DOSE-DEPENDENT EFFECTS OF VITAMIN E ON BONE IN AN ORCHIDECTOMIZED RAT MODEL OF OSTEOPOROSIS

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NOMENCLATURE

1,25[OH]2D	1, 25-dihydroxyvitamin D
2D	2-dimensional
3D	3-dimensional
ALP	Alkaline Phosphatase
BMA	Bone Mineral Area
BMC	Bone Mineral Content
BMD	Bone Mineral Density
CTx	C-telopeptide of collagen cross-links
DXA	Dual Energy X-Ray Absorptiometry
DHT	Dihydrotestosterone
Dpd	Deoxypyridinoline
FDA	Food and Drug Administration
FE	Finite Element
HU	Hindlimb Unloaded
ICTP	Cross-linked C-telopeptide of type-I collagen
IGF-I	Insulin Like Growth Factor-I
IL-1	Interleukin-1
IL-6	Interleukin-6
Micro-CT	Micro-Computerized Tomography

NTx	N-telopeptide of collagen cross-links
OC	Osteocalcin
ORX	Orchidectomized
PICP	Carboxy-terminal propeptide of type-1 collagen
PTH	Parathyroid Hormone
Sham	Sham-operated
TNF-Alpha	Tumor Necrosis Factor-Alpha
TRT	Testosterone Replacement Therapy

CHAPTER I

INTRODUCTION

Research Problem

Osteoporosis is a disease which is characterized by low bone mass and impaired trabecular structures, making bone weaker and susceptible to fractures (National Osteoporosis Foundation, 2004). Osteoporosis has received increasing attention as a major public health threat due to the fact that in the United States, an estimated 10 million individuals suffer from osteoporosis and about 34 million individuals tend to have low bone mass placing them at high risk (National Osteoporosis Foundation, 2004). The health care expenditure for osteoporosis was $17 billion in 2001 (National Osteoporosis Foundation, 2004), and the amount of expenditure is rising rapidly due to the increased elderly population and longevity (National Osteoporosis Foundation, 2004; Seeman, 2001; Gullberg et al., 1997).

The spotlight has always been on women and osteoporosis; however, an estimated 2 million American men also suffer from osteoporosis and another 12 million are at risk of developing osteoporosis (National Osteoporosis Foundation, 2004). While researchers and pharmaceuticals companies are focusing and investing billions of dollars and tremendous efforts in order to develop therapies for preventing bone loss in women, male osteoporosis remains inadequately researched (Bilezikian, 1999).

The pathogenesis of male osteoporosis, in part, may be due to decreased calcium absorption (National Institutes of Health, 2003) and bone formation (Francis, 2000). Moreover, age-related declines in levels of sex hormones, insulin-like growth factor 1, and growth hormone are believed to contribute to bone loss in men (Francis, 2000; Bilezikian, 1999). Hypogonadism, e.g. low serum testosterone level, is associated with 20% of vertebral fractures (Bailie et al., 1992) and 50% of hip fractures in men (Stanley et al., 1991; Jackson and Spiekerman, 1989).

There are limited treatment options for male osteoporosis. Alendronate (National Institutes of Health, 2003; Romagnolo et al., 2003) and parathyroid hormone (PTH) (National Institutes of Health, 2003) are the only medications approved by the Food and Drug Administration (FDA) for the treatment of osteoporosis in men. However, studies have shown that alendronate can contribute to side effects, such as abdominal pain, bone and joint pain, constipation, diarrhea, indigestion, muscle pain and nausea, more often in men than in women (Makins and Ballinger, 2003). Moreover, the cost of PTH is unaffordable if used long term (Deal and Gideon, 2003). Other treatment such as testosterone replacement therapy (TRT) (Romagnolo et al., 2003), is only recommended for men with hypogonadism. However, TRT treatment has negative side effects such as cancer, sleep apnea, and erthrocytosis (Tan and Salazar, 2004; Slater and Oliver, 2000).

Ideally, it would be beneficial to offer a natural supplement therapy that could be easily incorporated into the diet, which can also reduce the incidence and risk of osteoporosis in men.

Vitamin E, a potent antioxidant (Crary and McCarty, 1984), has osteoprotective effects. Vitamin E has been shown to suppress the production of certain cytokines such as

interleukin (IL)-1, IL-6 and tumor necrosis factor-alpha (TNF-α) (Jialal et al., 2001; Beharka et al., 2000; van Tits et al., 2000; Wang et al., 1994) that have been linked to increased bone loss (Jilka et al., 1996; Jilka et al., 1995; Manolagas and Jilka, 1995). Vitamin E has also been found to protect bone cells from oxidative damage as a result of lipid peroxidation (Xu et al., 1995). Arjmandi and colleagues (Arjmandi et al., 2002) also demonstrated that vitamin E improved bone quality in the aged but not in young adult male mice. A recent study by Smith et al. (Smith et al., 2005) also showed the protective effect of high dose vitamin E on the bones of hindlimb unloaded male rats by preventing the loss of trabecular number (Th.N) and bone surface normalized to tissue volume (BS/TV). If vitamin E could be shown to effectively reverse bone loss in a rat model of male osteoporosis, it would provide a relatively inexpensive alternative therapy for male osteoporosis.

The **hypothesis** of this study was that vitamin E, in a dose-dependent manner, reverses bone loss in an orchidectomized rat model of male osteoporosis. To test this hypothesis, there are three specific aims:

Specific Aim 1: To examine the dose-dependent effect of vitamin E on reducing bone loss and maintaining bone quality in an orchidectomized rat model of male osteoporosis. Bone quality was assessed by measuring bone mineral area (BMA), bone mineral density (BMD), and bone mineral content (BMC) using dual energy x-ray absorptiometry (DXA).

Specific Aim 2: To assess the dose-dependent effect of vitamin E on bone turnover in an orchidectomized rat model of male osteoporosis. Urinary excretion of

deoxpyridiniline (Dpd), a specific marker of bone resorption, and serum osteocalcin (OC), a marker of bone formation, were assessed.

Specific Aim 3: To evaluate the dose-dependent effect of vitamin E on trabecular and cortical microarchitectural properties in an orchidectomized rat model of male osteoporosis. Trabecular and cortical bone structures were evaluated using micro-computerized tomography (micro-CT).

CHAPTER II

REVIEW OF LITERATURE

Prevalence and Incidence of Osteoporosis in Men

Osteoporosis is a disease characterized by low bone mass and impaired trabecular structures, making bone weaker and susceptible to fractures (National Osteoporosis Foundation, 2004). Currently, approximately 2 million men suffer from osteoporosis and another 12 million men are at risk of developing osteoporosis in the United States (National Osteoporosis Foundation, 2004). Based on the National Osteoporosis Foundation, the total number of men diagnosed with osteoporosis and those at risk of developing osteoporosis is expected to increase to over 17 million by 2010 and to over 20 million by 2020 (National Osteoporosis Foundation, 2004).

The prevalence of male osteoporosis in non-Hispanic white and Asian at the age of 50 years is 7%, in non-Hispanic black is 4% and in Hispanic is 3%. In addition, the prevalence of osteopenia in non-Hispanic white and Asian at the age of 50 years is 35%, in non-Hispanic black is 19% and in Hispanic is 23% (National Osteoporosis Foundation, 2004). In fact, about 30% of hip fractures occur in men (Amin and Felson, 2001; Seeman, 2001) and one in eight men will have an osteoporotic fracture at the age of 50 years (Campion and Maricic, 2003). Furthermore, since men tend to have greater peak bone mass and size to provide a greater protection against fracture (Seeman, 2001), they

get bone fractures 10 years later than the women (Amin and Felson, 2001). However, hip fracture mortality in men one year after incidence is 31% compared to only 17% in women (Amin and Felson, 2001).

Risk Factors for Osteoporosis in Men

The development of osteoporosis in men is primarily related to aging (i.e., bone loss due to increase in age), ethnicity (i.e., non-Hispanic white has higher risk of osteoporosis than other races), and genetic factors (Conde and Aronson, 2003). However, statistics show that 30 to 60 percent of osteoporosis in men is associated with one or more of the secondary risks factors (Klibanski et al., 2000).

The most common secondary risk factors for male osteoporosis are excessive use of glucocorticoid, hypogonadism, smoking, and alcohol over consumption. Glucocorticoids are steroids that are widely used by the elderly to treat inflammatory diseases (Rackoff and Rosen, 1998). Studies have shown that long-term glucocorticoid therapy is associated with bone loss due to decreases in osteoblast proliferation and biosynthetic activity, as well as increase in bone resorption (Canalis et al., 2003), sex-steroid deficiency, decreased intestinal calcium absorption and secondary hyperparathyroidism (Lafague-Proust et al., 2003). A clinical study conducted by Dykman et al. (1985) on 161 ambulatory patients, who had rheumatic disease and received long-term treatment with prednisone, showed that patients with cumulative prednisone dose of less than 10 g, 10 to 30 g, and over 30 g had 23%, 40% and 78% incidence of osteopenia, respectively. These patients also developed 22%, 33% and 53% incidence of fracture, respectively (Dykman et al., 1985). LoCascio et al. (1990) also

showed that patients receiving glucocorticoid therapy for 5 to 7 months or an average of 5 years developed an estimate 20% or 63% of trabecular volume bone loss. The findings of these two studies (LoCascio et al., 1990; Dykman et al., 1985) supported the theory of Wolinsky-Friedland (Wolinsky-Friedland, 1995) that glucocortocoid-induced bone loss is associated with total cumulative dose of glucocorticoid and the duration of such therapy.

Hypogonadism is defined as the reduction or absence of secretion of hormones from the sex glands (National Institutes of Health, 2003). In fact, hypogonadism is found in an estimated 30% of men with vertebral fractures (National Institutes of Health, 2003), and 50% of men with hip fractures (Francis, 1999). Francis et al. (1986) investigated the pathogenesis of osteoporosis in men and found that hypogonadism-induced bone loss is due to the reduction of plasma 1, 25-dihydroxyvitamin D (1,25[OH]2D), estrogen and calcitonin which lead to malabsorption of calcium and reduced bone formation. They also concluded that hypogonadism causes both cortical and trabecular loss and alters trabecular architecture (Francis et al., 1986).

Smoking is considered one of the risk factors for osteoporosis, although its mechanism of action on bone remains in question (National Osteoporosis Foundation, 2004; National Institutes of Health, 2003). However, the findings of several studies have (Tanaka et al., 2005; Walker et al., 2001; Fang et al., 1991) suggested that nicotine and other chemicals found in cigarettes may cause bone loss due to their direct toxic effects on osteoblast cells, the cells that stimulate bone formation, or the inhibition of calcium absorption. A study showed that smoking was associated with a reduction in BMD, which can lead to fracture (Nelson et al., 2002). A study conducted by Kanis et al. (2005) from ten prospective cohorts found that the smoking history was associated with a

significantly increased risk of fractures compared to individuals with no history of smoking. In addition, the risk ratios of fractures were also significantly higher in men than in women, except the hip fracture.

Excessive alcohol intake is another well-recognized cause of secondary osteoporosis in men (National Osteoporosis Foundation, 2004; National Institutes of Health, 2003). Excessive alcohol intake can result in testosterone deficiency in men and women (Isidori and Lenzi, 2005; Kim et al., 2003). Studies (Kasperk, 1997; Francis et al., 1986) have demonstrated that low testosterone levels are linked to decreased activity of osteoblasts and calcium absorption. Moreover, alcoholics have also been shown to have a high level of cortisol (Kim et al., 2004) which has been linked to decreased bone formation and increased bone resorption (Medras and Jankowska, 2000). Several studies have demonstrated that excessive consumption of alcohol is associated with the increased risk of fractures, especially in hip (Hoidrup et al., 1999; Fujiwara et al., 1997; Felson et al., 1988). Increased incidence of osteoporosis due to excessive intake of alcohol is more obvious in men than in women (Hoidrup et al., 1999; Felson et al., 1988).

