducted a study to compare the effects of HLU to ovariectomy and

concluded that the degree of bone loss due to HLU was greater than that induced by estrogen deficiency.

Once bone loss has occurred during a period of decreased weight bearing activity, restoration of bone is critical. Studies have shown that the recovery of bone may not be complete and may take longer than the time of the original loss of bone (Giangregorio, 2002). Animal studies evaluating the recovery of bone following unloading, have shown that after 3 weeks of unloading, partial bone recovery was seen within 2 weeks of normal activity (Abram, 1988). A study by Wan and colleagues (2000) observed that the bone mass changes following 21 days of HLU in young growing rats (i.e. 7-week-old) were restored by day 21 of re-ambulation. In contrast, Weinreb and others (1997) reported the rate of bone loss during HLU is more rapid than its recovery during reloading in 4 week old rats. Both the human and animal studies suggest that recovery from skeletal unloading can be accomplished to a certain degree; however, studies have shown that bone loss was not completely recovered with up to six months of weight-bearing activity (LeBlanc, 1990).

Pharmaceutical Agents

Over the past 20 years, several pharmaceutical agents have been approved for the treatment of osteoporosis. Among the most prescribed are the potent antiresorptive agents, bisphosphonates (Close, 2006). Bisphosphonates have shown to be effective in preventing bone loss associated with estrogen deficiency (McClung, 1998), glucocorticoid treatment (de Nijs, 2006), and immobilization (Yang, 2005). Bisphosphonates act by binding to the hydroxyapatite crystals on the bone surface and

inhibiting osteoclastic activity, which decreases bone resorption (Chapurlat, 2006). The three Food and Drug Adminstration (FDA) approved bisphosphonates for the treatment of osteoporosis are alendronate, risedronate, and ibandronate that can be consumed daily, weekly or even monthly for convenience (Chapurlat, 2006).

The advent of bisphosphonates has had a tremendous impact on the prognosis of treating osteoporosis and/or osteopenia. Alendronate, a now weekly treatment for osteoporosis, was approved by the FDA in 1995. The Fracture Intervention Trial first demonstrated alendronate's ability to reduce risk of hip fracture by 51% in women with osteoporosis (Black, 2000). LeBlanc and colleagues (2002) showed that alendronate protected against bone loss during 17 weeks of bed-rest. Risedronate has also been approved for the prevention and treatment of osteoporosis and has been shown to prevent bone loss in postmenopausal women (Chapurlat, 2006) and reduce the risk of fractures in women with osteoporosis (Durchschlag, 2006). In 2001, the Hip Intervention Program (HIP), showed that risedronate significantly reduced risk of hip fracture in elderly women with osteoporosis (McClung, 2001). Mosekilde and colleagues (2000), showed that both risendronate and alendronate protected against loss of bone density induced by 28 days of HLU. The newest bisphosphonate, ibandronate, has also been shown to prevent bone loss (Tanko, 2003) and reduce risk of fracture (Chestnut, 2004). Bisphosphonates are generally well tolerated, however, side effects such as myalgia, esophagitis and uveitis have been reported (Chapurlat, 2006). Additionally, there are no trials comparing bisphosphonates with regard to fracture efficacy or with any other drug with a fracture point (Nelson, 2003).

Another treatment option for osteoporosis is the group of agents known as selective estrogen receptor modulators (SERMs). These compounds bind to and activate estrogen receptors. The concept of SERMs comes from the observation that tamoxifen, an effective therapy of breast cancer, has estrogen-like effects on the skeleton (Turken, 1989). Researchers found tamoxifen (20 mg/day) in postmenopausal women with breast cancer to prevent loss of bone in the lumbar spine (Love, 1992). Another SERM, raloxifene, now approved for prevention and treatment of osteoporosis, was the first SERM to be studied for the primary purpose of treatment of osteoporosis. Raloxifene blocks estrogen in a similar manner as tamoxifen and inhibits bone resorption in both trabecular and cortical bone by blocking the activity of cytokines (South-Paul, 2001). Kanis and colleagues (2003) reported that treatment with 60 mg/day raloxifene significantly reduced the risk of vertebral fractures in postmenopausal women after 3 years. However, similar to estrogen, raloxifene is associated with increased risk of venous thromboembolic disease (SERMs).

Calcitonin, an endogenous hormone secreted by the thyroid gland, aids in calcium homeostasis and is also used as a treatment for osteoporosis (Woolf, 2003). Its main skeletal effect is to inhibit osteoclastic bone resorption by shrinking osteoclast size within minutes of administration (Inserillo, 2002). Previous studies have shown calcitonin to increase bone density and reduce vertebral fractures (Prestwood, 2000). The PROOF study demonstrated that 200 IU salmon calcitonin nasal spray per day significantly reduced the risk of vertebral fractures by up to 36% in postmenopausal women with previous fractures (Chestnut, 2000). However, the potency of calcitonin is significantly less than bisphosphonates or estrogen (South-Paul, 2001) and once treatment is

16

discontinued, gains in BMD are quickly reversed (Prestwood, 2000). Additionally, Muff and colleagues (1999) reported 40% of patients developed antibodies during the first six months of treatment with salmon calcitonin which renders it ineffective.

Estrogen replacement therapy (ERT) has also been considered a treatment for osteoporosis. Numerous studies have shown ERT, started within 10 years of menopause, to prevent loss of bone at the hip and spine (Prestwood, 2000). In the Postmenopausal Estrogen/Progestin Intervention (PEPI) trial, women treated with HRT had increased hip and spine BMD (Marcus, 1999). The Bone, Estrogen, and Strength Training (BEST) Study showed a significant increase in BMD at the femoral neck, trochanter, lumbar spine, and total body when exercise and HRT were combined during the early postmenopausal period. ERT has been associated with a 30% rate of endometrial hyperplasia per year and endometrial cancer at a lower rate (Prestwood, 2000). Today the primary concern with regard to ERT is whether the potential benefit on bone health is worth the potential risk of breast and endometrial cancer (Sharma, 2003 and South-Paul, 2001).

The last pharmaceutical treatment option for osteoporosis is PTH. PTH, known for its ability to alter gene expression of the osteoblast, is essential for the maintenance of calcium homeostasis through direct influence on bone and kidney, and indirect actions on the gastrointestinal tract (Jüppner, 2000). PTH assists release of calcium from the bone by stimulating bone resorption, promotes calcium reabsorption in the kidneys, and enhances calcium absorption in the gastrointestinal tract. In young growing animals, PTH was shown to have a potential role in skeletal development (Kronenberg, 2006), accretion of peak bone mass (Hock, 1992), and increased bone formation (Dempster, 1993).

Clinical studies of the effects of intermittent PTH therapy have been very promising. PTH has been reported to decrease the incidence of vertebral fracture in postmenopausal women compared to those on placebo (Srivastava, 2002). There was also a reduced rate of new or worsening back pain with those on PTH when compared to those receiving a placebo. Hadsman and others (2003) conducted a clinical trial on postmenopausal, osteoporotic women with daily injections of PTH. Following a 12 month treatment period, significant increases were seen in both vertebra and hip BMD. An additional study investigating PTH and alendronate in postmenopausal osteoporosis reported PTH to significantly enhance BMD when compared to alendronate (Body, 2003). Researchers also reported fracture rates to be significantly lower in those receiving PTH compared to those on alendronate.

Previous studies conducted on animals have found PTH to prevent bone loss induced by HLU. Moriyama and colleagues (2002) concluded that intermittent administration of human PTH (1-34) may prevent cancellous bone loss and increase BV/TV in hindlimb unloaded rats. Similar results were reported in six month old male rats (Bloomfield, 2002) in which PTH produced a significant increase in total and cancellous bone BMD and cancellous BV/TV following 28 days of HLU. In a more recent study, Turner and colleagues (2006) reported intermittent PTH to prevent bone loss by increasing bone formation rate in HLU male rats. Although some of these pharmaceutical treatments may prevent and even reverse bone loss, they still have a possible risk of adverse side effects. Finding a natural treatment to restore and prevent bone loss could possibly be safer, more convenient and less expensive.

Nutritional Supplements, Functional Foods and Bone

Numerous studies have been conducted on dietary supplements and functional foods with antioxidant and anti-inflammatory properties to find a natural alternative for the prevention and treatment of osteoporosis. For instance, calcium, the principal cation of bone mineral (Heaney, 2003) is required for the bone formation phase of bone remodeling (Dawson-Hughes, 2003). Inadequate intake of calcium results in reduced calcium absorption and an increased secretion of PTH which leads to a loss in bone (Dawson-Hughes, 2003). Long-term (i.e. 4 year) effects of calcium supplementation (~670 mg/calcium/day) on bone in young women increased bone accretion during the pubertal growth spurt only (Matkovic, 2005). A comprehensive review of clinic trials (Dawson-Hughes, 1991) revealed that in the early postmenopausal period when bone loss is accelerated, calcium supplementation (500-2000 mg/day) slows bone loss in the radius, but has no effect on the spine. Another 4 year study demonstrated that calcium supplementation (750 mg/day) prevented loss of BMD, secondary hyperparathyroidism and bone turnover in elderly men and women (Peacock, 2000). To date, no studies have shown calcium supplementation to reverse bone loss.

In addition to calcium, the ability of vitamin D to prevent and reverse bone loss has been investigated extensively. Vitamin D, recognized for its role in calcium homeostasis, is a secosteroid that is activated in the skin when exposed to sunlight. During bone mineralization, active vitamin D (1,25-dihydroxyvitamin D) maintains calcium and phosphorous in a supersaturated state, resulting in passive bone mineralization (Holick, 2003). Vitamin D deficiency may have a role in osteopenia, worsen osteoporosis and increase muscle weakness, leading to increased risk of fall and

fracture (Holick, 2006). Deficiency can be prevented with adequate sunlight and supplementation if needed. Ruohola and colleagues (2006), measured serum 25-hydroxyvitamin D concentrations as a risk factor for bone stress fracture in 756 military recruits and found that 22 recruits had stress fractures and low serum 25(OH)D, concluding that low serum concentrations of vitamin D may be predisposing for stress fractures. Supplementation of both vitamin D and calcium in adult women over 45 years old significantly reduced loss of BMD over 30 months when compared to placebo (Di Daniele, 2004). Vitamin D analogs may also be suitable for the treatment of osteoporosis due to their ability to increase intestinal absorption of calcium and have an antiresorptive effect on bone (Erben, 2001).

In addition to research on vitamin D, many studies have focused on the effects of vitamin K on bone. Vitamin K supplementation has been reported to improve bone turnover profile (Bugel, 2003). Supplementation of vitamin K for as little as 6 weeks has shown to increase markers of bone formation during spaceflight (Vermeer, 1998). Iwamoto and colleagues (2003) evaluated the effects of supplementation of both vitamins D and K in 6 week old female rats on a low-calcium diet and found increased intestinal calcium absorption, renal calcium reabsorption, and cancellous bone, and reduced hypocalcemia. Vitamin K analogs have also been investigated for their potential to reduce osteoporotic fractures by significantly increasing BMD in postmenopausal women (Iwamoto, 2001).

Vitamin E, a fat-soluble vitamin, functions to protect the membrane of cells by preventing the oxidation of unsaturated fatty acids in the phospholipids of cellular membranes (Knight, 2000). Arjmandi and colleagues (2002) reported that aged mice

receiving 30 days of vitamin E supplementation (500 mg/day) experienced enhanced

bone quality as well as increased bone dry weight, osteocalcin, and IGF-1 compared to

animals consuming an adequate vitamin E diet. Vitamin E has also been shown to

protect against reductions in trabecular thickness, double-labeled surface, and rate of

bone formation to bone volume in animals following HLU (Smith, 2005). Although,

vitamin E has been shown to have osteoprotective properties in experimental models such

as the aged mouse and HLU rat, vitamin E supplementation may not be beneficial in

control animals.

Aside from individual nutrient supplementation, functional foods, such as soy,

green tea, pomegranates and dried plums, have also been the focus of much research. To

evaluate the effects of soy protein and its isoflavones on postmenopausal women,

subjects were randomly assigned to consume soy-containing foods or kept on a control

diet (Arjmandi, 2005). Following 1 year, both groups positively enhanced alkaline

phosphatase, IGF-1, and osteocalcin, however, both groups experienced significant

decreases in whole body and lumbar BMD. Soy and its isoflavones have been shown to

enhance tibial BMC and BMD in 9 month old osteopenic ovariectomized rats

(Devareddy, 2006) by stimulating bone formation (Soung, 2006). Recent human studies

have also shown that oral consumption of green tea may increase BMD (Cabrera, 2006).

As for pomegranates, Mori-Okamoto and others (2004) reported pomegranate extract to

reverse OVX-induced loss of BMD after only 2 weeks. While these dietary supplements

and functional foods have the focus of numerous studies for the prevention and reversal

of bone loss, it seems that the most promising data has been seen in dried plums.

Dried Plum and Bone

Dried plums (*Prunus domestica L.*) contain significant amounts of phenolic compounds that contribute to their color and taste (Raynal, 1989). Dried plums are a known rich source of antioxidants as indicated by their high oxygen radical absorbance capacity (ORAC) rating used to evaluate commonly consumed fruits and vegetables (Kayano, 2003). In addition, dried plums contain a significant amount of micronutrients such as boron, vitamin K and potassium (Anderson, 1994).

Animal and human studies have indicated that dried plums have osteoprotective properties (Arjmandi, 1999; Arjmandi, 2001; Franklin, 2006; Deyhim, 2005; Smith, 2005). Previous studies found that dried plums protected against (Arjmandi, 2001; Franklin, 2006) and even reversed (Arjmandi, 1999; Deyhim, 2005) bone loss in animal models of osteopenia associated with gonadal hormone deficiency. High dose dried plum (25%, w/w) completely prevented loss of vertebral and femoral BMD from occurring following OVX (Arjmandi, 2001). Deyhim and colleagues (2005) reported that osteopenic OVX animals on a dried plum diet as low as 5% recovered lost femoral and vertebral bone density to that of sham operated animals. Improvements were also seen in overall yield and ultimate force and dried plum was able to significantly enhance trabecular microarchitecture when compared to controls. In males, dried plum prevented orchidectomy (ORX)-induced decrease in femoral and vertebral BMD, and trabecular bone loss (Franklin, 2006). The protective effects of dried plum appeared to be mediated via an increase in IGF-1 and decrease in bone resorption. Most recently, Smith and colleagues (2005) reported dried plum to enhance recovery of bone mass and microarchitecture similar to PTH treatment in male rats following ORX. These studies

provide strong support of the efficacy of dried plum in preventing and reversing bone loss in animal models of gonadal hormone deficiency.

