12-2003

The Osteogenic Effects of 12 Weeks of Oral Supplementation of Androstenedione in Middle-Aged Men.

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The Osteogenic Effects Of Twelve Weeks Of Oral Supplementation

Of Androstenedione In Middle-Aged Men

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A thesis
presented to
the faculty of the Department of Physical Education, Exercise, and Sports Sciences
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Master of Arts in Exercise Science

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by
Troy M. Wills
December 2003

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Keywords: androstenedione, estrogens, resistance training, osteoporosis

ABSTRACT

The Osteogenic Effects of 12 Weeks of Oral Supplementation

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by

Troy M. Wills

Recent evidence suggests that declining bone mineral density (BMD) in males is related to declining circulating estrogens. The majority of endogenous plasma estrogens in males result from peripheral aromatization of plasma androgens. Thus, it was hypothesized that dietary supplementation with an aromatizable androgen (androstenedione) may stimulate increases in BMD.

BMD (measured by dual energy x-ray absorptiometry) and biochemical markers of bone turnover (1, 25 OH2 Vitamin D, calcitonin, deoxypyrodinoline, and parathyroid hormone) were assessed before and after 12 weeks of dietary androstenedione supplementation (200 mg/d). Twenty-four volunteers were randomized into either an androstenedione supplementation or placebo groups. Study volunteers also performed high intensity resistance training (RT) during the treatment period.

Androstenedione supplementation significantly increased plasma estradiol-17ß levels by 82%. However, the increase in estradiol-17ß did not impact bone turnover. The RT regimen did stimulate significant, local increases in BMD. Spine BMD was significantly increased by 6% for both treatment groups.

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CHAPTER 1

INTRODUCTION

Osteoporosis is recognized as a serious public health problem as a result of the mortality and morbidity associated with the complications related to osteoporotic fractures (Barrett Connor, 1995; Cooper, Atkinson, Jacobsen, O'Fallon, & Melton, 1993; Cooper, Atkinson, O'Fallon, & Melton, 1992; Melton et al., 1997; Ray, Griffin, & Baugh, 1990). For instance, studies (Cooper et al., 1993; Ray et al., 1990; White, Fisher, & Laurin, 1987) following elderly patients after they have suffered an osteoporotic fracture have reported similar one-year mortality rates of approximately 20-30%. Along with this, there is a concern of the growing economic burden that this disease is presenting by way of health care costs. Ray, Chan, Thamer, and Melton (1997) reported that in the United States alone, medical expenditures for the treatment of osteoporotic fractures in persons 45 yr. and older were estimated to be $13.8 billion in 1995. Of this estimate, approximately $10.3 billion (75.1%) was afforded to the treatment of white women. This is a clear example that explains why this disease is predominantly recognized only as a problem in aging, white women. However, of the remaining 24.9% of medical expenditures related to this disease, Ray et al. (1997) report that 18.4% was associated with the treatment of white men, which equates to approximately $2.5 billion in medical costs. Numbers such as this, in conjunction with the rate of growth in our population (Spencer, 1989), has given many the evidence needed to warn that the future health care costs of osteoporosis related to men may become as serious of an economic problem as presently seen in women (Seeman, 1995).

Albright, Smith, and Richardson (1941) were the first to recognize a clear and concise relationship between the failure of the ovaries in women and the development of osteoporosis. From this, these researchers hypothesized that the lack of estrogen (E) production brought about

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by ovarian failure played the principle role in the their subjects diminishing formation and maintenance of bone. Since then, the validity of this theory has been proven through research (Greendale, Edelstein, & Barrett Connor, 1997; Heshmati et al., 2002; Khosla, Melton, Atkinson, & O'Fallon, 2001; Khosla et al., 1998; Richelson, Wahner, Melton, & Riggs, 1984; Riggs et al., 1998) yielding results that show strong negative relationships between levels of circulating E and bone loss in human subjects. The extent of this problem in females has led to the administration of estrogen replacement therapy (ERT) in many postmenopausal women who display low bone mineral density (BMD) or frailty. Research (Aitken, Hart, & Lindsay, 1973; Greendale, Espeland, Slone, Marcus, & Barrett Connor, 2002; Lane, Haupt, Kimmel, Modin, & Kinney, 1999; Moore, Bracker, Sartoris, Saltman, & Strause, 1990; Preisinger et al., 1995; Recker, Saville, & Heaney, 1977) has shown that the use of ERT is very effective in combating the progression of this disease by supporting bone formation and maintenance in postmenopausal women. Yet, the most common treatment employed to reduce bone loss in men suffering from low BMD or osteoporosis is testosterone replacement therapy (TRT). The use of TRT benefit aging men in this regard by increasing lean mass (muscle) (Bhasin et al., 1997; Hajjar, Kaiser, & Morley, 1997; Tan, Shiu, Pang, & Kung, 1998; Zgliczynski et al., 1996), and, in some cases, increasing biochemical markers of bone formation (Falahati Nini et al., 2000; Wang et al., 1996). However, Falahiti-Nini et al. performed a study and found that only the elderly men in their study who received ERT experienced a decline in markers of bone resorption and increases in measurements of bone formation in comparison to those receiving TRT. This led Falahiti-Nini et al. to conclude that E was the dominant sex steroid responsible for the regulation of bone resorption and that ERT should be used in men as well as women.

Men lose substantial amounts of bone over the course of their lifetime (Garn, Rohmann, & Wagner, 1967; Jones, Nguyen, Sambrook, Kelly, & Eisman, 1994; Mazess et al., 1990; Meema, 1963; Newton John & Morgan, 1970; Riggs et al., 1981), rather than the exaggerated acute period of bone loss generally experienced by women following menopause. This is primarily a result of the circulating levels of E in men having no dependence upon ovarian production. Rather, E production in men is chiefly dependent on the aromatization of circulating androgens, such as testosterone (T) and its precursors, to E (MacDonald, Madden, Brenner, Wilson, & Siiteri, 1979; Taxel et al., 2001; Weissberger & Ho, 1993). Interestingly, this may be the critical factor regarding bone loss with age in men because it has been documented that levels of circulating androgens decline with age as well (Davidson et al., 1983; Denti et al., 2000; MacDonald et al.; Nankin & Calkins, 1986; Vermeulen, Rubens, & Verdonck, 1972). Therefore, a better-suited treatment for bone loss in men may actually be to provide those suffering from low BMD with an androgen or an androgen precursor that is easily aromatized to E once in the peripheral circulation.

Recently, oral supplementation of androstenedione (AN) has gained popularity as an ergogenic-aid, which could possibly increase T production and lean mass in males when combined with a resistance-training program. This premise arises from AN being a direct precursor of T via the delta-4 pathway ($\Delta 4$) (Norman & Litwack, 1987). However, several studies (Broeder et al., 2000; Brown et al., 2000; King et al., 1999; Rasmussen, Volpi, Gore, & Wolfe, 2000) that investigated this claim resulted in findings that suggested supplementation of oral AN in a male population has no significant effects on T production. In fact, the most common finding of the studies (Broeder et al., 2000; Brown et al., 2000; King et al., 1999; Rasmussen et al., 2000) involving oral AN supplementation in males was significant increases in

estrogens. Broeder et al. (2000) hypothesized that this occurrence, which was displayed in their male subjects receiving oral AN, was peripheral aromatization of AN to E once an abundance of oral AN has been introduced to the circulation. While these findings may not be conducive to what the initial proposed use of the supplement was, it is feasible that the increase in E resulting from the oral supplementation of AN may be harnessed to benefit the maintenance of bone in aging men.

<div align="center">Statement of the Problem</div>

The purpose of this study was to determine if 12 weeks of oral AN supplementation by the subjects of the "Andro Project" by Broeder et al. (2000) positively influenced these subjects' biochemical markers of bone resorption/formation and measurements of bone density. All previous investigations of oral AN supplementation involving male subjects have produced findings that suggest this supplement results in elevated levels of E. Most likely the elevated E levels are the result of an increased aromatization of AN and testosterone once above normal peripheral circulation levels and/or through first-pass liver effects of AN supplementation. From this, it is reasonable to assume that E levels in men ingesting AN will increase with extended use. By taking into account the significant amount of research (Greendale et al., 1997; Heshmati et al., 2002; Khosla et al., 2001; Ravaglia et al., 2000; Richelson et al., 1984; Riggs et al., 1998) that has established the positive relationship between bone health and circulating E, it can be postulated that these findings may offer insight into an innovative technique to treat bone loss in aging men. Therefore, by using frozen urine and blood serum specimens previously taken during the "Andro Project", this study will attempt to determine the osteogenic effects on bone of oral AN supplementation in 12 subjects while consuming 200 mg/d of the 3, 17-dione (DIONE) form of AN compared to 12 placebo subjects.

<p style="text-align:center">Hypotheses</p>

The following hypotheses are proposed for this study:

H_{A1}: Twelve weeks of oral AN supplementation in subjects from the "Andro Project" will

cause a decrease in the presence of markers of bone resorption in those subjects.

H_{A2}: Twelve weeks of oral AN supplementation in subjects from the "Andro Project" will

cause an increase in the presence of markers of bone formation in those subjects.

H_{A3}: Twelve weeks of oral AN supplementation in subjects from the "Andro Project" will

cause increases in BMD as measured by DEXA.

<p style="text-align:center">Assumptions</p>

For the purposes of this study the following assumptions will be made:

1. The subjects took the supplement given to them by the researcher according to the guidelines set by the researcher.

2. The subjects observed the regulation of fasting 12 hours prior to each blood draw.

3. Changes in BMD and/or bone markers of formation/resorption that occurred in response to the resistance-training program used during this study were similar in all subjects. This assumption was based on all subjects being similar in regards to health status, and records of all training depicted that both groups (placebo, AN) performed similar amounts of work and increased strength similarly as well.

4. All urine and blood serum samples were properly handled and stored.

As in all studies, the researchers will face varying limitations within the study's base. These limitations are as followed:

1. Subjects volunteered for the study in which the urine and blood samples were taken, and volunteers can often affect the internal validity a study. For instance, some of the subjects in this study had previous resistance-training experience and some had not, which could possibly cause variations in the subjects' response to resistance-training in regards to increases in BMD and rates of bone turnover.

2. The number of samples used for this study is relatively small, and all were taken from relatively healthy men from 35-65 years old that were generally from the same geographical area. This limits the researcher's ability to generalize data toward a large population such as men of out of the age range of 35-65, of abnormal health, or women.

3. Because the subjects involved in this study primarily had normal bone density values at the start of the study, this may limit our ability to observe significant improvements in bone turnover or bone density responses related to either the RT program or oral AN supplementation.

4. It is possible because we randomly selected subjects for the Placebo and Androstenedione groups respectively, that subject data for a given variable may not form a standard curve. Thus, statistical corrections may be required to meet the statistical assumptions of parametric data analyses procedures.

5. Because lifestyle factors such as smoking, alcohol consumption, and dietary intakes of calcium and vitamin D intake can influence rates of bone turnover, it must considered the subjects lifestyles in regard to these aspects could have affected their BMD

measurements and/or the biochemical markers of bone turnover that will be used for this study.

<div align="center">Delimitations</div>

The results from the "Andro Project" produced the following delimits. These delimits are defined in Table 1.

Table 1: Delimits of the Study/Subject Characteristics**.**

Item	Delimit
Population	❖ 24 males aged 35-65 years
Fitness Status	❖ Trained and Untrained in Resistance Exercise
Supplement Intervention*	❖ Androstenedione (200 mg/d), n = 12 ❖ Placebo (cornstarch), n = 12
Resistance Training Intervention	❖ 12 wk (3 sessions per week) ❖ training intensity: 60-70 % of 1 RM for wk 1, and 80-95 % of 1 RM for wk 2-12. ❖ whole body multi-joint lifts primarily (i.e. bench press, leg press) ❖ total weight lifted and time of each session were recorded ❖ all sessions were observed and assisted by personal trainers
Dietary Markers	❖ Three-day dietary recall (one weekend day, two weekdays) given to each subject randomly throughout study and then analyzed using the Dine Nutrient Analysis software (Dine Systems, Inc., Amherst, NY). ❖ A food frequency intake survey was given and explained by a registered dietician to each subject and then analyzed using the Nutritionist 4 software (N-Square Computing, Salem, OR). ❖ The values for total calories, protein, carbohydrates, fat, vitamin D, calcium, smoking, and alcohol consumption gathered from the subjects' specimens that will be used in this study will be analyzed to determine their effect on data collected from BMD measurements, and biochemical markers of bone turnover.
Blood Chemistry Markers	❖ Blood samples were taken from all subjects prior to and during the study on a monthly basis to assure no subject had exceeded standard clinical limits for hematocrit, hemoglobin, and prostate-specific antigen.

Table 1: continued.

Item	Delimit
Hormonal Markers	❖ Baseline and monthly values for total testosterone, free testosterone, estradiol-17β, luteinizing hormone, and follicle-stimulating hormone were taken from the subjects in this study. ❖ Baseline and post-treatment values for estrone, androstenedione, and DHEAS were also taken from each subject. ❖ Primarily, this study will use the values for estradiol-17β and estrone to compare their relationship with the measurements of BMD, and the values gathered from the biochemical markers of bone turnover.
Bone Turn-over Markers	❖ The immunoassay kits for intact parathyroid hormone and deoxypyrodinoline will be used to determine rates of bone resorption in response to increased estrogen levels. ❖ The immunoassay kit for calcitonin will be used to determine rates of bone formation in response to increased estrogen levels. ❖ The immunoassay kit for 1, 25 OH2 Vitamin D will be used to determine the effect that increased levels of estrogens had on Vitamin D status (a strong determinant of healthy bone and bone formation).
Bone Density Markers/Body Composition	❖ Bone density and body composition measurements taken by dual-photon x-ray absorption (DEXA) at baseline and post-treatment periods will be used in this study.
Work Performed and Strength Markers	❖ Measurements of total body strength and one repetition max (1 RM) for the bench press and leg extension that were taken at baseline and post-treatment periods will be used to demonstrate the consistency at which both placebo and androstenedione groups increased in a similar manner in regards to strength as a result of resistance training. ❖ Values displaying total weight lifted and work performance (kg lifted/min) during the training period will be used to display that both the placebo and androstenedione groups performed similar amounts of work during the study.

CHAPTER 2

REVIEW OF LITERATURE

Osteoporosis and reduced bone mineral density is already recognized as an international health and economic concern for aging women. In recent years, though, the growing number of medical expenditures afforded to the care of men suffering from osteoporotic fractures has brought attention to the need for treatment of the disease in this population as well. An abundance of research has given clear results that the role of normal estrogen production or formation is predominant in maintaining bone mass in both sexes. The purpose of this study is to determine whether the 12 weeks of oral androstenedione supplementation contributed to positive changes in bone formation.

In this chapter, literature is reviewed from various research related to the problem that focuses on: (a) prevalence and economic cost of osteoporosis in white males, (b) lifestyle factors that influence the development of osteoporosis, (c) the influence of exercise and the intake of calcium and vitamin D in the onset and treatment of osteoporosis, (d) aging and bone loss, (e) estrogens influence on bone loss with age in males, (f) benefits of estrogen replacement therapy, (g) androstenedione supplementation, and (h) oral androstenedione supplementation as a possible means to protect aging men from bone loss.

