EFFECT OF MORINGA OLEIFERA ON BONE DENSITY IN POST MENOPAUSAL WOMEN


A Thesis
by
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**Abstract**

EFFECT OF MORINGA OLEIFERA ON BONE DENSITY IN POST MENOPAUSAL
WOMEN

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Introduction: Osteoporosis is a clinical condition of low bone mineral density (BMD) that can lead to fractures with hip fractures accounting for more than 300,000 hospitalizations each year. The loss of ovarian estrogen after menopause plays a large role in why women are the most affected. Consuming foods that are rich in bone building vitamins and minerals may help provide important bone protection from this estrogen loss. Moringa Oleifera (M. Oleifera) is a tropical plant and contained in the leaves are nutrients such as beta-carotene, all of the essential amino acids, vitamin C, potassium, and calcium, and multiple important micronutrients that are critical for bone health. Therefore, the study objective is to determine the effects of M. Oleifera on the structure and function of bone in post-menopausal women ingesting 1g of M. Oleifera daily for 12 weeks. Methods: Post-menopausal women (aged 60-70) were split into either the control ($N$=10) or experimental ($N$=10) group with each group consuming 1g daily of a cabbage placebo or M. Oleifera, respectively. No changes to daily habits including food or exercise were made and a three day diet log was recorded. DEXA scans were recorded on each individual before and after the intervention. The main focus was on the hip neck and total hip BMD and bone mineral content (BMC), as well as the whole body BMC and BMD. Results: No significant differences in

starting height ($p$=.107), weight ($p$=.547), body fat percentage ($p$=.620), or lean mass ($p$=.857) were observed between the groups. The only significant difference at the onset of the intervention was a higher right total hip BMD and BMC of the placebo group; this difference persisted throughout the intervention ($p$=.037). No significant interaction of the M. Oleifera on bone density was found with no difference in total body BMD between the two groups. Significant differences were found between pre-total body BMD and the post total body BMD with the average for the entire subject group dropping from a BMD of 1.046 g/cm² to 1.034 g/cm² ($p$=.030); which is a -1.11% drop. Amount of exercise and BMD also significantly correlated with the more exercise per week equally higher total body BMD ($p$=.044). Conclusion: Overall, no relationship between consuming M. Oleifera and an increase in bone density was found. This was mitigated by a lack of power that the study found. The -1.11% decrease in total body bone density is extremely high but could be explained by seasonal changes, medications taken, menopause age, and higher starting bone density. Future studies should look to continue this study for a longer period of time, take blood samples to measure hormone level changes, add exercise to examine its effect, increase the length of study. Developing novel ways to help prevent or slow osteoporosis and osteopenia can have a major impact on the well-being of millions of women.

**Acknowledgments**

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<center>**Introduction**</center>

Consuming a healthy and balanced diet is an important feature in daily life, as it helps improve overall health indices and can help reduce disease rates. Healthy diets that provide the recommended daily allowances of vitamins and minerals can help promote natural body growth and health. Currently, a majority of our country does not eat a healthy diet that contains the proper vitamins and minerals the body needs (Krebs-Smith, Guenther, Subar, Kirkpatrick, & Dodd, 2010). The United States, instead replaces 10% of daily calories with fast food and junk food. This excess of saturated fat, sugar, and sodium along with a low amount of necessary nutrients has shown to elicit negative health consequences such as increased obesity rates, disease rates, and medical costs (Center for Disease Control, 2013). One of those impacts has been on bone health. Millions of Americans suffer from low bone mass and this leads to millions suffering from secondary affects such as broken bones, decreased mobility, or even a loss or fear of completing daily activities (National Osteoporosis Foundation, 2002). These secondary affects can impact not only the single individual but the society as a whole by increased medical costs and a less productive work force. Consumption of the proper vitamins and minerals is imperative in order to prevent low bone density or other bone diseases. These preventative measures can help reduce future negative health and financial consequences for those directly involved and the society in general.

**Nutrition and Bone Health**

Consuming a healthy diet that contains adequate and proportional amounts of vitamins and minerals is extremely important in preventing bone disorders such as osteopenia and

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osteoporosis (Nieves, 2005).  As stated earlier, the average American citizen currently does not meet the federal dietary recommendations, and over 90% intake more empty calories than are recommended  (Krebs-Smith et al., 2010).  Moreover, consumption of soda in the US has risen 86% from 1970 to 1997 (Vartanian, Schwartz, & Brownell, 2007). The increase in these discretionary calories from soft drinks are currently replacing the intake of essential nutrients. In the long run, these negative dietary habits compromise bone density as the intake of colas (dark sodas) are found to cause lower bone mineral density (BMD) in older women, an observation attributed to high phosphoric acid content (Tucker et al., 2006). It appears that a diet that is low in important nutrients combined with excess discretionary calories from sodas and fast foods can cause negative health outcomes including bone health. Indeed, a study found that women that consumed dark vegetables have fewer fractures than women who do not but instead replace vegetables  with acid-forming fluids such as carbonated beverages (Lin et al., 2013).

The specific vitamins and nutrients in the diet associated with an increase in bone density later in life include calcium, vitamin D, magnesium, potassium, vitamin C, zinc, and fiber. (Gunn, Weber, McGill, & Kruger, 2015). Also, high consumption of fruit earlier in life positively correlates with high bone density in later life (New, Bolton-Smith, Grubb, & Reid, 1997). Further, another study that looked at the diet of women hospitalized with a hip fracture compared with healthy controls found that those with a higher-quality diet were less likely to suffer from a hip fracture. High-quality scores are typically characterized by a high consumption of plant-based foods (e.g., fruit, vegetables, and legumes), fish or white meat, whole grains, fiber, moderate alcohol intake, and a low consumption of red and processed meat. This type of diet is rich in antioxidants (e.g., vitamins A, C, and E, carotenes, zinc, and selenium), cations (magnesium, potassium, and calcium), vitamin K, folate, and polyunsaturated fatty acids (PUFA), and poor in saturated fatty acid (SFA) (Zeng et al., 2014). Going forward, future studies on bone density

should focus on examining the impact of a diet that is rich in fruits, vegetables, whole grains, and lean proteins on bone health.

**Bone Disease and Clinical Impacts**

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fractures. Clinically, it is defined as being 2.5 standard deviations lower than the average bone density at any given age (Fonseca, Moreira-Goncalves, Coriolano, & Duarte, 2014). Osteopenia (an early state of osteoporosis) or osteoporosis increase the likelihood of fractures, which consequently may cause significant negative impacts on daily life (Dickinson, 2014). Hip fractures, which are one of the three main fractures associated with osteoporosis, are by far the most devastating, accounting for more than 300,000 hospitalizations every year. Collectively, these hip fractures are associated with chronic pain, increased dependence, reduced mobility, deformity, depression, loss of self-esteem, increased rates of hospitalization, and heavy personal socioeconomic burden (Dickinson, 2014).These effects not only impact the individual and those closest to them but they can also influence society as a whole. Economically, hip fractures are adding increased pressure to an already tight U.S. medical budget.  Hip fractures represent only a small portion of osteoporotic fractures (14%), but they account for as much as 72% of the cost expenditure of all fractures. This cost is projected to rise to over 18.2 billion dollars annually by 2025 (Burge et al., 2007). This amount of money equates to roughly $ 55 dollars per American per year. This increase in the medical costs of hip fractures is exacerbated by the fact that the demographic group of 50 year-olds in the U.S.is predicted to increase by 60% between 2000 and 2025, eventually reaching 121.3 million people (Burge et al., 2007). Such a demographic increase in the population will inevitably lead to a corresponding increase in the percentage of people suffering from osteoporotic fractures, including hip fractures,

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and subsequently, an upsurge in medical costs for individuals and the society at large. This trend signifies the importance of investing in and developing programs that target prevention of bone fracture and technologies that evaluate bone density as of the social and economic impact of these fractures continue to grow.