To date, one short-term clinical trial has reported on the efficacy of dried plum on bone metabolism (Arjmandi, 1999). Postmenopausal women consuming either 100 g dried plums or 75 g dried apples a day for 3 months experienced an increase in serum bone-specific alkaline phosphatase (BSAP) and insulin-like growth factor-I. These findings indicate that dried plum may have potential anabolic effects on bone.

While the dried plum studies to date have all examined the effects of dried plum on bone loss induced gonadal by hormone deficiency, no studies have focused on the ability of dried plum to reverse bone loss in other models of osteopenia or osteoporosis. Therefore, the purpose of this study is to evaluate the effects of dried plum on bone mass, microarchitecture, strength and bone metabolism during re-ambulation following HLU.

CHAPTER III

MATERIALS AND METHODS

Animals and Diets

Seventy-two, 6-month old female Sprague-Dawley rats (Harlan, Indianapolis, IN) were individually housed in an environmentally controlled laboratory upon arrival. After five days of acclimation, rats were divided into eight groups of 10-12 rats each. Animals were either hind limb unloaded (HLU=6 groups) or kept as ambulatory controls (AMB=2 groups) for 3 weeks. During the HLU period, all groups were fed a semi-purified powder casein-based AIN-96M control diet (Reeves, 1993). Ambulatory rats were matched-fed to the mean food intake of the HLU animals to minimize potential group differences in body weight. At the end of 3 weeks of HLU, 1 AMB and 1 HLU group was anesthetized with a ketamine/xylazine cocktail (70 and 3 mg/kg body weight), scanned using DXA (Hologic QDR 4500-A Elite DXA, Hologic Waltham, MA) and necropsied. Bone specimens were harvested for analysis of the changes in trabecular and cortical bone microarchitecture. Following the unloading period, the HLU animals were returned to normal ambulation (i.e. re-ambulation) for 12 weeks. During the re-ambulation period animals were either fed diets supplemented with one of three doses of dried plums (5%, 15% or 25% w/w) or the control diet. An additional group was fed the control diet and

received daily subcutaneous PTH injections (80 μg/kg bw; 3 x wk; Bachem, Inc.) to serve as a positive control.   The dried plum diets were adjusted for total energy, carbohydrate, protein, fat, fiber, calcium and phosphorus concentrations (**Table 1**). Animals had free access to deionized water and were weighed weekly throughout both the HLU and re-ambulation periods.

At the end of the 12-week treatment period, twelve-hour urine was collected. Animals were anesthetized for whole body DXA scans and bled through the abdominal aorta. Blood samples were collected and serum was separated by centrifugation and stored at -20°C. The femurs, tibiae and lumbar vertebrae were removed, cleaned of adhering soft tissue and appropriately stored for analysis. All procedures associated with the project adhered to the Oklahoma State University Institutional Animal Use and Care guidelines.

DXA Assessment

DXA scans on whole body and excised bone were evaluated to compare the influence of HLU and the effectiveness of dried plum in reversing bone loss. BMD, BMC, and BMA were assessed using DXA at initial, post-HLU and final time points. Bone density of the excised femur and lumbar vertebra were further evaluated using the Regional High Resolution software package (Hologic QDR 4500-A Elite DXA, Waltham, MA) designed for studying small animal bones.

Micro-computed Tomography

The vertebra and femur were scanned using high resolution micro-computed tomography (Micro CT40 Scanner, SCANCO, Medical Switzerland). Trabecular bone parameters including bone volume expressed as a percentage of total bone volume (BV/TV), trabecular number (Tb.N.), separation (Tb.Sp.), and thickness (Tb.Th.), structure model index (SMI) and trabecular connectivity were evaluated using μCT scanner. Medullary area, cortical area, porosity and thickness were assessed to determine the structural integrity of the cortical-rich femoral midshaft. All scans were performed using a 1024 X 1024 matrix resulting in an isotropic voxel resolution of 22 um³. An integration time of 70 milliseconds per projection was used with a rotational step of 0.36 degrees resulting in total acquisition time of approximately 150 minutes/sample.

Trabecular bone was assessed at both the distal femur metaphysis and the vertebral body. The distal femur was scanned from the growth plate in the proximal direction to acquire 350 slices (~16 μm/slice) for the assessment of the microarchitecture of trabecular bone. Contours were semi-automatically placed to incorporate the secondary spongioso beginning 25 slices (400 μm) from the growth plate and 150 images in the volume of interest (VOI). Trabecular parameters were analyzed with a scan of the lumbar vertebra in a cranial-caudal direction. A total of 530 transverse slices were acquired of the vertebral body. The region of interest (ROI) included all secondary spongiosa in the vertebral body with the exception of the last 25 slices from either growth plate (~300 slices). To assess cortical bone, the midshaft was assessed by scanning 34 slices at the midpoint and then 30 slices analyzed in the VOI.

Three-point Bending Test of the Femur

Femurs, which were stored in phosphate-buffered saline (PBS), were tested using a three-point bending apparatus (TA.XT2i, Stable Microsystems, Inc.) to evaluate mechanical properties of cortical bone. Samples were allowed to equilibrate to room temperature prior to testing. Femur length was measured with a Vernier caliber from the proximal end to the distal chondyles. The external diameter of the femur was measured at the midshaft by taking two measurements 90° apart (i.e. medial - lateral and anterior - posterior). Each femur was positioned on the 3-point bending apparatus so that the posterior surface rests on the lower supports and anterior surface touches the upper supports. During this test, the anterior surface was under compression at a displacement rate of 3 mm/min and the posterior surface is under tension. The load displacement curve was recorded in real time throughout the test so that break load and modulus could be determined from the curve. Following the test, cortical thickness was assessed by taking four measurements, 90° apart, at the point of fracture. The ultimate load, yield load and the stiffness of the specimen was measured from the load-displacement curve.

Biochemical Markers of Bone Resorption and Formation

Serum indicators of bone formation, osteocalcin, alkaline phosphatase (ALP) and insulin growth factor-1 (IGF-1), were measured to determine the alterations in osteoblast activity induced by HLU and treatments. Serum osteocalcin, a noncollagenous protein secreted by ostesoblasts and indicator of matrix mineralization, was assessed by double antibody immunoradiometric assay (IRMA – Immunotropics, Inc., San Clemente, CA)

specific for rats. The intra- and inter-assay coefficients of variation were 2.0% and 5.0%, respectively. In addition, ALP, an indicator of matrix maturation, was measured using a COBAS Fara II clinical analyzer by a colorimetric method using a kit from Roche Diagnostics (Roche Diagnostic Systems, Indianapolis, IN). The intra- and inter-assay coefficients of variation were 2.0% and 1.9%. Serum IGF-1, known to be involved in osteoblast proliferation, was also determined using a commercially available radioactive immunoassay (RIA) (Nichols Institute Diagnostics, San Clemente, CA). IGF-1 was extracted from serum samples by an acid-ethanol extraction overnight and then analyzed the following day. The intra- and inter-assay coefficients of variation were 8.4% and 3.0%.

To examine the effects of HLU and dried plum on bone resorption, urinary excretion of deoxypyridinoline (DpD), a product of collagen degradation, were assessed and expressed per unit of creatinine and total per 12 hours. Urinary creatinine concentrations were measured based on a colormetric assay (Roche Diagnostic Systems, Indianapolis, IN). The intra- and inter-assay coefficients of variation were 1.0% and 2.6%, respectively. Urinary DpD was assessed utilizing a competitive enzyme immunoassay (Pyrilinks-D, Metra Biosystems, Mountain View, CA). The the intra- and inter-assay variability were 8.4% and 4.8%, respectively.

Statistical Analysis

Data analysis involved computation using PC SAS statistical software (version 8.02; SAS Institute Inc., Cary, NC) and were presented as means $\pm$ standard error. ANOVA model was performed using the generalized linear model (GLM). When F

values were significant, post hoc analysis using Fisher's least square means separation

tests were preformed to determine differences between groups. Differences of $P<0.05$

were considered significant in all statistical analysis.

Table 1. Diet Composition (g/kg diet) for the Control (AIN-93M), Low Dose (LD=5%), Medium Dose (MD=15%), and High Dose (HD=25%) Dried Plum Diets

Ingredients	Control (AIN-93M) (g/kg diet)	LD (5%) dried plum (g/kg diet)	MD (15%) dried plum (g/kg diet)	HD (25%) dried plum (g/kg diet)
Carbohydrates				
Cornstarch	465.7	425.7	345.7	265.7
Maltodextrin	155.0	155.0	155.0	155.0
Sucrose	100.0	100.0	100.0	100.0
Dried plum	--	40.0	120.0	200.0
Total	720.7	720.7	720.7	720.7
Protein				
Casein	140.0	138.5	135.50	132.5
Dried plum	--	1.5	4.5	7.5
Total	140.0	140.0	140.0	140.0
Fat				
Soybean oil	40.0	39.75	39.25	38.75
Dried plum	--	0.25	0.75	1.25
Total	40.0	40.0	40.0	40.0
Fiber				
Cellulose	50.0	45.5	36.5	27.5
Dried plum	--	4.5	13.5	22.5
Total	50.0	50.0	50.0	50.0
Vitamin Mix	10.0	10.0	10.0	10.0
Mineral Mix				
Mineral mix (Ca-P deficient)	13.4	13.4	13.4	13.4
Ca carbonate	9.88	9.79	9.6	9.43
Ca (dried plum)	--	0.036	0.108	0.18
K phosphate	5.6	5.48	5.24	5.0
Na phosphate	3.44	3.32	3.08	2.84
P (dried plum)	--	0.054	0.162	0.27
K citrate	0.9	0.9	0.9	0.9
Sucrose	1.78	2.02	2.78	2.98
Total	35.0	35.0	35.0	35.0
L-cysteine	1.8	1.8	1.8	1.8
Caloric density (kJ/g diet)	17.6	17.5	17.5	17.4

.

CHAPTER IV

RESULTS AND DISCUSSION

Alterations in Bone Mass, Structure, Strength, and Biochemical Markers Induced by

HLU

The primary focus of this project was to evaluate the ability of dried plum to

enhance bone recovery following skeletal unloading. Understanding the influence of

HLU on bone mass, structure, biomechanical properties and bone metabolism in 6-month

old female rats is first required. Therefore, data on the alterations on bone parameters are

presented to establish the baseline changes in bone induced by HLU.

Following 3 weeks of HLU, body weight was significantly reduced in the HLU

group compared to the AMB control group (**Table 2**). Tibial and vertebral BMC and

BMD were significantly decreased by HLU (Table 2). Trabecular bone volume (BV/TV)

and thickness (TbTh) were reduced in the distal femur metaphysis and vertebral body ($p<$

0.0001) and femoral trabecular separation (TbSp) was increased ($p< 0.05$) (**Table 3**).

Significant increases in SMI were observed in both the distal femur and vertebral body

after HLU resulting in a more rod-like structure and vertebral connectivity density (Conn

Density) was significantly higher in HLU when compared to AMB (Table 3). Cortical

bone strength as demonstrated by ultimate and yield load based on femur 3-pointing

bending was decreased by HLU (**Table 4**). Vertebral trabecular bone compressive

strength was also reduced as indicated by ultimate and yield load and stress (Table 4). Bone formation was not altered by HLU based on OC, ALP and IGF-I, but a significant increase in total 12 hour urinary DpD excretion, a marker for bone resorption was observed (Table 2).

Body Weight and DXA

Over the course of the 90 day treatment period there were no significant differences observed in body weight between the groups (**Table 5**). No differences were noted in food intake due to animals being match-fed to the mean intake of the group with the lowest intake adjusted weekly (*data not shown*).

Following the 90 day treatment, the higher dose of dried plum enhanced the recovery of BMC (Table 5) and BMD **(Figure 1)**. In particular, BMC and BMD at both the tibia and vertebra were increased to a greater extent ($p< 0.05$) in those receiving dried plum than the HLU group consuming the control diet. BMD of the tibia was by enhanced by 7.6% in HD compared to 5.5% in the HLU-control group at the end of re-ambulation and was similar to that observed in the PTH-treated group (Figure 1). The HD of dried plum also increased the recovery of vertebral BMC (Table 5) and BMD (Figure 1) as well. The LD and MD treatments did not provide any additional benefit on tibial BMC or BMD above that of the control diet, but the MD diet enhanced the recovery of vertebral BMD (Figure 1). HD and PTH treatments were significantly increased beyond that of the AMB control, suggestive of an anabolic effect with HD dried plum (Figure 1).

Microarchitecture of Trabecular and Cortical Bone

Dried plum also had positive effects on trabecular bone microarchitecture of the distal femur and lumbar vertebra. Femur BV/TV was significantly enhanced by both HD and PTH when compared to HLU (**Figure 2A**). Vertebral BV/TV was also improved by MD dried plum compared to animals on the control diet (HLU-control) and similar to the PTH treated group (Figure 2a). TbTh, assessed at the femoral metaphysis, was not improved by any dose of dried plum and only PTH treatment significantly improved TbTh (**Figure 2B**). Increases in vertebral TbTh were observed in MD, and HD treatments, over and above that of the animals consuming the control diet, but not to the extent of the PTH. TbSp decreased in both MD and HD dried plum however, no group was significantly different from HLU control (**Figure2C**). There were no changes in TbN in the femur or vertebra in response to either dried plum or PTH. (**Figure 2D**).