Prevalence and Economic Cost of Osteoporosis in White Males

Osteoporosis refers to the reduction in mass and density of bone that results in a compromise of skeletal integrity and mechanical support as defined by McCance and Huether (1998) (McCance & Huether, 1998). It is estimated that as many as 20 to 25 million people in the U.S. are affected by this disease (Peck et al., 1988). A precise determination of the number of persons afflicted by this disease is difficult to obtain due to its diagnosis usually being

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established only after the occurrence of an osteoporotic fracture. However, concern for the health-care costs coupled with the growing prevalence of such fractures is the focal point of a significant amount of research (Barrett Connor, 1995; Ray et al., 1997; Schneider & Guralnik, 1990). Therefore, the majority of research in this area has estimated the number of those suffering from osteoporosis on the basis of reported osteoporotic fractures (Barrett Connor). More specifically, fractures of the hip, vertebrae, and distal forearm have all been distinguished as primary results of osteoporosis or below average BMD in the elderly (Cooper et al., 1992; Melton, Ilstrup, Beckenbaugh, & Riggs, 1982; Owen, Melton, Johnson, Ilstrup, & Riggs, 1982). Data collected by Cummings et al.(1990) showed an inverse relationship amongst the risk of hip fracture over a 2-year period and bone mineral density (BMD) at any site of the femur in 9703 non-black women (>65 yr.), giving evidence toward the feasibility of such estimates. Others (Cooper et al., 1993; Ray et al., 1990; White et al., 1987) have identified the morbidity and mortality rates associated with osteoporotic fractures as a significant health concern. For instance, Ray, Griffin, and Baugh (1990) found a 23.7% one-year mortality rate of 2130 patients who had suffered a hip fracture and were 65 years of age or older. This estimate corresponds with others that have been attained through similar studies (Cooper et al., 1993; White et al.) deeming osteoporosis as a major public health concern for the elderly. Furthermore, the economic costs of this disease in regards to health care are a major concern (Barrett Connor, 1995; Ray et al., 1997; Schneider & Guralnik, 1990).

Of those suffering from this disease, it has been well-established through documentation of treated osteoporotic fractures that women over the age of 45 years are those most commonly affected by it (Cooper, Atkinson, O'Fallon & Melton, 1992; Cummings et al., 1990; Melton et al., 1997). For example, Melton et al. (1982) used the medical records over a 50-year period of

one community to find that the number of women who suffered hip fractures nearly quadrupled that of men and only 5% of those fractures occurred in persons less than 50 years of age. Additionally, it has been determined that whites are at a greater risk of developing the bone fragility associated with osteoporosis more so than blacks due to genetic differences in BMD (Baron et al., 1994; Kellie & Brody, 1990; Rodriguez, Sattin, & Waxweiler, ; Taaffe et al., 2001). Wright et al. (1995) observed that various biochemical factors such as increased growth hormone (GH) secretion in black men provide a genetic predisposition toward higher absolute and relative BMD in their race. Thereby, allowing for a decline in BMD with age to be of less concern. Due to findings like these, osteoporosis has essentially been recognized as a health concern for only white women. Statistics from the third National Health and Nutrition Survey (NHANES III, 1988-1991, U.S.) support this conclusion, estimating that the prevalence of osteoporosis in white women is as much as 2.5 times higher than in black women and 1.2 times higher than in Mexican women (Looker et al., 1995). However, as the number of aging persons within the U.S. increases (Schneider & Guralnik, 1990), the health care costs for the treatment of osteoporotic fractures in white men is gradually becoming an economic burden (Seeman, 1995).

The growth of the elderly population provides a primary reason of the subsequent rises in health care costs associated with osteoporosis in the U.S. (Schneider & Guralnik, 1990). Spencer (1989) used data compiled by the U.S. Census Bureau to predict that the number of persons in the U.S. who were 65 or older would rise from approximately 30 million in 2000 to 52 million by 2020 and continues to grow to 68 million by 2040. In 1986, the estimated total annual cost of osteoporotic fractures per year was between $7 and $10 billion in the U.S (Peck et al., 1988). A comprehensive study by Ray et al. (1997), which took into account the expenditures afforded to all health care variables related to osteoporosis, found this estimate to have risen to $13.8 billion

in 1995. Demonstrating osteoporosis' predominance in whites, Ray et al. (1997) attributed 75.1% and 18.4% of these costs to white women and men, respectively. Their report also illustrates the idea that white women are the majority of those affected by osteoporosis and much of the preventive treatment in this area should be directed toward them. Nevertheless, it must be recognized that the previously mentioned 18.4% of total health-care costs attributed to osteoporosis in men by Ray et al. equate to approximately $2.5 billion dollars (Ray et al.).

These expenditures invite even more need for concern when estimates based on the rise of the elderly population project that medical expenses toward the care of osteoporotic fractures would continue to increase (Ray et al., 1997; Schneider & Guralnik, 1990; Seeman, 1995). Cummings et al. (1990) used nationally collected data to estimate the potential rises in these costs and speculated that total annual costs for hip fractures alone could reach $240 billion by 2040 when inflation was accounted for. Additionally, Seeman (1995) stated that the projected number of hip fractures in men worldwide in the year 2025 would be 1.2 million. This amount is similar to the global number of fractures reported in women in 1990, which was approximately 1.4 million (Seeman, 1995). These calculations suggest that not only will the problem of osteoporosis in women reach a tremendous magnitude, but that the effects of this disease in white men will evolve into a problem that is proportional to the one currently seen in white women.

<u>Lifestyle Factors that Influence the Development of Osteoporosis</u>

In most cases, the onset of osteoporosis is the result of a combination of factors that are influenced by lifestyle (Krall & Dawson Hughes, 1999; Poor, Atkinson, O'Fallon, & Melton, 1995; Rapuri, Gallagher, Kinyamu, & Ryschon, 2001), age (Mazess et al., 1990), genetics (Kelly et al., 1993), and, most importantly, gender (Garn et al., 1967). As a people age, their lifestyle

becomes a key determinant of their relative risk in developing a number of diseases with aging. In regards to the development of osteoporosis, several studies (Abbott, Nadler, & Rude, 1994; Harris & Dawson Hughes, 1994; Krall & Dawson Hughes, 1999; Poor et al., 1995) have shown that factors such as caffeine intake, alcohol consumption, and smoking contribute to bone loss. In a study performed by Rapuri, Gallagher, Kinyamu, and Ryschon (2001), it was shown that elderly women with a caffeine intake >300 mg/d had a significant increase in bone loss over a 3-year period in comparison to elderly women with a caffeine intake <300 mg/d. Additionally, Hernandez-Avila et al. (1991) found a positive relationship with caffeine intake and the risk of hip fracture in women (34-59 yr.). They also found alcohol consumption to be independently associated with the increased risk of hip and wrist fractures in these women. Christiansen hypothesized that the adverse effect that alcohol consumption has on bone mineral density (BMD) comes as a result of its toxic action on bone cells and warned that chronic alcohol abuse is a risk factor for men and women in the development of osteoporosis. Abbott et al. (1994) explain that many alcoholics display a depletion of magnesium (Mg), a mineral that represents approximately one third of total bone mass, due to renal Mg-wasting that leads to an increase in urinary Mg excretion. While the reasons for the negative effect that increased alcohol consumption has on bone are not precisely understood, it is a proven risk factor for reduced BMD (Christiansen).

The positive relationship between hip fracture and cigarette smoking has been documented as well (Cornuz, Feskanich, Willett, & Colditz, 1999; Krall & Dawson Hughes, 1999). Women (34-59 yr.) who smoked or had formerly smoked in a study by Cornuz et al. (1999) showed a linear increased risk for hip fracture with greater cigarette consumption and experienced greater incidence of hip fractures than those who had never smoked. Krall and

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Dawson Hughes (1999) observed similar findings in 402 elderly men and women (> 65 yr.) studied during a 3-year placebo-controlled study of calcium (Ca) and vitamin D supplementation. Through measurements of Ca intake and urinary Ca excretion, they determined that the 1.7 % decrease in Ca absorption seen in smokers was a result of smoking decreasing the intestines' ability to absorb Ca. While these factors do influence the development of osteoporosis, there are other elements of one's lifestyle that have a much stronger effect on this disease.

<u>The Role of Calcium and Vitamin D Intake, and Exercise in the</u>

<u>Onset and Treatment of Osteoporosis</u>

A primary element of Ca absorption is, of course, whether or not an individual's diet supplies that person with sufficient amounts of dietary Ca, the largest constituent of solid particles in bone (Manore & Thompson, 2000). Also, adequate intake of Vitamin D, a nutrient necessary for the body to absorb Ca, is an integral factor in the development of healthy bone. In many cases, it has been shown that those who suffer from osteoporotic fractures have reported low intakes of both of these nutrients (Jowsey, 1976). For instance, osteoporotic fractures are a major health concern in Asia (Barrett Connor, 1995; Seeman, Young, Szmukler, Tsalamandris, & Hopper, 1993). The role that Ca intake may contribute to this fact is displayed in Japan which is one of the highest dietary Ca-deficient nations in the world (Fujita, 1990). Fujita reports that the average Ca intake for the Japanese individual is 300 mg/d, which is half the recommended daily allowance (RDA) of 600 mg/d in Japan. He also points out that this amount is less than half of the RDA value in the U.S., which is 1000 mg/d. Along with this, Fujita explains that the vitamin D intake of the elderly there is well below RDA values, and this is intensified by the nation's limited solar exposure in northern regions, which is the other primary source of vitamin

D in humans (Manore & J., 2000). However, this problem is not limited to Japan. Chapuy, Preziosi, Maamer, et al. (1997) studied 1569 male and female French citizens (mean age= 50 ± 6 yr.) from 20 different French cities and found the mean intakes of Ca (849 ± 481 mg/d) and vitamin D (3.4 ± 7.6 μg/d) were lower than the present RDA values of 1000 mg/d (Wickham et al., 1989) and 10 μg/d (Holbrook, Barrett Connor, & Wingard, 1988), respectively. Additionally, they found a strong relationship ($r = 0.72$) between vitamin D status and reported exposure to sunlight. This supports other evidence (Quesada Gomez, Alonso, & Bouillon, 1996) of the positive influence sunlight has on maintaining vitamin D status and it's prevention of low BMD.

It has been reported that Ca intake begins to decline at approximately 20 years of age (Odland et al., 1972). Also, the rate at which Ca is absorbed by the body declines with age (Bullamore et al., 1970). Vitamin D intake is often inadequate as well in the elderly due to the limitation of this nutrient from natural dietary sources and the tendency of those who are frail to receive less sunlight than what is needed to compensate for their dietary shortcomings (Kessenich & Rosen, 1996; Sato, Asoh, & Oizumi, 1998). Sato et al. (1998) examined the plausibility of these factors affecting the BMD of a diseased, elderly population by studying a group of 46 ambulatory women who had been diagnosed with Alzheimer's Disease (AD) and lived in a nursing home. They found the Ca intake, vitamin D status (dietary intake, and serum blood levels of 25-hydroxyvitamin D), sunlight-exposure, as well as the BMD of the AD group to be significantly lower than those of age-matched controls. More specifically, approximately 80 % of the AD group displayed deficient blood levels of 25-hydroxyvitamin D (< 10 ng/ml), less than adequate intakes of Ca and vitamin D, and the entire group was either completely deprived of sunlight or was exposed to sunlight < 15 min/wk. The negative, synergistic effect that the low dietary intakes of calcium and vitamin D along with the lack of sunlight seemed to

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have on the vitamin D status in these women gave adequate evidence for the researchers to label these variables as primary contributing factors to the subjects reduced BMD. It also can be assumed that the same factors may induce bone loss in other elderly individuals suffering from disease.

One of the key components toward the onset of osteoporosis with age is the peak bone mass (PBM) attained by an individual, which is usually met between the ages of 25-40, but is heavily influenced during childhood and adolescence (Marieb, 1992a). The higher PBM a person obtains is important because it allows for any ensuing loss of bone mass with age to be better tolerated (Seeman et al., 1993). Ruiz, Mandel, and Garabedian (1995) found that children and adolescents (n = 151; 7-15.3 yr.) in their study who had dietary Ca intakes < 1000 mg/d more often displayed lower BMD at the vertebral and femoral sites in comparison to those with adequate Ca intakes. Furthermore, the duration of weekly physical activity proved to be an independent determinant of BMD at both sites for all children but especially for girls (vertebral: r^2 = .657, femoral: r^2 = .591).

A person's activity level positively influences absolute PBM (Aloia, Vaswani, Yeh, & Cohn, 1988; Barrett Connor, 1995; New, 2001; Paganini Hill, Chao, Ross, & Henderson, 1991), and assists in maintaining BMD with aging (Evans, 1995; New, 2001; Paganini Hill et al., 1991). In 1870, a German scientist, J. B. Wolff, established a theory that defined the relationship in which bone responds to the mechanical stimuli placed upon it (Chamay & Tschantz, 1972). Wolff's law states that a bone grows proportional in response to the forces or stresses placed upon it (Marieb, 1992a). The scientific basis to this theory is that bone modifies its structure under mechanical load by appositional growth in the concavity and resorption in the convexity (Chamay & Tschantz). Chamay and Tschantz tested this theory by using injured dogs that had

undergone a surgical procedure that caused a consistent injury within the ulnas of the animals, by measuring the injured bones' response to different types of mechanical overload induced by treadmill exercise. They found that hypertrophy of the dogs' ulnas continued until the bone had adapted to the mechanical overload placed upon it, reaffirming and supporting the findings of Wolff. This also gives support to the role that an individual's activity level plays in minimizing the onset of osteoporosis with age by stimulating bone formation and, thereby, allowing the weight-bearing bones to undergo reasonable amounts of stress without fracture.

An abundance of research (Evans, 1995; Greendale, Barrett Connor, Edelstein, Ingles, & Haile, 1995; Kirchner, Lewis, & O'Connor, 1996; McCartney, Hicks, Martin, & Webber, 1995; Menkes et al., 1993; Nelson et al., 1994) has been collected showing the positive relationship between activity levels and BMD. For instance, Greendale et al. (1995) studied the BMD measurements of a cohort of 1703 men and women (mean age = 73 yr.) taken over a 3- year period to determine the effect exercise has on BMD acutely and over time. They found that the BMD measurements of the hip were significantly higher in those who participated in strenuous ($p = .0001$) and moderate ($p = .0004$) exercise in comparison to those who exercised less. These findings led them to infer that exercise provides a protective effect on BMD of the hip in men and women. Another study of similar nature on white premenopausal women (39 ± 1.39 yr.) by Aloia et al. (1988) found that current activity levels were positively correlated ($r = .41$) with BMD of the spine and total body Ca levels ($r = .51$). They deduced from their data that the level of physical activity might be the principle determinant of PBM in this population. Also, a study by Grisso et al. (1997) used the various factors that are associated with hip fracture such as body mass, medications being taken, presence of disease, and activity levels to determine the significance each may have had in contributing to hip fractures in men. They used data collected

regarding these factors from 356 men (> 45 yr.), who had suffered a hip fracture and compared it with that of healthy, age-matched controls. They found that increased levels of physical activity were markedly protective against hip fractures, displaying physical activity's positive influence on BMD in men as well. The importance of this factor lies in the relationship between the absence of weight-bearing activity and BMD (Donaldson et al., 1970; Krolner & Toft, 1983).