The most common technology used for determining bone density is the dual-energy X-ray absorptiometry (DEXA) (Provyn, Clarys, Wallace, Scafoglieri, & Reilly, 2008). This machine works by passing x-rays through the body with the densest bone letting the least amount of x-rays through. This equipment determines the variations in bone density with two different results. Bone mineral content (BMC) indicates the number of bone particles (in grams) in the scanned region, and bone mass density (BMD) which divides the scanned area by the BMC (how many particles are in the given region) and provides the data in $g/cm^2$. The BMD is the most common representation of these scans and a normal or average BMD is 1.0. The DEXA can also provide the, z and t-score, body fat, and lean mass percentages in a few selected places such as the femoral head, lumbar spine, and total body (Provyn et al., 2008). These scans are one of the easiest and quickest measures of bone density and provide an accurate method for analyzing overall bone strength and have been shown to have quality accuracy for all measurements.

**Low Bone Density Factors**

Prevention of osteoporosis in females is imperative as they face the greatest danger given their longer life expectancy, smaller bone mass, and loss of the bone preserving action of estrogen (Greenway, Walkley, & Rich, 2012). Menopause, the permanent cessation of menses due to the loss of ovarian folliculo-genesis (The World Health Organization, 1995), which occurs on average around 51 years of age, with an age range varying between 40 and 60 years (Treloar, 1981). One impact of menopause is the dramatic loss of the ovarian hormone, estrogen, an

important preserver of bone health. Estrogen works to help break apart osteoclasts, reduce inflammatory proteins that encourage osteoclastogensis, encourages osteoblast proliferation, and modulates the calcium levels in the blood. These effects of estrogen help maintain and encourage bone density throughout life (Khosla et al., 1998).

As women age, serum estrogen will drop putting females at an especially high risk for fractures and bone diseases. Estrogen starts to decrease slightly as early as age thirty but is drastically reduced post-menopause (Finkelstein et al., 2013) and the loss of estrogen correlates to a slow loss of bone density even before menopause is reached (Khosla et al., 1998). Roughly, 8.2 million women suffer from osteoporosis and an additional 27.3 million women having some form of low bone mass (Cosman et al., 2014). Therefore, the risk of suffering from a broken bone is extremely high for women. Most importantly though, after the age of 50, a woman is just as likely to die from a hip fracture as she is from breast cancer (Price, Langford, & Liporace, 2012). This clearly shows the importance of monitoring bone density and to encourage those at risk to partake in healthy bone-promoting activities.

Due to the physiological roles of estrogen, other than the bisphosphonates discussed earlier, one of the most common ways to treat low bone density is with estrogen replacement therapies (ERT). Studies have shown the increase in bone density with ERT (Gambacciani & Levancini, 2014). ERTs work to put estrogen back into circulation in the body which maintains the osteoclast/osteoblast ratio which will increase bone density (Gambacciani & Levancini, 2014). The two main combinations of hormone replacement therapies are estrogen only and estrogen plus progestin, the latter of which is given for women who have not had a hysterectomy (Gynecologists, 2015). There are two main ways to distribute the ERT; locally or systemically. The ideal route for increasing bone density is systemic via a patch, pill, or gel.

However, like bisphosphonates, use of ERTs are limited due to the side effects shown with taking ERT. Exogenous estrogen only treatments thicken the uterine lining and increase the chance of uterine cancer. Combination treatments have been found to increase the risk of breast cancer, deep- vein thrombosis, heart attack, stroke, and gall bladder disease (Bae & Kim, 2015; Gambacciani & Levancini, 2014; Rahnama, Jastrzębska-Jamrogiewicz, Jamrogiewicz, & Trybek, 2014; Zhao, Xu, & Zhao, 2015). For this reason, the 2015 European Code against Cancer currently recommends limiting or avoiding ERT use due to their link with cancers (Friis et al., 2015). The cost/benefit analysis is one that needs to be looked at individually for each person by a trained physician. Women currently on ERT show low persistence or compliance with taking the drugs for the recommended time due to side effects. These results showed no difference in continuation of the therapy between the high or low dose group as well as with how the drugs were administered (oral versus cream) (Kyvernitakis et al., 2015). Overall, ERT have shown some beneficial responses to increasing bone density but there are quite a few risks associated with consuming these drugs. Developing an all-natural regimen for protecting and building bone mass could greatly reduce the prevalence of cancer in women by offering an alternative to ERT.

One easy and safe way to improve bone density is to consume natural foods that contain necessary vitamins and minerals. Previous studies have shown the positive benefits to consuming healthy diets to improve bone density (Kim, Bu, Sung, & Choi, 2013; Macdonald, New, Golden, Campbell, & Reid, 2004; New et al., 1997; Palacios, 2006; Zeng et al., 2014). These diets have found relationships with specific vitamins and minerals such as potassium, magnesium, vitamin D, vitamin C, vitamin K, calcium, zinc, boron, and silicon. These articles show that a diet rich in these vitamins and minerals will help to improve bone density. One other article found that two main factors were associated with fracture rates: consuming nutrient dense food (fruits, vegetables, and whole grains), and consuming energy dense foods (soft drinks, potato chips, and

desserts). This article showed that the higher the nutrient dense diet the lower the fracture risk as well as the higher the energy dense diet the higher the fracture risk (Langsetmo et al., 2011). From these articles, it can be assumed that post-menopausal women that have low BMD or fractures also consume less of these nutrient rich foods, which shows the necessity for consuming these nutrients.

**Moringa Oleifera**

Moringa Oleifera (M. Oleifera) is a tropical plant native to northern India, Pakistan, the Himalayan region, Africa, Central America, and Arabia and is exceptionally rich in a variety of nutrients and medicinal phytochemicals. M. Oleifera contains various nutrients such as vitamin D, calcium, phosphorus, magnesium, and others that are associated with an increase in bone density (Palacios, 2006). M. Oleifera also contains micronutrients that play a critical role in bone health and in calcium absorption such as boron, vitamin C, magnesium, potassium, phosphorous and others (Issa, 2012; Price et al., 2012). For instance, boron stabilizes and extends the half-life of vitamin D (Ghanizadeh et al., 2012); magnesium (Mg) affects the activities of osteoblasts and osteoclasts, as well as bone homeostasis by modulating the concentration of parathormone and the activated form of vitamin D (McNaughton, Bolton-Smith, Mishra, Jugdaohsingh, & Powell, 2005). Mg also contributes to bone structural development (Mahdavi-Roshan, Ebrahimi, & Ebrahimi, 2015); vitamin K reduces bone turn over, improves bone strength and plays an essential role in osteocalcin carboxylation (Bügel, 2008; Trumbo, Yates, Schlicker, & Poos, 2001)) and vitamin C increases bone density and strength by increasing collagen formation and promotes healing during fracture (Leveille et al., 1997; Morton, 1991). Collectively, these micronutrients in M. Oleifera are expected to significantly impact bone health.