SMI and Conn density were also analyzed using μCT in trabecular rich regions of the distal femur and vertebra (**Table 6**). HD dried plum resulted in a more plate-like structure as indicated by the decrease in SMI of the femur, however, these alterations were not to the level of PTH treated group (Table 6). No other treatments were able to significantly alter femur SMI or reverse the increase in vertebral SMI (Table 5). Femoral and vertebral bone Conn Density was not altered by dried plum, but PTH decreased Conn Density of the femur (Table 5).

Microarchitecture of the cortical bone was assessed in the femoral mid-diaphysis using μCT. No significant differences between groups were demonstrated in response to either the dried plum or PTH treatment on cortical porosity, thickness, area or medullary

area (Table 6). These results indicate that the primary effect of dried plum following HLU was on trabecular bone.

Biomechanical Effects on Femur and L4 Vertebra

Three-point testing of the femur demonstrated that cortical bone strength recovery was unaltered by dried plum and PTH. There were no significant differences in ultimate and yield load, ultimate and yield stress and modulus of elasticity in HLU-control and HLU groups on dried plum diets at 90 days of re-ambulation (**Table 7)**. Stiffness was the only biomechanical parameter of cortical bone that tended (*p<0.055)* to be increased by HD and PTH.

In addition to assessment of cortical bone strength, compression testing was preformed on the trabecular bone of the lumbar vertebra. The ultimate load the trabecular bone could withstand was comparable in the HD and PTH-treated groups, although the effect was not significantly greater than the animals consuming the control diet (**Table 8**). Similar results were observed with regard to ultimate stress. Yield load tended to be enhanced by MD, HD and PTH (*p<0.0627*), but there were no significant changes in compressive stiffness, yield stress, or modulus of elasticity (Table 8).

Biomarkers of Bone Metabolism

Serum osteocalcin, ALP and IGF-1 and urinary DpD were assessed as markers of bone formation and resorption (**Table 9**). Neither osteocalcin nor IGF-I were significantly affected by any of the doses of dried plum or PTH. Serum ALP, associated with bone formation, was increased (*p<0.05*) in the HD diet group following 90 days of

treatment, while all other groups were not significantly different. Bone resorption, as assessed by urinary DpD per unit of creatinine, was not altered by either dried plum or PTH at the end of the study.

Discussion

The objective of this study was to evaluate the dose-dependent effects of dietary dried plum supplementation on bone recovery following hindlimb unloading (HLU). Skeletal unloading using the HLU model has been shown to induce significant bone loss in both young and adult male and female rats (Perrien, 2006; Hefferan, 2003; Bloomfield, 2002). In our study, 3 weeks of HLU induced a significant decrease in body weight, bone mineral content (BMC) and density (BMD), and bone volume (BV/TV) and an increase in trabecular separation (TbSp). Similar results were found in 6-month-old male rats (Bloomfield, 2002) with significantly reduced BMC and BMD following up to 28 days of HLU, but 6-month-old female retired breeders lost primarily cortical as opposed to trabecular bone (Allen, 2003). Hefferan and colleagues (2003) reported significant decreases in BV/TV and TbN and increased TbSp in both male and female rats (6-months-old) after only 2 weeks of HLU. Others observed decreases in BV/TV, TbTh and TbN in as little as 13 days of HLU (Barou, 2002).

In terms of bone recovery during re-ambulation, we observed that dried plum enhanced recovery of bone mass and trabecular bone microarchitecture in both the hindlimbs and the vertebra. Several studies evaluating the effects of dried plum on bone in models of gonadal hormone deficiency have been reported in the literature (Arjmandi, 2001; Deyhim, 2005; Franklin, 2006); however, this is the first to determine its effectiveness in enhancing bone recovery in HLU female rats. Previous animal and

human studies have shown dried plum to prevent (Arjmandi, 2001; Franklin, 2006; Deyhim, 2005) and even reverse bone loss (Arjmandi, 1999) in part by increasing the rate of bone formation. We have reported similar results here supporting the beneficial properties of dried plum on bone following recovery from HLU.

Dried plum at the high dose significantly enhanced the recovery of femoral and vertebral BMD, as well as vertebral BMC. These findings are similar to reports from Deyhim and colleagues (2005) in which 60 days of dried plum reversed the loss of femoral and vertebral BMD in osteopenic OVX rats but even doses as low as 5% (w/w) were effective in reversing bone loss in OVX. An additional study in osteopenic orchidectomized (ORX) male rats demonstrated that dried plum diet at the highest dose increased BMD of the femur and vertebra compared to ORX animals consuming the control diet (Smith, 2005). These findings in male and female gonadal hormone deficient rats combined with our observations following HLU indicate that dried plum can reverse the detrimental effects on BMD.

To further evaluate the ability of dried plum to reverse bone loss, we compared the effects of dried plum on BMC and BMD to that of the anabolic agent PTH. We found that while the higher dose of dried plum enhanced the recovery of BMD in both the tibia and the vertebra, the vertebra was the only site in which BMD was enhanced to the point of the PTH. Cranney and colleagues (2006) reported significant increases in lumbar spine, femoral neck and total hip BMD following 1 year PTH treatment in postmenopausal women. An additional study with ovariectomized (OVX) rats also found PTH to reverse lost BMD to the level of sham (Fox, 2006). Other agents such as bisphosphonates have been used in the treatment of osteoporosis due to their ability to

increase BMD but may have limited ability to restore bone once the loss has occurred

(Watts, 2003). Mosekilde and colleagues (2000), reported both risedronate and

alendroante prevent BMD loss in immobilized female rats. Additionally, raloxifene, has

been shown to increase both femoral and lumbar BMD in postmenopausal women

(D'Amelio, 2003). However, currently the only FDA approved agent with anabolic

potential to restore bone is PTH, making the similarities between dried plum and PTH's

effect on BMD remarkable.

As we have reported, dried plum enhanced BV/TV and tended to improve TbTh

and TbSp during the re-ambulation following HLU. Similar findings were reported in 6-

month-old male rats following ORX (Smith, 2005) in that the HD dried plum induced an

increase in BV/TV and decrease TbSp. In contrast to our findings, dried plum did

increase TbN while reversing bone loss due to ORX, but these differences may have

resulted more from the model than the effect of dried plum. The influence of dried plum

on trabecular bone microarchitecture was similar but, in cases perhaps not as pronounced

as PTH. PTH, has been shown to normalize BV/TV in OVX rats over a 12 month

treatment period (Fox, 2006) and significantly enhance BV/TV after 15 days of HLU

(Moriyama, 2002). Additionally, Ma and colleagues (1995) demonstrated that in 6-

month-old female rats PTH to significantly increased TbTh and TbN following 30 days

of HLU.

Cortical bone strength appeared to be unaltered by the dried plum diet. There

were no significant differences in ultimate and yield load, ultimate and yield stress and

modulus elasticity on dried plum diets at 90 days of re-ambulation. Stiffness was the only

biomechanical parameter of cortical bone that tended ($p<0.055$) to be increased by HD

and PTH. This differs from previous dried plum research in which Deyhim and colleagues (2005) reported an improvement in femoral yield and ultimate force in OVX animals on a dried plum diet. Additionally, in a prevention study, Franklin (2006) observed that MD and HD prevented the decrease in ultimate load in the femur of ORX rats. These researchers also reported that 90 days of dried plum consumption improved vertebral force and stiffness. Compared to PTH, previous studies have shown 5 weeks of PTH increased cortical thickness and improved ultimate force in animals with OVX induced bone loss (Rhee, 2004). Collectively, these studies are suggestive that dried plum's primary effect is on trabecular bone and not on cortical bone.

In terms of bone metabolism, our findings indicate that at 90 days, dried plum enhanced bone formation and had no effect on bone resorption. No changes were observed in osteocalcin and IGF-I in our study; however, there was a significant increase in serum ALP. It should be mentioned that the ALP assay available for the rodent does not assess bone-specific ALP. Therefore, these results should be confirmed by further analyses at the tissue level. Other studies by Arjmandi and colleagues (1999) in OVX animals and Franklin et al (2006) in ORX animals have shown that dried plum diet increased serum IGF-I, which is associated with bone formation. In terms of bone resorption, urinary DpD was unchanged in our study at the end of the re-ambulation period. Similar results were reported in a 3 month clinical trial with postmenopausal women consuming 10-12 dried plums per day (Arjmandi, 2002). In that study, both IGF-I and ALP, indicators of increased bone formation, were significantly increased and markers of bone resorption were unaffected. These findings indicate that dried plum may increase bone formation and have no effect on bone resorption. Since PTH treatment

38

tends to increase bone resorption but have a greater effect on enhancing bone formation, the mechanism which dried plum improves bone may be somewhat different than that of PTH.

We conclude that recovery of bone is enhanced by the higher doses of dried plum in the HLU rat to a greater extent than observed in animals on the control diet and is comparable to PTH treatment. In this study, it appears that dried plum's primary effect is on trabecular bone rather than cortical bone and that dried plum enhances bone formation and has no effect on bone resorption.

Table 2. Alterations in Body Weight, Bone Mineral Content (BMC) and Density (BMD), and Biochemical Markers in Response to Hindlimb Unloading in 6-month-old Female Rats

Parameters	AMB	HLU	P-Value
Body Weight			
Pre-HLU (g)	293 ± 3	292 ± 5	0.8987
Post-HLU (g)	303 ± 4	272 ± 5	0.0002
Tibia			
BMC (g)	0.3454 ± 0.0086	0.3215 ± 0.0059	0.0352
BMD (g/cm^2)	0.2176 ± 0.0030	0.2093 ± 0.0016	0.0233
Vertebra			
BMC (g)	0.1626 ± 0.0053	0.1468 ± 0.0028	0.0146
BMD (g/cm^2)	0.2654 ± 0.0044	0.2444 ± 0.0029	0.0007
Serum			
Osteocalcin (ng/ml)	106.53 ± 8.86	135.27 ± 14.47	0.1216
Alkaline Phosphatase (ukat/L)	1.35 ± 0.19	1.38 ± 0.13	0.9066
Urinary Deoxypyridinoline (nmol/12hr)	75.31 ± 25.01	571.11 ± 162.25	0.0374

AMB—ambulatory control rats; HLU—hindlimb unloaded rats; BMC- bone mineral content; BMD- bone mineral density
Data are means ± SE. Parameters with $P<0.05$ indicates a significant difference between AMB and HLU groups. (n=9 rats/group)

Table 3. Alterations in Trabecular and Cortical Bone Parameters as Assessed by μCT at the Distal Femur Metaphysis and Vertebral Body in Response to Hindlimb Unloading in 6-month-old Female Rats

Parameters	AMB	HLU	P-Value
Distal Femur			
BV/TV (%)	38.48 ± 1.87	22.68 ± 1.77	0.0001
TbN (1/mm^3)	5.41 ± 0.16	4.95 ± 0.15	0.0538
TbTh (mm)	0.082 ± 0.002	0.07 ± 0.002	0.0001
TbSp (mm)	0.17 ± 0.007	0.20 ± 0.007	0.0199
Conn Density (1/mm^3)	149.85 ± 9.76	140.95 ± 9.26	0.5172
SMI	0.82 ± 0.17	1.44 ± 0.16	0.0003
Vertebra			
BV/TV (%)	39.43 ± 2.54	31.22 ± 2.41	0.0314
TbN (1/mm^3)	4.43 ± 0.14	4.20 ± 0.13	0.2432
TbTh (mm)	0.09 ± 0.003	0.08 ± 0.003	0.0085
TbSp (mm)	0.22 ± 0.01	0.24 ± 0.01	0.2445
Conn Density (1/mm^3)	59.86 ± 4.07	80.56 ± 3.86	0.0018
SMI	-1.21 ± 0.34	-0.05 ± 0.33	0.0255

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means ± SE. Parameters with *P<0.05* indicate a significant difference between AMB and HLU groups (n=9 rats/group)

Table 4. Effects of HLU on Cortical and Vertebral Bone Strength Assessed by 3-point Bending and Compression Testing in 6-month-old Female Rats

Parameters	AMB	HLU	P-Value
Femur			
Ultimate Load (N)	135.27 ± 3.80	124.33 ± 1.21	0.0464
Yield Load (N)	110.50 ± 6.70	93.41 ± 2.23	0.0226
Stiffness (N/mm)	134.23 ± 1.46	134.24 ± 1.92	0.9953
Ultimate Stress (N/mm^2)	200.64 ± 5.26	198.39 ± 9.26	0.9307
Yield Stress (N/mm^2)	170.22 ± 9.74	148.82 ± 7.21	0.1145
Modulus Elasticity (1/mm^2)	5667.14 ± 236.24	6052.87 ± 375.05	0.3875
Vertebra			
Ultimate Load (N)	234.64 ± 12.12	166.40 ± 4.45	0.0001
Yield Load (N)	167.34 ± 15.04	120.14 ± 5.86	0.0111
Stiffness (N/mm)	709.17 ± 68.75	597.33 ± 48.31	0.2044
Ultimate Stress (N/mm^2)	35.14 ± 1.65	26.56 ± 0.79	0.0003
Yield Stress (N/mm^2)	25.08 ± 2.21	19.12 ± 0.77	0.0229
Modulus Elasticity (1/mm^2)	684.34 ± 59.32	627.91 ± 043.91	0.4572

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means $\pm$ SE. Biomechanical parameters with *P<0.05* indicate a significant difference between AMB and HLU groups (n=9 rats/group)

Table 5. Alteration in Body Weight and Bone Mineral Content (BMC) in Response to Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH=80 µg/kg) After 90 Days of Treatment.

Parameter	AMB-Control	HLU-Control	HLU-LD	HLU-MD	HLU-HD	HLU-PTH	P-Value
Initial Body Weight(g)	279.3 ± 7.4	277.2 ± 6.0	277.2 ± 6.0	277.2 ± 6.0	277.2 ± 6.0	277.2 ± 6.0	277.2 ± 6.0
Final Body Weight (g)	301.9 ± 10.7	308.5 ± 8.7	308.6 ± 8.4	306.89 ± 8.7	307.3 ± 8.4	307.3 ± 8.4	307.3 ± 8.4
Tibia BMC (g)	0.3407 ± 0.0095[b,c]	0.3407 ± 0.0095[b,c]	0.3407 ± 0.0095[b,c]	0.3407 ± 0.0095[b,c]	0.3407 ± 0.0095[b,c]	0.3407 ± 0.0095[b,c]	0.3407 ± 0.0095[b,c]
Vertebra BMC (g)	0.1684 ± 0.0050[c]	0.1684 ± 0.0050[c]	0.1684 ± 0.0050[c]	0.1684 ± 0.0050[c]	0.1684 ± 0.0050[c]	0.1684 ± 0.0050[c]	0.1684 ± 0.0050[c]

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means ± SE. Parameters with $P<0.05$ indicate a significant difference between groups (n=12 rats/group)

43

Table 6. Alterations in Non-metric Trabecular and Cortical Bone Microarchitectural Parameters in Response to 90 Days of Treatment with Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH=80 µg/kg).