The likelihood of decreased physical activity and the susceptibility of debilitating disease are heightened with age (Schneider & Guralnik, 1990). This decline in weight-bearing exercise that is often associated with chronic illness has been shown to have a deteriorating effect on the BMD of individuals (Krolner & Toft, 1983). Donaldson et al. (1970) studied the effects of long-term "bed-rest" (30-36 wk) on the rates of bone loss in three healthy male volunteers (21-22 yr.) in an attempt to simulate the rates of mineral loss and skeletal changes that occur during sustained periods of immobilization. Total-body Ca losses for the subjects during the "bed rest" period were estimated at 4.2% of the body calcium store. Urinary Ca loss reached a maximum value during the 7th week at 70% above baseline values with the subjects continuing to remain in negative Ca balance up to the final week of "bed rest." This suggests that bone mineral loss is perpetual throughout periods of immobilization. Also, measurements of the os calcis bone by gamma ray transmission scan in all subjects revealed a mean loss of 34.3% of its bone mineral content during the 12th week through the final week of "bed rest." Additionally, declines in bone mineral content of the os calcis bone were greater with increased duration of immobilization. From this observation, Donaldson et al. (1970) deduced that weight-bearing bone is the primary source of mineral loss during sustained "bed rest" and may propagate the risk of fracture in these bones once an individual becomes ambulatory. In another study, male and female subjects (18-60 yr.) hospitalized and treated for lower back with therapeutic "bed rest" pain were studied by

Krolner and Toft (1982) to determine declines in lumbar BMD as a result of this therapy. Unlike the previous study, subjects did participate in a physical activity program administered by physical therapists. However, all physical activity was performed with the subjects in a recumbent position to negate any vertebral loading. The estimated loss of BMD in the second, third, and fourth lumbar vertebrae for all subjects during the "bed rest" period (mean = 27 days) was 0.9% per week. Krolner and Toft (1982) suggest that this gives evidence to the susceptibility of individuals who undergo recurrent periods of "bed-rest" being predisposed to spinal osteoporosis.

It is clear that inadequate physical activity and intakes of Ca and Vitamin D have a negative effect on BMD. Yet, there is evidence that shows introducing these elements to the lifestyles of individuals, who are deficient in regards of their exercise and dietary needs, can aid in maintaining and increasing their BMD (Adams, Kantorovich, Wu, Javanbakht, & Hollis, 1999; Aloia et al., 1994; Dawson Hughes et al., 1991; Nelson et al., 1994). For example, regular exercise is a primary means of treatment for low BMD in any population ("Exercise programs for the elderly. Council on Scientific Affairs.," 1984). Snow-Harter, Bouxsein, Lewis, Carter, and Marcus (1992) proposed that RT and cardiovascular exercise (CE) are both, similarly effective in increasing or restoring BMD in men and women. However, their results suggesting this conclusion were derived from only using the vertebral and femoral regions to measure changes in BMD, which do not take into account the impact of training on the wrist (a site that is often fractured in those with osteoporosis). Additionally, the RT regimen used during this study was of relatively low-intensity and/or of less than one year in duration, either of which may not provide adequate stimuli to initialize substantial bone formation. For instance, Snow-Harter et al. found similarly, modest increases in BMD of the lumbar spine in young women (n = 52, mean

age = 19.9 yr.) who either participated in a RT or jogging exercise program for 8 months. However, the RT program never exceeded efforts greater than 75% of their initial one-repetition maximal effort (1RM) until the final 2 months of the 8-month program, which was circuit-based in nature (e.g. a type of program often used to increase muscular endurance). More recent studies (Kerr, Morton, Dick, & Prince, 1996; Menkes et al., 1993; Nelson et al., 1994) have found that high-intensity (i.e. heavy loading) RT regimens provide a significant increase in total body BMD that cannot be obtained by CE, alone, due to the acute stress that this type of training introduces directly to the skeleton. In one study, intervention of a 1-year, high-intensity RT program in postmenopausal women resulted in significant increases in BMD of the femur ($p = .02$) and spine ($p = .04$) (Nelson et al., 1994). In contrast, the BMD of the age-matched controls declined during the same period. To better understand the different effects that low- and high-intensity RT may have on BMD status, Kerr et al. (1996) separated their sedentary, postmenopausal, female subjects (n = 48) into a strength training (ST) group and an endurance training (ET) group during their 1-year study. The RT exercises were the same for both groups, along with the number of times they exercised per week and total training volume. However, the ST group performed fewer repetitions and used higher loads for each exercise, while the ET group used lower loads and performed more repetitions (i.e. ST: 3 sets x 8 reps at 10 kg vs. ET: 3 sets x 20 reps at 4 kg). Also, the subjects only used one side of their body to perform the RT exercises, leaving the side that wasn't exercised as a control. Upon the study's completion, the exercise side of the ST group's BMD was significantly higher than their control side at the trochanter-, intertrochanter-, and Ward's triangle-sites of the hip, as well as the radius ultra distal site of the forearm. Interestingly, the only significant site-specific change in BMD observed in the ET group at the end of the study was at the radius mid of the forearm. These findings suggest that the bone mass

of post-menopausal women who are estrogen-deficient are only increased through a RT regimen that requires the performance of high-load low repetitions in contrast to one that consists of low-load high repetitions. Furthermore, the results give insight to the superior nature in which high-intensity RT can comprehensively combat the low BMD at all appendicular sites of inactive individuals in comparison to endurance activities such as treadmill walking, and cycling.

Researchers have also measured the BMD and biochemical markers that signify bone formation and bone resorption in subjects taking synthetic Ca and vitamin D supplements individually (Adams et al., 1999; Ooms et al., 1995; Orwoll, Oviatt, McClung, Deftos, & Sexton, 1990) and simultaneously (Baeksgaard, Andersen, & Hyldstrup, 1998; Chapuy et al., 1992; Peacock et al., 2000) over extended periods of time to determine their value in combating osteoporosis. With most finding that, like physical activity, the role that the supplemented intake of Ca and Vitamin D may contribute in counteracting declines in BMD is often relative to the degree of deprivation displayed by the subject (Adams et al., 1999; Dawson Hughes et al., 1991). More specifically, supplementation of these nutrients in those suffering from low BMD is often trivial if an individual's regular diet supplies that person's necessary requirements, and their low BMD is most likely the result of other factors. For instance, 1634 elderly women (mean age = 84 ± 6 yr.) that previously had low dietary intakes of Ca and vitamin D displayed a 43% lower incidence of hip fracture after receiving daily supplements of Ca (1200 mg) and vitamin D (20 μg) for 18 months in comparison to age-matched controls who received placebo during the same period in a study by Chapuy et al. (1992). The women receiving supplements also showed a 2.7% increase in BMD of the proximal femur, whereas the placebo group's BMD measurement of this site declined by 4.6%. This gives credibility to the value supplementing synthetic forms of these nutrients in those with less than adequate dietary intakes of them may play in impeding

bone loss. However, Kessenich and Rosen (1996) found that 53 osteoporotic females (mean age = 71.7 ± 1.7 yr.) continued to display vitamin D insufficiency as indicated by blood serum levels of 25-hydroxyvitamin D through winter months despite the supplementation of this nutrient. They concluded that the lack of sunlight experienced by these women during this time was the culprit to their low vitamin D status and one of the contributors to their low BMD along with the decrease in exercise that frequently accompanies winter. Their study divulges insight into the complexity of the onset-factors of osteoporosis in that single treatment remedies are often insufficient in treating this disease. Orwoll et al. (1990) performed the first longitudinal study of its kind, in which the rate of normal bone loss in 77 healthy men (30-87 yr.) was monitored over a 3-year period. In addition to this, the subjects were either given daily Ca (1000 mg/d) and Vitamin D (25 µg/d) supplements or identical placebos to determine whether supplementing these nutrients has any relevance in decreasing the normal bone loss that accompanies age. The major finding of this study was that in this population of men who were well nourished and free of medical conditions that may affect bone metabolism, there was a substantial degree of bone loss with age regardless of Ca and Vitamin D supplementation. Specifically, the results of the study suggested that in normal men there is 1-%-yearly decline in BMD at, both, the proximal radial- and distal radial-sites of the forearm and a 2.3% -yearly loss of vertebral BMD after the age of 30. These findings were consistent in the treatment and placebo groups. Subjects in this study were stringently chosen on criteria that would eliminate any extrinsic factors that may affect bone metabolism from decreasing the validity of the results. For example, subjects who smoked or regularly consumed large amounts of alcohol were not allowed to take part in the study. This allows one to assume that bone loss is perpetual with age in men, and such lifestyle factors only intensify the normal decline in BMD. Another one of the factors that would prohibit

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applicants from participating in this study was if they were regularly physically active, due to exercise's known effect on bone metabolism. While this allows for suspicion toward whether exercise could have positively effected the observed declines in bone mass, it can only be assumed that exercise may have lessened the rate of BMD loss and, yet, the men would have still continued to lose bone mass with age. With this in mind, it is clear that there are more substantial elements that contribute to bone loss with age.

<u>Aging and Bone Loss</u>

There is a decline in BMD that accompanies aging in men and women (Cummings et al., 1990; Garn et al., 1967; Garnero & Delmas, 1998; Mazess et al., 1987; Mazess et al., 1990; Ruegsegger, Dambacher, Ruegsegger, Fischer, & Anliker, 1984). However, the rate and extent of decline in BMD is much more progressive and conceptually understood in women rather than men (Albright, Smith, & Richardson 1941; Meema, 1963; Richelson et al., 1984; Riggs et al., 1981). For instance, it has been made clear by scientific research (Albright et al.; Garn et al., 1967; Meema; Riggs et al., 1981) that women between the ages of 45-55 yr. experience a distinct, accelerated decline in BMD. To determine whether the decline in BMD displayed by middle-aged women is ethnically and/or geographically bias, Garn et al. (1967) compared the cortical characteristics of the second metacarpal of men and women (n = 7190; 8-90 yr.) from the U.S., Guatemala, and El Salvador. Beginning at age 40, they found a common trend of cortical bone loss at this site in both sexes regardless of ethnicity or location. However, females displayed the most dramatic decline in bone loss, which was estimated to be 39% between the 5th and 10th decades of life in comparison to 15% in males. Similarly, Smith and Rizek (1966) studied the BMD of the spine, femur, and hand in healthy women (45-90 yr.) residing in Puerto Rico (n = 200) and Michigan (n = 2063: black = 137, white = 1926). Their findings revealed that

nearly 50% of all women over the age of 45 displayed a significant degree of vertebral atrophy. While it was shown that the decline in BMD was progressive after the age of 45 in all populations, those of Caucasian origin proved to be principally prone to diminishing levels of vertebral BMD. Further, white women studied from Michigan showed a significantly distinct decline in femoral thickness occurring at approximately 53 yr. of age that continued to progress with age thus, establishing substantial evidence for the predominant risk of osteoporosis and osteoporotic fracture in white women with age.

In men, bone diminution is inherent with age as well (Garn et al., 1967; Mazess et al., 1990; Meema, 1963; Suominen, Heikkinen, Vainio, & Lahtinen, 1984; Wishart, Need, Horowitz, Morris, & Nordin, 1995). Yet, a particular age of onset in which rapid bone loss begins to occur (as seen in women) is yet to be defined in this population (Garn et al.; Mazess et al., 1990; Suominen et al.). A substantial amount of research in this area suggests that bone loss in most men is initiated gradually and may only become of concern after the 8th decade of life. The relevance of this assumption is warranted through the findings of a population-based study by Cooper et al. (1992) that found the age-incidence of vertebral fractures in men to equate that of women for individuals over the age of 84. Some of the earliest research in this area performed by Meema (1963) revealed that a gradual decline in the cortical thickness of the radius was present with age in both sexes. However, the average lifetime loss of cortical bone was three times greater in women than men. Also, while women in his study began to display considerable cortical atrophy between the ages of 45-55 yr., the reduction of cortical thickness in males was not detected until the ages of 55-65 yr. Mazess et al. (1990) found similar evidence supporting the gradual loss of bone in males by measuring the BMD of the spine (n = 315) and femur (n = 282) in healthy white male subjects. When compared with BMD measurements of the same sites

of age- and race-matched female subjects from a previous study performed by the same researchers (Mazess et al., 1990), BMD loss of the spine in men at the age of 70 was estimated to be 85% less than that in women at this age. Additionally, the loss of BMD at the femoral neck of the men was approximately 21% upon reaching the 8th decade in comparison to 25-30% in females. Interestingly, the researchers of both of these studies related the occurrence of self-reported menopause, the period of life in females where hormonal changes prompt ovulation and menstruation to cease (Marieb, 1992b), with the losses of bone found in their female populations. Like other research (Falahati Nini et al., 2000; Ohta, Makita, Komukai, & Nozawa, 2002; Sarrel, 2002; Yoshimura, Kasamatsu, Sakata, Hashimoto, & Cooper, 2002) that has observed the interactions of these two occurrences, their findings indicated that the change in gonadal function is the single most important factor in the etiology of osteoporosis in females.

Albright et al. (1941) pioneered the way in which postmenopausal osteoporosis is presently understood by being the first to suggest that the decline in estrogen production as a result of ovarian failure brought about by menopause is chiefly responsible for the accelerated bone loss experienced by women between the ages of 45-55 yr. Since then, a plethora of research (Falahati Nini et al., 2000; Gnudi, Mongiorgi, Figus, & Bertocchi, 1990; Khosla et al., 1998; Ohta et al., 2002; Sarrel, 2002; Yoshimura et al., 2002) supporting this claim has been made available through observations of the inverse relationship between BMD and declines in circulating estrogens of women after menopause. In fact, Richelson, Wahner, Melton, and Riggs (1984) actually showed that the reduced BMD displayed by postmenopausal women is predominantly caused by estrogen deficiency rather than age. In their study, they measured the BMD at several sites of women (mean age = 54 yr.) who had undergone oophorectomy 15-25 yr. prior to normal menopause ($\approx$ 50 yr.) and compared their BMD measurements to those of

perimenopausal (mean age = 52 yr.) and postmenopausal women (mean age = 73 yr.). Their findings revealed that the degree of bone loss of oophorectimized women in comparison to the perimenopausal women of approximately the same age was nearly as great as the difference seen between the latter and women who had gone through menopause approximately 20 yr. earlier. These results led Richelson et al. to conclude that the similar time-periods of estrogen deficiency in which the oophorectimized and postmenopausal women had both undergone to be the primary source of bone loss in this population in contrast to simply aging. However, the role that estrogens may play in the gradual decline of bone loss experienced in men has only recently begun to be explored.

Most often, it is postulated that the frequency in which men are diagnosed as osteoporotic is less than that of women comes as a result of the male population, generally, reaching higher absolute PBM measurements (Seeman, 1995). This occurrence is the outcome of an elongated prepubertal growth period in males, which can last up to a two years longer than that of females, and largely contributes to the stature differences observed between sexes (Preece, Pan, & Ratcliffe, 1992).Additionally, the sustenance of BMD is partly reliant upon an individuals overall body mass as well, with those displaying lower absolute body mass values to be at a greater risk of bone loss (Barondess, Nelson, & Schlaen, 1997; Bevier et al., 1989; Felson, Zhang, Hannan, & Anderson, 1993; Grisso et al., 1997; Mazess et al., 1990; Takada, Washino, & Iwata,). Hannan et al. (2000) used data collected from individuals (n = 800) participating in the renowned Framingham Study to find lower baseline weights and weight loss to both be significantly associated with BMD loss over a 4-year period in both sexes. Because, ordinarily, men have greater absolute body mass values; it can be assumed that women are naturally more at risk for lower bone density and substantial bone loss by this factor alone. In regards to the

specific contributions that the fat and lean components of an individuals body mass have in maintaining BMD or reducing its loss, research in this area has primarily resulted in findings that suggest both provide comparable benefits (Barondess et al.; Bevier et al.; Nguyen, Howard, Kelly, & Eisman, 1998). Support to these findings is given by monozygotic (n = 57) and dizygotic (n = 55) female twins (52.8 ± 13 yr.) studied by Nguyen et al. (1998). The intra-pair analyses used to compare the individual effects that the two main components of body mass have on the lumbar spine, femoral neck, and total body BMD of their subjects revealed strikingly similar positive correlations for fat mass (r = 0.37, 0.26, 0.59, respectively) and lean mass (r = 0.38, 0.27, 0.32, respectively) at these skeletal sites. These results and others like them produced from similar studies (Barondess et al.; Bevier et al.; Seeman et al., 1996) suggest that the added load each of these components create for the skeleton to bear is recognized as simply that, and the subsequent appositional growth of bone in response to them is much like the response seen as a result of RT. Nonetheless, the higher levels of lean mass and lower incidence of reduced bone mass that are generally observed in males in comparison to females has spawned researchers to investigate the influence that muscle mass and its most closely associated steroid hormone, testosterone (T), may have on bone maintenance/loss (Falahati Nini et al., 2000; Khosla et al., 2001; Khosla et al., 1998; Scopacasa et al., 2000; Van Den Beld, De Jong, Grobbee, Pols, & Lamberts, 2000).