Despite the long history in use of the tree, information on the optimal dose of M. Oleifera needed to promote bone density in human is scanty. There is however, useful information that can be gathered from animal and cell models. In one study, animals (rats, mice, and rabbits) were given 15 times more than the recommended daily dose of M. Oleifera leaf powder for adults, which is around suggested to be 1,600mg daily. The amount given was found to be equivalent to a child consuming 375 grams daily, and no adverse side effects were seen on any of the study animals (Boven & Morohashi, 2002; Stohs & Hartman, 2015). In another study that used mice, the dosage level of 30mg/kg per day was found to elicit a beneficial effect while no toxicities were seen at this dosage (Faizi et al., 1998). Another study using rats determined that there were no dangerous toxicity levels associated with M. Oleifera at or below 1,000 mg/kg, while supertoxicity levels were seen above 3,000 mg/kg (Asare et al., 2012). In recent human studies, supplementation levels of 500 mg/day in breastfeeding women (Estrella, Jacinto Bias III, David, & Taup, 2000) and up to 8000mg/day in men with broken jaws (Singh et al., 2011) were used, with no adverse effects seen in either study.

Based on these earlier animal studies, a nutritional program that involves ingestion of a M. Oleifera powder could lead to increase bone strength post-menopausal women. More research is needed to determine if M. Oleifera can induce bone specific changes seen by an increase in bone mass density or bone mineral content. Given this knowledge, the purpose of this study is to determine the effects of M. Oleifera on the structure and function of bone in post-menopausal women ingesting 1 gram of M. Oleifera daily for 12 weeks seen with the use of the DEXA.

## Statement of the Problem

Proper nutrition has been proven to promote bone health, including development and growth. However, with age the bones begin decay and weaken, in part, due to loss of hormones such as estrogen. The loss of bone mass puts people, particularly women, at a greater risk for bone diseases such as osteoporosis and osteopenia. M. Oleifera is an edible plant that has been shown to be effective in exerting a variety of health benefits and has a variety of nutrients and minerals that might help slow bone mass loss. However, to date, no comprehensive human research study has examined the effects of M. Oleifera on bone mineral density. Therefore, the purpose of this study is to determine the effects of M. Oleifera on the structure and function of bone in post-menopausal women ingesting 1 gram of M. Oleifera daily for 12 weeks.

## Hypothesis

I hypothesize that supplementation of M. Oleifera in the daily diet of postmenopausal women will promote bone mineral density and bone mineral content, within three months of intervention.

## Significance of the Study

Due to the medical costs and risks associate with current treatments for low bone mass, it is imperative that alternative therapies that are affordable and safer are explored. The present study investigates the effectiveness of using a whole leaf plant-based supplement used to improve the bone density score in postmenopausal women. This information will give doctors, nutritionists, and therapists the necessary information to discuss the possible benefits of M. Oleifera with their at-risk patients.

**Review of Literature**

Proper nutrition is an important component of daily life, as it helps improve bone strength in young individuals and maintain bone strength in old individuals. In addition to either improving or maintaining bone strength, a proper diet containing a rich diversity in vitamins and minerals can provide a reduction in bone diseases and complications such as osteopenia and osteoporosis. Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fractures, and is clinically defined as being 2.5 standard deviations lower than the average bone density at any given age (Fonseca et al., 2014). Females, given their longer life expectancy, smaller bone mass, and loss of the bone preserving action of estrogen face the greatest danger to these diseases (Greenway et al., 2012).

One of the main clinical and economic consequences of osteoporosis is skeletal bone fracture with the most common breaks in weak parts of bones such as the wrist, spine, and hip (Department of Health & Human Services, 2004). Of the three main fractures, hip fractures are by far the most devastating, accounting for more than 300,000 hospitalizations every year. Collectively, these fractures are associated with chronic pain, increased dependence, reduced mobility, deformity, depression, loss of self-esteem, increased rates of hospitalization, and heavy personal socioeconomic burden (Dickinson, 2014). In the first year after hip fracture mortality rate increases 20-24% from all causes (Cooper, Atkinson, Jacobsen, O'Fallon, & Melton, 1993).

More women than men have osteoporosis or osteopenia (low bone mass), particularly postmenopausal women, with roughly, 8.2 million women suffering from osteoporosis and an additional 27.3 million women having low bone mass (Cosman et al., 2014). After the age of 50 a woman is just as likely to die from a hip fracture as she is from breast cancer (Price et al., 2012). This increase in mortality rate is in part due to loss of ovarian estrogen, which plays a protective role on bone. These bone diseases, however, are controllable if not even preventable through an

increase in healthy eating, vitamin supplementation, and or exercise. Consumption of the proper nutrients daily, especially for older women, can drastically improve bone health and reduce the risk of fracture (Khosla et al., 1998). This review will discuss how and why bones become strong, the proper nutrition related to bone strength, and the effect M. Oleifera has on the body.

**Bone Strength Determinants**

   The process of bone growth is dictated by involuntary factors such as genetics and hormone levels. Once humans have reached physical maturity, the bones continue to adapt and change for a variety of reasons including hormone and chemical levels. Osteoblasts, osteocytes and osteoclasts are the three cell types involved in the development, growth and remodeling of bones. Osteoblasts are bone-forming cells, osteocytes are mature bone cells and osteoclasts are cells that break down and reabsorb bone (Carter, Van der Meulen, & Beaupre, 1996). As the body grows during childhood, the osteoblasts form new bone at the epiphyseal plate at the ends of the bone. Once maturity is reached the osteoblasts no longer increase the length of the bone but may still work by increasing the diameter of the bone which is called appositional growth (Carter et al., 1996). During adulthood, a person who is healthy and active will have osteoblasts that constantly create new bone while the osteoclasts remove old bone cells and make sure the bones do not become too bulky. This becomes an issue with age, however, as the osteoblast activity decreases and the osteoclast activity continues working at the same pace (Carter et al., 1996). This homeostatic process of bone formation and degradation is what many osteoporotic drug therapies are working toward controlling (Burr, 1997).

   Bone strength determinants can be broken down into whole bone geometry, which is the bone size and cortical thickness, the microarchitecture, which is made up of cortical porosity,

trabecular connectivity, trabecular shape, and lastly, the tissue properties of the bone such as the collagen cross linking, cellular density, and osteocyte network (Fonseca et al., 2014).