Parameter	AMB-Control	HLU-Control	HLU-LD	HLU-MD	HLU-HD	HLU-PTH	P-Value
Distal Femur Metaphysis							
SMI	$0.54 \pm 0.25^{b,c}$	0.98 ± 0.25^{b}	$0.71 \pm 0.22^{b,c}$	$0.73 \pm 0.25^{b,c}$	0.25 ± 0.24^{c}	-0.53 ± 0.23^{d}	<0.0001
Conn Density (1/mm^3)	111.29 ± 10.52^{b}	101.42 ± 10.52^{b}	112.30 ± 9.11^{b}	116.92 ± 10.52^{ab}	122.06 ± 9.74^{ab}	67.04 ± 9.74^{c}	0.0001
Vertebral							
SMI	$-2.11 \pm 0.52^{b,c}$	-1.32 ± 0.37^{b}	-1.68 ± 0.26^{b}	$-2.28 \pm 0.37^{b,c}$	-2.03 ± 0.267^{b}	-3.15 ± 0.29^{c}	<0.0001
Conn Density (1/mm^3)	$33.43 \pm 7.02^{b,c}$	46.44 ± 4.98^{b}	40.58 ± 3.52^{b}	42.24 ± 4.95^{b}	$35.72 \pm 3.51^{b,c}$	28.24 ± 4.07^{c}	<0.0001
Femur Mid-diaphysis							
Cortical Porosity (%)	1.11 ± 0.05	1.15 ± 0.04	1.20 ± 0.01	1.24 ± 0.07	1.25 ± 0.07	1.21 ± 0.05	0.6615
Cortical Thickness (mm)	0.72 ± 0.01	0.71 ± 0.01	0.72 ± 0.01	0.71 ± 0.01	0.73 ± 0.0082	0.72 ± 0.01	0.8462
Cortical Area (mm^2)	6.36 ± 0.18	6.47 ± 0.16	6.52 ± 0.11	6.44 ± 0.13	6.62 ± 0.14	6.57 ± 0.13	0.8338
Medullary Area (mm^2)	38.99 ± 12.32	56.06 ± 9.93	59.37 ± 7.73	43.40 ± 10.69	27.51 ± 9.24	46.82 ± 10.10	0.2090

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means $\pm$ SE. Parameters with P<0.05 indicate a significant difference between groups (n=12 rats/group)

44

Table 7. Recovery of Cortical Bone Biomechanical Properties with 90 Days of Treatment with Either Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH=80 µg/kg) as Assessed by 3-point Bending.

Parameter	AMB-Control	HLU-Control	HLU-LD	HLU-MD	HLU-HD	HLU-PTH	P-Value
Cortical Bone							
Ultimate Load (N)	138.18 ± 3.57	139.95 ± 2.86	140.62 ± 2.75	140.32 ± 2.86	145.43 ± 2.75	142.97 ± 3.51	0.6016
Yield Load (N)	99.82 ± 3.65	104.96 ± 2.79	101.3908 ± 2.6778	102.31 ± 2.79	105.25 ± 2.79	102.28 ± 3.41	0.1913
Stiffness (N/mm)	134.86 ± 1.99	141.71 ± 1.63	141.58 ± 1.56	141.88 ± 1.62	140.50 ± 1.56	143.30 ± 1.99	0.055
Ultimate Stress(N/mm^2)	240.01 ± 28.19	224.46 ± 23.02	240.57 ± 22.12	272.77 ± 23.02	219.43 ± 22.12	251.89 ± 28.19	0.6224
Yield Stress (N/mm^2)	161.58 ± 24.29	167.78 ± 19.84	173.48 ± 19.06	201.55 ± 19.84	151.26 ± 19.07	179.31 ± 24.29	0.5927
Modulus of Elasticity (N/mm^2)	6040.40 ± 420.61	6477.07 ± 343.42	6145.48 ± 329.95	6428.10 ± 343.42	6351.99 ± 329.95	6295.70 ± 420.61	0.9588

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means ± SE. Parameters with $P<0.05$ indicate a significant difference between groups (n=12 rats/group)

Table 8. Alterations in Vertebral Biomechanical Properties in Response to Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH= give the dose) as Assessed by Compression Testing.

Parameter	AMB-Control	HLU-Control	HLU-LD	HLU-MD	HLU-HD	HLU-PTH	P-Value
Trabecular Bone							
Ultimate Load (N)	208.09 ± 8.00^c	214.84 ± 14.70^c	212.08 ± 9.09^c	$227.75 \pm 11.95^{b,c}$	$250.73 \pm 16.33^{a,b,c}$	279.51 ± 13.25^a	0.0014
Yield Load (N)	156.90 ± 13.56	159.18 ± 18.20	152.61 ± 11.78	144.04 ± 14.56	189.02 ± 19.44	205.32 ± 15.71	0.0627
Stiffness (N/mm)	631.74 ± 60.89	666.54 ± 51.11	600.34 ± 30.37	706.58 ± 63.55	755.97 ± 69.84	757.78 ± 25.25	0.2074
Ultimate Stress(N/mm^2)	30.68 ± 1.35^b	31.58 ± 1.99^b	30.94 ± 1.32^b	34.42 ± 2.12^b	$35.56 \pm 2.67^{a,b}$	40.75 ± 1.75^a	0.0045
Yield Stress (N/mm^2)	23.20 ± 2.15	23.46 ± 2.66	22.20 ± 1.56	21.83 ± 2.46	27.21 ± 3.39	29.89 ± 2.16	0.1562
Modulus of Elasticity (N/mm^2)	655.72 ± 54.46	709.95 ± 49.31	629.99 ± 35.18	763.51 ± 65.40	752.89 ± 75.34	785.64 ± 25.74	0.2602

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means $\pm$ SE. Parameters with $P<0.05$ indicate a significant difference between groups (n=12 rats/group)
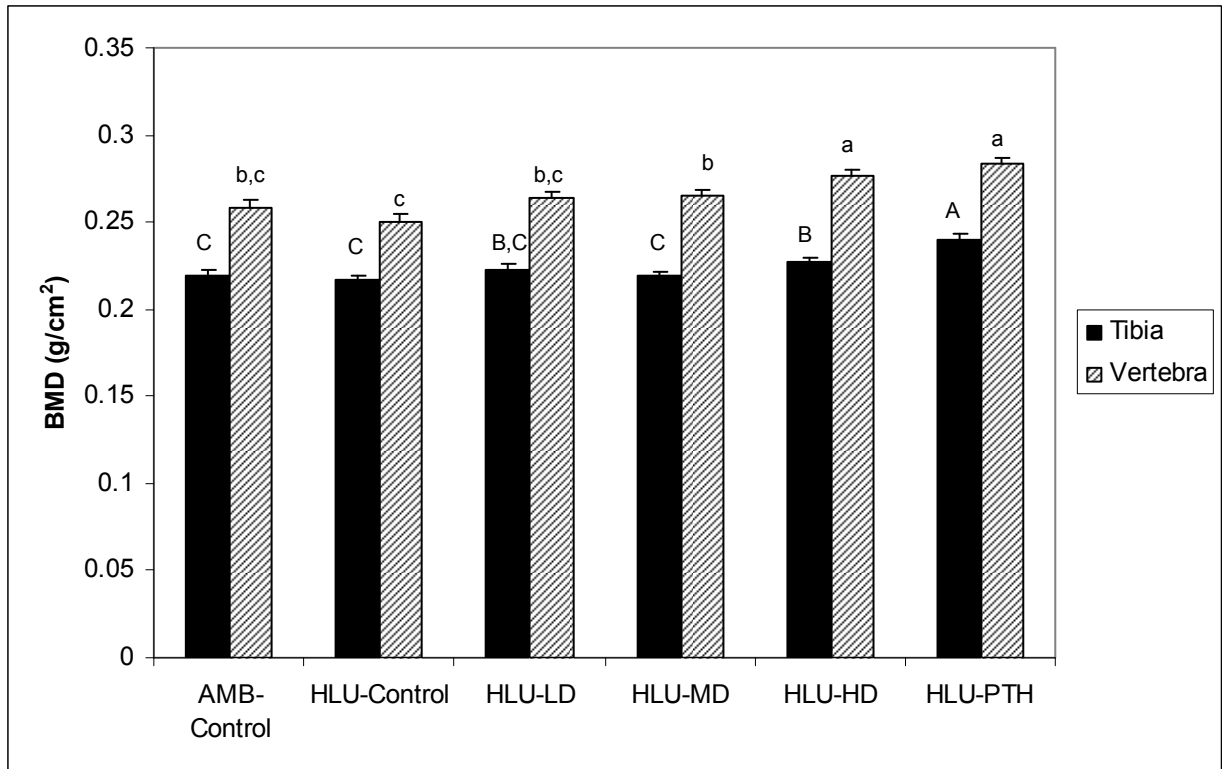
Table 9. Bone Formation and Resorption Markers Response to Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH=80 µg/kg) Following 90 Days of Recovery from HLU.

Parameter	AMB-Control	HLU-Control	HLU-LD	HLU-MD	HLU-HD	HLU-PTH	P-Value
Serum							
Osteocalcin (ng/mL)	75.98 ± 14.64	100.55 ± 13.56	91.49 ± 10.82	65.39 ± 10.36	67.78 ± 10.92	92.59 ± 16.05	0.2161
ALP (U/L)	32.88 ± 3.10^{b}	33.90 ± 2.7^{b}	34.54 ± 2.43^{b}	36.08 ± 2.53^{b}	44.54 ± 2.43^{a}	32.00 ± 3.10^{b}	0.0133
IGF-I (ng/mL)	238.85 ± 35.39	199.55 ± 15.26	207.92 ± 14.66	232.38 ± 24.22	233.42 ± 21.02	264.85 ± 14.79	0.3113
Urinary							
DpD/Cr (nmol/mmol)	26.97 ± 6.49	9.21 ± 5.18	22.13 ± 4.96	16.74 ± 4.96	17.23 ± 5.1807	25.58 ± 7.01	0.2659

AMB—ambulatory control rats; HLU—hindlimb unloaded rats;
Data are means $\pm$ SE. Parameters with $P<0.05$ indicate a significant difference between groups (n=12 rats/group)

47

Figure 1. Alterations in Tibial and Vertebral Bone Mineral Density (BMD) in Response to Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH=80μg/kg)

Data are means $\pm$ SE. Parameters with *P<0.05* indicate a significant difference between groups.

Figure 2. Alterations in the (A) Trabecular Bone Volume (BV/TV), (B) Trabecular Thickness (TbTh), (C) Trabecular Separation (TbSp) and (D) Trabecular Number (TbN) at the Distal Femur Metaphysis and the Lumbar Vertebra in Response to Low Dose (LD=5%), Medium Dose (MD=15%), High Dose (HD=25%) Dried Plum or Parathyroid Hormone (PTH=80 μg/kg) during a 90 Day Re-ambulation Period.

A.
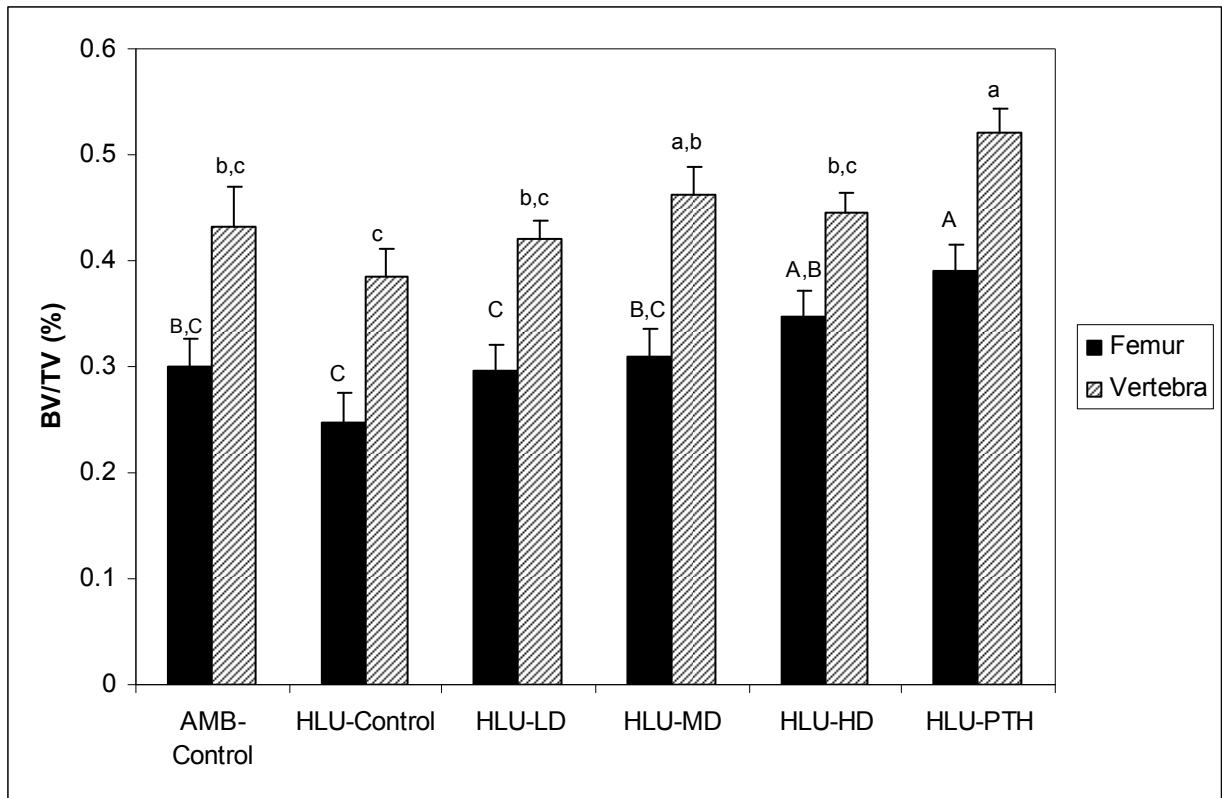
Data are means $\pm$ SE. Parameters with *P<0.05* indicate a significant difference between groups.