Treatment Options for Male Osteoporosis

Limited medical treatment options are available for male osteoporosis. Bisphosphonates that include alendronate, risedronate and etidronate are medications that inhibit osteoclast-mediated bone resorption and prevent bone loss (Ebeling, 2004; Mathoo et al., 2004; McClung, 2003). Among these medications, alendronate and risedronate have been approved by the FDA as treatment options for glucocortocoid-

induced osteoporosis (National Osteoporosis Foundation, 2004; National Institutes of Health, 2003).

Orwoll et al. (2000) conducted a two-year placebo-controlled trial study on 241 men with osteoporosis. Patients treated with 10 mg alendronate/day had increased BMD in the lumbar spine and the femoral neck by 7.1% and 2.5%, respectively, compared to the 1.8% and 0.1% increase in those patients treated with placebo. Ringe et al. (2001) also demonstrated from a two-year prospective study on 134 men with osteoporosis that patients treated with 10 mg alendronate/day increased their BMD of the lumbar spine by 10.1% and the femoral neck by 5.2%. However, patients treated with alfacalcidol at 1 μg/day only showed an increased BMD in the lumbar spine by 2.8% and the femoral neck by 2.2% (Ringe et al., 2001). These two studies clearly documented that daily oral intake of 10 mg alendronate is an effective treatment for osteoporosis in men.

Reid et al. (2001) conducted placebo-controlled study to demonstrate the positive effect of risedronate. Risedronate at a dose of 5 mg/day was found to significantly increase the BMD of the lumbar spine, femoral neck, and femoral trochanter by 4.8%, 2.1% and 2.6%, respectively. However, the BMD decreased significantly by 3.4%, 3.3%, and 3.4% in the lumbar spine, femoral neck, and trochanter, respectively for individuals in the placebo group. Therefore, daily intake of risedronate increased bone density and decreased vertebral fracture risk in men receiving corticosteroid therapy (Reid et al., 2001).

Although intakes of aledronate and risedronate were shown to significantly reduce the risk of vertebral fracture, such treatments can contribute to common side effects e.g.

abdominal pain, bone and joint pain, constipation, diarrhea, indigestion, muscle pain and nausea more often in men than in women (Makins and Ballinger, 2003).

Serum levels testosterone decline gradually and progressively with aging in men. However, such hypogonadism-induced osteoporosis in men can be improved by TRT treatment (Kaufman et al., 2000). Francis et al. (1986) reported that hypogonadism patients treated with testosterone had significant increases in total and free plasma (1,25[OH]2D), and an improved calcium absorption which can potentially lead to reduction on bone resorption. Katznelson et al. (1996) examined the effect of TRT treatment on bone structure in 29 hypogonadal men. Such treatment at a dose of 100 mg/week for 6 to 18 months increased spinal and trabecular BMD by 5% and 14%, respectively. Kenny et al. (2001) in another study showed that treatment with testosterone at 5 mg/day prevented bone loss at the femoral neck compared to the control group that had a 1.6% decrease in femoral neck BMD. However, Snyder et al. (1999) noticed increases of BMD values in both the testosterone treated and placebo groups in a three-year clinical study, although the increase was not significantly greater in subjects treated with testosterone.

Although TRT is shown to have a positive effect against bone loss and vertebral fracture, it also has many side effects in men. The most alarming risk associated with TRT is prostate cancer development. Holmang et al. (1993) reported that patients treated with testosterone at 160 mg/day for 8 months had increased mean prostate volume by 12%. Other disorders that may accompany TRT in men include sleep apnea (Snyder et al., 1999) and erthrocytosis (Clague et al., 1999; Snyder et al., 1999; Jockenhovel et al., 1997; Sih et al., 1997; Drinka et al., 1995). In most studies, between 6% and 25% of the

treatment subjects developed elevated hematocrit readings ranging from 2.5% to 5% over the baseline value (Ferrando et al., 2002; Clague et al., 1999; Synder et al., 1999; Tenover, 1992). Therefore, men with prostate cancer should not take testosterone replacement therapy (Morales, 2005). TRT cannot be recommended for older men with normal or low normal testosterone levels, either, although it may be warranted in older men with mildly or markedly decreased testosterone levels (Gruenewald and Matsumoto, 2003). All men considering testosterone replacement therapy should undergo a thorough prostate cancer screening prior to starting this therapy and consider the long-term safety of the treatment.

Recently, teriparatide (recombinant human PTH 1-34) has been approved by FDA for the treatment of osteoporosis in men (National Osteoporosis Foundation, 2004). PTH is the major regulator of calcium and phosphate metabolism, mainly through activation of the PTH/PTHrP receptor (Hodsman et al., 2005). Several studies (Dempster et al., 2001a; Parisien et al., 1990; Eriksen et al., 1986) have shown that PTH is responsible for increased rate of bone turnover. An 18 month placebo-controlled trial done by Kurland et al. (2000) on 23 men with idiopathic osteoporosis had demonstrated that the BMD of the lumbar spine and femoral neck of patients treated with 400 IU PTH were increased by 13.5% and 2.9%, respectively. Another 11 month clinical trial done by Orwoll et al. (2003) on 437 men had also demonstrated that patients treated with 20 µg and 40 µg of teriparatide significantly increased the BMD of lumbar spine by 5.9% and 9.5%, respectively. Teriparatide treatment has not only been shown to increase lumbar spine BMD, but also to increase trabecular connectivity of iliac crest as assessed by micro-computed tomography (Dempster et al., 2001b).

However, PTH should only be considered as treatment for men with high risk of fracture and severe osteoporosis due to the high cost of treatment, which is approximately $600 per month; drug delivery method administered by injection (Deal and Gideon, 2003); and the development of osteosarcoma (Orwoll et al., 2003; Neer et al., 2001; Kurlan et al., 2000). Several teriparatide trials were terminated prematurely because of the findings of induced osteosarcoma in an ongoing carcinogenicity study in rats (Orwoll et al., 2003; Neer et al., 2001; Kurlan et al., 2000). Others undesirable adverse effects such as marrow fibrosis, tunneling resorption, nausea, and headache have also been encountered in clinical trials (Orwoll et al., 2003).

Biochemical Markers of Bone Metabolism

Bone formation markers are the direct or indirect expression products of active osteoblasts during different phases of osteoblast development and reflecting different aspects of osteoblast function and bone formation. For clinical purposes, biomakers of bone formation can be assessed using bone-specific alkaline phosphatase (ALP), osteocalcin (OC), carboxy-terminal propeptide of type-1 collagen (PICP) and amino-terminal propeptide of type-1 collagen (PINP). However, the majority of bone resorption biomarkers are degradation products of bone collagen. Biomarkers of bone resorption can be assessed by measuring urinary hydroxyproline, free and total pyridinolines (Pyd), free and total deoxypyridinolines (Dpd), N-telopeptide of collagen cross-links (NTx), and serum C-telopeptide of collagen cross-links (CTx), cross-linked C-telopeptide of type-I collagen (ICTP), and tartrate-resistant acid phosphatase (TRAP).

Several studies have examined the correlation between biochemical markers of bone turnover and bone mineral density. A study done by Kenny et al. (1998) showed that NTx and CTx, the biomarkers of bone resorption, have negative correlation with BMD of the whole body, femur, and spine (Kenny et al., 1998). Krall et al. (1997) also demonstrated that serum OC and urinary NTx had negative correlation with BMD value of all skeletal sites except spine. Additionally, Khosla et al. (1998), and Szulc et al. (2001) suggested that biochemical markers are appropriate tools to assess bone metabolism and to predict the status of current osteoporosis in men.

Biochemical markers can also be used to predict fracture for osteoporotic men. Studies done by Luukinen et al. (2000) in 300 men of over 70 year old and Resch et al. (1992) in 27 men with spinal fracture, reported that PICP (Luukinen et al., 2000) and ALP (Resch et al., 1992), tend to be lower in men with osteoporotic related fractures. Ohishi et al. (2000) also reported that ICTP, a specific bone resorption marker, was positively correlated to fracture incidence.

Micro-computed Tomography (micro-CT)

The common clinical approach for osteoporosis diagnosis today is to measure BMD using DXA. To understand the mechanical strength of a bone structure and the evolution of age-related progression of the disease, it is necessary to further study the bone structure using a method such as the 3-dimensional (3D) micro-CT. The use of micro-CT allows the 3D assessment of structural characteristics of trabecular and improves our ability to understand the pathophysiology of osteoporosis; to test the efficacy of pharmaceutical intervention; and to estimate bone biomechanical properties.

The technique used to determine the microstructure can only provide 2-dimensional (2D) histomorphometric information. However, the 3D micro-CT will allow for nondestructive 3-dimension evaluation of bone structures. Moreover, the structure can be quantitatively evaluated with various parameters including the orientation, connectivity, and shape of trabecular.

Barou et al. (2002) completed a study to determine bone loss and changes in trabecular architecture on a rat model of disuse osteoporosis by using a 3D micro-CT, and also to compare the results with those by using DEXA and bone histomorphometry for bone mass. The results showed that DEXA and 3D micro-CT detected bone loss earlier than standard bone histomorpometry. In addition, all bone mass and architectural parameters measured with these three techniques correlated significantly except the trabecular thickness. Another study conducted by Muller et al. (1998) on 63 cylindrial human transiliac bone specimens also showed highly significant correlations between the 2D conventional histology and 3D micro-CT method. The findings of these two studies (Barou et al., 2002; Muller et al., 1998) confirmed that the 3D micro-CT is a nondestructive, fast, and very precise procedure that allows for the measurement of cancellous and compact bone in unprocessed biopsies or small bones, as well as a fully automatic determination of 3D morphometric indices.

The structure model type and trabecular thickness are two important characteristics that describe cancellous bone architecture. The structure model type can be assessed by calculating the structure model index (SMI). The SMI is calculated by means of the 3D image analysis based on a differential analysis of the triangulated bone surface. It has been described that trabecular structure changes radically from plate-like

to rod like during aging (Grote et al., 1995; Vogel et al., 1993) or remodeling (Kinne et al., 1995). For an ideal plate and rod structure the SMI value is 0 and 3, respectively (David et al., 2003). Ding and colleague (2000) evaluated age-related changes in the structure model type in human tibial cancellous bone. The results showed that SMI was significantly higher in old-age group compared with middle- and young-age groups. This finding supported the theory of Grote et al. (1995) and Vogel et al. (1993) that the structure model type changed towards more rod-like in the elderly.

McCalden et al. (1997) and Dempster et al. (1993) showed that human trabecular thickness decreased with age for femoral and lumbar cancellous bone. However, trabecular thickness did not decrease with age in iliac cancellous bone (Dempster et al., 1993) or vertical trabeculae from vertebral cancellous bone (Bergot et al., 1988).

Orchidectomized (Orx) Rat Model of Male Osteoporosis

The Orx adult male rat model for studying bone has been widely used (Lerouxel et al., 2004; Audran et al., 2001; Libouban et al., 2001). It is well established that androgens withdrawal induced by Orx results in decreased bone mass in experimental animals (Vanderschueren et al., 1993; Wink and Felts, 1980) and also in humans (Daniell, 1997; Stepan et al., 1989). Androgen deficiency is associated with accelerated bone turnover and imbalance between bone resorption and bone formation, which results in bone loss (Libouban et al., 2002; Vanderschueren et al., 1993; Wink and Felts, 1980). Several studies also reported that Orx results in decreased BMC (Morear et al., 2001), BMD (Gunness and Orwoll, 1995), bone strength (Danielsen et al., 1992), whole body weight and lean body mass (Morear et al., 2001).