The first factors that influence bone development are individual genes. The genetic code dictates whether naturally thicker, stronger, or stunted bones will develop. These genetic factors are unchangeable and are just one factor dictating bone health. Besides the unchangeable genetic predispositions, there are a large number of hormonal and chemical changes that have a potent effect on bone strength. Sex hormone levels, specifically estrogen, have been found to be a large player in bone strength and health (Ducher et al., 2011). 17β-estradiol (E2; a form of estrogen), has been found to have a positive correlation with bone density in polycystic ovary syndrome patients (Katulski et al., 2014). The role of sex hormones was also found in men. A study using older Swedish men found that serum E2 and testosterone were inversely related, whereas serum sex hormone binding globulin was directly related to fracture risk. This study also reported that E2 was a much better independent predictor of bone loss than testosterone (Mellstrom et al., 2008). These results show the importance of sex hormones and why increasing age is a large risk factor for decreased bone strength.

This formation of bone requires a much longer amount of time for growth compared to the muscular system. Bone formation and degradation is a slow and continuous process that requires ample amounts of time in order to see significant differences in the body. A muscular system can see drastic changes in strength or muscle size within three to four weeks but bone growth takes more time in a growth environment for there to be changes. There are various studies that show this estimated time required for bone growth during exercise regimens. A 2014 review article compares a large body of work that focuses on different training modalities and how those impact bone (Gregov & Šalaj, 2014). The studies included range from 10 weeks to 48 months in duration and look at women, men, young and old participating in exercises from

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vibration platforms to strength training to aerobic exercise (Gregov & Šalaj, 2014). These articles show that depending on the population and intensity of the intervention, significant increases in BMD can be seen in as little as ten to fourteen weeks (Guadalupe-Grau et al., 2009; Helge et al., 2010; Johannsen, Binkley, Englert, Neiderauer, & Specker, 2003). Despite this possible intervention length the most common amount of time used for a bone strength intervention was around 6-10 months (Gregov & Šalaj, 2014).

Other studies have examined the effect of nutritional interventions on bone density changes as well. One study studying elderly women (mean age 84.8 years) found that by increasing calcium and vitamin D fortified plain cheese, after 1.5 months, their PTH levels decreased, the insulin-like growth factor-1, osteocalcin, and type-1 procollagen levels increased (Bonjour et al., 2009). A reduction in PTH and increase in growth factor and osteocalcin are the first steps needed for bone growth to occur.  It was also shown that the bone turnover markers decreased after 1, 1.5, and 2 months of a dairy intervention in similar studies (Bonjour, Benoit, Payen, & Kraenzlin, 2013; Bonjour et al., 2009; Bonjour, Benoit, Rousseau, & Souberbielle, 2012). Another study by Heaney et al. (1999) looked at an increase in calcium and vitamin D via an increase in dairy consumption in men and women aged 55-85 (Heaney et al., 1999). In 12 weeks' time this study found Mg and phosphorus increases along with reductions in PTH, and bone resorption marker, N-telopeptide excretion (Heaney et al., 1999). This study also demonstrates the shown precursor steps that precede bone stability. These articles show the speed at which one growth markers can be seen in the body after a nutritional supplementation. This gives a good timeline for the potential for positive bone changes within weeks or months rather than just years.

**Dual Energy X-Ray Absorptiometry**

The standard for bone mass assessment is dual energy x-ray absorptiometry (DEXA) (Bareither, Grabiner, & Troy, 2008; Daly et al., 2008; Greenway et al., 2012; Kato et al., 2006; Nielson et al., 2011; Raghuvanshi & Singh, 2012; Shedd et al., 2007; Vainionpaa, Korpelainen, Leppaluoto, & Jamsa, 2005; Witzke & Snow, 2000; Zribi et al., 2014). DEXA is able to measure whole body bone mineral content and bone mineral density as well as BMD at a few specific sites including femoral head, lumbar spine, and hip region. This machine works by passing x-rays through the body with the densest bone letting the least amount of x-rays through. This process will then be able to show which areas of the bone are the densest by measuring areal bone mineral density. Areal bone mineral density is a two-dimensional representation of bone density as it reports the amount of bone mineral content (BMC) in a given area scanned. This BMC is the first important output from a DEXA. The second output from the DEXA is bone mass density (BMD), which is the area of the scanned region divided by the BMC (how many particles are in the given region) and gives the BMD in $g/cm^2$. The BMD is the most common representation of these scans with a good or average BMD being at 1.0. The DEXA can also provide the, z and t-score, body fat, and lean mass percentages at a few select places such as the femoral head, lumbar spine, and total body.

DEXA is the easiest and quickest measure of bone density and provides an accurate method for estimating overall bone strength (Vaccaro, Busetto, Bernardini, Anselmi, & Zotti, 2012). When comparing the DEXA to other methods to determine BMD, it was found that the mean grey value was completely comparable to the DEXA (Vaccaro et al., 2012).Once the DEXA machine has been purchased, each scan, which will take around 8 minutes, is essentially free. The radiation given off during each scan is also very low, which allows for multiple scans to be completed at a time. A single DEXA scan produces 1 – 6 microseiverts (µSv) and a standard

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flight from New York to Los Angeles generates 16 µSv; and daily radiation levels (at sea-level) expose us to 12 – 16 µSv. Overall, the DEXA provides an easy, quick, accurate, and well-studied depiction of BMD and BMC.

**Nutrition and Bone Health**

One modifiable factor contributing to bone health is the level of certain vitamins and minerals in the body. These vitamin and mineral levels are dependent on a wide variety diet including a large amount of fruits and vegetables. By increasing vegetable and fruit intake a group of midlife women were able to reduce their net endogenous acid production as well as increase the pH of their urine; which means the body was under less oxidative stress (Gunn et al., 2015). Another article showed that the fragility fracture prevalence in participants who rarely or never consumed deep-colored vegetables was significantly higher than that of those who often consumed deep-colored vegetables: 17.6% versus 9.0% (Lin et al., 2013). In a study that only looked at the difference between animal and vegetable protein, the results showed that the animal protein group had a greater loss of bone than the vegetable group (Lanham-New, Lee, Torgerson, & Millward, 2007). One study that examined the roles of fruits on BMD found that daily increase of 100 g/1000 kcal fruit intake was associated with a 6.4% BMD increase in whole body, and 4.8% in femoral neck in women (Liu et al., 2015). A longitudinal study that looked at diet records and bone density over a four year period found an increase in BMD with increased potassium and magnesium in three hip sites and one forearm site (Tucker et al., 1999). Secondly, an increase in BMD in two hip sites was found for women who consumed high amounts of fruits and vegetables.

Beyond just fruit and vegetable intake two studies showed the affect overall meal quality can have on bone density. One study showed that higher quality diets that include fruits,

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vegetables, whole grains, and lean proteins correlated to lower fracture rates in elderly women (Zeng et al., 2014). Again, another article focusing on nutrient dense versus energy dense found that eating higher nutrient dense meals produced a lower incidence toward fracture. At the same time eating higher energy dense meals produced a higher tendency toward fractures (Langsetmo et al., 2011). Fruits and vegetables provide a very high amount of proper nutrients for bone health versus processed and fatty foods.