B.
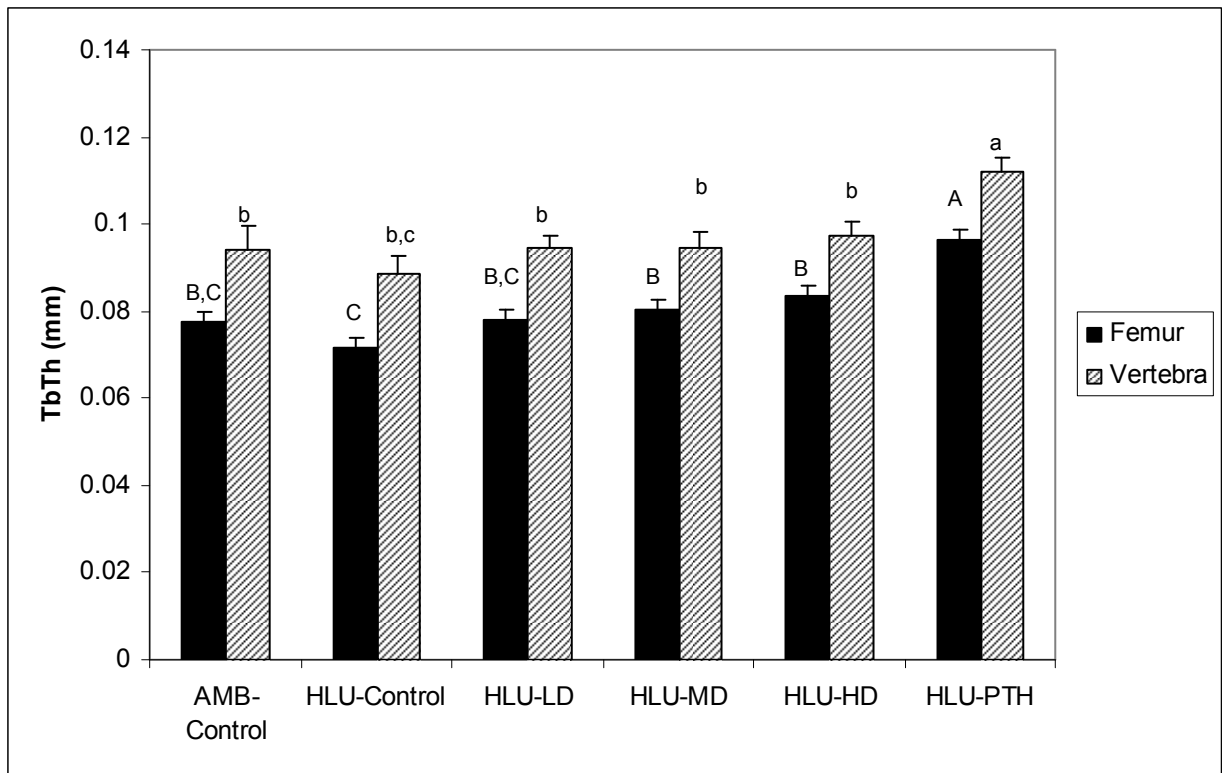
Data are means $\pm$ SE. Parameters with *P<0.05* indicate a significant difference between groups.

C.
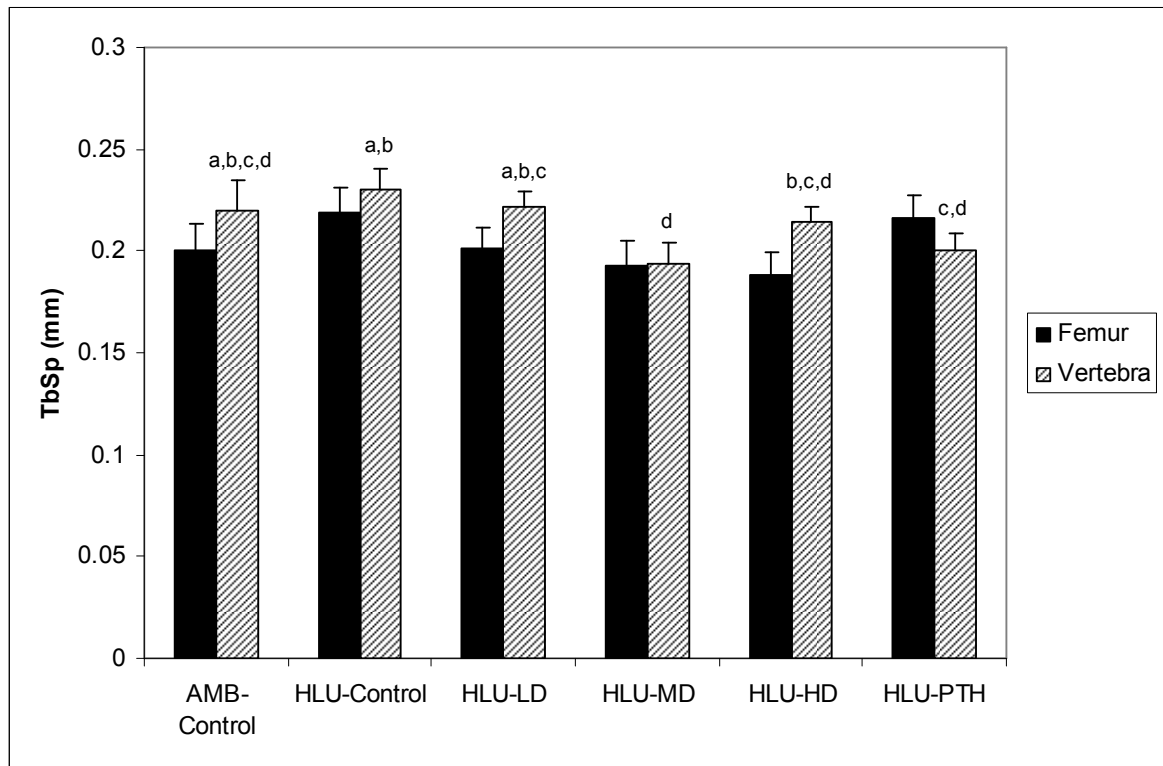
Data are means $\pm$ SE. Parameters with *P<0.05* indicate a significant difference between groups.

D.

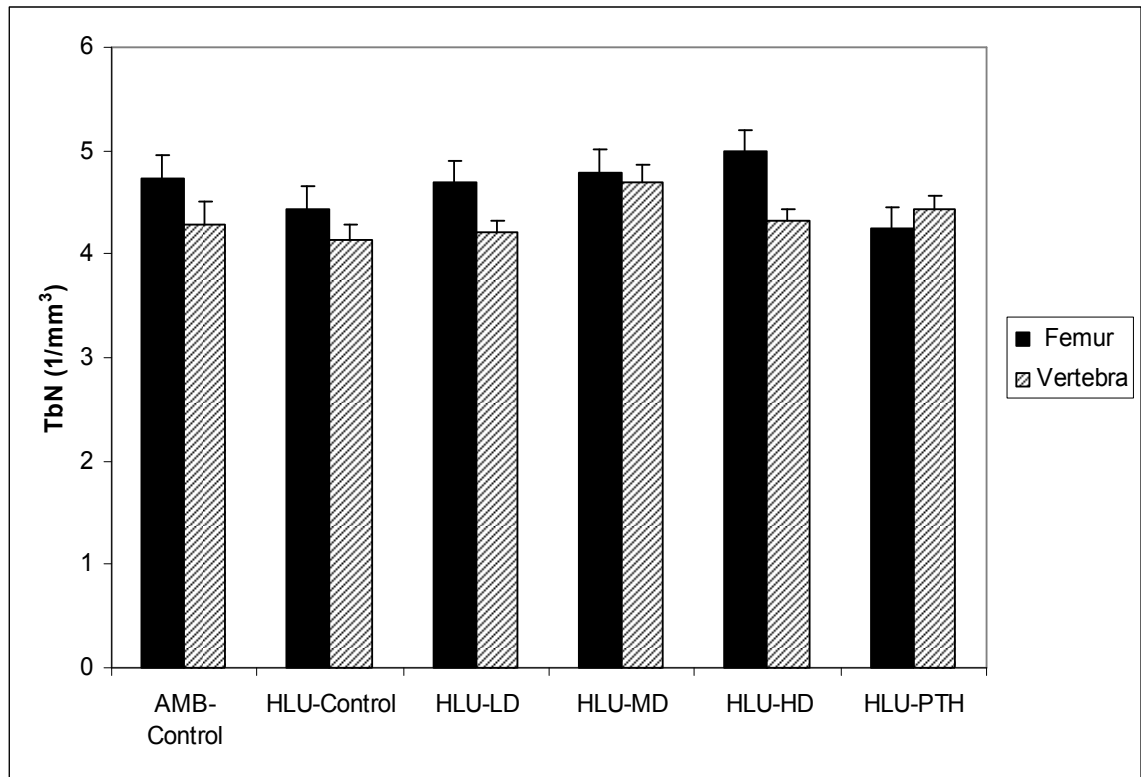Data are means ± SE. Parameters with *P<0.05* indicate a significant difference between groups.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

Recent studies have shown dried plum to prevent and reverse bone loss in animal models of gonadal hormone deficiency-induced osteoporosis. The objectives of this study were to determine: 1) if dried plums could enhance bone recovery following hindlimb unloading (HLU); 2) the most effective dose (5%, 15%, or 25%; w/w) of dried plum; and 3) how the effects of dried plum on bone compare to the anabolic effects of parathyroid hormone (PTH). Six month old female virgin Sprague-Dawley rats were either HLU (n=70) to induce bone loss or remained ambulatory (n=16) for 3 weeks. Following the HLU period and the confirmation of bone loss, the remaining groups were randomly assigned to treatments for 90 days: standard semi-purified (control) diet with either LD=5%, MD=15% or HD=25% (w/w) dried plum added or control diet plus parathyroid hormone administration (80 µg PTH/kg bw; 3 x wk Bachem, Inc). We reported that the higher doses of dried plum enhanced the recovery of BMD and BMC of both the tibia and vertebra compared to animals on the control diet, although only the vertebral site responded to the level of the PTH group. Trabecular bone volume (BV/TV) was enhanced in the HD group and was similar to the group

receiving intermittent PTH. Trabecular thickness (TbTh) of the vertebra increased in the MD and HD dried plum groups, although not to the level of PTH. Bone formation as indicated by serum alkaline phosphatase (ALP) was significantly higher in the HD group, but bone resorption was unaltered. These findings suggest that dried plum enhances bone recovery in osteopenic female rats perhaps by up-regulating bone formation. These effects appear to be somewhat similar to PTH in restoring trabecular bone, but perhaps acting through a different mechanism.

Conclusions

Hypothesis: Dried plums will dose-dependently enhance the recovery of bone following hindlimb unloading and have similar effects to the anabolic agent, parathyroid hormone (PTH).

We reject this hypothesis because dried plum did not have a dose-dependent response in the recovery on bone following HLU. Although, dried plum did enhance recovery positively, it was not a true dose-dependent response. High dose dried plum provided the greatest enhancement of bone recovery when compared to animals on the control diet and was often comparable to the effects of PTH. Significant increases were seen in BMC, BMD and bone microarchitecture in animals on HD dried plum while LD and MD dried plum provided no benefit. HD dried plum also aided in the recovery of trabecular bone as assessed by compression testing and significantly increased serum ALP.

Recommendations

Considering the findings of this study, future research should be continued in this area related to the effects of dried plum on bone. We have demonstrated that dried plum can enhance the recovery of bone, especially trabecular bone, even after significant deterioration has occurred. Future studies should be designed to determine how dried plum positively effects bone formation, including studies using dynamic bone histomorphometry. Recent findings demonstrated that in males, bone loss is prevented by up-regulating IGF-I and down-regulating the RANK pathway (Franklin, 2006). Further studies are needed to better understand how these effects are mediated and whether they are actually similar to PTH or present a novel pathway by which dried plum impacts bone metabolism.

Additionally, clinical trials are necessary to evaluate dried plum's effect in men and women. Studies are needed to determine: 1) the dose-dependent effects of dried plum on BMD and BMC in both postmenopausal women as well as age-related bone loss in both men and women; 2) the most efficacious dose of dried plum for each of the study populations; 3) the alterations in bone metabolism corresponding to the changes in BMD; and 4) if there are any possible adverse side effects in human subjects.

LITERATURE CITED

Abram, A. C., Keller, T. S., & Spengler, D. M. 1988. The effects of simulated weightlessness on bone biomechanical and biochemical properties in the maturing rat. J.Biomech., 21(9): 755-767.

Allen, M. R. & Bloomfield, S. A. 2003. Hindlimb unloading has a greater effect on cortical compared with cancellous bone in mature female rats. J.Appl.Physiol, 94(2): 642-650.

Aloia, J. F. 1981. Exercise and skeletal health. J.Am.Geriatr.Soc., 29(3): 104-107.

Arjmandi, B. H., Birnbaum, R., Goyal, N. V., Getlinger, M. J., Juma, S., Alekel, L., Hasler, C. M., Drum, M. L., Hollis, B. W., & Kukreja, S. C. 1998. Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. Am.J.Clin.Nutr., 68(6 Suppl): 1364S-1368S.

Arjmandi, B. H., Birnbaum, R. S., Juma, S., Barengolts, E., & Kukreja, S. C. 2000. The synthetic phytoestrogen, ipriflavone, and estrogen prevent bone loss by different mechanisms. Calcif.Tissue Int., 66(1): 61-65.