Hypogonadism is one of the risk factors for male osteoporosis. Hypogonadal men with lower BMD (Devogelaer et al., 1992; Finkelstein et al., 1987), lean body mass (Wang et al., 2004) and higher bone turnover (Szulc et al., 2003) had also been reported. The Orx model has been characterized as a representative model for bone studies of androgen replacement in hypogonal men (Venken et al., 2005; Vanderschueren et al., 2000). Moreau et al. (2001) measured whole body weight, lean and fat mass, whole BMC in the Orx rat model by DXA and showed that, except for the fat mass, the other three parameters had significantly decreased in Orx rats. Vanderschueren et al. (2000) conducted a study to evaluate the effects of androgen replacement on body composition and bone in an aged male Orx rat model. They found that Orx rats had significantly lower BMC and BMD than the control animals. These decreases in BMC and BMD were prevented by testosterone administration. As to the body composition measurements, Orx induced a decrease in lean body mass, but not in body weight and fat mass.

Erben et al. (2000) investigated the effects of androgen deficiency on bone and hormonal status in aged Orx rats for nine months. The results showed that Orx induced a decrease in serum testosterone and estradiol. Gill et al. (1998) also demonstrated Orx caused fall in serum testosterone levels by 80% in male rats. Such reduction in testosterone levels could be prevented by testosterone replacement therapy (Gill et al., 1998). Wakley et al. (1991) demonstrated that the dosing of testosterone at 10 mg per day could result in the prevention of bone loss in Orx rats. Vanderput et al. (2002) also showed that trabecular bone loss in aged Orx rats was prevented by administering estradiol and 5 α dihydrotestosterone (DHT) for four months. These finding suggested

that the Orx adult male rats can serve as a model to examine the effects of both testosterone and estrogen deficiency on bone structure and metabolism.

Previous Studies of Vitamin E and Bone

Vitamin E is a free-radical scavenger with anti-inflammatory properties (Crary and McCarty, 1984). Vitamin E has been shown to suppress the production of certain cytokines such as IL-1, IL-6, and TNF-α (Jialal et al., 2001; Beharka et al., 2000; van Tits et al., 2000; Wang et al., 1994) that are linked to increased bone loss (Jilka et al., 1996; Jilka et al., 1995; Manolagas and Jilka, 1995). Xu et al. (1995) showed that vitamin E protects bone cells in chick cartilage from damages caused by lipid peroxidation, and helps maintain normal bone modeling. They also suggested that the supplemental vitamin E increases bone mass by lowering the concentration of free radicals that would stimulate bone resorption. Arjmandi et al. (2002) reported that vitamin E supplementation at 500 mg/kg diet enhanced the synthesis of bone matrix proteins in old mice as evidenced by higher levels of osteocalcin mRNA, a bone matrix protein, and IGF-I, an important local regulator of bone metabolism. Furthermore, vitamin E was demonstrated to have a pronounced effect on the bones of aged male mice by increasing the mRNA level of type-1 alpha-collagen, a predominant bone matrix protein, and total protein content (Arjmandi et al., 2002). A recent study by Smith et al. (2005) investigated the effects of three different doses of vitamin E (15, 75, and 500 IU/kg diet) on bones in hindlimb unloaded (HU) and normal loading (AMB) male rats. The HU animals fed 15 IU vitamin E diet and the AMB animals fed 500 IU vitamin E had decreased bone surface normalized to tissue volume (BS/TV) and trabecular number

(Tb.N). However, both 75 IU and 500 IU vitamin E had protective effects on bones in HU animals by preventing the loss of trabecular number (Tb.N) and bone surface normalized to tissue volume (BS/TV).

These findings suggest that vitamin E protects bone integrity with its antioxidant properties. However, no study has determined if vitamin E supplementation can reverse bone loss in men or animal models of male osteoporosis. Male osteoporosis is a major public health concern which has hardly been investigated with limited treatment options. If it is found that vitamin E could effectively reverse bone loss in a rat model of male osteoporosis, it can potentially provide a relatively inexpensive alternative therapy for osteoporosis.

CHAPTER III

RESEARCH DESIGN AND METHODS

Animals and Diets

Forty 12-month old male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed and kept in an environmentally controlled laboratory (the Oklahoma State University Laboratory for Animal Research, Stillwater). After three days of acclimation, the rats were either sham-operated (Sham; 1 group) or orchidectomized (Orx; 3 groups) with 10 rats in each group. Initially after the surgery, all rats were fed an AIN-93M casein-based control diet (Harlan, Madison, WI) for 120 days to establish bone loss in these animals. One hundred-twenty days from the date that bone loss was established, the Sham group and one Orx group, which served as the control group, received 75 IU supplemental vitamin E per kg diet. The remaining two Orx groups received diets containing either 250 or 500 IU supplemental vitamin E per kg diet (Table 1) for 90 days. The rats were pair-fed to the mean food intake of the group that was consuming the least but all rats had free access to deionized water. Food intake was determined every three days and body weight was determined every week. These rats were fed for 90 days and then sacrificed at the end of the treatment.

Collection of Urine and Blood Samples

At the end of the 90 day treatment period, rats were fasted and placed in metabolic cage. Urine was collected in acid-washed tube for 12 hours. Prior to necropsy, rats were anesthetized with a mixture of ketamine and xylazine at 70 mg and 3 mg/kg body weight, respectively. After whole body DXA (QDR-4500A Elite, Hologic Waltham, MA) scans were performed, the animals were bled from their abdominal aortas for blood sample collection. Urine and serum were centrifuged at 4000 rpm for 20 minutes at 4 ^{o}C, aliquotted and stored at -20 ^{o}C until analysis.

Serum and Urine Biomarkers of Bone Metabolism

Bone biochemical markers that were measured included urinary excretion of Dpd, a specific marker of bone resorption, and serum OC as a marker of bone formation. Dpd was measured by utilizing a competitive enzyme immunoassay in a microtiter stripwell format (Metra DPD EIA Kit, Quidel Corporation, CA) and a microplate reader (ELx808 Ultra Microplate Reader, Bio-Tek Instrument Inc, VT). Urinary creatinine concentration was determined colorimetrically by utilizing ACE Clinical Analyzer (Alfa Wassermann, West Caldwell, NJ). Serum OC was assessed by utilizing two site immunoradiometric assay (IRMA) (Immunotropics, Inc., San Clemente, CA).

Bone Density Assessment

Whole body scanning was conducted on under anesthetized rats by using DXA before the first surgery and 120 days after the first surgery to confirm bone loss. 120 days after bone loss was established, whole body BMC and BMD were assessed prior to

necropsy. After necropsy, the fourth lumbar vertebrae and right femurs were scanned to determine BMC, BMD and BMA for final evaluation.

Assessment of the Trabecular and Cortical Bone Structures

The microarchitectural trabecular and cortical bone structures in distal femur and femoral midshaft were evaluated using micro-CT (Micro-CT40, Scanco Medical, Switzerland). The distal femur was scanned from the growth plate in the proximal direction, beginning and ending at 330 slices with a thickness of about 16μm/slice. The trabecular bone morphometric parameters assessed with the micro-CT included the bone volume over total volume (BV/TV), trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular thickness (Tb.Th). Non-metric parameters included structural model index (SMI), and connectivity density (Conn.D). To analyze cortical bone volume, thickness (Co.Th), porosity (Co.P) and medullary area (M.Area), contours were also placed on 34 slices (480 μm) in the midshaft region from the growth plate.

Biomechanical Testing of Distal Femur Using Finite Element Analysis

Three-dimensional finite element (FE) analysis is one of the best ways to assess stress and strain distribution in trabecular bone structures (Cattaneo et al., 2001; Muller and Ruegsegger, 1995). The distal femur was scanned using mirco-CT to determine 3D geometry, the apparent density and the elastic properties using FE analysis (Shefelbine et al., 2005). The FE modeling and calculations were performed in order to determine the mechanical properties of the bone specimen such as the average strain, total force,

physiological force, stiffness of the trabecular cores, size of independent stiffness, and average von miss stress (MPa).

Statistical Analysis

Analysis of variance (ANOVA) was conducted using SAS Version 8.2 (SAS Institute, Cary, NC) with PROC GLM MIXED to determine the treatment effects. Data analysis involved computation of mean and standard error for each the treatment group. If post hoc analysis showed statistical significance, Fisher's least square means separation test was used to determine and compare the significant differences among the means of various treatment groups. In all statistical comparisons, differences with $P < 0.05$ were considered significant.

Table 1: Composition of the experimental diets

	Sham	Orx		
Vitamin E *(IU/kg diet)*	**75**	**75**	**250**	**500**
Ingredients *(g/kg mix)*				
Corn Starch	466	466	466	466
Casein	140	140	140	140
Dextrinized Corn Starch	155	155	155	155
Sucrose	100	100	100	100
Soybean Oil	40	40	40	40
Fiber	50	50	50	50
Mineral Mix‡	35	35	35	35
Vitamin Mix†	10	10	10	10
Additional Vitamin E	---	---	0.117	0.283
I-Cystine	1.80	1.80	1.80	1.80
Choline Bitartrate	2.50	2.50	2.50	2.50
Tert-butylhydroquinone	0.008	0.008	0.008	0.008

The composition of these experimental diets was based on the AIN-93M (Harlan Teklad; Madison, WI). Vitamin E levels were adjusted using alpha-tocopherol.

†The vitamin mixture (TD #94047) obtained from Harlan Teklad (Madison, WI) consisted of (g/kg): nicotinic acid, 3.0; calcium pantothenate, 1.6; pyridoxine HCl, 0.7; thiamin HCl, 0.6; riboflavin, 0.6; folic acid, 0.2; D-biotin, 0.02; vitamin B-12 (0.1% in mannitol), 2.5; DL-α-tocopheryl acetate (500 IU/g), 15; vitamin A palmitate (500,000 IU/g), 0.8; cholecalciferol (500,000 IU/g), 0.2; phylloquinone, 0.075; and sucrose, 974.705.

‡The mineral mixture (TD #79055) obtained from Harlan Teklad (Madison, WI) was a modification of the AIN 76 lacking calcium and phosphorus but with sucrose as a diluent.

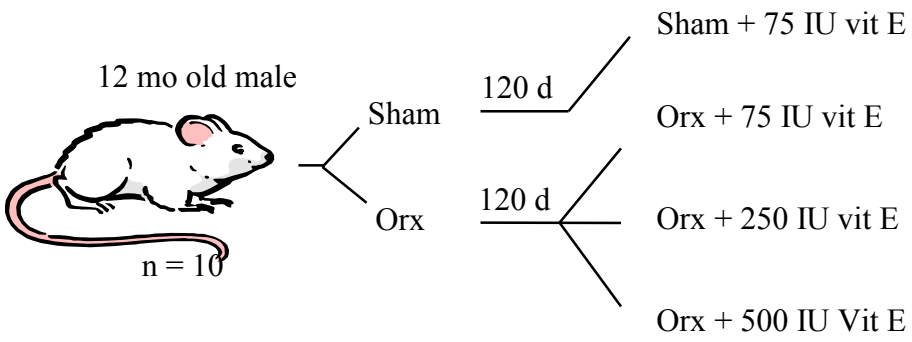

Figure 1: Experimental Design. Sham; Sham operated, Orx; Orchidectomized. Rats were feed an AIN-93M diet for 120 days to establish bone loss. Thereafter, rats were assigned to their corresponding treatment groups for 90 days.

CHAPTER IV

RESULTS

Food Intake, Body Weight, and Coagulating Gland Weight

The effects of Orx and supplemental doses of vitamin E on food intake, body and coagulating gland weights of male rats are presented in Table 2. Food intake and body weight gain were similar among all treatment group. The mean coagulating gland weights in the Orx groups were significantly lower than that of the Sham group ($P < 0.001$), which indicated the success of surgery and the lack of effects of vitamin E on coagulating gland.