One very important nutrient for bone health is vitamin D. Vitamin D and its hormonal form, 1,25-dihydroxyvitamin D, elevate serum calcium and phosphorus levels necessary for bone mineralization. A 2010 study by The Osteoporotic Fractures in Men (MrOS) showed that serum vitamin D levels below 20ng/ml were associated with greater rates of hip bone loss and hip fracture (Cauley et al., 2010). This study was found to be inconsistent with other vitamin D studies including one by the same research group in 2012. The 2012 study proved that there was no association of fractures with lower BMD or BMC with abnormal Vitamin D levels (Barrett-Connor et al., 2012; Roddam et al., 2007). One discrepancy between studies could be because the first study by Cauley et al. (2010) followed over 1600 men over a mean period of 5.3 years and sampled a larger proportion of non-Caucasian men, who consistently have lower vitamin D serum levels whereas the second study used mostly healthy, Caucasian, and non-obese individuals which are factors associated with higher vitamin D levels (Cauley et al., 2010). These differences between the two studies could be one reason as to why the association between vitamin D levels and bone health are inconsistent. Additionally, vitamin D levels seem to be closely linked to calcium levels. Studies have shown that when analyzed separate from calcium, there is no relationship between vitamin D supplementation and fracture risk (Avenell, Gillespie, Gillespie, & O'Connell, 2005). This is why most will recommend taking vitamin D and calcium together in order to facilitate the benefits of both.

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Calcium, by itself, is also an important nutrient for bone health because it is the primary building block of bone. Blood calcium homeostasis is tightly controlled by maintaining the parathyroid and osteocalcin hormones. Calcium levels are held within a tight window between 9-11mg/100ml of blood for normal homeostasis (Mundy & Guise, 1999). When this level becomes too low, the parathyroid gland will secrete PTH to break down bone to raise the level of calcium in the blood. Oppositely, when the calcium levels are too high in the blood the thyroid gland will secrete calcitonin to deposit excess calcium in the bones (Mundy & Guise, 1999). A regular and constant flux of PTH will slowly break down the bone, which can cause osteoporosis or osteopenia. This homeostatic control of calcium shows the importance of calcium in the diet.

Another important element for bone growth is phosphorus. Hydroxyappatite, which is one of the primary mineral compounds in osseous tissue, is composed of calcium and phosphate. One study found significant increases in BMD at specific sites such as femoral neck and the lumbar spine with phosphorus supplementation. This article did not, however, find significant results when looking at whole body BMD (Lee, Kim, Kim, Seo, & Song, 2014) A second article found a 4.2% increase in BMC and a 2.1% increase in BMD (Lee & Cho, 2015). There are, however, negative side effects to consuming too much phosphorus intake as a too high concentration can throw off the calcium balance and cause no bone formation to occur. Therefore, the stated ratio should be around 0.60 of calcium to phosphorus (Lee & Cho, 2015).

Other micronutrients also help play a role in bone development. For instance, boron stabilizes and extends the half-life of vitamin D (Ghanizadeh et al., 2012). Magnesium (Mg), with 67% of total body Mg found in the skeletal system, affects the activities of osteoblasts and osteoclasts, as well as bone homeostasis by modulating the concentration of parathyroid hormone (PTH) and the activated form of vitamin D (Hayhoe, Lentjes, Luben, Khaw, & Welch, 2015; McNaughton et al., 2005). Mg also contributes to bone structural development (Department of

Health & Human Services, 2004). Vitamin K reduces bone turn over, improves bone strength and plays an essential role in osteocalcin carboxylation (Bügel, 2008). Vitamin C increases bone density and strength by increasing collagen formation and promoting healing during fracture (Leveille et al., 1997; Morton, 1991). Collectively, these micronutrients are expected to significantly impact bone health.

Oppositely, one very important component of diets can severely impact bone health. The build-up of acid in the body can potentially have drastic bone health effects and needs to be monitored.  This production of acid in the body can occur when consuming foods that are high in acid (animal proteins/grains) and not ingesting foods high in bicarbonate (fruits and vegetables). This causes an increase in non-carbonic acid in the blood which will then combine with sulfur from the animal proteins to form sulfuric acid (Tabatabai et al., 2015). This will then form an increasing level of acid build-up in the system.

 Renal function as well as some cardiorespiratory functions will help dispel this extra acid but renal function will decline as the body ages (Tabatabai et al., 2015). This will then cause a drop of pH in the body which the body will work to raise in order to maintain homeostasis. The bones serve as a constant base reservoir with large amounts of calcium salts (phosphates, carbonates, and hydroxides) constantly available. As the body continues to have a high acid content based on a diet without fruits and vegetables the bone will release basic calcium salts in order to counteract the acid levels. This results in a progressive drop in bone mass over time. Acidic fruits such lemons, oranges, and even yogurts could potentially increase an acid load in the system but this potential build-up is buffered by the magnesium and potassium found in these fruits as well (Liu et al., 2015). This acidic load can also be reduced by the calcium already in the fruit or in calcium fortified fruit beverages (Franklin, Masih, & Thomas, 2014). This leads to even acidic fruits and vegetables leading to a positive calcium balance. In order to reduce the

chance of chemical levels or acid problems becoming an issue it is important to ingest large amount of fruits and vegetables as well as limit unprocessed and fatty foods.

**Moringa Oleifera**

M. Oleifera is a tropical plant native to northern India, Pakistan, the Himalayan region, Africa, Central America and Arabia and is exceptionally rich in a variety of nutrients and medicinal phytochemicals. Specifically, it contains all the essential amino acids, beta-carotene, vitamin C, potassium, calcium, as well as other smaller micronutrients (Radek & Savage, 2008). M. Oleifera has comparable levels of calcium and protein to powdered milk, which is around 13 mg/g of calcium (Lucey & Singh, 1997). More importantly, unlike pharmaceutical products or milk, which only contain one or two important elements essential for bone health, M. Oleifera contains multiple micronutrients that play a critical role in bone health and in calcium absorption, such as boron, vitamin C, magnesium, potassium, phosphorous and others (Issa, 2012; Price et al., 2012).

The M. Oleifera plant has been widely used in its various forms including the seeds, leaves, oil, sap, bark, roots, and flowers. The leaves, however, are the most widely used due to their high nutritional content. The leaves are reported to have high antioxidant compounds including ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani, Kumar, & Khan, 2013; Vongsak, Sithisarn, & Gritsanapan, 2013). Various common uses are known for M. Oleifera including anti-inflammatory, antihypertensive, antioxidant, antidiabetic, anti-hyperlipidemic, and cardio-protectant activities (Anwar, Latif, Ashraf, & Gilani, 2007; Mbikay, 2012).