Arjmandi, B. H. 2001. The role of phytoestrogens in the prevention and treatment of osteoporosis in ovarian hormone deficiency. J.Am.Coll.Nutr., 20(5 Suppl): 398S-402S.

Arjmandi, B. H., Khalil, D. A., Lucas, E. A., Georgis, A., Stoecker, B. J., Hardin, C., Payton, M. E., & Wild, R. A. 2002. Dried plums improve indices of bone formation in postmenopausal women. J.Womens Health Gend.Based.Med., 11(1): 61-68.

Arjmandi, B. H., Lucas, E. A., Juma, S., Soliman, A, Stoecker, B. J., Khalil, D. A., Smith, B. J., and Wang, C. Dried plums prevent ovariectomy-induced bone loss in rats. JANA 4[Spring 2001], 50-56. 2003.

Arjmandi, B. H., Lucas, E. A., Khalil, D. A., Devareddy, L., Smith, B. J., McDonald, J., Arquitt, A. B., Payton, M. E., & Mason, C. 2005. One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. Nutr.J., 4: 8.

Armstrong, J. W. & Chapes, S. K. 1994. Effects of extracellular matrix proteins on macrophage differentiation, growth, and function: comparison of liquid and agar culture systems. J.Exp.Zool., 269(3): 178-187.

Arnaud, S. B., Sherrard, D. J., Maloney, N., Whalen, R. T., & Fung, P. 1992. Effects of 1-week head-down tilt bed rest on bone formation and the calcium endocrine system. Aviat.Space Environ.Med., 63(1): 14-20.

Banu, J. & Kalu, D. N. 2002. Effects of cerivastatin and parathyroid hormone on the lumbar vertebra of aging male Sprague-Dawley rats. Bone, 31(1): 173-179.

Bemben, D. A., Buchanan, T. D., Bemben, M. G., & Knehans, A. W. 2004. Influence of type of mechanical loading, menstrual status, and training season on bone density in young women athletes. J.Strength.Cond.Res., 18(2): 220-226.

Bikle, D. D., Halloran, B. P., Cone, C. M., Globus, R. K., & Morey-Holton, E. 1987. The effects of simulated weightlessness on bone maturation. Endocrinology, 120(2): 678-684.

Bikle, D. D., Halloran, B. P., & Morey-Holton, E. 1997. Space flight and the skeleton: lessons for the earthbound. Endocrinologist., 7(1): 10-22.

Bikle, D. D. & Halloran, B. P. 1999. The response of bone to unloading. J.Bone Miner.Metab, 17(4): 233-244.

Bikle, D. D., Sakata, T., & Halloran, B. P. 2003. The impact of skeletal unloading on bone formation. Gravit.Space Biol.Bull., 16(2): 45-54.

Black, D. M., Thompson, D. E., Bauer, D. C., Ensrud, K., Musliner, T., Hochberg, M. C., Nevitt, M. C., Suryawanshi, S., & Cummings, S. R. 2000. Fracture risk reduction with alendronate in women with osteoporosis: the Fracture Intervention Trial. FIT Research Group. J.Clin.Endocrinol.Metab, 85(11): 4118-4124.

Bloomfield, S. A., Allen, M. R., Hogan, H. A., & Delp, M. D. 2002. Site- and compartment-specific changes in bone with hindlimb unloading in mature adult rats. Bone, 31(1): 149-157.

Body, J. J., Gaich, G. A., Scheele, W. H., Kulkarni, P. M., Miller, P. D., Peretz, A., Dore, R. K., Correa-Rotter, R., Papaioannou, A., Cumming, D. C., & Hodsman, A. B. 2002. A randomized double-blind trial to compare the efficacy of teriparatide [recombinant human parathyroid hormone (1-34)] with alendronate in postmenopausal women with osteoporosis. J.Clin.Endocrinol.Metab, 87(10): 4528-4535.

Brixner, D. 2006. Assessment of the prevalence and costs of osteoporosis treatment options in a real-world setting. Am.J.Manag.Care, 12(7 Suppl): S191-S198.

Bugel, S. 2003. Vitamin K and bone health. Proc.Nutr.Soc., 62(4): 839-843.

Cabrera, C., Artacho, R., & Gimenez, R. 2006. Beneficial effects of green tea--a review. J.Am.Coll.Nutr., 25(2): 79-99.

Carmeliet, G., Vico, L., & Bouillon, R. 2001. Space flight: a challenge for normal bone homeostasis. Crit Rev.Eukaryot.Gene Expr., 11(1-3): 131-144.

Carvalho, D. C., Garlipp, C. R., Bottini, P. V., Afaz, S. H., Moda, M. A., & Cliquet, A., Jr. 2006. Effect of treadmill gait on bone markers and bone mineral density of quadriplegic subjects. Braz.J.Med.Biol.Res., 39(10): 1357-1363.

Cauley, J. A., Lui, L. Y., Ensrud, K. E., Zmuda, J. M., Stone, K. L., Hochberg, M. C., & Cummings, S. R. 2005. Bone mineral density and the risk of incident nonspinal fractures in black and white women. JAMA, 293(17): 2102-2108.

Chaiamnuay, S. & Saag, K. G. 2006. Postmenopausal osteoporosis. What have we learned since the introduction of bisphosphonates? Rev.Endocr.Metab Disord..

Chapin, R. E., Ku, W. W., Kenney, M. A., McCoy, H., Gladen, B., Wine, R. N., Wilson, R., & Elwell, M. R. 1997. The effects of dietary boron on bone strength in rats. Fundam.Appl.Toxicol., 35(2): 205-215.

Chapurlat, R. D. & Delmas, P. D. 2006. Drug insight: Bisphosphonates for postmenopausal osteoporosis. Nat.Clin.Pract.Endocrinol.Metab, 2(4): 211-219.

Chesnut, C. H., III, Silverman, S., Andriano, K., Genant, H., Gimona, A., Harris, S., Kiel, D., LeBoff, M., Maricic, M., Miller, P., Moniz, C., Peacock, M., Richardson, P., Watts, N., & Baylink, D. 2000. A randomized trial of nasal spray salmon calcitonin in postmenopausal women with established osteoporosis: the prevent recurrence of osteoporotic fractures study. PROOF Study Group. Am.J.Med., 109(4): 267-276.

Chesnut, C. H., III & Rosen, C. J. 2001. Reconsidering the effects of antiresorptive therapies in reducing osteoporotic fracture. J.Bone Miner.Res., 16(12): 2163-2172.

Close, P., Neuprez, A., & Reginster, J. Y. 2006. Developments in the pharmacotherapeutic management of osteoporosis. Expert.Opin.Pharmacother., 7(12): 1603-1615.

Collet, P., Uebelhart, D., Vico, L., Moro, L., Hartmann, D., Roth, M., & Alexandre, C. 1997. Effects of 1- and 6-month spaceflight on bone mass and biochemistry in two humans. Bone, 20(6): 547-551.

Cosman, F. 2005. The prevention and treatment of osteoporosis: a review. MedGenMed., 7(2): 73.

Cranney, A., Papaioannou, A., Zytaruk, N., Hanley, D., Adachi, J., Goltzman, D., Murray, T., & Hodsman, A. 2006. Parathyroid hormone for the treatment of osteoporosis: a systematic review. CMAJ., 175(1): 52-59.

Dalsky, G. P. 1989. The role of exercise in the prevention of osteoporosis. Compr.Ther., 15(9): 30-37.

Dawson-Hughes, B. 1991. Calcium supplementation and bone loss: a review of controlled clinical trials. Am.J.Clin.Nutr., 54(1 Suppl): 274S-280S.

Dawson-Hughes, B. 2003. Interaction of dietary calcium and protein in bone health in humans. <u>J.Nutr.</u>, 133(3): 852S-854S.

de Nijs, R. N., Jacobs, J. W., Lems, W. F., Laan, R. F., Algra, A., Huisman, A. M., Buskens, E., de Laet, C. E., Oostveen, A. C., Geusens, P. P., Bruyn, G. A., Dijkmans, B. A., & Bijlsma, J. W. 2006. Alendronate or alfacalcidol in glucocorticoid-induced osteoporosis. <u>N.Engl.J.Med.</u>, 355(7): 675-684.

Dehority, W., Halloran, B. P., Bikle, D. D., Curren, T., Kostenuik, P. J., Wronski, T. J., Shen, Y., Rabkin, B., Bouraoui, A., & Morey-Holton, E. 1999. Bone and hormonal changes induced by skeletal unloading in the mature male rat. <u>Am.J.Physiol</u>, 276(1 Pt 1): E62-E69.

Dempster, D. W., Cosman, F., Parisien, M., Shen, V., & Lindsay, R. 1993. Anabolic actions of parathyroid hormone on bone. <u>Endocr.Rev.</u>, 14(6): 690-709.

Devareddy, L., Khalil, D. A., Smith, B. J., Lucas, E. A., Soung, d. Y., Marlow, D. D., & Arjmandi, B. H. 2006. Soy moderately improves microstructural properties without affecting bone mass in an ovariectomized rat model of osteoporosis. <u>Bone</u>, 38(5): 686-693.

Deyhim, F., Stoecker, B. J., Brusewitz, G. H., Devareddy, L., & Arjmandi, B. H. 2005. Dried plum reverses bone loss in an osteopenic rat model of osteoporosis. <u>Menopause.</u>, 12(6): 755-762.

Donaldson, C. L., Hulley, S. B., Vogel, J. M., Hattner, R. S., Bayers, J. H., & McMillan, D. E. 1970. Effect of prolonged bed rest on bone mineral. <u>Metabolism</u>, 19(12): 1071-1084.

Donovan, D. S., Jr., Papadopoulos, A., Staron, R. B., Addesso, V., Schulman, L., McGregor, C., Cosman, F., Lindsay, R. L., & Shane, E. 1998. Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. <u>Am.J.Respir.Crit Care Med.</u>, 157(6 Pt 1): 1892-1899.

Durchschlag, E., Paschalis, E. P., Zoehrer, R., Roschger, P., Fratzl, P., Recker, R., Phipps, R., & Klaushofer, K. 2006. Bone material properties in trabecular bone from human iliac crest biopsies after 3- and 5-year treatment with risedronate. <u>J.Bone Miner.Res.</u>, 21(10): 1581-1590.

Epstein, S. 2006. Update of current therapeutic options for the treatment of postmenopausal osteoporosis. <u>Clin.Ther.</u>, 28(2): 151-173.

Erben, R. G. 2001. Vitamin D analogs and bone. <u>J.Musculoskelet.Neuronal.Interact.</u>, 2(1): 59-69.

Fang, N., Yu, S., & Prior, R. L. 2002. LC/MS/MS characterization of phenolic constituents in dried plums. <u>J.Agric.Food Chem.</u>, 50(12): 3579-3585.

Fernandes, P., Rodrigues, H., & Jacobs, C. 1999. A model of bone adaptation using a global optimisation criterion based on the trajectorial theory of wolff. Comput.Methods Biomech.Biomed.Engin., 2(2): 125-138.

Finkelstein, J. S., Klibanski, A., Arnold, A. L., Toth, T. L., Hornstein, M. D., & Neer, R. M. 1998. Prevention of estrogen deficiency-related bone loss with human parathyroid hormone-(1-34): a randomized controlled trial. JAMA, 280(12): 1067-1073.

Fox, J., Miller, M. A., Newman, M. K., Metcalfe, A. F., Turner, C. H., Recker, R. R., & Smith, S. Y. 2006. Daily treatment of aged ovariectomized rats with human parathyroid hormone (1-84) for 12 months reverses bone loss and enhances trabecular and cortical bone strength. Calcif.Tissue Int., 79(4): 262-272.

Frankel, A. E. 1993. Immunotoxin therapy of cancer. Oncology (Williston.Park), 7(5): 69-78.

Franklin, M., Bu, S. Y., Lerner, M. R., Lancaster, E. A., Bellmer, D., Marlow, D., Lightfoot, S. A., Arjmandi, B. H., Brackett, D. J., Lucas, E. A., & Smith, B. J. 2006. Dried plum prevents bone loss in a male osteoporosis model via IGF-I and the RANK pathway. Bone.

Garrett, I. R., Boyce, B. F., Oreffo, R. O., Bonewald, L., Poser, J., & Mundy, G. R. 1990. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J.Clin.Invest, 85(3): 632-639.

Giangregorio, L. & Blimkie, C. J. 2002. Skeletal adaptations to alterations in weight-bearing activity: a comparison of models of disuse osteoporosis. Sports Med., 32(7): 459-476.

Globus, R. K., Bikle, D. D., & Morey-Holton, E. 1986. The temporal response of bone to unloading. Endocrinology, 118(2): 733-742.

Going, S., Lohman, T., Houtkooper, L., Metcalfe, L., Flint-Wagner, H., Blew, R., Stanford, V., Cussler, E., Martin, J., Teixeira, P., Harris, M., Milliken, L., Figueroa-Galvez, A., & Weber, J. 2003. Effects of exercise on bone mineral density in calcium-replete postmenopausal women with and without hormone replacement therapy. Osteoporos.Int., 14(8): 637-643.

Grano, M., Mori, G., Minielli, V., Barou, O., Colucci, S., Giannelli, G., Alexandre, C., Zallone, A. Z., & Vico, L. 2002. Rat hindlimb unloading by tail suspension reduces osteoblast differentiation, induces IL-6 secretion, and increases bone resorption in ex vivo cultures. Calcif.Tissue Int., 70(3): 176-185.

Heaney, R. P. & Weaver, C. M. 2003. Calcium and vitamin D. Endocrinol.Metab Clin.North Am., 32(1): 181-viii.

Hefferan, T. E., Evans, G. L., Lotinun, S., Zhang, M., Morey-Holton, E., & Turner, R. T. 2003. Effect of gender on bone turnover in adult rats during simulated weightlessness. J.Appl.Physiol, 95(5): 1775-1780.