As seen with bone, blood serum levels of T gradually decrease with age in men (Davidson et al., 1983; Khosla et al., 1998; Nankin & Calkins, 1986; Stearns et al., 1974; Van Den Beld et al., 2000). Also, studies (Davidson et al., 1983; Nankin & Calkins, 1986; Stearns et al., 1974; Van Den Beld et al., 2000; Vermeulen, Rubens, & Verdonck, 1972) have shown that a significant decline in T levels (resulting from Leydig cell dysfunction) begins to occur at

approximately the 6th decade of life in men and become more pronounced by the 8th decade,

which is generally the stage of life at which men are most susceptible to osteoporosis (Cooper et

al., 1992; Ebbesen et al., 1999; Jones et al., 1994; Seeman, 1995). Stearns et al. (1974) studied

the decline in testicular function of 283 men (18-96 yr.) to find that free- and unbound-T fell

below the normal lower limit of young males in 40% of their subjects over the age of 50. In

addition to this, total T levels declined significantly in 29 % of their subjects who were over the

age of 70. This provides feasibility to T playing an integral role in BMD maintenance with age.

Meier, Orwoll, Keenan, and Fagerstrom (1987) explored this possibility by measuring the bone

mineral content (BMC) of the radial and vertebral sites of their healthy male subjects (n = 62;

30-92 yr.) in relationship to T levels. As expected, the researchers found their subjects to

gradually lose bone with age at rates of approximately 2 %, and 3.4 % per decade at the vertebral

and radial sites, respectively. In regards to the affect that levels of T played in this decline, the

only measurement to display a significant relationship was that of free T to vertebral BMC ($r =$

.458, p < .0003). However, free T did not add to the affect of age on vertebral BMC once a

multiple regression analysis was performed. Findings such as these from the majority of related

research (Falahati Nini et al., 2000; Khosla et al., 2001; Khosla et al., 1998; Riggs et al., 1998;

Scopacasa et al., 2000; Van Den Beld et al., 2000) give basis to the conclusion most have

reached pertaining to the effect of T on bone. This conclusion being that while T has been shown

to have some biochemical effect on bone metabolism (Riggs et al., 1998), its strongest influence

may be indirectly promoting bone maintenance by increasing body mass via its role in the

growth and/or maintenance of lean mass. What is of interest is that more recent research

(Falahati Nini et al.; Khosla et al., 2001; Khosla et al., 1998; Van Den Beld et al.) involving the

effect that levels of sex steroids have in influencing bone loss in males strongly suggests that, as

seen in the female population, declining levels of estrogens may be the chief determinant of bone loss with age in males.

<div align="center">Estrogens Influence on Bone Loss with Age in Males</div>

As previously mentioned, a substantial amount of research (Falahati Nini et al., 2000; Khosla et al., 1998; Khosla et al., 2001; Riggs et al., 1998; Scopacasa et al., 2000; Van Den Beld et al., 2000) has been directed toward determining the magnitude of influence T, the primary male sex hormone, has upon the varying rate of bone loss displayed by males and females with age. In doing so, it has been determined that T, although a positive influence in the bone health of males, is not the limiting factor that causes men to undergo the gradual, less exaggerated decline in bone mass with age that they display in comparison to women (Falahati Nini et al.; Khosla et al., 1998; Khosla et al., 2001; Riggs et al., 1998; Scopacasa et al.; Van Den Beld et al.). Yet, there is accumulating evidence (Falahati Nini et al.; Khosla et al., 2001; Khosla et al., 1998; Riggs et al., 1998; Scopacasa et al.; Van Den Beld et al.) that relates losses of bone mass with age to declining levels of estrogens in males as it already has been specified to do so in females.

A number of studies (Khosla et al., 1998; Khosla et al., 2001; Scopacasa et al., 2000; Van Den Beld et al., 2000) measuring the endogenous sex steroids of individuals to establish their role toward BMD are cross-sectional in nature and make it difficult to make assumptions toward the influence these hormones generate over time. Nonetheless, many have produced convincing results that suggest estrogens have an independent effect on increasing BMD in males (Khosla et al., 1998; Khosla et al., 2001; Scopacasa et al.; Van Den Beld et al.). Van Den Beld et al. (2000) found estrone (E_1) to have a stronger, independent effect than T on total-body BMD in 403 healthy, elderly male subjects (73-94 yr.). Also, these researchers obtained

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measurements of sex hormone binding globulin (SHBG) as well as the amount of T, E_1, and estradiol-17β (E_2) fragments bound and unbound to SHBG. This was done as a means to assist in determining whether being bound to SHBG affects the bioavailability of these hormones. Their data showed that total E_2 levels were strongly positively related to BMD at all sites measured, but non-SHBG-bound E_2 actually displayed a stronger relationship in this regard. The researchers hypothesized from this, and the fact that SHBG was positively correlated with age in their subjects, that the SHBG-bound form of estrogen may be less bioavailable to its target tissues. This is conceivable because Nankin and Calkins (1986) found SHBG-bound T display less bioavailability than free forms of T. This makes it quite feasible to assume the gradual bone loss experienced by men with age may not only result from declines in circulating estrogens entirely but also be contributed to by subsequent decreases in the bioavailability of this hormone.

Recently, Greendale et al. (1997) were able to use the sex steroid measurements from a cohort of male (n = 534) and female (n = 457) Caucasian subjects (50-89 yr.) from the Rancho Bernardo Study (1984-1991) to make an evaluation of the bearing that the various hormones in question have on BMD in a longitudinal manner. Among the sex steroids measured in both sexes of their subjects participating in the study, bioavailable levels of E_2 were the only to show a significantly positive relationship with all BMD sites measured (ultradistal- and midshaft radius, lumbar spine, and total hip) in women and men. The authors interpreted this as evidence suggesting E_2 to have a "global" effect on BMD. The significance of E_2 toward bone maintenance is also supported by the findings of Ravaglia et al. (2000) who found estradiol-17β to be significantly correlated ($p < .05$) with spinal BMD of 129 men (20-95 yr.). Still, it must be recognized that bone formation/loss are processes that occur over extended periods and, as previously stated, are influenced by a number of factors that may skew cross-sectional

measurements being used to determine the relationships in question. Even after adjustments for such things as smoking, physical activity, and body size have been made (as most of the mentioned literature has done), it is still imprudent to assume that the positive influence of estrogens on bone in men as shown by the findings of these studies is truly valid without more conclusive evidence.

One way that has been used to determine the direct influence that relatively high or low levels of estrogen values have on the changes (formation or loss) in bone that are present upon these measurements being taken is by also measuring the biochemical values for factors that are most closely related to bone resorption (i.e. calcitonin) and bone formation (i.e. osteocalcin). An abundance of research (Garnero & Delmas, 1998; Power & Fottrell, 1991; Woodhead, 1990) has been performed disclosing the high levels of accuracy at which measurements of biochemical markers such as parathyroid hormone (PTH), and osteocalcin depict rates of bone resorption and bone formation, respectively, in human subjects. Additionally, several other biochemical markers have been used in conjunction with the latter (Bettica et al., 1992; Garnero & Delmas, 1998; Gertz et al., 1994; Heikkinen, Vaheri, Kainulainen, & Timonen, 2000; Seibel et al., 1993; Zaninotto, Bernardi, Ujka, Bonato, & Plebani, 1998) to determine whether rates of bone loss exceed rates of bone formation or vice versa in human subjects more precise and, thus, allow conclusions that address the role various sex steroids have in influencing either positive/negative bone turnover to possess more validity once drawn. Khosla et al. (2001) recently performed a longitudinal study in which BMD was measured at the lumbar spine, mid-distal radius, and ulna of primarily Caucasian young (n = 88, 22-39 yr.) and elderly (n = 130, 60-90 yr.) men over a 3-year period. Levels of total and bioavailable E and T were also measured in all subjects as well as biochemical markers for bone resorption (N-telopeptide of type I collagen, NTX), and bone

formation (osteocalcin). Again, their results revealed that bioavailable levels of E_2 seemed to most markedly influence rates of bone loss in both groups of subjects in comparison to the other sex steroids measured. The researchers specifically noted the inverse correlations between the elderly subjects whose bioavailable E_2 levels fell below 40 pmol/liter and urinary ($r = -.43$, $p < .001$) and serum ($r = -.29$, $p < .05$) NTX levels in comparison to those with bioavailable E_2 levels above 40 pmol/liter. Khosla et al. (2001) continued to report that elderly subjects who retained values of bioavailable $E_2 > 40$ pmol/liter throughout the study displayed little or no loss of bone at the appendicular sites. These data were used to support their hypothesis that the decline of bioavailable levels of E_2 below 40 pmol/liter may be the major cause toward the onset of increased bone resorption and subsequent bone loss in elderly men. The authors also added that this subset of men would benefit most from estrogen replacement therapy (ERT) in contrast to T replacement therapy (TRT), which has formerly been proposed as the optimal treatment to combat bone loss in men.

<div align="center">Benefits of Estrogen Replacement Therapy</div>

One of the most common and successful means to reduce or stabilize the loss of bone displayed by women after menopause is the use of ERT (Felson et al., 1993; Lindsay, Hart, Forrest, & Baird, 1980; Recker et al., 1977; Worley, 1981). Aitken et al. (1973) first initialized this treatment by administering a synthetic form of estrogen, mestranol to a group of women who had undergone premenopausal oopherectomy within the previous 3 years prior to treatment. They also gave a placebo to another group of women with matching criterion in order to establish a control for the effects of the treatment to be compared with. The findings revealed that women in the treatment group either gained or maintained BMD at the second metacarpal site upon annual examination in comparison to the placebo group, which displayed BMD values

significantly lower in comparison. In following years, evidence of the positive affect ERT has on

BMD of postmenopausal or oophorectimized women has been compiled by others as well

(Felson et al., 1993; Lindsay et al., 1980; Recker et al., 1977; Worley, 1981). For example,

Lindsay et al. (1980) found the height of oophorectimized women (n = 58) receiving mestranol

(69 µg/day) to decline by 4% over a 9-year period in comparison to a 38% reduction in height of

those receiving placebo (n = 42). A major study regarding the benefits of ERT to

postmenopausal women was performed by Felson et al. (1993). In their study, they used a cohort

of white, postmenopausal women (n = 670, 68-96) from the Framingham Study to determine the

major effects of previous ERT on the subjects BMD measurements of sites most often affected

by osteoporosis. They stratified the women into three groups, which included those who had

undergone ERT for 1-6 yr. (n = 143), $\geq$ 7 years (n = 69), and those who had never received ERT

(n = 458). The key finding that these researchers discovered was that ERT was only effective in

maintaining or increasing BMD in those who received treatment for $\geq$ 7 years. Specifically,

women who had received ERT for only 3-4 years showed essentially no differences in BMD

than the untreated women, and the differences that were displayed by women treated for 5-6

years in comparison to the untreated women were insignificant. However, women who had

received treatment for 7 or more years generally showed higher BMD values at all sites in

comparison to the untreated group, with those who had been treated for > 10 years displaying

significantly (p < .05) higher BMD values at all sites but the spine. Felson et al. (1993) used

these and other data to suggest that ERT should be administered for $\geq$ 7 years immediately

following menopause in order for it to be effective in maintaining bone mass in women from a

long-term prospective. The authors did make clear that if used in a long-term manner, ERT is

very protective in maintaining bone mass in postmenopausal women and its use is warranted in this population.

TRT is much more used in aging men than ERT due to the number of age-related health problems that arise in this population as a result of declining levels of T and subsequent losses of lean mass (Cohen, 2001; Hermann & Berger, 2001; Janssens & Vanderschueren, 2000; Lamberts, Van Den Beld, & Van Der Lely, 1997; Roy et al., 2002). Research related to the benefits and safety of TRT have found it to support increases in lean mass (Bhasin et al., 1997; Katznelson et al., 1996; Wang et al., 1996) and be well tolerated in regards to any adverse changes in the blood profiles of those receiving treatment (Hajjar et al., 1997; Tan et al., 1998; Zgliczynski et al., 1996). In fact, Zgliczynski et al. (1996) found significant declines in the total ($p < .002$) and low-density lipoprotein ($p < .05$) cholesterol of 22 hypogonadal men receiving TRT over a 12-week period. Also, this treatment has been shown to facilitate increases in the BMD of male subjects suffering from primary and secondary hypogonadism over extended use (Hajjar et al., 1997; Katznelson et al., 1996; Leifke et al., 1998; Wang et al., 1996). Leifke et al. (1998) measured the BMD of 32 T-substituted males over 3.2 ± 1.7 years to find that the final values of BMD were significantly higher ($p < .05$) than the baseline measurements of all subjects after TRT. Data taken from a similar study by Katznelson et al. (1996) also support the positive effect TRT may have on BMD in hypogonadal men by finding a significant decline ($p = .04$) in the bone resorption marker deoxypyridinoline (DPD) taken from urinary excretions of hypogonadal men ($n = 36$, 22-69 yr.) undergoing TRT for 18 months. This is a very conceivable occurrence with scientific evidence (Colvard et al., 1989; Kasperk et al., 1989; Schweikert, Wolf, & Romalo, 1995) showing androgen receptors on human osteoblastic cells that are active in bone metabolism. Also, Wang et al. (1996) performed a study that, in addition to measuring

longitudinal changes of lean mass and BMD in hypogonadal males during TRT, also obtained measurements of bone resorption and formation markers to determine the effects that this type of therapy has on bone metabolism. Their findings revealed that the primary marker used to determine bone resorption, NTX/creatinine ratio, decreased significantly ($p = .0304$) over a 6-month period in those receiving TRT ($n = 67$) and osteocalcin (bone formation marker) to increase significantly ($p = .0001$) during this time. This suggests that TRT could comprehensively combat the age-related bone loss in men. However, it must be pointed out that E_2 levels increased significantly ($p = .0001$) as well during this period. The authors did not hypothesize or comment upon what effect this may have had upon their data. Nonetheless, it seems apparent that this most likely occurred as a result from the aromatization of exogenous T to E, which exogenous T has shown the capacity to do when administered. Interestingly, Falahiti-Nini et al. performed a study that controlled for exogenous T and E production and aromatization of administered sex steroids while measuring markers for bone resorption and formation in elderly males subjects (mean age = 68 yr.) who were either receiving no hormone replacement therapy (HRT) ($n = 14$), ERT only ($n = 15$), TRT only ($n = 15$), or ERT and TRT ($n=15$). Falahiti-Nini et al. did this by administering a long-acting GnRH agonist that suppresses endogenous production of T and E, and the aromatase inhibitor letrozole to all their subjects. Then, the subjects were each started on standard TRT (5 mg/d) and ERT (.0375 mg/d) for a 3-week period to simulate the circulating levels of T and E_2 found in normal men. After this, subjects either continued or discontinued each therapy on the basis of what group that individual had been assigned for an additional 3-week period. Their findings revealed that ERT showed the strongest relationship in inhibiting markers of bone resorption (DPD, NTX) in comparison to TRT, which had no significant effect. Specifically, the two-factor ANOVA used to compare

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subjects taking ERT versus those not and those undergoing TRT versus those not disclosed a clear effect of ERT on DPD excretion (p = .005) unlike TRT, where no effect was found (p = .232). Similar effects were found for ERT (p = .0002) and TRT (p = .085) on NTX excretion by the same analysis. However, ERT and TRT showed significant effects (p = .002 and .013, respectively) in maintaining serum levels of osteocalcin in those subjects receiving them when the same analysis was performed, suggesting that both therapies and/or sex steroids contribute to bone formation. By these results, which were found under stringent methods to control for the physiological factors of endogenous sex steroids and their aromatization as well as those administered, the authors were able to conclude that E is the dominant sex steroid regulating bone resorption in men. Moreover, their results provide relevance to the use of ERT or other methods that influence bone maintenance by the same mechanism as an appropriate means to combat the age-related decline of bone mass in men.