*Nutritional Content*

Determining the exact macro and micro nutrients as well chemical components in M. Oleifera is important for future studies. One study on M. Oleifera has found that the dried leaves of M. Oleifera offer the most health benefits with roughly three to five times more vitamins and minerals in dried leaves versus the fresh leaves (Fuglie, 2002). The dried M. Oleifera leaves contain all of the essential amino acids with arginine (1,325mg), leucine (1,950mg), lysine (1,325mg), and phenylaline (1,388mg) accounting for the most abundant amino acids per 100g of edible portion (Fuglie, 2002). M. Oleifera dried leaves contained 2003mg of calcium per 100g of edible portion while milk only gives 120mg of calcium for the same dose and also contains up to 25 times more iron than spinach, nine times the protein of yogurt, and ten times the vitamin A of carrots (Fuglie, 2002).

Other studies have examined the properties found in M. Oleifera as well. Another study by Valdez-Solana et al. (2015) found that the most abundant macro-elements found in their analysis were calcium, magnesium, and potassium (Valdez-Solana et al., 2015). Also, in one of the batches of M. Oleifera they found similar protein amounts as Fugile et al. (2002), but in another batch they found much lower protein amounts, which they state is contrary to other studies. Furthermore, to explain the lack of protein found in one batch they cite it could be due to the specific geography or climate of the crop location (Valdez-Solana et al., 2015). Other researchers found that the protein content in M. Oleifera actually rivaled that of eggs (Fahey, 2005). Overall, the nutritional content in M. Oleifera covers a wide breadth of vitamins, minerals, and vegetable based protein. This variety also gives potential evidence for increasing bone health as calcium, magnesium, potassium, vitamin A, iron, and protein are all included at high levels in M. Oleifera.

*Human Studies*

M. Oleifera has long been used as a traditional food source and medicine in the cultures where it grows naturally. The use of M. Oleifera in reported human studies, however, is limited. The majority of studies on M. Oleifera's effects on humans have analyzed the anti-lipidemic and -diabetic properties of M. Oleifera which has shown that M. Oleifera provides vast and substantial protective benefits (Arun Giridhari, Malathi, & Geetha, 2011; Kumar Gupta et al., 2013; Kumari, 2010; Nambiar, Guin, Parnami, & Daniel, 2010; William, Lakshminarayanan, & Chegu, 1993). Other studies have explored the use of M. Oleifera on blood pressure and even milk lactation in postpartum women which also proved to be beneficial for both blood pressure and milk lactation (Estrella et al., 2000; Kushwaha, Chawla, & Kochhar, 2014). These studies highlight the potential benefits that M. Oleifera can offer to the human body.

To date the authors have found only one study that examined the role of M. Oleifera on bone structure and function. M. Oleifera was given to study its effects on the healing process of mandibular fractures. The M. Oleifera intervention showed a decrease in swelling and tenderness and an increase in jaw mobility over the placebo group (Singh et al., 2011). Unfortunately, there were no x-ray scans administered to show any structural changes. Also, the exact amount of M. Oleifera prescribed was not listed in the article. There were no adverse effects found in any of the studies mentioned above.

*Animal/ In Vitro Studies*

Even though few human studies have studied M. Oleifera there have been many that used animal and in vitro subjects. These studies have examined similar aspects as the human studies, looking at the antioxidant, antihypertensive, anti-hyperglycemic, and anti-dislipidemic properties

of M. Oleifera. The main difference between these studies comes from the way the M. Oleifera is administered. Most of the human studies use a leaf powder while the animal and in vitro studies mostly use a solubilized aqueous or alcohol solution. Overall, however, the results seem to be similar to human studies when analyzing the antioxidant, dislipidemic, cardioprotective, and anti-cholesteric properties of M. Oleifera (Chumark et al., 2008; Jaiswal, Rai, Kumar, Mehta, & Watal, 2009; Jung, 2014; Nandave, Ojha, Joshi, Kumari, & Arya, 2009; Ndong, Uehara, Katsumata, & Suzuki, 2007; Panda, Kar, Sharma, & Sharma, 2013; Santos, Argolo, Paiva, & Coelho, 2012; Sasikala, Rooban, Priya, Sahasranamam, & Abraham, 2010; S Sreelatha, Jeyachitra, & Padma, 2011; Sreelatha & Padma, 2010; Tiloke, Phulukdaree, & Chuturgoon, 2013; Yassa & Tohamy, 2014).

Only one animal model study has examined the efficacy of M. Oleifera on bone strength. This study completed by Nkuwana et al. (2014) used a total of 2400 day old broiler chickens and divided them randomly into four different groups; control, starter (1-5 g of M. Oleifera/kg feed), grower (3-15 g/kg feed) and finisher (5-25g/kg feed) (Nkukwana, Muchenje, Masika, Hoffman, & Dzama, 2014). The researchers found that there was a significant positive relationship between the tibia weight, dried tibia weight, and the phosphorus content on the tibia compared to the amount of M. Oleifera in the feed. There was also a trend that associated higher calcium with the higher M. Oleifera concentrations. These results show that M. Oleifera was able to improve the tibia integrity and inorganic content of the broiler chickens (Nkukwana et al., 2014).

*Toxicity Levels*

The M. Oleifera tree has been consumed by the local communities in Africa and Asia as an important vegetable in their diet for thousands of years. Despite this long history, determining a set amount of M. Oleifera needed to see bone density benefits is unknown. However, useful information can be gathered from animal and cell models. In one study, animals (rats, mice, and

rabbits) were given 15 times the recommended daily dose of M. Oleifera leaf powder (800mg twice daily), which is equivalent to a child consuming 375 grams daily, and no adverse side effects were seen (Boven & Morohashi, 2002). In another study that used mice, the dosage level of 30mg/kg was found to elicit a beneficial effect while no toxicities were seen at this dosage (Faizi et al., 1998). Another study using rats showed that there were no dangerous toxicity levels associated with M. Oleifera at or below 1,000 mg/kg, while supratoxicity levels were seen above 3,000 mg/kg (Asare et al., 2012).

In more recent human studies, supplementation levels of 500 mg/day in breastfeeding women (Estrella et al., 2000) and up to 8000mg/day in men with broken jaws (Singh et al., 2011) were used, with no adverse effects seen in either study. Another study examined the effects of a one-time bolus of 5000mg of M. Oleifera and there were no adverse effects seen after this ingestion (William et al., 1993). Another study used 7000mg daily for 40 days without any problems indicated (Kumari, 2010). Therefore, the long history of this tree along with the animal, cell, and human models that are available indicate that at a dosage of 20mg/kg there should have no adverse effects on the subjects.

**Conclusion**

Osteoporosis and osteopenia are two bone diseases that can cause significant harm to females that have reached and surpassed menopause (Daan & Fauser, 2015). The importance of getting the proper nutrition is paramount for bone health. General nutrition such as eating more fruits and vegetables has been found to relate to positive bone effects (Gunn et al., 2015). More specifically, getting enough vitamin D along with calcium is associated with increases in bone density levels (Lips et al., 2010). M. Oleifera has been shown to contain all of these important vitamins and minerals (Fuglie, 2002). M. Oleifera also has shown to have various other beneficial

roles in general health such as antioxidant, anti-hypertensive, anti-cholesteric, and anti-hyperlipidemic properties. The previous studies show very positive benefits for overall health improvements but there is still a gap in the research that related to its benefits on bone structure and function. With bone density being of utmost importance to millions of older women in the US, it is imperative that more research is done on the bone density effects of M. Oleifera. Therefore, the purpose of this study is to determine the effects of M. Oleifera on the structure and function of bone in post-menopausal women ingesting 1 gram of M. Oleifera daily for 12 weeks.