Bone Mineral Content (BMC), Density (BMD) and Area (BMA)

The effects of Orx and supplemental doses of vitamin E on the whole body, right femur, and the fourth lumbar BMC, BMD and BMA are presented in Table 3. The four test groups did not show difference in BMA readings for the whole body ($P = 0.417$), right femur ($P = 0.4715$) and the fourth lumbar ($P = 0.0958$). However, the Orx groups had significantly ($P < 0.05$) lower BMD and BMC values for these three regions than the Sham group, although the three Orx groups showed no differences in the values. Therefore, the significant losses of BMD and BMC in the whole body, right femur and 4th lumbar due to orchidectomy were not prevented by vitamin E treatments.

Serum Osteocalcin and Urinary Deoxypyridinoline (Dpd)

No differences in the levels of serum osteocalcin, a marker of bone formation, were noted among the four treatment groups (Table 4). Similarly, neither orchidectomy nor vitamin E supplementation had any effect on urinary Dpd, a specific marker of bone resorption.

Trabecular Microarchitectural Properties in Distal Femur

The effects of Orx and supplemental doses of vitamin E on trabecular microarchitectural parameters on distal femurs are presented in Table 5. Orchidectomy caused decreases in the values of BV/TV (Figure 2), Tb.N (Figure 3), Tb.Th (Figure 4) and Conn.D (Figure 6) by 55.9%, 38.3%, 11.9% and 46.87%, respectively. However, the value of Tb.Sp (Figure 5) increased by 34.3% due to Orx. Since the three Orx groups showed no differences in the values of BV/TV, Tb.N, Tb.Th, Conn.D and Tb.Sp, it is concluded that supplemental doses of vitamin E have no effect in preventing Orx-induced unfavorable alterations of these parameters. The results showed that there was no Orx-induced changes in SMI ($P = 0.0906$).

Cortical Microarchitectural Properties in Femoral Midshaft

The effects of Orx and supplemental doses of vitamin E on cortical microarchitectural parameters in femoral midshaft of male rats are presented in Table 6. Orchidectomy did not cause changes in Co.P ($P = 0.0925$) and M.Area ($P = 0.9108$) in test animals, but significantly reduced Co.Th (Figure7) and Co.Area (Figure8) by 14.1% and 12.3%, respectively. Vitamin E treatment had no effect on Orx-induced reduction of

Co.Th and Co.Area except for the 250 IU treatment group that slightly improved the Co.Area.

Finite Element Analysis (FE) of Distal Femur

The effects of Orx and supplemental doses of vitamin E on the biomechanical properties of the distal femur of male rats by finite element analysis (FE) are shown in Table 7. Orx caused significant reduction in the values of average strain, total force, stiffness, physiological force and size independent stiffness by 55.3%, 83.9%, 62.8%, 83.9%, 81.8%, respectively. MPa increased by 68.8% due to Orx. The von mises force represents the amount of stress within the bone when a force is applied. Vitamin E had no effect on any of the FE parameters. The Orx groups with higher doses of vitamin E tended to have greater stress within them when this force was applied.

Table 2: Effects of orchidectomy (Orx) and supplemental doses of vitamin E on food intake, body and coagulating gland weights

	Sham	**Orx**			
Vitamin E ***(IU/kg diet)***	**75**	**75**	**250**	**500**	***P value***
Average food intake (g/day)	16.5±0.21	16.0±0.21	16.0±0.21	16.1±0.21	0.2734
Body weights (g)					
Initial	488.6±10.1	486.7±9.54	487.7±9.54	488.2±9.54	0.9992
Final	481.7±15.2	464.7±14.4	484.4±14.4	502.8±14.4	0.3351
Coagulating gland (g)	1.34±0.11[a]	0.18±0.11[b]	0.22±0.14[b]	0.18±0.10[b]	0.0001

Values are means ± standard errors of the mean, n = 10/group.

[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Table 3: Effects of orchidectomy (Orx) and supplemental doses of vitamin E on bone mineral density (BMD), bone mineral content (BMC), and bone mineral area (BMA)

	Sham	Orx			
Vitamin E ***(IU/kg diet)***	**75**	**75**	**250**	**500**	***P value***
BMD (g/cm^2)					
Whole body	0.187±0.002[a]	0.177±0.002[b]	0.174±0.002[b]	0.174±0.002[b]	0.001
Right femur	0.277±0.004[a]	0.249±0.004[b]	0.252±0.004[b]	0.242±0.004[b]	<0.0001
4th lumbar	0.256±0.004[a]	0.223±0.004[b]	0.224±0.004[b]	0.219±0.004[b]	<0.0001
BMC (g)					
Whole body	15.863±0.26[a]	14.548±0.25[b]	14.709±0.25[b]	14.432±0.25[b]	0.001
Right femur	0.717±0.017[a]	0.625±0.017[b]	0.635±0.017[b]	0.608±0.017[b]	0.0003
4th lumbar	0.203±0.005[a]	0.173±0.005[b]	0.166±0.005[b]	0.163±0.005[b]	<0.0001
BMA (cm^2)					
Whole body	84.80±1.25	82.29±1.18	84.33±1.18	82.84±1.18	0.417
Right femur	2.584±0.042	2.507±0.040	2.505±0.040	2.508±0.040	0.4715
4th lumbar	0.794±0.015	0.777±0.014	0.741±0.014	0.746±0.014	0.0958

Values are means ± standard errors of the mean, n = 10/group.

[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Table 4: Effect of orchidectomy (Orx) and supplemental doses of vitamin E on serum osteocalcin, and urinary deoxypyridinoline

	Sham	Orx			
Vitamin E ***(IU/kg diet)***	**75**	**75**	**250**	**500**	***P value***
Serum (ng/mL)					
Osteocalcin	13.0±1.65	17.9±1.57	18.4±1.57	16.0±1.57	0.0992
Urine (nmol/mmolcreatinine)					
Deoxypyridinoline	22.8±6.08	38.3±5.76	28.9±5.76	32.3±5.76	0.3197

Values are means ± standard errors of the mean, n = 10/group.

Table 5: Effects of orchidectomy (Orx) and supplemental doses of vitamin E on trabecular microarchitectural parameters in distal femur

	Sham	Orx			
Vitamin E *(IU/kg diet)*	**75**	**75**	**250**	**500**	***P value***
Distal Femur					
BV/TV *(1)*	0.137±0.008[a]	0.061±0.008[b]	0.056±0.008[b]	0.054±0.008[b]	<0.001
Tb.N *(1/mm)*	2.095±0.151[a]	1.293±0.151[b]	1.281±0.151[b]	1.438±0.151[b]	0.0029
Tb.Th *(mm)*	0.085±0.002[a]	0.075±0.002[b]	0.073±0.002[b]	0.071±0.002[b]	0.0013
Tb.Sp *(mm)*	0.521±0.069[b]	0.793±0.069[a]	0.823±0.069[a]	0.737±0.069[a]	0.0233
Conn.D *(1/mm^3)*	26.625±2.032[a]	14.147±2.032[b]	14.659±2.032[b]	13.987±2.032[b]	0.0005
SMI	1.668±0.126	1.903±0.126	1.818±0.125	2.141±0.126	0.0906

Values are means ± standard errors of the mean, n = 6/group.

[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Bone volume as percentage of tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp), connectivity density (Conn.D), and structure model index (SMI).

Table 6: Effects of orchidectomy (Orx) and supplemental doses of vitamin E on cortical microarchitectural parameters in femoral midshaft

	Sham	Orx			
Vitamin E *(IU/kg diet)*	**75**	**75**	**250**	**500**	***P value***
Femoral midshaft					
Co.Th *(mm)*	0.737±0.018[a]	0.633±0.018[b]	0.610±0.018[b]	0.602±0.020[b]	0.0001
Co.Area *(mm^2)*	2.039±0.064[a]	1.789±0.064[b]	1.871±0.064[ab]	1.716±0.071[b]	0.0165
Co.P *(%)*	1.010±0.428	1.348±0.428	2.517±0.428	2.042±0.469	0.0925
M.Area *(mm^2)*	11.113±0.461	11.331±0.461	11.580±0.461	11.265±0.505	0.9108

Values are means ± standard errors of the mean, n = 6/group.

[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Cortical thickness (Co.Th), cortical area (Co.Area), cortical porosity (Co.P), and medullary area (M.Area).

Table 7: Effects of orchidectomy (Orx) and supplemental doses of vitamin E on biomechanical properties in distal femur by finite element analysis (FE)

	Sham	Orx			ANOVA
Vitamin E *(IU/kg diet)*	**75**	**75**	**250**	**500**	***P value***
Average Strain, (mm)	0.238±0.02[a]	0.106±0.02[b]	0.093±0.02[b]	0.061±0.02[b]	<0.0001
Total Force, (N)	5539±632[a]	891±632[b]	667±632[b]	447±632[b]	<0.0001
Stiffness, (N/m x 10^3)	21697672± 1441571[a]	8070482± 1441571[b]	6945527± 1441571[b]	6737329± 1441571[b]	<0.0001
Physiological Force, (N)	16.617±1.90[a]	2.674±1.90[b]	2.001±1.90[b]	1.342±1.90[b]	<0.0001
Size Independent Stiffness, (N/m)	361.819±6.72[a]	65.983±6.72[b]	50.418±6.72[b]	33.577±6.72[b]	<0.0001
Corr. Von Mises Stresses Average, (MPa)	17.925±13.44[b]	57.446±13.44[a b]	75.010±13.44[a]	76.243±13.44[a]	<0.0001

Values are means ± standard errors of the mean, n = 6/group.

[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

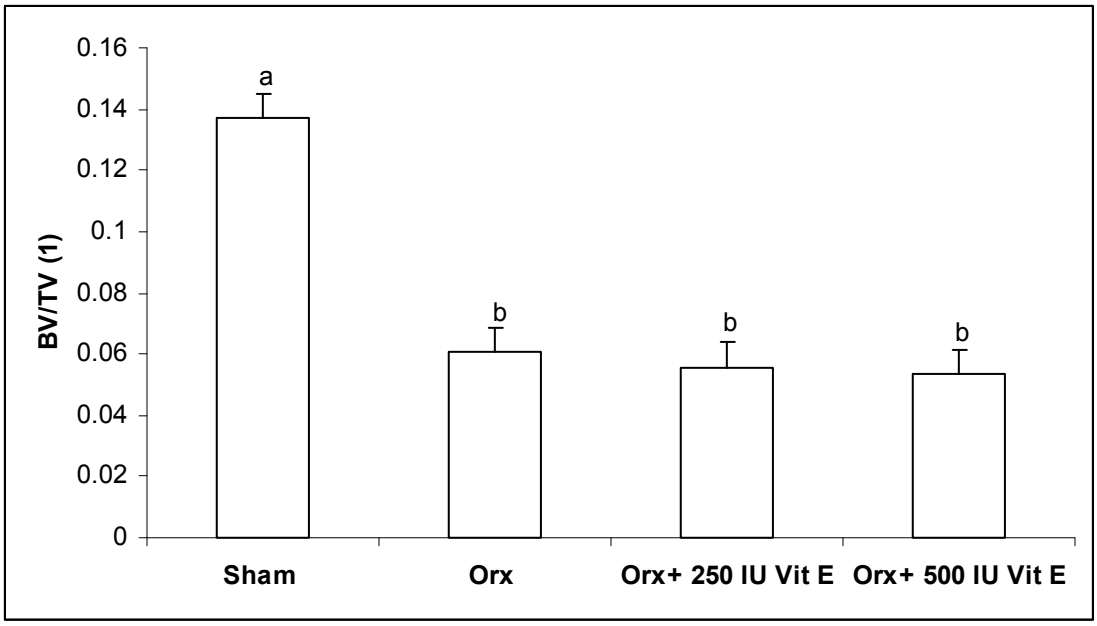

Figure 2: Effects of Orx and supplemental doses of vitamin E on cancellous bone volume as a percentage of tissue volume (BV/TV) in distal femur. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