Androstenedione Supplementation

The publicity that oral supplementation of androstenedione (AN) has received as a possible approach to increase levels of T and, subsequently, lean mass in young and aging men has prompted researchers to investigate the physiological implications of its consumption in these populations (Broeder et al., 2000; Brown et al., 2000; King et al., 1999; Rasmussen et al., 2000). Initially, it is feasible that such administration may enhance endogenous T production because of AN being a direct precursor to T via the Δ4 pathway (Norman & Litwack, 1987). While the Δ5 pathway is the primary route of endogenous T production, the Δ4 pathway does contribute to T levels in men as well (Norman & Litwack, 1987). However, all studies (Broeder et al.; Brown et al.; King et al.; Rasmussen et al.) regarding the short- and long-term supplementation of oral AN have failed to find any significant results implicating the

enhancement of T production by the additional precursor, with most finding E levels to rise during periods of supplementation that most likely result from the peripheral aromatiztion of this (AN) compound (Broeder et al.; Brown et al.; Horton & Tait, 1966; King et al.; Rasmussen et al.).

Some of the earliest research performed regarding the possible interconversion of oral AN to T in human subjects was performed by Horton and Tait (1966). The researchers did so by accounting for normal secretions and interconversions of T and its major precursors (i.e. AN) by standardized blood draws and urine specimens from normal adults (21-33 yr.) prior to and after the infusion of AN (7-10 μc) into the peripheral or gastrointestinal circulation of the subjects. By controlling for normal androgen production and clearance, the authors were able to discover that both oral and intravenous administered AN contribute only negligibly to total T levels of males. In fact, their study showed that only 5.9% and 1.8% of intravenous and oral AN, respectively, entered the plasma as T. Their findings also reveal that nearly 89% of orally administered AN is actually excreted in the urine as T glucoronide. Interestingly, the interconversion rate of T to AN was much greater in both sexes upon infusion of AN, contributing to approximately 36% of plasma AN levels. This may perhaps illustrate the increased likelihood of physiological down-regulation of endogenous T production once additional AN is introduced to the normal circulation of humans. However, prior to this study Mahesh and Greenblatt (1962) found oral consumption of 50 or 100 mg of AN to bring about an acute increase in the T levels of female subjects. Further, a more recent study performed by Earnest, Olson, Broeder, Breuel, and Beckham (2000) also resulted with findings that suggested supplementing oral forms of AN (3, 17-dione, and 4-androstene-3β, 17β-diol) may increase circulating levels of T for a short period following consumption in males. While the increase in T did not reach a significant value in the

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latter study, Earnest et al. (2000) found circulating levels of total T and free T to increase by 6% and 12%, respectively, in 8 healthy males over a 90-minute period following the oral consumption of 200 mg of 3, 17-dione (DIONE). The results of the these studies (Earnest et al., 2000; Mahesh & Greenblatt, 1962), which seem to substantiate the claims of AN facilitating increases in T levels, and the growing popularity and use of this product gave reason to investigate the benefits and possible side effects of this compound over an extended period of time.

In that the recent marketing of AN indicated its use could possibly improve athletic performance through increases in muscular size and strength, the initial studies to evaluate this hypothesis and the changes this supplement influenced in regards to blood serum values of T and E incorporated a RT regimen within their study protocol. For instance, King et al. (1999) measured serum values of sex steroids over an 8-week period in 20 young, healthy males (19-29 yr.) who participated in a comprehensive RT program and were randomly assigned to either an AN group (300 mg/d, n = 10) or placebo group (250 mg/d of rice flour, n = 10). Along with baseline and biweekly measurements of T (free and total) and E (E_1, E_2, and estriol), King et al. tested the one-repetition max (1RM) of their subjects on all exercises performed within the protocol of their RT regimen, and took body composition (BC) measurements at baseline, 4-wk, and 8-wk during their study. Upon the conclusion of the study, their data revealed AN supplementation essentially contributed to no changes in blood serum levels of free or total T, strength, or BC when coupled with RT during the 8-wk period when compared with the same values taken from the placebo group. The most critical finding, though, was in regards to the increases in E_1, and E_2 displayed by the AN group during the supplementation period. After 2 weeks of AN supplementation the E_2 concentration of the AN group was significantly greater (p

< .05) than baseline and placebo group values, which did not change throughout the study. This difference in E_2 concentration continued at measurements taken during the 5th and 8th weeks of the study as well. King et al. also point out that the mean values for E_2 for the AN group (2nd wk- 310 $\pm$ 20 pmol/L, 5th wk- 300 $\pm$ 30 pmol/L, 8th wk- 280 $\pm$ 20 pmol/L) were all approximately 20% greater than those normally found in their subjects' population. In addition to the changes observed in E_2 concentration, the AN group also displayed similar increases in E_1 values.

Specifically, the E_1 concentration in the AN group increased by approximately 31% from baseline values by the 2nd week of the study. However, this value continued to decrease during subsequent measurements and approached baseline values by the 8th week. The absence of change in T concentration and subsequent marked increases observed in E_1 and E_2 of the AN group led King et al. (1999) to hypothesize that a large quantity of supplemented AN is aromatized to estrogens in this population. These finding lessen the likelihood that this substance may act as a catalyzing stimulus to increases in muscle mass and strength that RT normally provides young, healthy males with. This is the only study that has been performed in regards to the hormonal and physical changes influenced by long term AN supplementation in young, healthy males to date thus far that was performed in a stringent enough manner that would yield reliable and accurate results. Therefore, one must assume that AN supplementation provides no anabolic effects to young men undergoing a RT program. Rasmussen et al. (2000) added evidence to this premise by directly analyzing the muscle fibers of normal young males (n = 7, 32 $\pm$ 4 yr.) prior to and in the postabsorptive state preceding 5-7 days of AN supplementation (100 mg/d). The researchers could find no differences in protein synthesis in those receiving AN versus six other subjects (31 $\pm$ 3 yr.) acting as controls but did find a significant increase in protein breakdown (p = .086) and a more negative trend (p = .093) in net muscle balance, a

measure of net protein deposition, in subjects receiving AN. Rasmussen et al. suggests that this is an indication of AN supplementation actually having more of a catabolic effect on muscle metabolism of young men in contrast to the anabolic impact it has been suggested to have. In addition to this, the researchers also found the E_2 levels of the AN group to increase significantly ($p = .05$) in the last hour of an additional study in which blood samples were measured in 10-minute intervals over a 2-hour period following AN supplementation. These findings led Rasmussen et al. to concur with King et al. (1999) in their findings that the majority of orally supplemented AN is aromatized to E_2 in a young male population.

Similar to the amount of research involving AN supplementation in young males, there is a limited body of knowledge addressing this compounds affect on middle-aged men as well. Yet, the two studies (Broeder et al., 2000; Brown et al., 2000) that comprehensively monitored the hormonal profiles of middle-aged, male subjects during long-term oral AN supplementation resulted in similar findings, in regards to E_2 concentration, as those performed on younger males had. Of the two studies previously mentioned involving AN supplementation in middle-aged males, Broeder et al. performed the longest in duration by monitoring 50 males subjects (35-65 yr.) who were made of groups that either ingested 200 mg/d of the 3, 17-dione (DIONE) or 4-androstene-3β, 17β-diol (DIOL) forms of AN and one that received a placebo during a 12-week RT regimen. Along with standard measurements taken for changes in strength, and BC, the hormone profiles of, both, the DIONE and DIOL groups responded to oral AN in a similar pattern that those receiving AN in King et al.'s (1999) study had. After one month of AN supplementation, Broeder et al. (2000) found the E_2 levels of the DIONE and DIOL groups to increase by approximately 50% and 20%, respectively, from baseline values, with changes in the DIONE group reaching a significant value ($p < .05$). This change was essentially maintained in

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the DIONE group throughout the remainder of the study but increased to a significant value (p < .05) by the 12th week in the DIOL group as well. Also, both groups receiving oral forms of AN displayed significantly (p < .05) higher levels of E_2 at the completion of the study when compared with their own pretreatment values and those of the placebo group. Of interest, the response of the DIONE's group free and total T concentration did show an initial increase. In fact, total T in the DIONE group was significantly higher (p .05) from the DIOL and placebo group values by the end of the 1st month of supplementation, and free T had increased by 4.2% at this time as well in the DIONE group. However, the rise in free T would, eventually, dissipate to pretreatment values by the 12th week, and values for total T actually fell almost 20% below pretreatment measurements at this time in the DIONE group. Again, these data led Broeder et al. (2000) to propose that supplementation of this T precursor had led to increases in peripheral aromatization thereby augmenting E synthesis and the subsequent rises observed in E_2 concentration. By using statistical analysis to determine that declines in measurements of luteinizing hormone (LH) were responsible for 33.9% of the negative change in total T of the DIONE group, the authors were also able to substantiate the idea that oral AN supplementation may actually down-regulate endogenous T production in middle-aged men. This would also explain the below-baseline values of total T in the DIONE group following 12 weeks of treatment.

In the study performed by Brown et al. (2000), subjects (30-56 yr.) were stratified into groups to represent the 4th (n = 20), 5th (n = 20), and 6th (n = 16) decades of life. Then, they were randomly assigned to one of two treatment groups, which consisted of those receiving 300 mg/d of AN or a placebo for a 4-week period. The subjects were not required to participate in a RT program and were asked to maintain normal activity levels. As in previous studies,

measurements of sex steroids and related compounds were taken on a systematic basis. However, the relatively brief nature of the study allowed the researchers to evaluate these values on a weekly basis. The results of this study were strikingly similar to what Broeder et al. (2000) had observed during the initial 4 weeks of their study.

By the end of the first week, all groups (4th, 5th, and 6th decades) that were receiving oral AN showed significant ($p < .05$) increases in E_2 concentration from baseline values. This degree of change persisted throughout all weekly measurements of the study in all groups, giving more relevance to the concept of this T precursor being extremely susceptible to undergoing aromatization to E_2 once consumed orally in males, young and aging. While no groups showed any substantial variance in total T during the supplementation period, all groups that received oral AN displayed and maintained significant ($p < .05$) increases in free T. In fact, serum free T values increased by 37, 51, and 46% in the 4th, 5th, and 6th decade groups, respectively, who were receiving AN. Whether these values would have remained with continued supplementation can only be speculated on. Nonetheless, if data from Broeder et al. (2000) are taken into account, one can only assume that these values would have not been maintained as result of the physiological response to down-regulate endogenous T production once an abundance of precursor remained present.

The results of the previous studies present undeniable evidence that oral supplementation of AN yields very little, if any, anabolic benefits toward protein synthesis and, subsequent, increases in muscle tissue when used by males. Conversely, the data (Broeder et al., 2000; Brown et al., 2000; King et al., 1999; Rasmussen et al., 2000) does indicate that E_2 concentrations will rise significantly upon the ingestion of oral AN in virtually all healthy males because of the supplements tendency to easily aromatize. This brings forth an interesting concept

in which the possibility that these rises in circulating E could possibly support positive bone formation in aging men suffering from declines in BMD.

<p style="text-align:center">Oral Androstenedione Supplementation as a</p>

<p style="text-align:center">Possible Means to Protect Aging Men from Bone Loss</p>

With the unquestionable benefits that administered E provide in regards to supporting bone health in men and women (Falahati Nini et al., 2000; Felson et al., 1993; Heikkinen et al., 2000; Lindsay et al., 1980; Recker et al., 1977; Worley, 1981), it is reasonable to assume that a supplement such as oral AN, which easily aromatizes to E in the body, may provide similar advantages. This assumption gathers more credibility when the fact that the principle provider of circulating E in castrated males and postmenopausal women is the aromatization of circulating androgens (MacDonald et al., 1979; Taxel et al., 2001) is taken into account. In fact, there is evidence (Marshall, Crilly, & Nordin, 1977; Schweikert et al., 1995) that AN may play a specific role in the formation of E within the actual bone cell.

Schweikert et al. (1995) used the spogiosa taken from bone cells of 11 women (47-91 yr.) and 4 men (59-73 yr.) undergoing hip replacement surgery to perform *in vitro* experiments accessing the effect different concentrations (10 to 150 nM) of 1 β- ^{3}H-androstendione or non-radioactive AN (NRAN) had on the formation of E in these cells. The researchers found that E formation increased upon the introduction of NRAN, with aromatase activity reaching a maximal velocity of 0.1 pmol/g bone/h when this factor was analyzed by using a Lineweaver-Burk plot. More specifically, the apparent K_m of aromatase activity ranged between 6 to 50 nM of NRAN in all but one subject, with Schweikert et al. noting this being similar to the range found in other extragonadal tissues. The researchers also found E formation only to be suppressed upon the

introduction of known aromatase inhibitors. These findings prompted Schweikert et al. to hypothesize that local aromatization of AN could quite possibly be a major contributor of estrogen in bone. Further, the significance of these findings are intensified when taken in account with a study by Marshall et al. (1977) that revealed the average levels of AN and E_1 to be lower in osteoporotic women in comparison to normal postmenopausal women. In men, though, the aromatization of AN may play a role of stronger or equal magnitude in E production and subsequent bone formation.

MacDonald et al. (1979) performed a study in which they attempted to quantify the various sources of extraglandular formation of E_1 and E_2 in normal males. They did this by infusing healthy young males (n = 4, 26-35 yr.) with tracer doses of AN, T, E_1, and E_2 after measuring 24-hr plasma secretions of each prior to infusion, and then taking systematic urine collections for the 72 hr following infusion. In their subjects, E_1 production was solely accounted for by: 1) aromatization of AN, 2) conversion of E_2 formed from the aromatization of plasma T, and 3) conversion of E_2. Additionally, only 12 µg or less of the mean value for E_2 (44 µg/24 hr) could not be accounted for by extraglandular formation from plasma steroid precursors. These results provide confirmation of the idea that the extraglandular aromatization of plasma precursors is the principle origin of E in men. Furthermore, a more recent study by Taxel et al. (2001) administered the aromatase inhibitor anastrozole (2.0 mg/d) to 15 eugonadal men over a 9-wk period to find the bone resorption marker of C-telopeptide of type 1 collagen (CTX) to show a progressive, significant increase over the tri-weekly measurements ([3rd]- 11%, [6th]- 24%, and [9th]- 33%). These rises were coupled with slight increases of NTX, a bone resorption marker, and a significant decline in bone formation as determined by measurements of OC which declined by 30 % at the final measurement.