## Methodology

### *Experimental Design*

Twenty four untrained post-menopausal women (60-70 years old) will be randomly assigned to either the control group (no program) or the experimental group (M. Oleifera supplement group). Both groups will ingest 1000mg each day (either M. Oleifera or a cabbage placebo) for 12 weeks. The bone scans will be conducted pre-supplementation and after 12 weeks to determine what adaptations the M. Oleifera will cause in the bone. The bone mass density, bone mineral content, and any anthropomorphic changes will be recorded to determine if there are any specific bone changes.

The subjects' first visit to the laboratory will serve as the information and testing session. The information session will consist of the informed consent document, health history and physical activity questionnaire. This first testing session will consist of the first DEXA scans. The participants will then be given six weeks' worth of their supplement. The subjects will come back to the testing site again at six weeks to pick up their second half of the supplements and they will be instructed on how to fill out a three day diet log. The diet log will be analyzed and averaged through the USDA Super Tracker online program. The third and last visit will be 12 weeks after the start of supplementation and will consist of the second DEXA measurements as well as turning in the diet record that will determine normal diet nutrients as well as caffeine intake.

### *Dual Energy X-Ray Absorptiometry (DEXA) Measurements.*

DEXA scanning allows for the measurement of whole body bone density as well as the specific density of the hip and femoral head. The subject will lay on the DEXA machine scanner motionless for 6-10 minutes while the scan is performed. This first scan will measure the whole

body bone density as well as the basic body composition. The subject will then be asked to lay motionless for another 6-10 minutes while another DEXA scan is completed, which will measure the bone density and characteristics of the hip and femoral head. The subject will lay with both hips abducted 15° while the whole legs are rotated inward 25°. This helps separate the femur and hips to get a clear scan of the hips. This will be aided by placing the feet on either side of a given foam plastic pyramid and strapped into place. The risks associated with a DEXA scan include exposure to small amounts of radiation. DEXA scanning utilizes radiation to obtain an image of the body. Everyone receives a small amount of unavoidable radiation from the environment each year. Some of this radiation comes from space and some from naturally-occurring forms of radioactive water and minerals. The DEXA scan technique gives the body the equivalent of about 4 extra days' worth of this natural radiation. The radiation received from the DEXA scan is only from the individual scan and does not include exposure received from other tests. The BMD, *t*-score and *z*-scores of the whole body will be recorded as well as the BMD, *t*-score, and *z*-scores of the right and left total hip and femoral necks. These data points will be used for analysis to find if there were any significant differences between the two groups after the intervention period.

### *M. Oleifera Supplementation*

The supplementation of the M. Oleifera will begin after the first visit which will contain the signing of consent, the pQCT scans, and the DEXA scans. Once the subject goes through these scans the subject will be randomly assigned to either the placebo group or the M. Oleifera group. The M. Oleifera group will be given whole leaf M. Oleifera powder in capsule form at 1000mg/50kg daily for 12 weeks. M. Oleifera harvested and processed under optimal conditions will be weighed on a scale and packed into capsules under sterile conditions. Each capsule will contain 500 mg of M. Oleifera. Each participant, in the treatment group, regardless of their

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weight, will take two capsules daily for 12 weeks. The subjects will be instructed to consume their capsules once daily orally. If they miss a day they should not double the dosage the next day. The control group will be given a placebo capsule consisting of cabbage, which has significantly lower levels of the vitamins and nutrients found in M. Oleifera, and subjects will take an equivalent dosage of the cabbage as the M. Oleifera supplement. The cabbage placebo will be packaged into one capsule containing the same 1000mg amount. The placebo group will be given the exact same instructions for consumption as the M. Oleifera group, and the study will be conducted in a double blind manner. The dietary records will be collected and analyzed to determine any significant differences between the two groups as far as normal daily consumed nutrients.

*Statistical Analysis*

Initial mean bone mass characteristics and descriptive statistics will be compared using unpaired student's *t*-test. A paired samples *t*-test will be used to determine any differences between the pre and post total body and left and right hip BMD DEXA measurements for the entire groups. A multivariate ANOVA will then be then be used to determine if there were any significant group related differences for the DEXA scan results. Descriptive statistics, partial eta squared, and observed power will be determined by the multivariate ANOVA. A binomial regression will be used to determine the differences in the change score for the total body BMD. A bivariate correlation will be used to determine any significant correlations between data points. Lastly, the diet record data will be analyzed using an independent samples *t*-test to determine any differences between the two groups. Statistical analysis will be computed through computer programs available in the Statistical Package for the Social Sciences, version 12.0J (SPSS). The statistically significant level will be set at 0.05.

## Results

No starting significant differences were found between the group of women in the placebo group ($N$=10) or the M. Oleifera group ($N$=9) in regards to body anthropometrics (Table 1) for total subject anthropometric data. There was a very wide range in body fat percentages ranging from 8% up to 46% total body fat for the subject group at the start of the intervention. Subjects tended to be highly educated with only 4 of the 20 subjects not completing a graduate level degree. None of the subjects were currently smoking and the study participants averaged around 1-2 drinks per week. The two groups exercised slightly below 3-4 times a week on average with only one subject exercising less than 1 times per week.

The daily medicines and vitamins and supplements were recorded and there were no notable differences in type or amount of consumed between the groups. The most common medicines taken were blood pressure medicines with only 6 subjects taking blood pressure medicine with 4 being from the M. Oleifera group and 2 being from the placebo group. The next most common drug recorded was for cholesterol. The vitamins and minerals were also recorded for this study. Both of these medicines were evenly distributed between the two subject groups. Nine out of the 19 women that finished the study took a daily multivitamin with that number being evenly split between M. Oleifera group ($N$=5) and the placebo group ($N$=4). Ten out of the 19 women consumed a vitamin D supplement with 5 being from M. Oleifera and 5 from the placebo group (Table 2).

No significant differences in starting height ($p$=.107), weight ($p$=.547), body fat percentage ($p$=.620), or lean mass ($p$=.857) were observed between the groups. There was also no significant changes throughout the intervention period for lean mass or body fat percentage (Figure 1 and 2). There also were no significant differences between the two group's diets including average kcals, protein, carbohydrate, fat, sodium, potassium, vitamin D, vitamin C,

vitamin E, or vitamin A consumed (Table 3). There was however a moderate positive correlation ($r$=.550) between sodium intake and the pre BMD of the pelvis for the entire group ($p$=.015) which showed that higher sodium intakes in the diet correlate to higher BMD of the pelvis. Another moderate positive correlation ($r$=.499) was found between copper intake and post BMD of the Total Left Hip scan ($p$=.03) which showed that higher copper intakes correlate to higher BMD of the total left hip. There were some significant differences found in the before bone measurements between the two groups. Before the intervention was started the placebo group had a significantly higher BMD of the pelvis ($p$=.037) than the M. Oleifera group with the placebo group having a BMD of 1.18 g/cm² compared to 1.06 g/cm². There was also a significant difference between the groups when looking at the BMC ($p$=.042), BMD ($p$=.044), t-score ($p$=.044), and z-score ($p$=.049) of the total right hip before the intervention started with the placebo group having higher scores than the M. Oleifera group in all of the above areas.