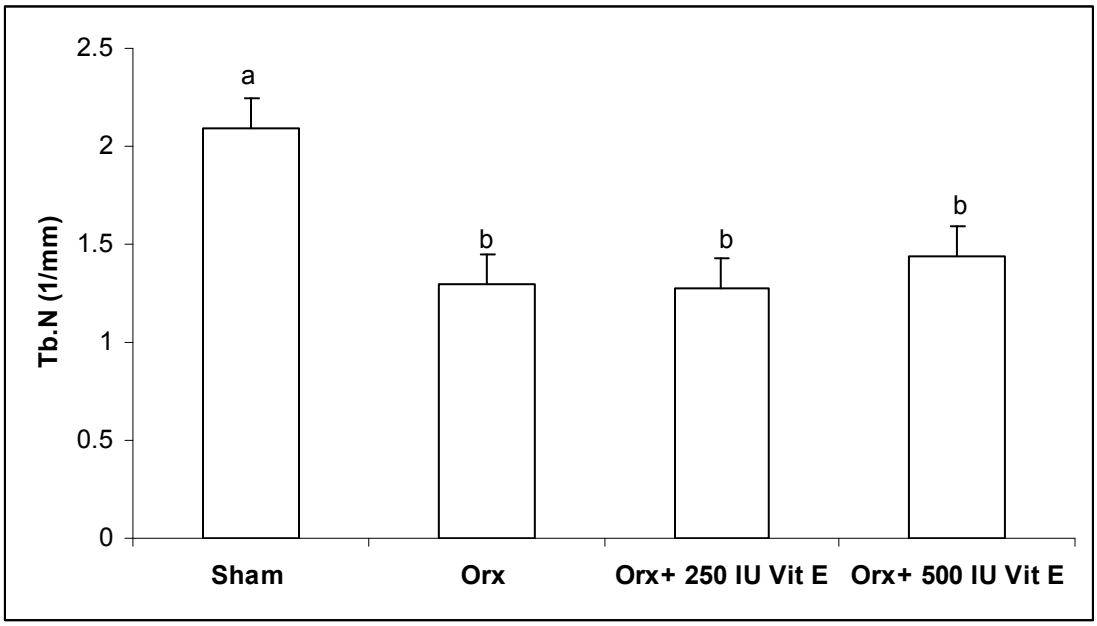

Figure 3: Effects of Orx and supplemental doses of vitamin E on trabecular number (Tb.N) in distal femur. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

Figure 4: Effect of Orx and supplemental doses of vitamin E on trabecular thickness (Tb.Th) in distal femur. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

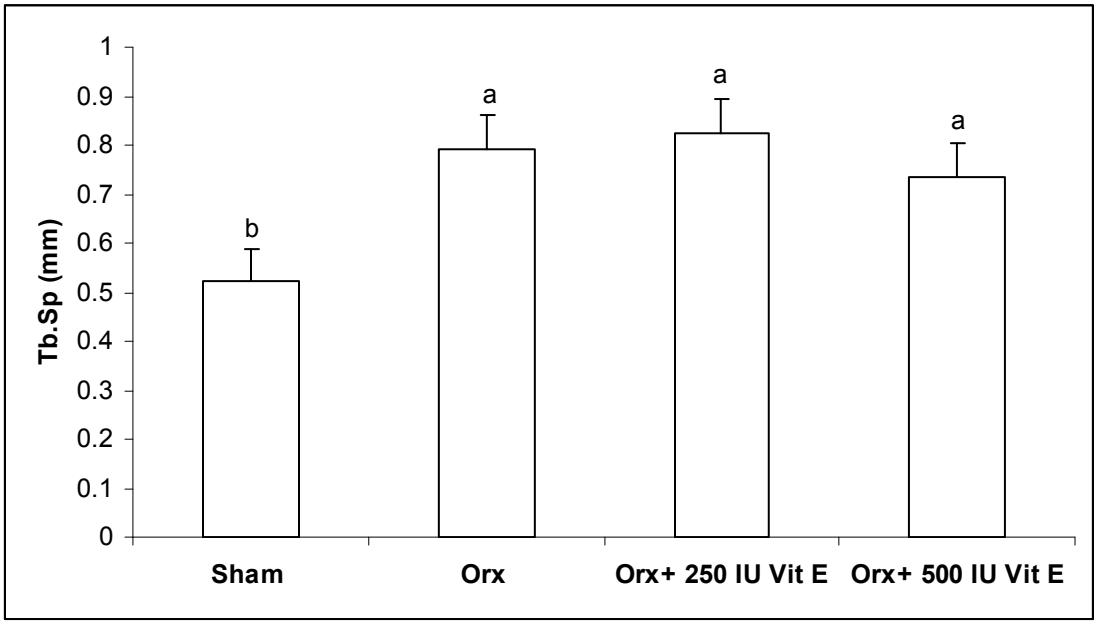

Figure 5: Effects of Orx and supplemental doses of vitamin E on trabecular separation (Tb.Sp) in distal femur. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

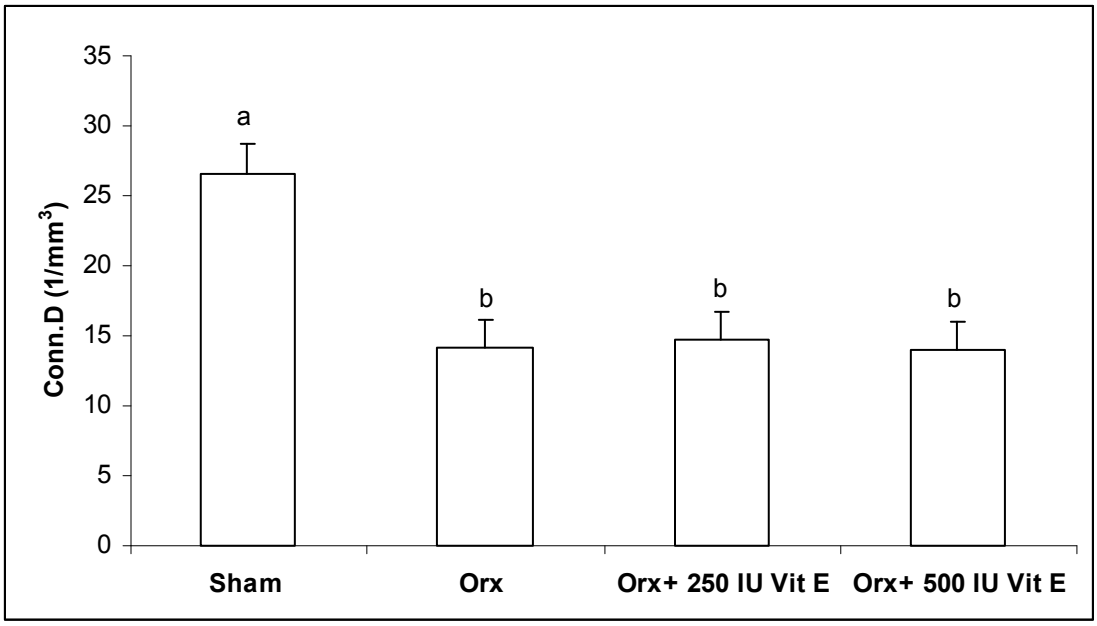

Figure 6: Effects of Orx and supplemental doses of vitamin E on connectivity density (Conn.D) in distal femur. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

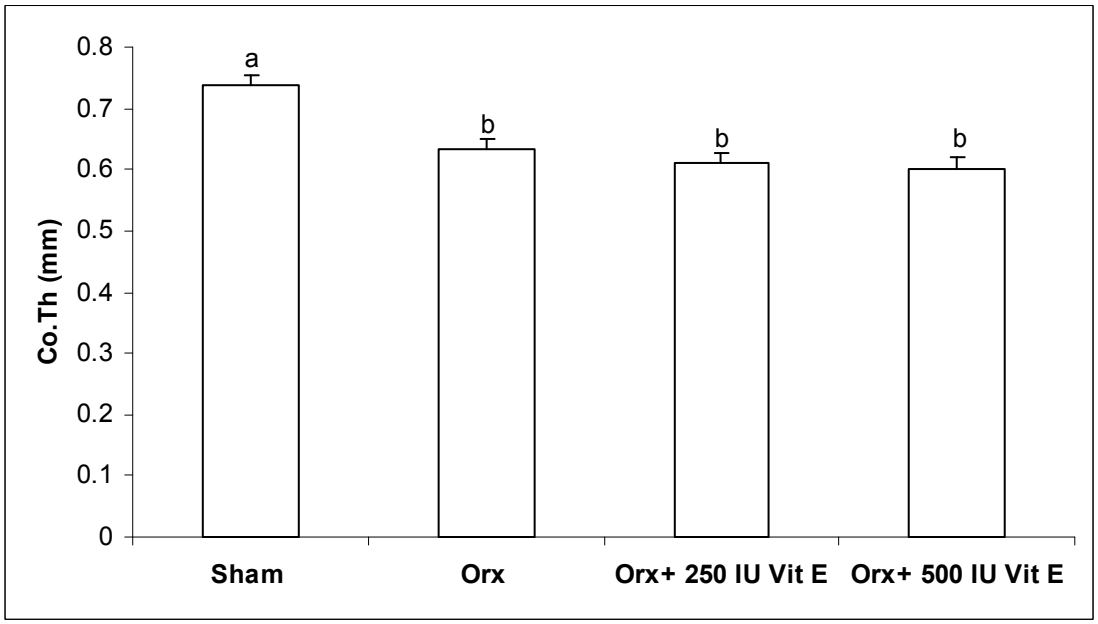

Figure 7: Effect of Orx and supplemental doses of vitamin E on cortical thickness (Co.Th) in femoral midshaft. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

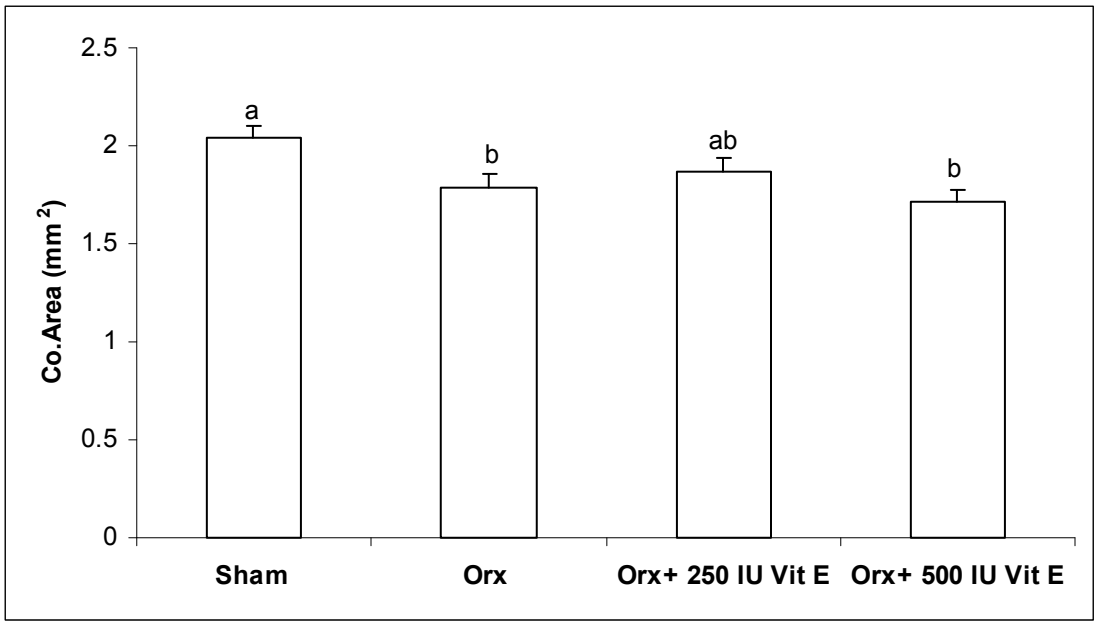

Figure 8: Effect of Orx and supplemental doses of vitamin E on cortical area (Co.Area) in femoral midshaft. Bars represent mean ± SE; n = 6 rats per group. Bars with different letters are significantly different ($P < 0.05$) from each other.