Hildebrand, T. and Ruegsegger, R. Quantification of bone microarchitecture with the structure model index. CMBBE 1, 15-23. 1-14-1997.
Ref Type: Generic

Hock, J. M. & Gera, I. 1992. Effects of continuous and intermittent administration and inhibition of resorption on the anabolic response of bone to parathyroid hormone. J.Bone Miner.Res., 7(1): 65-72.

Hodsman, A. B., Hanley, D. A., Ettinger, M. P., Bolognese, M. A., Fox, J., Metcalfe, A. J., & Lindsay, R. 2003. Efficacy and safety of human parathyroid hormone-(1-84) in increasing bone mineral density in postmenopausal osteoporosis. J.Clin.Endocrinol.Metab, 88(11): 5212-5220.

Holick, M. F. 2003. Evolution and function of vitamin D. Recent Results Cancer Res., 164: 3-28.

Holick, M. F. 2006. The role of vitamin D for bone health and fracture prevention. Curr.Osteoporos.Rep., 4(3): 96-102.

Hughes-Fulford, M., Tjandrawinata, R., Fitzgerald, J., Gasuad, K., & Gilbertson, V. 1998. Effects of microgravity on osteoblast growth. Gravit.Space Biol.Bull., 11(2): 51-60.

Inzerillo, A.M. and Zaidi, M. 2002. Osteoporosis: trends and interventions. Mt Sinai J Med. 69(4):220-31.

Ito, M., Azuma, H., Takagi, T., Kamimura, K., Komoriya, K., Ohta, T., and Kawaguchi, H. Preventive effects of sequential treatment with alendronate and 1 alpha-hydroxyvitamin D3 on bone mass and strength in ovariectomized rats. Bone 33, 90-99. 9-17-2002.

Iwamoto, J., Takeda, T., & Ichimura, S. 2001. Effect of menatetrenone on bone mineral density and incidence of vertebral fractures in postmenopausal women with osteoporosis: a comparison with the effect of etidronate. J.Orthop.Sci., 6(6): 487-492.

Iwamoto, J., Yeh, J. K., Takeda, T., Ichimura, S., & Sato, Y. 2003. Comparative effects of vitamin K and vitamin D supplementation on prevention of osteopenia in calcium-deficient young rats. Bone, 33(4): 557-566.

Iwamoto, J., Seki, A., Takeda, T., Sato, Y., Yamada, H., & Yeh, J. K. 2006. Comparative effects of alendronate and alfacalcidol on cancellous and cortical bone mass and bone mechanical properties in ovariectomized rats. Exp.Anim, 55(4): 357-367.

Juppner, H. 2000. Role of parathyroid hormone-related peptide and Indian hedgehog in skeletal development. Pediatr.Nephrol. , 14(7): 606-611.

Kanis, J. A., Johnell, O., Black, D. M., Downs, R. W., Jr., Sarkar, S., Fuerst, T., Secrest, R. J., & Pavo, I. 2003. Effect of raloxifene on the risk of new vertebral fracture in postmenopausal women with osteopenia or osteoporosis: a reanalysis of the Multiple Outcomes of Raloxifene Evaluation trial. Bone, 33(3): 293-300.

Kannus, P., Leppala, J., Lehto, M., Sievanen, H., Heinonen, A., & Jarvinen, M. 1995. A rotator cuff rupture produces permanent osteoporosis in the affected extremity, but not in those with whom shoulder function has returned to normal. J.Bone Miner.Res., 10(8): 1263-1271.

Kayano, S., Yamada, N. F., Suzuki, T., Ikami, T., Shioaki, K., Kikuzaki, H., Mitani, T., & Nakatani, N. 2003. Quantitative evaluation of antioxidant components in prunes (Prunus domestica L.). J.Agric.Food Chem., 51(5): 1480-1485.

Kerr, D., Ackland, T., Maslen, B., Morton, A., & Prince, R. 2001. Resistance training over 2 years increases bone mass in calcium-replete postmenopausal women. J.Bone Miner.Res., 16(1): 175-181.

Kim, H., Iwasaki, K., Miyake, T., Shiozawa, T., Nozaki, S., & Yajima, K. 2003. Changes in bone turnover markers during 14-day 6 degrees head-down bed rest. J.Bone Miner.Metab, 21(5): 311-315.

Krolner, B., Toft, B., Pors, N. S., & Tondevold, E. 1983. Physical exercise as prophylaxis against involutional vertebral bone loss: a controlled trial. Clin.Sci.(Lond), 64(5): 541-546.

Kronenberg, H. M. 2006. PTHrP and skeletal development. Ann.N.Y.Acad.Sci., 1068: 1-13.

Lang, T. F., LeBlanc, A. D., Evans, H. J., & Lu, Y. 2006. Adaptation of the proximal femur to skeletal reloading after long-duration spaceflight. J.Bone Miner.Res., 21(8): 1224-1230.

LeBlanc, A. D., Schneider, V. S., Evans, H. J., Engelbretson, D. A., & Krebs, J. M. 1990. Bone mineral loss and recovery after 17 weeks of bed rest. J.Bone Miner.Res., 5(8): 843-850.

LeBlanc, A. D., Driscol, T. B., Shackelford, L. C., Evans, H. J., Rianon, N. J., Smith, S. M., Feeback, D. L., & Lai, D. 2002. Alendronate as an effective countermeasure to disuse induced bone loss. J.Musculoskelet.Neuronal.Interact., 2(4): 335-343.

Lee, T. C. & Taylor, D. 1999. Bone remodelling: should we cry Wolff? Ir.J.Med.Sci., 168(2): 102-105.

Levy, F., Muff, R., Dotti-Sigrist, S., Dambacher, M. A., & Fischer, J. A. 1988. Formation of neutralizing antibodies during intranasal synthetic salmon calcitonin treatment of Paget's disease. J.Clin.Endocrinol.Metab, 67(3): 541-545.

Li, C. Y., Price, C., Delisser, K., Nasser, P., Laudier, D., Clement, M., Jepsen, K. J., & Schaffler, M. B. 2005. Long-term disuse osteoporosis seems less sensitive to bisphosphonate treatment than other osteoporosis. J.Bone Miner.Res., 20(1): 117-124.

Liberman, U. A. 2006. Long-term safety of bisphosphonate therapy for osteoporosis: a review of the evidence. Drugs Aging, 23(4): 289-298.

Locklin, R. M., Oreffo, R. O., & Triffitt, J. T. 1998. Modulation of osteogenic differentiation in human skeletal cells in Vitro by 5-azacytidine. Cell Biol.Int., 22(3): 207-215.

Love, R. R., Mazess, R. B., Barden, H. S., Epstein, S., Newcomb, P. A., Jordan, V. C., Carbone, P. P., & DeMets, D. L. 1992. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N.Engl.J.Med., 326(13): 852-856.

Lucas, E. A., Juma, S., Stoecker, B. J., & Arjmandi, B. H. 2000. Prune suppresses ovariectomy-induced hypercholesterolemia in rats. J.Nutr.Biochem., 11(5): 255-259.

Maeda, H., Kimmel, DB., Raab, DM, and Lane, NE. Musculoskeletal recovery following hindlimb immobilization in adult female rats. Bone 14, 153-159. 1993.
Ref Type: Generic

Majima, T., Tsutsumi, M., Nishino, H., Tsunoda, T., & Konishi, Y. 1998. Inhibitory effects of beta-carotene, palm carotene, and green tea polyphenols on pancreatic carcinogenesis initiated by N-nitorsobis(2-oxopropyl)amine in Syrian golden hamsters. Pancreas, 16(1): 13-18.

Marcus, R., Holloway, L., Wells, B., Greendale, G., James, M. K., Wasilauskas, C., & Kelaghan, J. 1999. The relationship of biochemical markers of bone turnover to bone density changes in postmenopausal women: results from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. J.Bone Miner.Res., 14(9): 1583-1595.

Martin, R. B. 1990. Effects of simulated weightlessness on bone properties in rats. J.Biomech., 23(10): 1021-1029.

Matkovic, V., Goel, P. K., Badenhop-Stevens, N. E., Landoll, J. D., Li, B., Ilich, J. Z., Skugor, M., Nagode, L. A., Mobley, S. L., Ha, E. J., Hangartner, T. N., & Clairmont, A. 2005. Calcium supplementation and bone mineral density in females from childhood to young adulthood: a randomized controlled trial. Am.J.Clin.Nutr., 81(1): 175-188.

McClung, B. & McClung, M. 2001. Pharmacologic therapy for the treatment and prevention of osteoporosis. Nurs.Clin.North Am. , 36(3): 433-40, viii.

Mehta, N. M., Malootian, A., & Gilligan, J. P. 2003. Calcitonin for osteoporosis and bone pain. Curr.Pharm.Des, 9(32): 2659-2676.

Misof, B. M., Roschger, P., Cosman, F., Kurland, E. S., Tesch, W., Messmer, P., Dempster, D. W., Nieves, J., Shane, E., Fratzl, P., Klaushofer, K., Bilezikian, J., & Lindsay, R. 2003. Effects of intermittent parathyroid hormone administration on bone mineralization density in iliac crest biopsies from patients with osteoporosis: a paired study before and after treatment. J.Clin.Endocrinol.Metab, 88(3): 1150-1156.

Moore, A. 2003. Hormone replacement therapy: dilemmas in 2002. Trans.Am.Clin.Climatol.Assoc., 114: 233-238.

Morey-Holton, E. R. & Globus, R. K. 1998. Hindlimb unloading of growing rats: a model for predicting skeletal changes during space flight. Bone, 22(5 Suppl): 83S-88S.

Morey-Holton, E. R. & Globus, R. K. 2002. Hindlimb unloading rodent model: technical aspects. J.Appl.Physiol, 92(4): 1367-1377.

Morey, E. R. & Baylink, D. J. 1978. Inhibition of bone formation during space flight. Science, 201(4361): 1138-1141.

Mori-Okamoto, J., Otawara-Hamamoto, Y., Yamato, H., & Yoshimura, H. 2004. Pomegranate extract improves a depressive state and bone properties in menopausal syndrome model ovariectomized mice. J.Ethnopharmacol., 92(1): 93-101.

Moriyama, I., Iwamoto, J., Takeda, T., & Toyama, Y. 2002. Comparative effects of intermittent administration of human parathyroid hormone (1-34) on cancellous and cortical bone loss in tail-suspended and sciatic neurectomized young rats. J.Orthop.Sci., 7(3): 379-385.

Mosekilde, L., Thomsen, J. S., Mackey, M. S., & Phipps, R. J. 2000. Treatment with risedronate or alendronate prevents hind-limb immobilization-induced loss of bone density and strength in adult female rats. Bone, 27(5): 639-645.

Mundy, G. R. 2002. Directions of drug discovery in osteoporosis. Annu.Rev.Med., 53: 337-354.

Naghii, M. R. & Samman, S. 1993. The role of boron in nutrition and metabolism. Prog.Food Nutr.Sci., 17(4): 331-349.

Nakatani, N., Kayano, S., Kikuzaki, H., Sumino, K., Katagiri, K., & Mitani, T. 2000. Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in prune (Prunus domestica L.). J.Agric.Food Chem., 48(11): 5512-5516.

Nelson, H. D. 2003. Postmenopausal osteoporosis and estrogen. Am.Fam.Physician, 68(4): 606, 610, 612.

Nelson, M. E., Fiatarone, M. A., Morganti, C. M., Trice, I., Greenberg, R. A., & Evans, W. J. 1994. Effects of high-intensity strength training on multiple risk factors for osteoporotic fractures. A randomized controlled trial. <u>JAMA</u>, 272(24): 1909-1914.

Nickols-Richardson, S. M., O'Connor, P. J., Shapses, S. A., & Lewis, R. D. 1999. Longitudinal bone mineral density changes in female child artistic gymnasts. <u>J.Bone Miner.Res.</u>, 14(6): 994-1002.

Oganov, V. S., Rakhmanov, A. S., Novikov, V. E., Zatsepin, S. T., Rodionova, S. S., & Cann, C. 1991. The state of human bone tissue during space flight. <u>Acta Astronaut.</u>, 23: 129-133.

Ohira, Y., Kawano, F., Wang, X. D., Sudoh, M., & Ishihara, A. 2003. Changes of bone morphology in response to hindlimb suspension of rats. <u>Biol.Sci.Space</u>, 17(3): 225-226.

Oxlund, H., Dalstra, M., Ejersted, C., & Andreassen, T. T. 2002. Parathyroid hormone induces formation of new cancellous bone with substantial mechanical strength at a site where it had disappeared in old rats. <u>Eur.J.Endocrinol.</u>, 146(3): 431-438.

Perrien, D. S., Akel, N. S., Dupont-Versteegden, E. E., Skinner, R. A., Siegel, E. R., Suva, L. J., & Gaddy, D. 2006. Aging alters the skeletal response to disuse in the rat. <u>Am.J.Physiol Regul.Integr.Comp Physiol</u>.

Prestwood, K. M. & Raisz, L. G. 2000. Prevention and treatment of osteoporosis. <u>Clin.Cornerstone.</u>, 2(6): 34-44.

Rattanakul, C., Lenbury, Y., Krishnamara, N., & Wollkind, D. J. 2003. Modeling of bone formation and resorption mediated by parathyroid hormone: response to estrogen/PTH therapy. <u>Biosystems</u>, 70(1): 55-72.

Rhee, Y., Won, Y. Y., Baek, M. H., & Lim, S. K. 2004. Maintenance of increased bone mass after recombinant human parathyroid hormone (1-84) with sequential zoledronate treatment in ovariectomized rats. <u>J.Bone Miner.Res.</u>, 19(6): 931-937.

Rogers, M. J. 2003. New insights into the molecular mechanisms of action of bisphosphonates. <u>Curr.Pharm.Des</u>, 9(32): 2643-2658.

Ruohola, J. P., Laaksi, I., Ylikomi, T., Haataja, R., Mattila, V. M., Sahi, T., Tuohimaa, P., & Pihlajamaki, H. 2006. Association between serum 25(OH)D concentrations and bone stress fractures in Finnish young men. <u>J.Bone Miner.Res.</u>, 21(9): 1483-1488.