The results of these studies make it reasonable to conceptualize that with the declines in T endured by aging men, an inadequate amount of steroid precursor to be aromatized to E may exist as well. The culmination of these two events could be a determining factor in the increased number of osteoporotic fractures sustained by aging males in that declines in T gradually lead to subsequent declines in lean and overall body mass, and decreases in E translate to eventual lower values of BMD. While previous studies evaluating the anabolic affect of oral AN have failed to generate promising benefits from the supplement in that respect, their findings may still bring value to the use of this supplement as a mean to promote E formation in men who are susceptible to osteoporosis. Also, further research involving oral AN may lead to findings that allow for it to be used as an alternative therapy for women suffering from or likely to suffer from osteoporosis.

CHAPTER 3

METHODS

The present study was designed to evaluate bone turnover markers of middle-aged men during 12 weeks of oral AN supplementation and RT. This chapter will explain the manner in which the researchers attempted to accurately follow and account for any changes that might have occurred in these subjects' rates of bone turnover that resulted from oral AN supplementation.

The Andro Project

The original purpose of the "The Andro Project" was to determine the effects of orally supplemented androstenedione or androstenediol on sex hormone and cholesterol profiles, body composition, and the muscular strength of 50 middle-aged male subject 35-65 yr.). Prior to the actual study, all subjects completed an informed consent approved by the institutional review board at East Tennessee State University and James H. Quillen-College of Medicine. In addition, all subjects underwent a prescreening blood draw that analyzed total testosterone levels, liver and kidney function, hemoglobin concentration, and prostate-specific antigen levels. Subjects who displayed levels of the specific measurements outside the normal clinical reverence values were disallowed from participating in the study. Each subject was randomly assigned to either AN (n = 15), AN-diol (n = 17), or placebo (n = 18) groups then were given the respectful supplement of their group to be taken in 100 mg doses two times a day (1 in A.M. and 1 in P.M.) everyday for 12 consecutive weeks. Upon the completion of the study, pill count procedures were used to determine each subject's supplement use compliance. During the study, blood serum measurements of dehydroepiandrosterone sulfates (DHEAS), AN, free testosterone, total testosterone, estrone, estrogen, luteinizing hormone, follicle stimulating hormone, and serum-

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hormone binding globulin were performed using standard immunoassay procedures prior to supplementation and after 12 weeks of supplementation. The post-treatment blood draws used for these measurements were performed within one hour of each subject's initial blood draw to ensure reliability of individual values. In addition, each subject competed a testing sequence prior to and upon the completion of the study that consisted of a complete body composition analysis [9 skinfold and 8 circumference measurements, single- and dual-frequency bioelectrical impedance, dual photon x-ray absorption (DEXA), which also provided bone density measurements] and maximum strength tests. Also, urine specimens were taken from each subject the day in which that subject underwent DEXA procedures during each pre- and post testing period. During the supplementation period, each subject was also required to participate in a comprehensive and progressive resistance-training program three days a week. A personal trainer, who also recorded the start and finish time of each session as well as the amount of weight lifted and repetitions performed during each session, supervised each resistance-training session.

Current Study General Description

The purpose of the current follow-up study is to specifically determine if AN supplementation in combination with high intensity resistance-training enhanced bone turnover and bone density in a positive manner. This follow-up study was developed because the findings of "The Andro Project" revealed that AN supplementation resulted in an increase of approximately 50% of the E_2 levels and similar increases in the E_1 levels of subjects. Thus, because estrogen related hormones play a major role in the attenuation of osteoclastic activity, we hypothesized that subjects in "The Andro Project" receiving oral AN supplementation would show a more positive rate of bone turnover and, possibly, greater increases in bone density

during the study than those receiving placebo. Therefore, it is our intention to use the stored blood serum and urine specimens of the subjects from the previous study to measure the biochemical markers of bone resorption/formation at each testing interval to determine the accuracy of this hypothesis. However, primarily due to cost concerns, only the specimens taken from the AN and placebo group will be compared in this manner.

<div align="center">Subjects</div>

The blood serum and urine specimens of 24 subjects between the ages of 35 and 65 years were randomly chosen from the original "Andro Project" subject pool of 37 middle-aged men (placebo – 12, AN – 12) will be used in this study. These subjects were chosen because financial limitations of the "Andro Project" only allowed for comprehensive hormonal assays to be conducted for 12 randomly-selected subjects from each group during this study. Because these subjects were randomly-selected, the present study used their data to negate the possibility that hormonal data would not be available to correspond with any data produced from the present study design. Statistical analyses were performed on the results of these subjects' hormonal profiles that were taken during the treatment period to determine how well they depicted the changes of the original subject pool. The results showed that these subjects' changes in hormonal profile, strength, and BMD were representative of those observed in the original subject pool of the "Andro Project."

<div align="center">Instruments</div>

The specimens for this study were taken after a 12-hour fast. Blood samples were taken 2 times (pre-treatment and post-treatment) during the 12-week study. The second blood draw was performed within one hour of each subject's initial blood draw to ensure reliability of individual

values. Following the initial hormone determinations in the "Andro Project", the remaining samples were returned to the researchers and stored at -80 degrees C until subsequent analysis in the present study. Urine specimens were collected from each of the subjects' pre- and post-AN treatment. For the present study, blood and urine samples were briefly removed from their storage area and sorted by group and treatment period (pre- or post-). Samples were returned to an ultra-cold freezer (-80 degrees C) and stored until subsequent measurement of analytes for bone metabolism.

<div align="center">Immunoassays</div>

Serum measurements of calcitonin and intact parathyroid hormone (intact-PTH) and urinary measurements of deoxypyridinoline (DPD) were measured using the IMMULITE calcitonin, Intact PTH and Pyrilinks-D chemiluminescent enzyme-labeled immunoassay kits (Diagnostic Products Corp., Los Angeles, CA), respectively. All assay procedures were performed on the IMMULITE Automated Immunoassay Analyzer (Diagnostic Products Corp., Los Angeles, CA). The assays use assay-specific, antibody, or antigen-coated plastic beads as the solid phase, an alkaline phosphatase-labeled reagent, and a chemiluminescent enzyme substrate. A proprietary "Test Unit" is used to house the bead, and serves as the reaction vessel for the immune reaction, the incubation, and washing processes, and the signal development.

The IMMULITE System automates the entire assay process and thus improves precision and accuracy. The instrument, first, incubates the sample with the alkaline phosphatase reagent. Then, the liquid reaction mixture in the "Test Unit" is spun at a high speed on its vertical axis. Following this, the entire fluid contents (the sample, excess reagent, and wash solution) are transferred to a coaxial waste chamber within the "Test Unit." The bead is left with no residual, unbound label. The bound label is then quantitated with a dioxetane substrate that produces light,

the light emission from this is detected by a photomultiplier tube (PMT) and the system's computer generates printed reports for each sample. All procedures regarding the chemiluminescent assays were performed in accordance with the manufacturer's instructions from each assay.

To determine whether the changes in E observed in the subjects for this study also affected vitamin D status, we anticipated using an enzyme-linked absorbent assay (ELISA) to measure 1, 25 $(OH)_2$ Vitamin D (ALPCO Diagnostics, Windham, NH) However, following measurement of serum levels of calcitonin and IPTH, it was determined that sample volume was not sufficient to perform the 1, 25 $(OH)_2$ Vitamin D measurements.

<div align="center">Statistical Procedures</div>

All data are presented as means ± one standard deviation. Values will be reported for the total sample population as well as for each of the two treatment groups. If no significant difference exists for a dependent variable between the placebo and AN groups for the pre-training value , a repeated measures ANOVA will be performed [Treatment (placebo versus AN) x Time (pre-training versus post-training)] for each dependent variable. If significant differences are found for a dependent variable comparison for the pretreatment measurement, a two way repeated measures ANCOVA will be performed using the pretreatment week data as the covariate. Repeated measures analyses of covariance will be conducted to test for interactions between DPD, estradiol-17β, estrone, body mass, fat free mass, age, total body strength, upper body strength, and lower body strength versus any significant increases in BMD that occur between pre- and post-training. In the case of significant effects ($\alpha \leq 0.05$) for either treatment or time in any of the ANOVA or ANCOVA models tested, the Tukey procedure was used post hoc

to discover between which levels of a variable (treatment and time) there is a significant

difference.

CHAPTER 4

RESULTS

The present study was designed to evaluate bone turnover markers of middle-aged men during 12 weeks of oral AN supplementation and RT. The outcomes of this study, which are reported in the following sections, are mean values $\pm$ one standard deviation (S.D.). Variable means are reported for each of the two treatment groups: AN and placebo.

Physical Characteristics of the Study Participants

The mean age, body mass index (BMI), and weight of the study participants is reported in Table 2. Table 3 includes means for both pre- and post- percent body fat and fat-free weight as determined by DEXA. There were no significant ($p > 0.05$) differences in any of these variables either across time or between treatment groups.

Table 2: Subject Characteristics.

	Placebo (N=12)	AN (N=12)
Age (yrs)	46.42 ± 8.90	43.25 ± 6.14
BMI (kg/m^2)	27.29 ± 3.38	28.04 ± 4.43
Weight (kg)	85.55 ± 15.35	88.05 ± 18.02

Values are means ± S.D.
BMI: Body Mass Index = body weight (kg)/height (m^2).

Table 3: DEXA Measurements of Body Composition.

	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
Body fat (%)	20.09 ± 8.77	19.84 ± 5.84	18.50 ± 6.69	19.32 ± 7.56
Fat-free weight (kg)	67.46 ± 8.39	68.94 ± 8.90	70.97 ± 14.35	73.11 ± 13.20

Values are mean ± S.D.

<u>Resistance Training Performance Measures</u>

Total body, as well as specific upper- and lower body, strength measurements are reported in Table 4. To best represent total body strength, 1-RM values for bench press, biceps curl, triceps press-down, leg press, and leg-extension were combined at both pre- and post-study testing points. The Upper-body strength variable was derived from the sum of bench press, biceps curl, and triceps press-down 1-RM tests. The lower-body strength variable was derived from the sum of the leg press and leg-extension 1-RM tests. Both the placebo and AN treatment groups significantly increased ($p < 0.05$) total body strength, upper body strength, and lower body strength from pre- to post testing points. The AN treatment compared to placebo was not associated with a positive impact on muscular strength development. In fact, post-training values for lower body strength were significantly ($p = 0.05$) higher in the placebo compared to the AN group. However, the differences between the two groups were very small in terms of physical function. The magnitude of the between group difference was five kg or 1.3%.

Table 4: Muscular Strength Measurements.

	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
Upper-Body Strength (kg)	336.67 ± 104.50	411.92 ± 110.55*	381.88 ± 111.71	451.92 ± 115.09*
Lower-Body Strength (kg)	273.13 ± 83.51	335.63 ± 102.28*†	268.96 ± 63.20	331.25 ± 54.94*
Total-Body Strength (kg)	609.79 ± 174.41	747.54 ± 191.98*	650.83 ± 156.39	783.17 ± 152.81*

Values are means ± S.D.
Upper body strength = sum of 1-RM for bench press, biceps curl, and triceps press-down.
Lower body strength = sum of 1-RM for leg-extension, and leg press.
Total body strength = sum of 1-RM for bench press, biceps curl, triceps press-down, leg-extension, and leg press.
*$p \leq 0.05$, indicates significant difference between pre- and post-training values.
†$p \leq 0.05$, indicates significant difference from AN group pre- and post- training values.

Bone Mineral Density

Pre- and post-training BMD values for both total body and specific body segments are

listed in Table 5. BMD of the spine increased significantly from pre- to post-training for both the

placebo and AN groups (p = 0.4 and 0.02, respectively). Pre- and post-training training spine

BMD for both treatment groups are displayed in Figure 1. No other BMD values changed

significantly between pre- and post-training and there were no between group differences for any

of the BMD measures. However, ANCOVA showed that post-lower body strength had a

significant and positive impact on post-leg BMD (p = 0.028). A similar relationship was found

between as post-upper body strength and post-arm BMD (p = 0.015) for both treatment groups.

Table 5: BMD MEASUREMENTS BY DEXA

BMD	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
Total-body (g/cm^2)	1.27 ± .07	1.27 ± .07	1.29 ± .12	1.30 ± .12
Spine (g/cm^2)	1.23 ± .11	1.29 ± .14*	1.26 ± .20	1.34 ± .23*
Pelvis (g/cm^2)	1.25 ± .10	1.24 ± .11	1.30 ± .16	1.30 ± .17
Trunk (g/cm^2)	1.04 ± .07	1.05 ± .08	1.07 ± .13	1.08 ± .14
Arm (g/cm^2)	1.08 ± .10	1.07 ± .08	1.11 ± .10	1.12 ± .11
Leg (g/cm^2)	1.37 ± .08	1.38 ± .08	1.39 ± .15	1.40 ± .13

Values are means ± S.D.
*$p \leq 0.05$, indicates significant difference between pre- and post-treatment values.

FIGURE 1: CHANGES IN SPINE BONE MINERAL DENSITY

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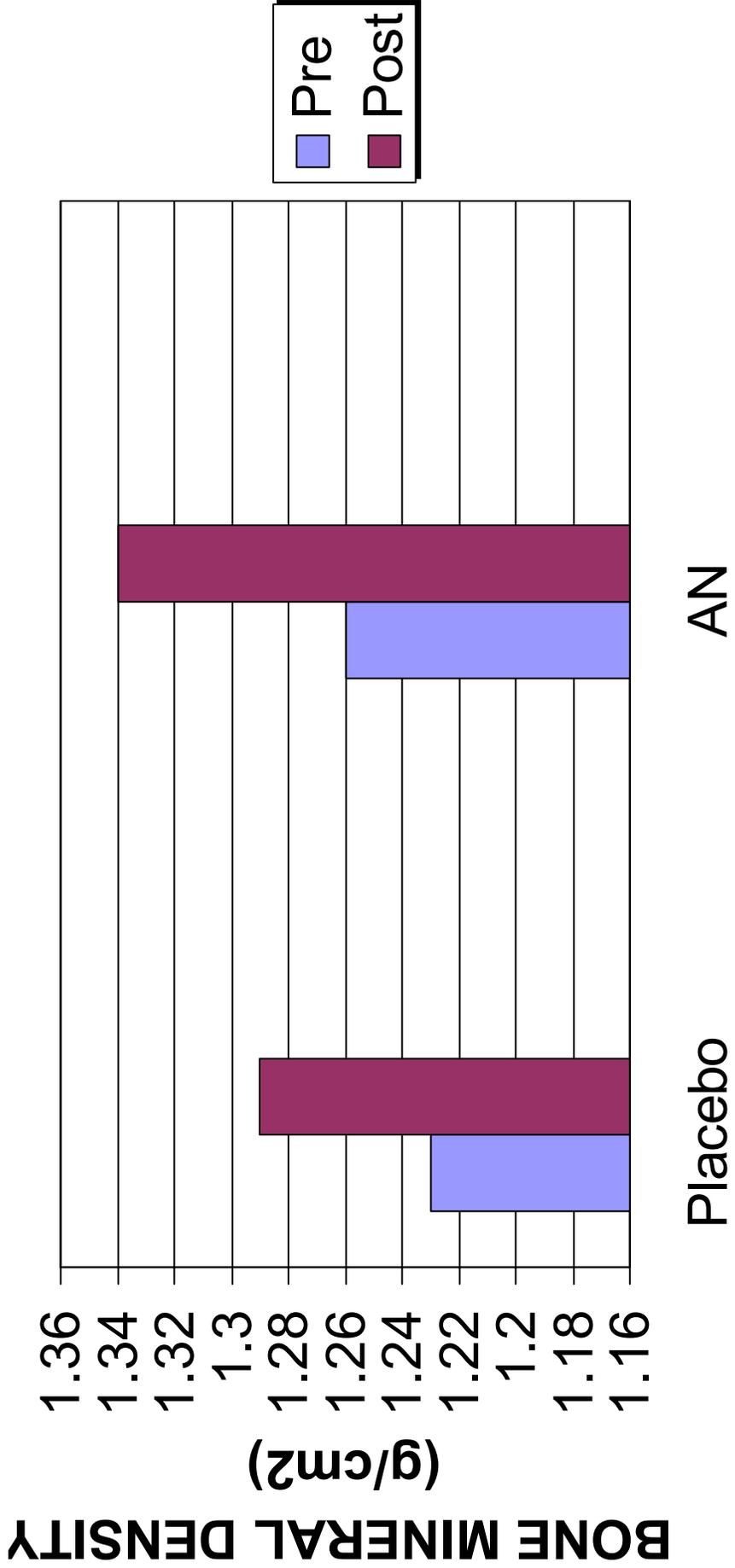
Hormonal Responses to Androstenedione and Resistance Training