CHAPTER V

DISCUSSION

Previous studies have demonstrated that vitamin E has some osteoprotective effects in male tail-suspended rat model of osteoporosis (Smith et al., 2005) as well as aged male mice (Arjmandi et al., 2002). However, the findings of this study indicate that vitamin E is unable to restore bone mass once the loss has occurred in Orx rat model of male osteoporosis. Among all analyzed parameters, vitamin E supplementation only provides modest bone protective effects on the cortical area (Co.Area) in femoral mid-shaft of Orx rats. These findings are inconsistent with previous observations by Smith et al. (2005) and Arjmandi et al. (2002) that had shown the bone-protective effects of vitamin E in male osteoporosis.

Although we cannot explain these paradoxical findings, a reasonable explanation could be because of the uses of different animal models of bone loss and the different experimental designs. Previous studies had utilized other animal models of osteopenia, such as HU male rats (Smith et al., 2005) and aged (i.e., 24-month-old) male mice (Arjmandi et al., 2002). To our knowledge, the present study is the first study that has examined if vitamin E supplementation can reverse bone loss in an Orx rat model of male osteoporosis. To conduct this study, we used Orx rat model of male osteoporosis. Initially after the surgery, all rats were fed an AIN-93 casein-based diet to establish bone loss and

the treatments with vitamin E supplementation were given after confirming that bone loss has occurred. However, in the study done by Smith et al. (2005), vitamin E treatments were initiated 9 weeks prior to unloading and continued during the 4 weeks of unloading period. In the study by Arjmandi et al. (2002), vitamin E treatments were initiated for 30 days on aged male mice (i.e., 24-month-old) in order to examine the short term influence of vitamin E treatments.

In the present study, the significant losses of BMD and BMC in the whole body, right femur and 4th lumbar due to orchidectomy were not prevented by vitamin E treatments. Serum OC, a marker of bone formation, tended to be increased by vitamin E supplementation, albeit not significantly ($P = 0.0992$). Urinary Dpd, a marker of bone resorption, was not significantly altered by Orx or vitamin E treatments. Though we cannot offer an explanation for this observation, we can say that vitamin E does have a modest bone protective effect by reducing bone resorption as seen by the Dpd values. For cortical microarchitectural parameters in femoral midshaft, vitamin E treatment had no effect on Orx-induced reduction of Co.Th and Co.Area except for the 250 IU treatment group that slightly improved the Co.Area. Unfavorable alterations of trabecular microarchitectural parameters in distal femur such as BV/TV, Tb.N, Tb.Th, Conn.D and Tb.Sp due to orchidectomy were also not prevented by vitamin E treatments.

On the contrary, Smith et al. (2005) demonstrated that vitamin E supplemented diets at both 500 IU and 75 IU had protective effects on bone in HU male rats by preventing the loss of trabecular number (Tb.N) and bone surface normalized to tissue volume (BS/TV). Besides the positive results in Tb.N and BS/TV, vitamin E showed no effect on any parameter of bone histomorphometry. The only finding by Smith et al.

(2005) that was consistent with this study was that serum ALP, a nonspecific marker of bone formation, which also tended to increase, though not to a level of statistical significance ($P = 0.0990$).

The study done by Arjmandi et al. (2002) demonstrated that high dose of vitamin E supplementation at 500 mg has a pronounced effect on the bones of aged male mice by increasing the mRNA level of type-1 alpha-collagen, a predominant bone matrix protein, and total protein content. By comparing the findings of this study with those of the previous studies by Smith et al. (2005) and Arjmandi et al. (2002), it is likely that the use of different animal models of bone loss could be the reason for not seeing the positive effects of vitamin E on bone.

Another possible reason to explain why the findings of the present study are inconsistent with those of the previous studies may be related to the amount of vitamin E added in AIN-93M diet. The AIN-93M is a formulated diet for rodents recommended by the American Institute of Nutrition with adequate vitamin E level. In this study, the AIN-93M diet was used as a control diet that already contained 75 IU/kg of vitamin E. It is reasonable to assume that vitamin E supplementation at 75 IU/kg had already reached a level to exert beneficial effects on bone. Therefore, feeding additional vitamin E in excess of 75 IU/kg may not result in further improvement of bone in Orx rats.

Vitamin E is one of the most commonly consumed vitamin supplements in the United States today due to the fact that a number of studies (Keaney et al., 1999; Pratico et al., 1998; Sigounas et al., 1997) have indicated an association between vitamin E supplementation and a lower risk of incidents of atherosclerosis and cancer in human. However, the studies by Micheletta et al. (2004), Simons et al. (1999), and Elliott et al.

(1995), also showed that vitamin E supplementation could have beneficial effects on cardiovascular disease in a high risk population only at the appropriate dose. Based on the findings of this study that vitamin E is unable to restore bone mass once the loss has occurred in Orx rat model of male osteoporosis, and also those of the other previously described studies (Micheletta et al., 2004; Simons et al., 1999; Elliott et al., 1995), it is clear that vitamin E supplementation should only be recommended for certain population. Furthermore, the appropriate dose of vitamin E treatment needs to be determined in order to achieve maximal benefits. An inappropriate or excessive consumption of vitamin E may most likely result in unnecessary expenditure in the already high medical cost today.

REFERENCES

Amin S, Felson DT. Osteoporosis in men. *Rheum Dis Clin North Am*. 2001;27:19-47.

Arjmandi B, Juma S, Beharka A, Bapna M, Akhter M, Meydani SN. Vitamin E improves bone quality in the aged but not in young adult male mice. *J Nutr Biochem.* 2002;13:543-549.

Audran M, Chappard D, Legrand E, Libouban H, Basle MF. Bone microarchitecture and bone fragility in men: DXA and histomorphometry in humans and in the orchidectomized rat model. *Calcif Tissue Int.* 2001;69(4):214-7.

Baillie SP, Davison CE, Johnson FJ, Francis RM. Pathogenesis of vertebral crush fractures in men. *Age Ageing*. 1992;21:139-141.

Barou O, Valentin D, Vico L, Tirode C, Barbier A, Alexandre C, Lafage-Proust MH. High-resolution three-dimensional micro-computed tomography detects bone loss and changes in trabecular architecture early: comparison with DEXA and bone histomorphometry in a rat model of disuse osteoporosis. *Invest Radiol*. 2002;37(1):40-6.

Beharka AA, Han SN, Adolfsson O et al. Long-term dietary antioxidant supplementation reduces production of selected inflammatory mediators by murine macrophages. *Nutr Res.* 2000;20:281-296.

Bergot C, Laval-Jeantet AM, Preteux F, Meunier A. Measurement of anisotropic vertebral trabecular bone loss during aging by quantitative image analysis. *Calcif Tissue Int.* 1998;43:143-149.

Bilezikian JP. Osteoporosis in men. *J Clin Endocrinol Metab*. 1999;84:3431-3434.

Campion JM, Maricic MJ. Osteoporosis in men. *Am Fam Physician.* 2003;67(7):1521-6.

Canalis E. Mechanisms of glucocorticoid- induced osteoporosis. *Curr Opin Rheumatol.* 2003;15:454–457.

Cattaneo PM, Dalstra M, Frich LH. A three dimensional finite element model from computed tomography data: a semi automated method. *Proc Inst Mech Eng.* 2001;215:203– 13.

Clague JE, Wu FC and Horan MA. Difficulties in measuring the effect of testosterone replacement therapy on muscle function in older men. *International Journal of Andrology*. 1999;22, 261–265.

Conde FA, Aronson WJ. Risk factors for male osteoporosis. *Urol Oncol.* 2003;21(5):380-383.

Crary EJ, McCarty MF. Potential clinical applications for high-dose nutritional antioxidants. *Med Hypotheses*. 1984;13:77-98.

Daniell HW. Osteoporosis after orchiectomy for prostate cancer. *J Urol.* 1997;157:439-444.

Danielsen CC, Mosekilde L, Andreassen TT. Long-term effect of orchidectomy on cortical bone from rat femur: bone mass and mechanical properties. *Calcif Tissue Int.* 1992;50(2):169-74.

David V, Laroche N, Boudignon B, Lafage-Proust MH, Alexandre C, Ruegsegger P, Vico L. Noninvasive in vivo monitoring of bone architecture alterations in hindlimb-unloaded female rats using novel three-dimensional microcomputed tomography. *J Bone Miner Res.* 2003;18(9):1622-31.

Deal C and Gideon J. Recombinant human PTH 1-34 (Forteo): an anabolic drug for osteoporosis. *Cleve Clin J Med*. 2003;70(7):585-6,589-90,592-4 passim.

Dempster DW, Cosman F, Kurland ES, Zhou H, Nieves J, Woelfert L, Shane E, Plavetic K, Muller R, Bilezikian J, Lindsay R. Effects of daily treatment with parathyroid hormone on bone microarchitecture and turnover in patients with osteoporosis: a paired biopsy study. *J Bone Miner Res*. 2001b;16:1846-1853.

Dempster DW, Ferguson-Pell MW, Mellish RWE, Cochran GVB, Xie F, Fey C, Horbert W, Parisien M, Lindsay R. Relationships between bone structure in the iliac crest and bone structure and strength in the lumbar spine. *Osteoporos Int*. 1993;3:90-96.

Dempster DW, Zhou H, Cosman F. PTH treatment directly stimulates bone formation in cancellous and cortical bone in humans. *J Bone Miner Res*. 2001a;16 (Suppl 1):S179.

Devogelaer JP, De Cooman S, Nagant de Deuxchaisnes C. Low bone mass in hypogonadal males. Effect of testosterone substitution therapy, a densitometric study. *Maturitas*. 1992;15(1):17-23.

Ding M, Hvid I. Quantification of age-related changes in the structure model type and trabecular thickness of human tibial cancellous bone. *Bone*. 2000;26(3):291-5.

Drinka PJ, Jochen AL, Cuisinier M, Bloom R, Rudman I, Rudman D. Polycythemia as a complication of testosterone replacement therapy in nursing home men with low testosterone levels. *J Am Geriatr Soc*. 1995;43(8):899-901.

Dykman TR, Gluck OS, Murphy WA, et al. Evaluation of factors associated with glucocorticoid-induced osteopenia in patients with rheumatic diseases. *Arthritis Rheum.* 1985;28:361–8.

Ebeling PR. Idiopathic or hypogonadal osteoporosis in men: current and future treatment options. *Treat Endocrinol.* 2004;3(6):381-91.

Elliott TG, Barth JD, Mancini GB. Effects of vitamin E on endothelial function in men after myocardial infarction. *Am J Cardiol*. 1995;76:1188–90

Erben RG, Eberle J, Stahr K, Goldberg M. Androgen Deficiency Induces High Turnover Osteopenia in Aged Male Rats: A Sequential Histomorphometric Study. *J Bone Miner Res.* 2000;15(6):10851098.

Eriksen EF, Mosekilde L, Melsen F. Trabecular bone remodeling and balance in primary hyperparathyroidism. *Bone.* 1986;7(3):213-21.

Fang MA, Frost PJ, Iida-Klein A, Hahn TJ. Effects of nicotine on cellular function in UMR 106-01 osteoblast-like cells. *Bone.* 1991;12(4):283-6.

Felson DT, Kiel DP, Anderson JJ, Kannel WB. Alcohol consumption and hip fractures: the Framingham Study. *Am J Epidemiol*. 1988;128:1102–1110.

Ferrando, AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, Lieberman SA, Tipton K, Wolfe RR, Urban RJ. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *American Journal of Physiological Endocrinology and Metabolism.* 2002;282:E601–E607.

Finkelstein JS, Klibanski A, Neer R, Greenspan SL, Rosenthal DI, Crowley WF. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. *Ann Intern Med.* 1987;106:354-61.

Francis RM. Male osteoprosis. *Rheumatol*. 2000;39:1055-1057.