Schneider, S. M., Amonette, W. E., Blazine, K., Bentley, J., Lee, S. M., Loehr, J. A., Moore, A. D., Jr., Rapley, M., Mulder, E. R., & Smith, S. M. 2003. Training with the International Space Station interim resistive exercise device. <u>Med.Sci.Sports Exerc.</u>, 35(11): 1935-1945.

Schoutens, A., Laurent, E., & Poortmans, J. R. 1989. Effects of inactivity and exercise on bone. <u>Sports Med.</u>, 7(2): 71-81.

Schultheis, L., Ruff, C. B., Rastogi, S., Bloomfield, S., Hogan, H. A., Fedarko, N., Thierry-Palmer, M., Ruiz, J., Bauss, F., & Shapiro, J. R. 2000. Disuse bone loss in hindquarter suspended rats: partial weightbearing, exercise and ibandronate treatment as countermeasures. J.Gravit.Physiol, 7(2): 13-14.

Seeman, E. & Delmas, P. D. 2001. Reconstructing the skeleton with intermittent parathyroid hormone. Trends Endocrinol.Metab , 12(7): 281-283.

Shackelford, L. C., LeBlanc, A. D., Driscoll, T. B., Evans, H. J., Rianon, N. J., Smith, S. M., Spector, E., Feeback, D. L., & Lai, D. 2004. Resistance exercise as a countermeasure to disuse-induced bone loss. J.Appl.Physiol, 97(1): 119-129.

Sharma, S. 2003. Hormone Replacement Therapy in menopause: current concerns and considerations. Kathmandu.Univ Med.J.(KUMJ.), 1(4): 288-293.

Sheng, M. H., Taper, L. J., Veit, H., Qian, H., Ritchey, S. J., & Lau, K. H. 2001. Dietary boron supplementation enhanced the action of estrogen, but not that of parathyroid hormone, to improve trabecular bone quality in ovariectomized rats. Biol.Trace Elem.Res., 82(1-3): 109-123.

Smith, B. J., King, J. B., Lucas, E. A., Akhter, M. P., Arjmandi, B. H., & Stoecker, B. J. 2002. Skeletal unloading and dietary copper depletion are detrimental to bone quality of mature rats. J.Nutr., 132(2): 190-196.

Smith, B. J., Lucas, E. A., Turner, R. T., Evans, G. L., Lerner, M. R., Brackett, D. J., Stoecker, B. J., & Arjmandi, B. H. 2005. Vitamin E provides protection for bone in mature hindlimb unloaded male rats. Calcif.Tissue Int., 76(4): 272-279.

Smith, S. M., Nillen, J. L., Leblanc, A., Lipton, A., Demers, L. M., Lane, H. W., & Leach, C. S. 1998. Collagen cross-link excretion during space flight and bed rest. J.Clin.Endocrinol.Metab, 83(10): 3584-3591.

Smith, S. M., Davis-Street, J. E., Fesperman, J. V., Calkins, D. S., Bawa, M., Macias, B. R., Meyer, R. S., & Hargens, A. R. 2003. Evaluation of treadmill exercise in a lower body negative pressure chamber as a countermeasure for weightlessness-induced bone loss: a bed rest study with identical twins. J.Bone Miner.Res., 18(12): 2223-2230.

Smith, S. M., Wastney, M. E., O'Brien, K. O., Morukov, B. V., Larina, I. M., Abrams, S. A., Davis-Street, J. E., Oganov, V., & Shackelford, L. C. 2005. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the mir space station. J.Bone Miner.Res., 20(2): 208-218.

Smith, S. M., Zwart, S. R., Block, G., Rice, B. L., & Davis-Street, J. E. 2005. The nutritional status of astronauts is altered after long-term space flight aboard the International Space Station. J.Nutr., 135(3): 437-443.

Soleas, G. J., Grass, L., Josephy, P. D., Goldberg, D. M., & Diamandis, E. P. 2002. A comparison of the anticarcinogenic properties of four red wine polyphenols. Clin.Biochem., 35(2): 119-124.

Soung, D. Y., Devareddy, L., Khalil, D. A., Hooshmand, S., Patade, A., Lucas, E. A., & Arjmandi, B. H. 2006. Soy affects trabecular microarchitecture and favorably alters select bone-specific gene expressions in a male rat model of osteoporosis. Calcif.Tissue Int., 78(6): 385-391.

South-Paul, J. E. 2001. Osteoporosis: part II. Nonpharmacologic and pharmacologic treatment. Am.Fam.Physician, 63(6): 1121-1128.

Srivastava, M. & Deal, C. 2002. Osteoporosis in elderly: prevention and treatment. Clin.Geriatr.Med., 18(3): 529-555.

Stacewicz-Sapuntzakis, M., Bowen, P. E., Hussain, E. A., Damayanti-Wood, B. I., & Farnsworth, N. R. 2001. Chemical composition and potential health effects of prunes: a functional food? Crit Rev.Food Sci.Nutr., 41(4): 251-286.

Takata, S. & Yasui, N. 2001. Disuse osteoporosis. J.Med.Invest, 48(3-4): 147-156.

Tanko, L. B., Mouritzen, U., Lehmann, H. J., Warming, L., Moelgaard, A., Christgau, S., Qvist, P., Baumann, M., Wieczorek, L., Hoyle, N., & Christiansen, C. 2003. Oral ibandronate: changes in markers of bone turnover during adequately dosed continuous and weekly therapy and during different suboptimally dosed treatment regimens. Bone, 32(6): 687-693.

Turken, S., Siris, E., Seldin, D., Flaster, E., Hyman, G., & Lindsay, R. 1989. Effects of tamoxifen on spinal bone density in women with breast cancer. J.Natl.Cancer Inst., 81(14): 1086-1088.

Turner, R. T., Evans, G. L., Cavolina, J. M., Halloran, B., & Morey-Holton, E. 1998. Programmed administration of parathyroid hormone increases bone formation and reduces bone loss in hindlimb-unloaded ovariectomized rats. Endocrinology, 139(10): 4086-4091.

Turner, R. T., Evans, G. L., Lotinun, S., Lapke, P. D., Iwaniec, U. T., & Morey-Holton, E. 2006. Dose response effects of intermittent parathyroid hormone on cancellous bone in hindlimb unloaded rats. J.Bone Miner.Res..

Turner, R. T., Lotinun, S., Hefferan, T. E., & Morey-Holton, E. 2006. Disuse in adult male rats attenuates the bone anabolic response to a therapeutic dose of parathyroid hormone. J.Appl.Physiol, 101(3): 881-886.

67

van der Wiel, H. E., Lips, P., Nauta, J., Kwakkel, G., Hazenberg, G., Netelenbos, J. C., & van der Vijgh, W. J. 1993. Intranasal calcitonin suppresses increased bone resorption during short-term immobilization: a double-blind study of the effects of intranasal calcitonin on biochemical parameters of bone turnover. J.Bone Miner.Res., 8(12): 1459-1465.

Vermeer, C., Wolf, J., Craciun, A. M., & Knapen, M. H. 1998. Bone markers during a 6-month space flight: effects of vitamin K supplementation. J.Gravit.Physiol, 5(2): 65-69.

Vico, L., Chappard, D., Alexandre, C., Palle, S., Minaire, P., Riffat, G., Morukov, B., & Rakhmanov, S. 1987. Effects of a 120 day period of bed-rest on bone mass and bone cell activities in man: attempts at countermeasure. Bone Miner., 2(5): 383-394.

Volpe, S. L., Taper, L. J., & Meacham, S. 1993. The relationship between boron and magnesium status and bone mineral density in the human: a review. Magnes.Res., 6(3): 291-296.

Wan, Y. M., Zhang, M. F., Cui, W., & Song, J. P. 2000. [Changes of femur minerals and serum BGP in hindlimb unloaded rats during convalescence]. Space Med.Med.Eng (Beijing), 13(4): 298-300.

Wang, L., Orhii, P. B., Banu, J., & Kalu, D. N. 2001. Effects of separate and combined therapy with growth hormone and parathyroid hormone on lumbar vertebral bone in aged ovariectomized osteopenic rats. Bone, 28(2): 202-207.

Wang, L., Orhii, P. B., Banu, J., & Kalu, D. N. 2001. Bone anabolic effects of separate and combined therapy with growth hormone and parathyroid hormone on femoral neck in aged ovariectomized osteopenic rats. Mech.Ageing Dev., 122(1): 89-104.

Watts, N. B. 2003. Bisphosphonate treatment of osteoporosis. Clin.Geriatr.Med., 19(2): 395-414.

Weinreb, M., Patael, H., Preisler, O., & Ben Shemen, S. 1997. Short-term healing kinetics of cortical and cancellous bone osteopenia induced by unloading during the reloading period in young rats. Virchows Arch., 431(6): 449-452.

Woolf, A. D. & Akesson, K. 2003. Preventing fractures in elderly people. BMJ, 327(7406): 89-95.

Wronski, T. J. & Morey, E. R. 1983. Effect of spaceflight on periosteal bone formation in rats. Am.J.Physiol, 244(3): R305-R309.

Xu, H., Watkins, B. A., & Seifert, M. F. 1995. Vitamin E stimulates trabecular bone formation and alters epiphyseal cartilage morphometry. Calcif.Tissue Int., 57(4): 293-300.

Yang, L. C., Majeska, R. J., Laudier, D. M., Mann, R., & Schaffler, M. B. 2005. High-dose risedronate treatment partially preserves cancellous bone mass and microarchitecture during long-term disuse. <u>Bone</u>, 37(3): 287-295.

Zerath, E. 1998. Effects of microgravity on bone and calcium homeostasis. <u>Adv.Space Res.</u>, 21(8-9): 1049-1058.

Zerwekh, J. E., Ruml, L. A., Gottschalk, F., & Pak, C. Y. 1998. The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. <u>J.Bone Miner.Res.</u>, 13(10): 1594-1601.

APPENDIX

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 1/13/2007

Date : Wednesday, January 14, 2004   Animal Care and Use Protocol (ACUP) No :   HE041

Proposal Title: Identification of bioactive component of dried plum involved in recovery of bone following
hindlimb unloading

Principal
Investigator:

Brenda Smith
Nutritional Sciences
423 HES
Campus

Reviewed and   Full Committee
Processed as:

Approval Status Recommended by Reviewer(s) :   Approved

Your protocol as revised is approved. You are approved for 60 rats for the next 3 years.

Signatures :

_____   Wednesday, January 14, 2004
Kent Olson, IACUC Chairperson   Date

cc: Department Head, Nutritional Sciences
     LAR

**Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications
to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to
initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to
monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance
document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.**

VITA

Ashley Lynn Ethriedge

Candidate for the Degree of

Master of Science

Thesis: DRIED PLUM ENHANCES RECOVERY OF BONE FOLLOWING
          HINDLIMB UNLOADING IN FEMALE RATS

Major Field: Nutritional Sciences

Biographical:

   Personal Data: Born in Stillwater, Oklahoma on June 10, 1980, the daughter of
          John and Audrey Ethriedge

   Education: Graduated from Memorial High School, Tulsa, Oklahoma in May
          1998; received Bachelor of Science degree in Dietetics from Oklahoma
          State University, Stillwater, Oklahoma in May 2002. Completed the
          requirements for the Master of Science degree with a major in Nutritional
          Sciences at Oklahoma State University in December 2006.

   Experience: Employed by Oklahoma State University, Department of Nutritional
          Sciences as a teaching assistant and graduate research assistant; Oklahoma
          State University, Department of Nutritional Sciences, 2002 to 2003.
          Employed by Hillcrest Medical Center as a Clinical Dietitian; Hillcrest
          Medical Center, 2004 to 2005. Employed by Oklahoma University Health
          Sciences Center, Section of Genetics as a Metabolic Dietitian; Oklahoma
          University Health Sciences Center, 2005 to current.

   Professional Memberships: Genetic Metabolic Dietitians International

Name: Ashley Lynn Ethriedge   Date of Degree: December, 2006

Institution: Oklahoma State University   Location: Stillwater, Oklahoma

Title of Study: DRIED PLUM ENHANCES RECOVERY OF BONE FOLLOWING
                 HINDLIMB UNLOADING IN FEMALE RATS.

Pages in Study: 71   Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Methods: Recent studies have shown dried plum to prevent and reverse bone loss in animal models of osteoporosis. The objectives of this study were to determine if dried plums could enhance bone recovery following hindlimb unloading (HLU); to determine the most effective dose (5%, 15%, or 25%; w/w) of dried plum; and to determine how the effects of dried plum on bone compare to the anabolic effects of parathyroid hormone (PTH). Six month old female virgin Sprague-Dawley rats were either HLU (HLU=6 groups of 8-12 rats) to induce bone loss or remained ambulatory (AMB=2 groups of 8-12 rats) for 3 weeks. Following the HLU period, two groups (i.e. one HLU and one AMB) were necropsied and bone evaluated to characterize the bone loss. The remaining groups were randomly assigned to the following treatments for 90 days: standard semi-purified AIN (control) diet with either LD=5%, MD=15% or HD=25% (w/w) dried plum added or control diet plus parathyroid hormone administration (80 µg PTH/kg bw; 3 x wk Bachem, Inc).

Findings and Conclusions: Tibial bone mineral content (BMC) and density (BMD) were enhanced significantly by the HD compared to animals on the control diet, although, not to the level of the PTH group. Compared to controls, recovery of vertebral BMD was significantly greater in the MD and HD groups, and the HD group was comparable to PTH. Evaluation of the distal femur using micro-computed tomography (Scanco Medical µCT 40) indicated that bone volume (BV/TV) in the HD group was similar to the PTH group and significantly higher than the control group. Trabecular thickness (TbTh) of the vertebra increased in the MD and HD dried plum groups, although not to the level of PTH. Trabecular separation (TbSp) and number (TbN) in the distal femur and TbN within the vertebra were unchanged by both dried plum and PTH. Bone formation as indicated by serum alkaline phosphatase (ALP) was significantly higher in the HD group, but bone resorption was unaltered. These findings indicate that bone recovery following HLU is enhanced by dried plum up-regulating bone formation and has somewhat similar effects to PTH.

                                        Dr. Brenda Smith
          Advisor's Approval: _____