Tables 6 and 7 provide a summary of mean plasma concentrations $\pm$ 1 S.D. of androgens, steroid hormone binding globulin, and estrogens at both pre- and post-training time points. Mean values are reported for the total sample population as well as separated by treatment group. In the placebo group, there were no significant pre-to post-training changes in any plasma hormone or binding globulin concentrations. Compared to pre-training values, the AN treatment group had significantly increased post-training levels of both plasma androstenedione and estradiol-17β. There also was a trend ($p \leq 0.10$) toward increased post-training levels plasma estrone levels. In the AN group, both free-testosterone and SHBG were significantly lower at post-compared to pre-testing ($p < 0.05$). In regards to plasma concentrations of estradiol-17β and androstenedione, the large magnitude of within group changes in the AN group of these variables resulted in significantly higher post-treatment values when compared to the placebo group ($p < 0.05$). Figures 2, and 3 display the different pre- and post-training values for circulating levels of androstenedione, and estradiol-17β, respectively.

Table 6: Serum Concentrations of Male Sex Hormones and Androstenedione.

	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
Total Testosterone (pmol/L)	433.92 ± 233.25	468.25 ± 207.63	508.75 ± 158.59	509.50 ± 147.80
Free Testosterone (nmol/L)	15.74 ± 5.57	13.50 ± 4.76	19.96 ± 5.50	16.94 ± 5.30*
Sex Hormone-Binding Globulin (nmol/L)	29.12 ± 19.45	28.93 ± 16.26	31.40 ± 12.32	25.96 ± 8.50*
Androstenedione (nmol/L)	1.63 ± .70	1.42 ± .89	1.93 ± .55	5.51 ± 3.20*†

Values are means ± S.D.

*$p \leq 0.05$, indicates significant difference between pre- and post-treatment values.

†$p \leq 0.05$, indicates significant difference from placebo group pre- and post- treatment values.

FIGURE 2: CHANGES IN CIRCULATING LEVELS OF ANDROSTENEDIONE
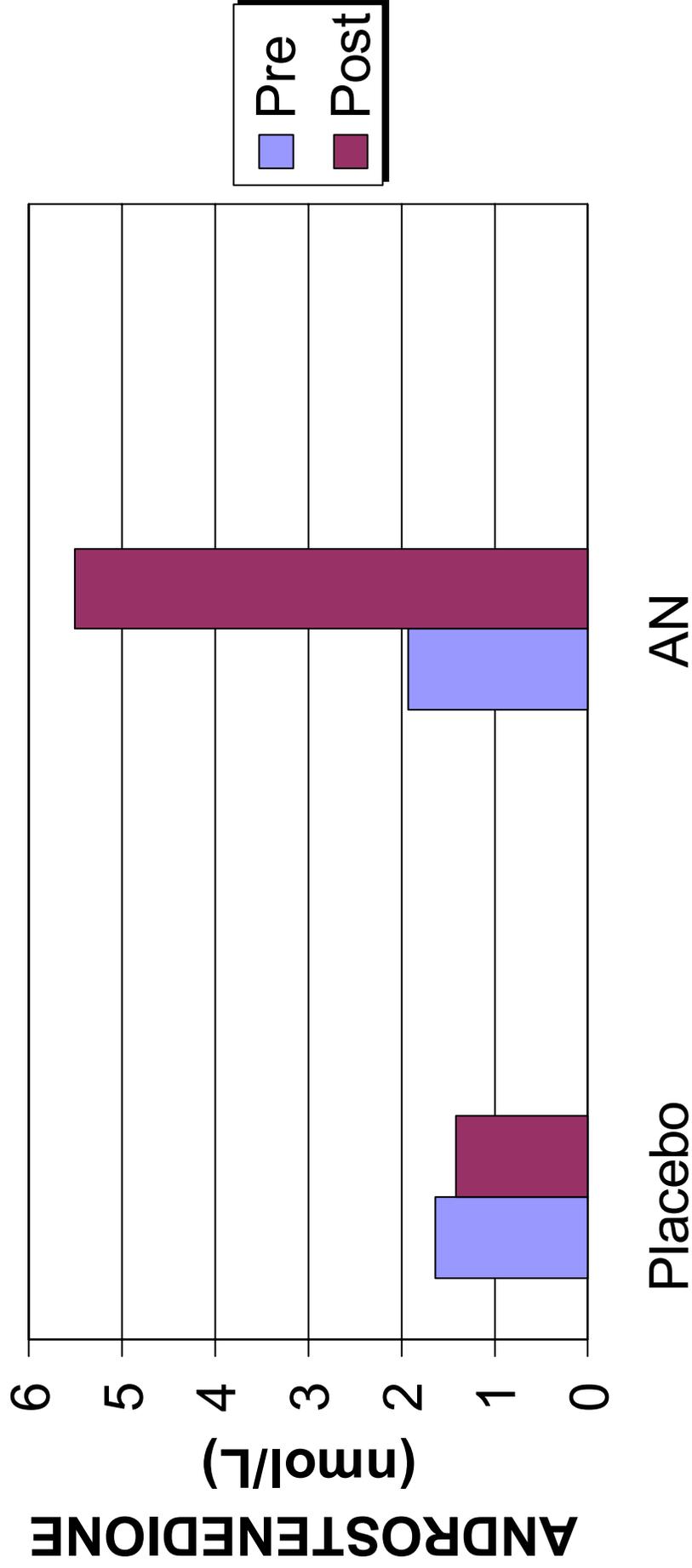
Table 7: Serum Concentrations of Estrogens.

	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
Estradiol (nmol/L)	36.58 ± 9.67	44.83 ± 17.57	37.25 ± 12.60	69.00 ± 25.85*†
Estrone (nmol/L)	13.86 ± 29.91	3.21 ± 1.15	3.41 ± 1.23	4.66 ± 2.02ψ

Values are means ± S.D.
*$p \leq 0.05$, indicates significant difference between pre- and post- treatment values.
†$p \leq 0.05$, indicates significant difference from placebo group pre- and post-treatment values.
ψ $p \leq 0.10$, indicates a significant trend toward a significant difference between pre- and post-treatment values.
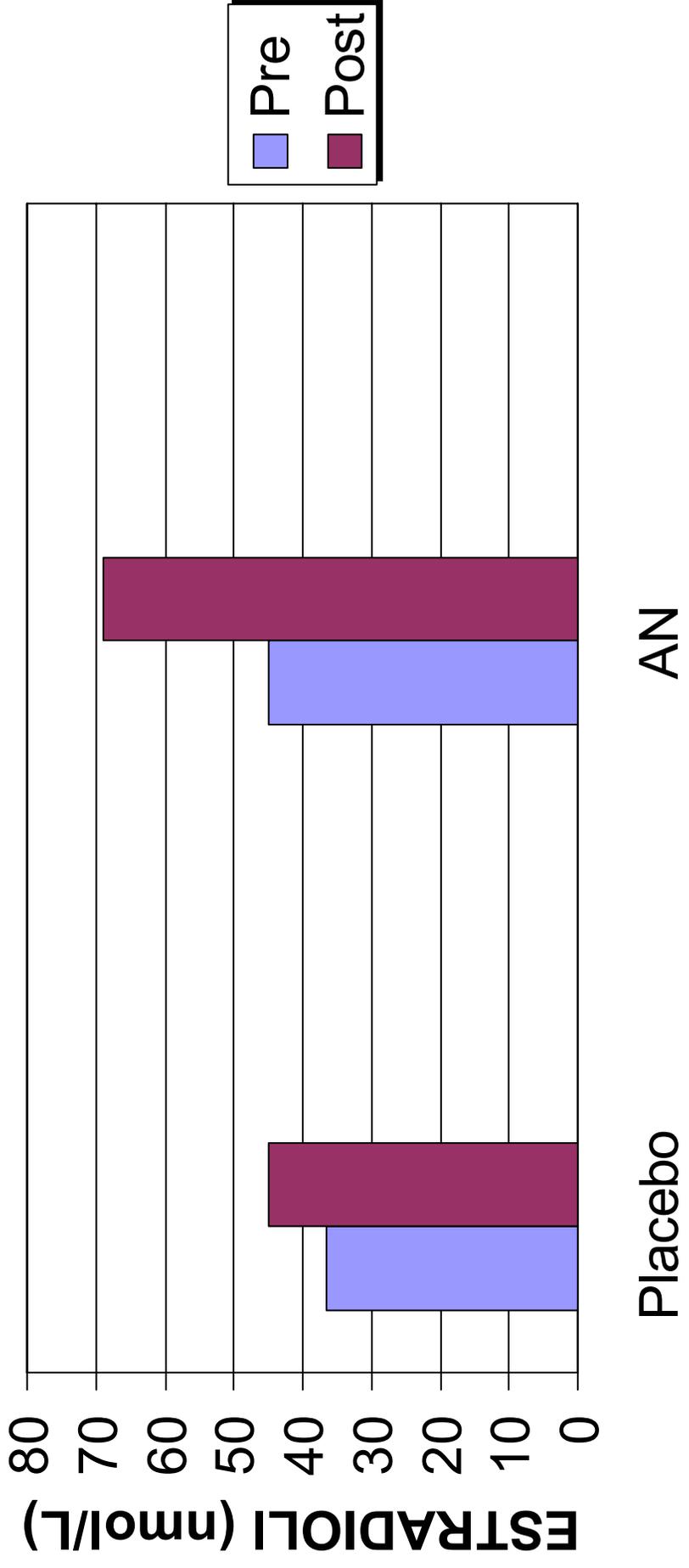
FIGURE 3: CHANGES IN CIRCULATING LEVELS OF ESTRADIOL

Markers of Bone Metabolism

Due both to insufficient sample volume and to indications that some plasma and urine samples may have degraded during storage, measurements of 1, 25 $(OH)_2$ Vitamin D were not conducted. In the case of iPTH, samples from only 7 of the 24 subjects (placebo: n = 2, AN: n = 5) provided enough blood serum for assays to be conducted for both pre- and post-treatment. In some subjects, there was enough specimen to conduct an assay but the measurements were below the sensitivity of the assay. For example, the normal expected range of the assay used for iPTH in the present study is 1.0 - 5.3 pmol/L. If for a particular sample the iPTH concentration was below 0 .7 pmol/L, that sample was assessed a value of *< 0 .7*. In that case the sample was considered to be degraded and was removed from the data set. As a result of limited numbers of samples (AN: n = 5, Placebo: n= 2) for the reasons stated above, no statistical comparisons between either pre- to post-training or between group plasma iPTH concentrations were made. Therefore, the measurements for mean and SD are given in Table 8, but no further statistics were run for this marker. Further, the assay for plasma calcitonin concentration produced values that were below the sensitivity of the assay (<2 ug/ml) for all but 4 of the 48 samples, suggesting that plasma calcitonin had degraded. Thus, no means or statistical analyses were conducted for this hormone.

Results from measurements of the bone resorption marker deoxypyridinoline (DPD) are given for both groups in Table 9. Only nine of the specimens from the placebo group and seven from the AN group were used for assay. DPD concentrations did not significantly change between or within either of the treatment groups. In an attempt to reduce both the impact of a large variance between samples at both pre- and post-training and the small sample size, a pre- to

post-training delta percent change variable (Δ) was created. Values are listed in Table 10. There were also no significant differences either between or within training groups for ΔDPD.

Table 8: Serum Concentrations of iPTH.

	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
iPTH (pmol/L)	3.10 ± 1.84	1.80 ± .42	4.50 ± 2.08	4.36 ± 2.49

Values are means ± S.D.

Table 9: Urine Concentrations of DPD.

	Placebo PRE (N=12)	Placebo POST (N=12)	AN PRE (N=12)	AN POST (N=12)
DPD (mg/dL)	46.03 ± 27.68	61.53 ± 43.32	39.60 ± 19.71	57.81 ± 33.53

Values are means ± S.D.

Table 10: Percent Change (Δ) in Urine Concentrations of DPD.

	Placebo (N=12)	AN (N=12)
ΔDPD (%)	73.85 ± 140.84	120.33 ± 247.49

Values are means ± S.D.

CHAPTER 5

Discussion

The purpose of this study was to determine if 12 weeks of oral AN supplementation had an impact on either biochemical markers of bone turnover or BMD. Measurements of biochemical markers of bone turnover that included DPD, iPTH, and calcitonin were obtained from blood and urine samples from twenty-four subjects from the "Andro Project" by Broeder et al. (2000). The markers of bone turnover for pre- and post-training periods were then compared with the hormonal profiles and BMD measurements of each subject's corresponding pre- and post-training values.

The results of this study indicate that oral supplementation of AN does not positively affect the rates of bone turnover in middle-aged men. Oral supplementation of AN did significantly increase levels of circulating estradiol-17β, and AN by 185 %, and 85%, respectively. It is possible, that the elevated levels of both estradiol-17β, and AN could stimulate a change in bone turnover. However, in this study the increases in estradiol-17β and AN were not associated with significant changes in biochemical markers of bone turnover or BMD. In the AN group, there was also a significant decline in circulating levels of serum free testosterone and SHBG from pre- to post-training periods. These changes did not have any impact on either of the variables (DPD and iPTH) used to determine changes in bone turnover.

The one significant change in BMD over the 12-week training period was spine BMD. Spine BMD significantly increased by 4%, and 6% in both the placebo and AN groups, respectively.

The increases observed in total-, upper-, and lower-body strength measurements from pre- to post-training were significant in both treatment groups. Increased muscular strength would result in increased loading of the skeleton, and this skeletal loading, independent of AN supplementation, may have significantly contributed to the stimuli that resulted in significant increases in spine BMD. The association between increases in spine BMD and strength measurements suggest that the specific design of the RT program used in the study by Broeder et al. (2000) may warrant consideration as a model for exercise programs directed toward combating low BMD.

The Effect of Resistance Training on Bone-Mineral Density

Interestingly, this study produced results that reinforce the findings of others (Kerr et al., 1996; Menkes et al., 1993; Nelson et al., 1994) regarding the positive effects of RT on BMD. The significant increase in spine BMD exhibited by the subjects participating in this study supports the use of RT programs as a means to alleviate the onset and possibly the symptoms of osteoporosis in men. The findings also give insight to the relative load that may be most beneficial in enhancing BMD.