Francis RM. The effects of testosterone on osteoporosis in men. *Clin Endocrinol (Oxf).* 1999;50:411-414.

Francis RM, Peacock M, Aaron JE, Selby PL, Taylor GA, Thompson J, Marshall DH, Horsman A. Osteoporosis in hypogonadal men: role of decreased plasma 1,25-dihydroxyvitamin D, calcium malabsorption, and low bone formation. *Bone.* 1986;7:261-268.

Fujiwara S, Kasagi F, Yamada M, Kodama K. Risk factors for hip fracture in a Japanese cohort. *J Bone Miner Res*.1997;12:998–1004.

Gill RK, Turner RT, Wronski TJ, Bell NH. Orchidectomy markedly reduces the concentration of the three isoforms of transforming growth factor B in rat bone, and reduction is prevented by testosterone. *Endocrinology.* 1998;139(2): 546-550.

Grote HJ, Amling M, Vogel M, Hahn M, Posl M, Delling G. Intervertebral variation in trabecular microarchitecture throughout the normal spine in relation to age. *Bone*. 1995;16:301–308.

Gruenewald DA, Matsumoto AM. Testosterone supplementation therapy for older men: potential benefits and risks. *Am Geriatr Soc*. 2003;51(1):101-15.

Gullberg B, Johnell O, Kanis JA. World-wide projections for hip fracture. *Osteoporos Int*. 1997;7:407-413.

Gunness M, Orwoll E. Early induction of alterations in cancellous and cortical bone histology after orchiectomy in mature rats. *J Bone Miner Res.* 1995;10(11):1735-44.

Hodsman AB, Bauer DC, Dempster DW, Dian L, Hanley DA, Harris ST, Kendler DL, McClung MR, Miller PD, Olszynski WP, Orwoll E, Yuen CK. Parathyroid Hormone and Teriparatide for the Treatment of Osteoporosis: A Review of the Evidence and Suggested Guidelines for Its Use. *Endocr Rev.* 2005;26(5):688-703.

Hoidrup S, Gronbaek M, Gottschau A, Lauritzen JB, Schroll M and the Copenhagen Centre for Prospective Population Studies. Alcohol intake, beverage preference and risk of hip fracture in men and women. *Am J Epidemiol*. 1999;149:933–1001.

Holmang S, Marin P, Lindstedt G, Hedelin H. Effect of long-term oral testosterone undecanoate treatment on prostate volume and serum prostate-specific antigen concentration in eugonadal middle-aged men. *Prostate*. 1993;23(2):99-106.

Isidori AM, Lenzi A. Risk factors for androgen decline in older males: lifestyle, chronic diseases and drugs. *J Endocrinol Invest*. 2005;28(3 Suppl):14-22.

Jackson JA, Spiekerman AM. Testosterone deficiency is common in men with hip fracture after simple falls. *Clin Res*. 1989;37:131-136.

Jialal I, Devaraj S, Kaul N. The effect of alpha-tocopherol on monocyte proatherogenic activity. *J Nutr.* 2001;131:389S-394S.

Jilka RL, Passeri G, Girasole G et al. Estrogen loss upregulates hematopoiesis in the mouse: a mediating role of IL-6. *Exp Hematol.* 1995;213:500-506.

Jilka RL, Weinstein RS, Takahashi K, Parfitt AM, Manolagas SC. Linkage of decreased bone mass with impaired osteoblastogenesis in a murine model of accelerated senescence. *J Clin Invest*. 1996;97:1732-1740.

Jockenhovel, F., Vogel, E., Reinhardt, W. & Reinwein, D. Effects of various modes of androgen substitution therapy on erythropoiesis. *European Journal of Medical Research*. 1997;2:293–298.

Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, Fujiwara S, Kroger H, McCloskey EV, Mellstrom D, Melton LJ, Pols H, Reeve J, Silman A, Tenenhouse A. Smoking and fracture risk: a meta-analysis. *Osteoporos Int.* 2005;16(2):155-62.

Kasperk CH, Wakley GK, Hierl T, Ziegler R. Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro. *J Bone Miner Res*. 1997;12:464-471.

Katznelson L, Finkelstein JS, Schoenfeld DA, et al. Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. *J Clin Endocrinol Metab*.1996; 81:4358.

Kaufman JM, Johnell O, Abadie E, Adami S, Audran M, Avouac B, et al. Background for studies on the treatment of male osteoporosis: State of the art. *Ann Rheum Dis*. 2000;59(10):765-72.

Keaney JF Jr, Simon DI,Freedman JE. Vitamin E and vascular homeostasis: implications for atherosclerosis. *FASEB J.* 1999;13:965–975.

Kenny AM, Gallagher JC, Prestwood KM, Gruman CA, Raisz LG. Bone density, bone turnover, and hormone levels in men over age 75. *J Gerontol.* 1998;53A:M419-M425.

Kenny AM, Prestwood KM, Gruman CA et al. Effects of transdermal testosteroneon bone and muscle in older men with low bioavailable testosterone levels. *J Gerontol A Biol Sci Med Sci*. 2001;56A:M266–M272.

Khosla S, Melton III LJ, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL. Relationships of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. *J Clin Endocrinol Metab.* 1998;83:2266-2274.

Kim MJ, Shim MS, Kim MK, Lee Y. Shin YG. Chung CH, Kwon SO. Effect of chronic alcohol ingestion on bone mineral density in males without liver cirrhosis. *Korean J Intern Med*. 2003;18(3):174-80.

Kim YH, Shim JC, Kelly DL, Lee JG, Seo YS, Conley RR. Cortisol response to buspirone in extended abstinent alcoholics. Alcohol Alcohol. 2004 Jul-Aug;39(4):287-9.

Kinney JH, Lane NE, Haupt DL. In vivo, three-dimensional microscopy of trabecular bone. *J Bone Miner Res.* 1995;10:264 –270.

Klibanski A, Campbell-Adams L, Bassford T, Blair SN, Boden SD, Dickersin K, et al. NIH consensus development conference statement: osteoporosis prevention, diagnosis, and therapy. March 27-29, 2000. Accessed December 11, 2002 at: www.consensus.nih. gov/cons/111/111_statement.htm.

Krall EA, Dawson-Hughes B, Hirst K, Gallagher JC, Sherman SS, Dalsky G. Bone mineral density and biochemical markers of bone turnover in healthy elderly men and women. *J Gerontol*. 1997;52A:M61-M67.

Kurland ES, Cosman F, McMahon DJ, Rosen CJ, Lindsay R, Bilezikian JP. Parathyroid hormone as a therapy for idiopathic osteoporosis in men: effects on bone mineral density and bone markers. *J Clin Endocrinol Metab*. 2000;85:3069-3076.

Lafague-Proust MH, Boudignon B, Thierry T. Glucocorticoid-induced osteoporosis: pathophysiological data and recent treatments. *Joint Bone Spine.* 2003;70:109–118.

Lerouxel E, Libouban H, Moreau MF, Basle MF, Audran M, Chappard D. Mandibular bone loss in an animal model of male osteoporosis (orchidectomized rat): a radiographic and densitometric study. *Osteoporos Int*. 2004;15(10):814-9.

Libouban H, Moreau MF, Legrand E, Audran M, Basle´ MF, Chappard D. Comparison of histomorphometric descriptors of bone architecture with dual-energy X-ray absorptiometry for assessing bone loss in orchidectomized rat. *Osteoporos Int*. 2002;13:422–428.

Libouban H, Moreau MF, Legrand E, Basle MF, Audran M, Chappard D. Comparison insight dual X-ray absorptiometry (DXA), histomorphometry, ash weight, and morphometric indices for bone evaluation in an animal model (the orchidectomized rat) of male osteoporosis. *Calcif Tissue Int.* 2001;68(1):31-7.

LoCascio V, Bonucci E, Imbimbo B, et al. Bone loss in response to long-term glucocorticoid therapy. *Bone Miner.* 1990;8:39–51.

Luukinen H, Kakonen SM, Pettersson K, Koski K, Laippala P, Luvgren T, Kivela SL, Vaananen HK. Strong prediction of fractures among older adults by the ratio of caroxylated to total serum osteocalcin. *J Bone Miner Res.* 2000;15:2473-2478.

Makins R, Ballinger A. Gastrointastinal side effects of drug. *Expert Opin Drug Saf.* 2003;4:421-429.

Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N Engl J Med.* 1995;332:305-311.

Mathoo JM, Cranney A, Papaioannou A, Adachi JD. Rational use of oral bisphosphonates for the treatment of osteoporosis. *Curr Osteoporos Rep*. 2004;2(1):17-23.

McCalden RW, McGeough JA, Court-Brown CM. Age-related changes in the compressive strength of cancellous bone. The relative importance of changes in density and trabecular architecture. *J Bone Joint Surg (Am).* 1997;79A:421-427.

McClung M. Use of highly potent bisphosphonates in the treatment of osteoporosis. *Curr Osteoporos Rep*. 2003;1(3):116-22.

Medras M, Jankowska EA. The effect of alcohol on bone mineral density in men. *Przegl Lek.* 2000;57(12):743-6.

Micheletta F, Natoli S, Misuraca M, Sbarigia E, Diczfalusy U, Iuliano L. Vitamin E supplementation in patients with carotid atherosclerosis: reversal of altered oxidative stress status in plasma but not in plaque. *Arterioscler Thromb Vasc Biol*. 2004;24:136–40.

Morales A. Monitoring androgen replacement therapy: testosterone and prostate safety. *J Endocrinol Invest*. 2005;28(3 Suppl):122-7.

Moreau MF, Libouban H, Legrand E, Basle MF, Audran M, Chappard D. Lean, fat and bone masses are influenced by orchidectomy in the rat. A densitometric X-ray absorptiometric study. *J Musculoskelet Neuronal Interact.* 2001;1(3):209-13.

Muller R, Ruegsegger P. Three-dimensional finite element modeling of non-invasively assessed trabecular bone structures. *Med Eng Phys.* 1995;17:126–33.

Muller R, Van Vampenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T, Ruegsegger P. Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. *Bone.* 1998;23(1):59-66.

National Institutes of Health. Osteoporosis in men. 2003; National Institutes of Health, Osteoporosis and Related Bone Diseases, National Resource Center, Washington, D.C.

National Osteoporosis Foundation. Men and osteoporosis. 2004; National Osteoporosis Foundation, Washington, D.C.

Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med*. 2001;344:1434-1441.

Nelson HD, Morris CD, Kraemer DF, Mahon S, Carney N, Nygren P, Helfand M. Osteoporosis in postmenopausal women: diagnosis and monitoring. In: Oregon Health and Science University Evidence-Based Practice Center (eds) Evidence report/technology assessment No. 28. Agency for Healthcare Research and Quality, Rockville, MD. 2002.

Newitt DC, Majumdar S, van Rietbergen B, von Ingersleben G, Harris ST, Genant HK, et al. In vivo assessment of architecture and micro-finite element analysis derived indices of mechanical properties of trabecular bone in the radius. *Osteoporos Int*. 2002;13:6– 17.

Ohishi T, Takahashi M, Kushida K, Ormura T. Biochemical markers and bone mineral density in patients with hip fractures in men. *Endocr Res.* 2000;26:275-288.

Orwoll E, Ettinger M, Weiss S, et al. Alendronate for the treatment of osteoporosis in men. *N Engl J Med.* 2000;343:604-10.

Orwoll ES, Scheele WH, Paul S, Adami S, Syversen U, Diez-Perez A, Kaufman JM, Clancy AD, Gaich GA. The effect of teriparatide [human parathyroid hormone (1-34)] therapy on bone density in men with osteoporosis. *J Bone Miner Res*. 2003;18:9-17.

Parisien M, Silverberg SJ, Shane E, de la Cruz L, Lindsay R, Bilezikian JP, Dempster DW. The histomorphometry of bone in primary hyperparathyroidism: preservation of cancellous bone structure. *J Clin Endocrinol Metab*. 1990;70(4):930-8.

Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nature Med.* 1998;4:1189–1192.