Changes in BMD and the Effect of Relative Load

Changes in BMD are often expected to be relatively small. In most studies (Braith, Mills, Welsch, Keller, & Pollock, 1996; Kerr et al., 1996; Lohman et al., 1995; McCartney et al., 1995; Nelson et al., 1994; Peterson et al., 1991; Pruitt, Taaffe, & Marcus, 1995) that observe the effects of a RT regimen on BMD, the minimum duration of RT which is required to stimulate significant increases in BMD is usually 6 months. In view of the fact that data for the present

study were collected for only 12 weeks, markers of bone metabolism were obtained by using the stored blood serum and urine specimens of the subjects to determine if changes had occurred in rates of bone turnover that may go undetected if BMD was solely relied upon to determine changes in bone metabolism. DPD, a marker of bone resorption, displayed no significant changes across time or between treatment groups.

In the present study, the significant increases in spine BMD were independent of any effects caused by oral AN supplementation because these changes occurred in both the AN and placebo treatment groups. It is reasonable to speculate that changes in spine BMD must have resulted from the RT program that both treatment groups underwent. This is a substantial finding because this suggests that a RT p may significantly and quickly increase BMD, reducing the risk of osteoporosis.

Changes in total-body BMD from the present study can be compared to data presented by Kerr et al. (1996). Kerr et al. who studied a population of females undergoing a RT program similar to the one in the present study. One important similarity between the two studies was that the subjects trained with loads approaching their 1-RM for certain exercises. In regard to the impact of skeletal loading through higher load RT, Kerr et al. included a training group which performed the RT program with lower relative loads. The relatively similar changes in BMD of the subjects in the study by Kerr et al. and the subjects of the present study reveal a universal effect that RT has on BMD when a relative load that approaches maximum is employed.

In the Kerr et al. (1996) study, the BMD of post-menopausal women (age = 60 ± 5 yr.) who were undergoing progressive RT (training at 60-80 % of 1-RM), was measured every 6 months for a 2-year period. They found that after the first 6 months of the study, the women's total body BMD had increased by approximately 0.7 %. In comparison, the subjects (males, $45 \pm$

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8 yr.) in the present study increased their total body BMD by approximately 0.33% during the 12-week RT program, regardless of AN supplementation. Thus, the rate of increase in total body BMD in the present study was proportional to the rate of increase in BMD that was reported for the female subjects in the Kerr et al. study. These data suggest that the positive effect that RT has on the BMD may be independent of sex.

The RT programs in both the present and the study by Kerr et al. (1996) were *progressive* RT programs in that the relative loads used in training approached maximal efforts. The similarity in training loads between these two RT programs may have been the determining factor in the magnitude of increases in total body BMD. Specifically, the subjects from the Broeder et al. (2000) study, and one group of subjects from the Kerr at al. study that performed RT exercises at 60-90% of their 1-RM gained total-body BMD at rates of .11 % per month, and .12 % per month, respectively. In comparison, the subjects of the Kerr et al. study that underwent RT that consisted of higher amounts of repetitions performed at a lower loads (40-60 % 1RM), displayed only modest gains in total-body BMD (0.03 % per month) after the initial 6 month training period. In summary, it appears that RT programs that include training at higher relative loads (60-90% 1RM) with lower numbers of repetitions (8-10 repetitions/set) are associated with the greatest increases in BMD. These findings are further supported by *Wolff's Law* that explains the adaptive response of bone when undergoing a mechanical load (Chamay & Tschantz, 1972).

<center>Application of Wolff's Law</center>

Wolff's Law states that "a bone will modify its structure through hypertrophy when it receives stress from a mechanical load" (Chamay & Tschantz, 1972). In other words, bones will adapt to the forces placed upon them, providing that the mechanical stimulus is of a sufficient magnitude. A *progressive* RT program begins with the beginning load employed during the

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initial training period (i.e. weeks 1-3) is a weight that can usually be performed for 8-10 repetitions (i.e. 70 %) with the relative load used increasing and the number of repetitions performed decreasing in future weeks. Therefore, regularly increasing the amount of resistance on bones through a *progressive* RT program such as the one used in the present study may have played a key role in the changes observed in absolute spine BMD and percent changes in total-body BMD.

Furthermore, the specific result of a significant increase in spinal BMD may have also been influenced by the exercises incorporated within the RT program. By using the reasoning of *Wolff's Law*, exercises that tend to direct axial pressure to the skeleton would induce greater increases in bone development. Examples of RT exercises that accomplish this and were used in the present study are back squat, military press, and the leg press. Refer to *Broeder et al. 2000* for a compete list of RT exercises that were used. The following section discusses the possible effect that these specific exercises may have had on bone development of subjects in the present study.

The Local Impact of Resistance Training on BMD

Wolff's Law states that, "Long bone tends to re-align itself in the axis of force and hypertrophy in zones specifically under compression (Chamay & Tschantz, 1972)." In the present study, only spine BMD significantly increased, and as previously stated it appears that 12 weeks of RT was a sufficient loading stimulus to stimulate the accretion bone in the spine. However, previous literature supports the assumption (McCartney et al., 1995) that 12 weeks of RT may not be a sufficient loading period to stimulate BMD increases most bone. It is interesting to see note that post-training BMD measurements for the leg showed signs of increase (≈ 0.01 g/cm^2, $p = 0.17$) from pre- to post-training in both groups.

A theory that would explain both the significant local increases in spine BMD and non-significant elevation in leg BMD is that these were the areas of the skeleton that had been subjected to the greatest loading forces during RT. As bipeds, the human spine is subject to tremendous loads. Essentially every movement in both the upper and lower body loads the spine. This loading is due to both the direct application of load to the skeleton and indirect loading by muscles that *pull* on the spine (Harms Ringdahl, 1986).

As described in Table 1, the exercises used in the RT program were multi-joint or compound movements. Therefore, more total body stress was induced by a particular exercise such as a back squat in comparison to an alternative exercise for quadriceps strength such as a leg-extension. Another factor that arises in the comparison of these two specific exercises is that the back squat places a load directly on the axis of the spine and femur, whereas the leg-extension isolates the quadriceps without applying a load to either of these areas of the skeleton. By applying Wolff's Law, it is obvious that the squat and other exercises that subject a direct load to the axis of the bones of the skeleton would be most beneficial in attempts to increase the BMD of an individual. Conversely, non-weight bearing RT exercises such as the leg-extension place significant loads on the skeleton only through the translation of muscular loading forces to the skeleton.

<center>Recommendations for Resistance Training Programs</center>

Findings reported in this study may help to provide a better understanding of the type of RT program that would be required to provide a sufficient skeletal loading dose to stimulate BMD accretion over a short period of time. First, the RT program should employ compound movements such as the back squat. Secondly, the program design should use relative workloads

greater than 70 %. Lastly, the exercises should be performed for multiple sets and the number of repetitions should elicit maximal efforts to better enhance increases in strength.

Data from the present study support the use of the RT program used in this study as a way to decrease the magnitude of bone loss that begins in men approximately between the 6th and 7th decade of life (Meema, 1963). Interactions between subject age and BMD changes were not evaluated in this study, but older subjects were part of the study population. The only assumption regarding the effect that this RT program had on men of a specific age that can be made is that the RT program used will increase spine BMD in men the age of our subjects (45 ± 8 yr.).

The results of this study combined with those of Kerr et al. (1996) provide the groundwork for the development of specific RT programs that may be effective in the treatment of low BMD or osteoporosis in males and females. By recognizing the importance of placing a reasonable load upon the skeleton to induce improvements in BMD through exercise, health-care providers may better assist those suffering from osteoporosis or low BMD to achieve better overall bone health either independently or in combination with bone sparing pharmacological therapies.

The Possible Relationship Between Oral Androstenedione Supplementation

And Bone Mineral Density

The common belief that oral supplementation of AN would produce an increase in circulating levels of testosterone in male subjects has not been supported by research (Broeder et al., 2000; Brown et al., 2000; King et al., 1999; Rasmussen et al., 2000). The findings of these studies (Broeder et al.; Brown et al.; King et al.; Rasmussen et al.) indicate that the impact of AN

supplementation has a much different effect. It appears that AN aromatizes to E once it enters the peripheral circulation (Broeder et al.), leading to elevated levels of circulating estradiol-17β, and estrone.

A reduction in bone loss or an increase in bone formation modulated by E has been demonstrated in numerous studies (Falahati Nini et al., 2000; Felson et al., 1993; Lindsay et al., 1980; Recker et al., 1977; Worley, 1981) in which subjects underwent ERT. This study explored a potential benefit of this paradoxical response to AN supplementation because it is plausible that the increased levels of circulating E could also have positively affected rates of bone turnover.

The Independent Effect of Androstenedione on Bone

In the present study, Subjects undergoing AN supplementation significantly increased circulating levels of AN. The importance of this lies in the fact that AN treatment of ovierectomised rats has been shown to result in reduced bone loss independently of E (Lea, Moxham, Reed, & Flanagan, 1998). It appears that bone has AN receptors and that AN can directly stimulate bone formation. The expression of androgen receptors on and within human bone cells has also been demonstrated (Abu, Horner, Kusec, Triffitt, & Compston, 1997). Thus, it conceivable that the AN may also independently mediate changes in bone turnover in humans. The results of the present study, however, do not indicate that the significant increases in AN that resulted from oral AN supplementation affected changes in either bone turnover or BMD.

<u>Increases in Estrogens as a Result of Androstenedione Supplementation</u>

Results of the present study were based on data from 24 subjects (placebo: n = 12, AN n = 12). As explained in Chapter 3, this was a sub-sample of the original subject pool of 33 participants (placebo: n = 18, AN: n = 15) in the "Andro Project" by Broeder et al. (2000).

In concordance with the findings from the original sample population, 12 weeks of AN supplementation combined w/ RT resulted in significant increases of circulating estradiol-17β. There was also a trend (p < 0.1) toward increased concentrations of plasma estrone.

The positive effect that estrogens have on BMD is well documented (Felson et al., 1993; Lindsay et al., 1980; Recker et al., 1977; Worley, 1981). In male subjects, the administration of ERT has proven to increase markers of bone formation and decrease markers of bone resorption (Falahati Nini et al., 2000). In the present study, AN supplemented subjects had increased amounts of circulating estrogens. It is possible that the increased amounts of circulating estrogens may have resulted in an improved profile in markers of bone turnover. If AN supplementation could be shown to positively affect rates of bone turnover, it may be introduced as an alternative to standard HRTs that many aging adults undergo to combat bone loss.

<u>The Effect of Increased Levels of Andtrostenedione, and Estrogens on Changes in Bone Resorption</u>

The results of the present study show that there were no significant changes in concentrations of DPD from pre- to post-training measurements or between treatment groups. This indicates that the significant increases in circulating levels of AN and estradiol-17β or the substantial rise in estrone did not influence osteoclast activity within the AN group. Due to the

lack of other biochemical markers of bone metabolism, the results of the present study cannot fully describe the osteogenic effect of AN supplementation in middle-aged men.

In the present study, it is unclear why there was not some change in bone turnover mediated by increased circulating levels of either E or AN. Perhaps the biochemical markers of bone metabolism that were originally included present study-design could have given a more precise illustration of what actually occurred in regards to the rates of bone turnover in these subjects. It could also be hypothesized that the physiological characteristics of the subjects in the study affected what role the increases AN and E had on bone turnover as well.

Rationale for the Lack of Effect of AN Supplementation on Bone Resorption

Several of the measurements of bone formation/resorption that had been proposed for the current study were unavailable. Therefore, assumptions regarding the effect that oral AN supplementation had on bone turnover were dependent on DPD, the sole marker that was available to the researchers. No significant differences were evident when measurements of this bone resorption marker were compared with the hormonal profile or BMD measurements for each treatment group. This finding was disappointing because the increases in estradiol-17β and estrone were substantial in the AN group (82% and 45%, respectively) and both have been negatively correlated with markers of bone resorption (Falahati Nini et al., 2000; Khosla et al., 1998; Riggs et al., 1998).

A possible explanation for the absence of change in bone resorption of the subjects in the present study is several-fold. First, the subjects in the AN group were only approaching their sixth decade of life (43.25 ± 6.14 yrs). Markers of bone resorption do not normally begin to increase in men until the age of 50 (Khosla et al., 1998). It is also known that the sixth decade of

life is when men begin to lose significant amounts of bone mass (Meema, 1963). Therefore, a decrease in bone resorption may have not occurred because the DPD concentration had not yet begun to increase and the physiological need to increase bone mass was unwarranted in these subjects.

It is plausible that the markers of bone metabolism that were not measured showed some changes. For example, it would have been interesting to observe the interactions of calcitonin, and iPTH and how they responded to the increases in circulating AN, and estrogens because these hormones are the two most identifiable markers of the bone matrix (Marieb, 1992a).

Bone Mineral Density Changes as a Result of Androstenedione Supplementation

In the present study, significant changes in BMD did not occur as a result of AN supplementation. Previous studies (Kerr et al., 1996; Menkes et al., 1993; Nelson et al., 1994) have shown that it would have normally taken 6 months or more for any major changes in BMD to occur as a result of RT exercise alone. However, by combining the proven effects that RT (Kerr et al.; Menkes et al.; Nelson et al.), and increased circulating E (Felson et al., 1993; Lindsay et al., 1980; Recker et al., 1977; Worley, 1981) have on BMD along with the possible effect AN supplementation might have on bone metabolism, it is conceivable that a significant increase in BMD may have occurred. However, any significant changes in BMD that transpired during the study proved to be independent of treatment. The same rationale for the lack of change in bone resorption could also be directed toward the lack of significant changes in this variable as well. Additionally, the relatively short duration of the study may have also played a role in the lack of change in BMD between treatment groups.

Study Design Recommendations

If this study were to be repeated with one of its sole purposes being to measure changes in BMD that result from AN supplementation, the study duration should be extended to a minimum of 6 months. This would be done to allow ample time for greater changes in BMD to occur.

Another possible issue that could be controlled for is to recruit subjects with previous RT experience. Thereby, the initial effect that RT may have on the bone metabolism or BMD of sedentary individuals could be eliminated or at the least minimized.

Recommendations for Future Research

A review of the design of the present study reveals several issues that should be considered in planning future research of this nature. First, adequate amounts of both blood and urine specimens should be obtained from each subject to ensure all planned assays can be conducted. In the present study, it was assumed that the specimen pool was sufficient for the assays that were to be performed. However, the shortage of specimens became apparent once assay procedures had begun. Second, in the present study the length of time that the samples were stored may have influenced the high rate of suspected degradation within the specimens. Prior to their use in the present study, the samples used had been stored for a period that exceeded 2 years. The storage period, itself, could have possibly affected the degradation that the samples seemed to have undergone (Power & Fottrell, 1991). Thawing of samples may also have affected the results of our study. Prior to the present study the samples had already been used for other measurements. This increases the number of times that the samples may have been thawed and allows for the possibility that some of the markers that were measured in the present study to

have degraded. Future research of this nature should specifically control the handling of samples specifically based on the criteria of assays that are to be conducted.

Summary

The results of the present study showed that 12 weeks of oral AN supplementation significantly increased circulating plasma levels of AN, and estradiol-17β by 185%, and 82%, respectively. However, the increased levels of these compounds did not have an impact on bone turnover. Mechanical loading of the skeleton through RT did stimulate significant, local increases in BMD of all subjects. Spine BMD was significantly increased by 6% for both treatment groups.

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