Rackoff PJ, Rosen CJ. Pathogenesis and treatment of glucocorticoid-induced osteoporosis. *Drugs Aging.* 1998;12:477–84.

Reid DM, Adami S, Devogelaer JP, Chines AA . Risedronate increases bone density and reduces vertebral fracture risk within one year in men on corticosteroid therapy, *Calcif Tiss Int*. 2001.69:242-247.

Resch H, Pietschmann P, Woloszczuk W, Krexner E, Bernecker P, Willvonseder R. Bone mass and biochmical parameters of bone metabolism in men with spinal osteoporosis. *Eur J Clin Invest.* 1992;22(8):542-545.

Ringe JD, Faber H, Dorst A. Alendronate treatment of established primary osteoporosis in men: results of a 2-years prospective study. *J Clin Endocrinol Metab.* 2001;86:5252-5.

Romagnoli E, Paglia F, Dionisi S, De Geronimo S, Pepe J, Di Virgilio R, Minisola S. Male osteoporosis: current treatment and future options. *Clin Ter.* 2003;154(1):49-53.

Schneider DL, Barrett-Connor EL. Urinary N-telopeptide levels discriminate normal, osteopenic, and osteoporotic bone mineral density. *Arch Intern Med*. 1997;157:1241-1245.

Seeman E. Unresolved issues in osteoporosis in men. *J Clin Endocrinol Metab*. 2001;2:45-64.

Shefelbine SJ, Simon U, Claes L, Gold A, Gabet Y, Bab I, Muller R, Augat P. Prediction of fracture callus mechanical properties using micro-CT images and voxel-based finite element analysis. *Bone.* 2005;36(3):480-8.

Sigounas G, Anagnostou A, Steiner M. dl-alphatocopherol induces apoptosis in erythroleukemia, prostate, and breast cancer cells. *Nutr Cancer.* 1997;28: 30–35.

Sih, R., Morley, J.E., Kaiser, F.E., Perry, H.M., 3rd, Patrick, P. & Ross, C. Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *Journal of Clinical Endocrinology and Metabolism*. 1997; 82: 1661–1667.

Simons LA, von Konigsmark M, Simons J, Stocker R, Celermajer DS. 1999. Vitamin E ingestion does not improve arterial endothelial dysfunction in older adults. *Atherosclerosis.*1999;143:193–99

Slater S, Oliver RT. Testosterone: its role in development of prostate cancer and potential risk from use as hormone replacement therapy. *Drugs Aging.* 2000;6:431-439.

Smith BJ, Lucas EA, Turner RT, Evans GL, Lerner MR, Brackett DJ, Stoecker BJ, Arjmandi BH. Vitamin E provides protection for bone in mature hindlimb unloaded male rats. *Calcif Tissue Int*. 2005;76(4):272-9.

Snyder PJ, Peachey H, Hannoush P et al. Effect of testosterone treatment on bone mineral density in men over 65 years of age. *J Clin Endocrinol Metab.* 1999;84:1966–1972.

Stanley HL, Schmitt BP, Poses RM, Deiss WP. Does hypogonadism contribute to the occurrence of a minimal trauma hip fractures in elderly men? *J Am Geriatr Soc*. 1991;39:766-771.

Stepan JJ, Lachman M, Zverina J, Pacovsky V, Baylink DJ. Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab. 1989;69:523–527.

Szulc P, Claustrat B, Marchand F, Delmas PD. Increased risk of falls and increased bone resorption in elderly men with partial androgen deficiency: the MINOS study. *J Clin Endocrinol Metab*. 2003;88(11):5240-7.

Szulc P, Garnero P, Munoz F, Marchand F, Delmas PD. Cross-sectional evaluation of bone metabolism in men. *J Bone Miner Res*. 2001;16(9):1642-50.

Tan RS, Salazar JA. Risks of testosterone replacement therapy in ageing men. *Expert Opin Drug Saf.* 2004;3(6):599-606.

Tanaka H, Tanabe N, Suzuki N, Shoji M, Torigoe H, Sugaya A, Motohasgi M, Maeno M. Nicotine affects mineralized nodule formation by the human osteosarcoma cell line Saos-2. *Life Sci.* 2005;June6.

Tenover, J.S. Effects of testosterone supplementation in the aging male. *Journal of Clinical Endocrinology and Metabolism*. 1992;75:1092-1098.

van Tits LJ, Demacker PN, de Graaf J, Hak-Lemmers HL, Stalenhoef AF. Alpha-tocopherol supplementation decreases production of superoxide and cytokines by leukocytes ex vivo in both normolipidemic and hypertriglyceridemic individuals. *Am J Clin Nutr.* 2000;71:458-464.

Vanderput L, Boonen S, Van Herck E, Swinnen JV, Bouillon R, Vanderschueren D. Evidence from the aged orchidectomized male rat model that 17beta-estradiol is a more effective bone-sparing and anabolic agent than 5alpha-dihydrotestosterone. *J Bone Miner Res.* 2002;17(11):2080-2086.

Vanderschueren D, Van Herck E, Schot P, Rush E, Einhorn T, Geusens P et al. The aged male rat as a model for human osteoporosis: evaluation by nondestructive measurements and biomechanical testing. *Calcif Tissue Int.* 1993;53:342–347.

Vanderschueren D, Vandenput L, Boonen S, Van Herck E, Swinnen JV, Bouillon R. An aged rat model of partial androgen deficiency: prevention of both loss of bone and lean body mass by low-dose androgen replacement. *Endocrinology*. 2000;141(5):1642-7.

Venken K, Boonen S, Van Herck E, Vandenput L, Kumar N, Sitruk-Ware R, Sundaram K, Bouillon R, Vanderschueren D. Bone and muscle protective potential of the prostate-sparing synthetic androgen 7alpha-methyl-19-nortestosterone: evidence from the aged orchidectomized male rat model. *Bone*. 2005;36(4):663-70.

Vogel M, Hahn M, Delling G. Relation between 2- and 3-dimensional architecture of trabecular bone in the human spine. *Bone.* 1993;14:199-203.

Wakley GK, Schutte HD, Hannon KS, Turner RT. Androgen Treatment prevents loss of cancellous bone in the orchidectomized rat. *J Bone Miner Res.* 1991;4:325-330.

Walker LM, Preston MR, Magnay JL, Thomas PB, El Haj AJ. Nicotinic regulation of c-fos and osteopontin expression in human-derived osteoblast-like cells and human trabecular bone organ culture. *Bone.* 2001;28(6):603-8.

Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Berman N, Hull L, Swerdloff RS. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J Clin Endocrinol Metab*. 2004;89(5):2085-98.

Wang Y, Huang DS, Liang B, Watson RR. Nutritional status and immune responses in mice with murine AIDS are normalized by vitamin E supplementation. *J Nutr.* 1994;124:2024-2032.

Wink CS, Felts WJL. Effects of castration on bone structure of male rats. A model of osteoporosis. *Calcif Tissue Int*. 1980;32:77–82.

Wolinsky-Friedland M. Drug-induced metabolic bone disease. *Endocrinol Metab Clin North Am.* 1995;24:395–420.

Xu H, Watkins BA, Seifert MF. Vitamin E stimulates trabecular bone formation and alters epiphyseal cartilage morphometry. Calcif. *Tissue Int.* 1995;57:293-300.

APPENDIX

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

Protocol Expires: 3/18/2006

Date : Wednesday, March 19, 2003 Animal Care and Use Protocol (ACUP) No : HE038

Proposal Title: Vitamin E dose-dependently reverses bone loss.

Principal
Investigator:

Cheng-I Wei
Nutritional Sciences
139 HES
Campus

Reviewed and Processed as: Full Committee

Approval Status Recommended by Reviewer(s) : Approved

Revised protocol approved for a total of 75 rats for 3 years.

Signatures :

Kent Olson, IACUC Chairperson

Wednesday, March 19, 2003
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.

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

Protocol Expires: 3/18/2006

Date : Thursday, November 13, 2003 Animal Care and Use Protocol (ACUP) No: HE038

Proposal Title: Vitamin E dose-dependently reverses bone loss.

Principal
Investigator:

Cheng-I Wei
Nutritional Sciences
139 HES
Campus

Reviewed and
Processed as: Full Committee **Modification**

Approval Status Recommended by Reviewer(s) : Approved

Modification approved for reduction in number of animals per original group and establishment of additional groups and experiments (no change in total number of animals).

Signatures : Kent Olson

Dr. Kent Olson, IACUC Chairperson

Thursday, November 13, 2003
Date

cc: Department Head, Nutritional Sciences
Research Director

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 procedures must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Modification approvals are valid for the duration of the protocol approval (see protocol expiration date). 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

Sheau Ching Chai

Candidate for the Degree of

Master of Science

Thesis: DOSE-DEPENDENT EFFECTS OF VITAMIN E ON BONE IN AN ORCHIDECTOMIZED RAT MODEL OF OSTEOPOROSIS

Major Field: Nutritional Sciences

Biographical:

Education: Graduated from Kelapa Sawit High School, Johor, Malaysia in 1996. Received Bachelor of Science degree in Nutritional Sciences and completed Nutritional Sciences Dietetic Internship from Oklahoma State University, Stillwater, Oklahoma in December 2002 and August 2004, respectively. Completed the requirements for the Master of Science degree with a Major in Nutritional Sciences at Oklahoma State University in December, 2005.

Experience: Graduate teaching assistant and research assistant in the Department of Nutritional Sciences, Oklahoma State University, Stillwater, Oklahoma in 2004-present and 2003-present, respectively.

Professional Memberships: American Society of Bone and Mineral Research, American Dietetic Association, Oklahoma Dietetic Association.

Name: Sheau Ching Chai Date of Degree: December, 2005

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: DOSE-DEPENDENT EFFECTS OF VITAMIN E ON BONE IN AN ORCHIDECTOMIZED RAT MODEL OF OSTEOPOROSIS

Pages in Study: 54 Candidate for the Degree of Master of Science

Major Field: Nutritional Science

Scope and Method of Study:

Vitamin E at a moderately high dose in the diet was shown previously to improve the biomechanical and biochemical properties of bone in old male mice. The present study was conducted to evaluate whether vitamin E can exert bone-sparing effects on osteopenic male rats using an orchidectomized rat model of osteoporasis. Forty 12-month old male Sprague-Dawley rats were either sham-operated (Sham) or orchidectomized (Orx), and fed control diet for 120 days to establish bone loss. Thereafter, rats were assigned to their corresponding treatment groups (n = 10): Sham and one Orx group received 75 IU vit E (controls), other Orx rats received 250 or 500 IU vit E per kg diet for 90 days. After 90 days of treatment, rats were necropsied and tissues were collected. Bone mineral content (BMC), and density (BMD) of whole body, 4^{th} lumbar vertebra and right femur were measured using DXA. Bone metabolism was assessed based on biochemical markers of urinary excretion of deoxpyridiniline (Dpd) as an indicator of bone resorption, and serum osteocalcin as an indicator of bone formation. Trabecular and cortical microarchitectural properties were assessed using micro-CT.

Findings and Conclusions:

The significant losses of BMD and BMC in the whole body, right femur and 4^{th} lumbar due to ORX were not prevented by vitamin E treatments. Neither Orx nor any of the vitamin E treatments altered serum osteocalcin level and urinary Dpd. Additionally, vitamin E supplementation has no effect in preventing Orx-induced changes of the trabecular microarchitectural parameters of distal femur. However, vitamin E supplementation provides modest bone protective effects on cortical area in femoral mid-shaft of Orx rats. Although vitamin E has been shown to have some osteoprotective properties in other animal models, so far the findings of the present study indicate that vitamin E is unable to restore bone mass once the loss has occurred in this rat model of male osteoporosis. However, vitamin E supplementation provides modest bone protective effects on the cortical area in femoral mid-shaft of Orx rats.

ADVISER'S APPROVAL: Cheng-I Wei, Ph.D.