When looking at specific group differences for total body bone density there were no significant differences between the M. Oleifera group and the placebo group after the intervention period (Figure 3). There was no significant difference between the groups when the raw change score (-1 for percent decrease, 0 for no change, and +1 for increase) was computed for the total body BMD changes ($p$=.945). No significant difference between groups with the total left and right hip BMD measurements was found but there were small differences in the groups with the total right hip showed a .07% increase in the M. Oleifera group with a -.01% decrease in the placebo group ($p$=.168) (Figure 4). The M. Oleifera group had no change in the total left hip BMD while the placebo group had a -.02% decrease in the total hip BMD ($p$=.674) (Table 4). The only group related significant difference was found in the post pelvis BMD with the placebo group being significantly higher with 1.19 g/cm² than the M. Oleifera group with 1.04 g/cm² ($p$=.035), which is a factor seen in the pre-scan data as well.

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When breaking down specific total group characteristics using an independent samples t-test analysis there were significant differences found between the pre-total body BMD DEXA results and the post total body BMD DEXA results with the average for the entire subject group dropping from a BMD of 1.046 g/cm² to 1.034 g/cm² ($p$=.030) (Figure 1). A significant change in the difference between the pre-total body t-scores and the post-total body t-scores was seen with the whole subject group dropping from -.81 to -.97 ($p$=.019) (Figure 2). The group difference did not significantly affect the rate of bone loss however, with the M. Oleifera group losing 1.08% bone mass and the placebo group losing 1.14% bone mass of the total body BMD during the 12 week period ($p$=.945). Also, a significant difference appeared when looking at the effect of exercise on total body BMD percent loss of the entire group. This result showed that the more times per week that a subject exercised the less bone density was lost ($p$=.044) (Figure 5). Significant negative correlations were seen with sodium ($r$=-.644; $p$=.003) and total body BMD percent change, as well (Figure 6).

**Discussion**

Post-menopausal women consuming M. Oleifera for 12 weeks do not obtain any benefit in hip bone mineral density BMD or whole body BMD compared to those consuming placebo, in fact, analysis of both groups combined determined that BMD decreased significantly over the 12 weeks. Specific analysis of the percent change from pre to post of these locations did not significantly differ between the two groups, even after accounting for the pre-scan bone density. Bone density of various regions did not significantly differ between M. Oleifera and placebo groups. The percent change of the total left and right hips for the entire subject group did not significantly vary from pre to post scans. Pelvic BMD was significantly different between groups prior to treatment and this difference persisted over the course of the study. Nutritional analysis revealed several significant correlations when measuring BMD at various sites with higher sodium and copper consumption positively correlating with BMD in the hip.

To our knowledge, this is the first study in the United States to look at the effects of M. Oleifera on bone density and the first to ever record the dosage of M. Oleifera used during the study. Human studies experimenting with M. Oleifera have only so far focused on the anti-lipidemic and -diabetic benefits of M. Oleifera (Arun Giridhari et al., 2011; Kumar Gupta et al., 2013; Kumari, 2010; William et al., 1993). To date, only one other human study has examined the effects of M. Oleifera on bone properties, notably bone healing properties on jaw fractures. This particular study demonstrated that M. Oleifera intervention caused a decrease in swelling and tenderness and an increase in jaw mobility over the placebo group. However, this study did not focus on bone density specific BMD measurements using empirical methods but rather relied on the personal testimony of patients for assessing efficacy. Furthermore, this earlier study was not a blind study nor did it specify the specific dosage used (Singh et al., 2011). The design of the present study builds or improves upon this earlier or original study by measuring in a more

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precise way: 1) bone density changes; 2) a specified dosage, and 3) was double blinded.

The existing variability between subjects made teasing out statistically significant differences between the groups difficult, including determining whether the effects attributed to supplementation occurred. For this reason, Pearson's correlation was also determined between groups and Cohen's effect size was calculated. Furthermore, the researchers conducted a post-hoc G-Power analysis based on the determined power of the pre bone total bone density effects on the post scans. This analysis shows how the variability in subject group affects the ability to find significant results. Based on the number of subjects ($N$=19) and the observed variability between subjects, the minimum sample size needed for the present study should have been 3,930 subjects in order to have a power of 0.80. This shows the extreme variability of the total subject group that we had, as we had subjects range in body fat percentage from 8% to 46% in total and BMI from 16.6 to 36. There were also people with osteopenia while there were others that were three standard deviations above normal BMD for their age. This variability drastically reduced the power and made this study unable to correctly determine the significant interactions M. Oleifera had on bone density. One reason that we were unable to determine the ideal sample size ahead of time is that there are currently no reported studies in the literature that have examined the effects of M. Oleifera on bone density in humans. This made the task of determining sample size and power very difficult.

Despite the inability to determine differences between groups several important results were seen with the total population pre to post change. A significant interaction was found with the amount of exercise per week and the BMD change from pre to post. The more often exercise was performed per week the smaller the decrease in BMD; with even an increase in BMD in the highest exercise per week group. The importance of exercise on bone density is paramount since bone loading is essential in bone growth (Gregov & Šalaj, 2014; Kai, Anderson, & Lau, 2003). A

nutritional intervention, such as M. Oleifera, along with an exercise regimen may likely lead to an even more pronounced improvement in bone density outcomes.

A second finding was the negative association of sodium intake and total body BMD changes. This relationship may exist due to poor diets that are high in sodium and low in essential vitamins and minerals. A diet that is predominantly fast food and pre-packaged tends to be higher in sodium and deficient in necessary vitamins and minerals. Research has shown that lower quality diets associate with lower BMD in an elderly population (Zeng et al., 2014).

The most important and unexpected finding was that the total body bone density of all subjects drastically or acutely decreased over the 12 week time period. Normally, most post-menopausal women lose up to 1% of their bone density during the whole year and not 12 weeks (Warming, Hassager, & Christiansen, 2002). Another earlier study found that women aged 80+ years lost $2.1 \pm 0.7\%$/year, compared with $1.1 \pm 0.2\%$/year among those aged 70–79 years and $0.6 \pm 0.1\%$/year among those aged 60–69 years (Nguyen, Sambrook, & Eisman, 1998). In our twelve-week time period the average bone loss was 1.11%, which is roughly a 4 times greater loss in total body BMD than previously reported.

While the >1% drop in BMD in only 12 weeks is alarming, seasonal changes might account for the drop. Seasonal changes can drastically affect bone density (Rapuri, Kinyamu, Gallagher, & Haynatzka, 2002; Storm et al., 1998). It is important to note that the initial scans were conducted between August and September, while the post scans were performed during November and December. It is well known that during the summer months, bone density can increase due to increased sun exposure and more opportunities for out-door physical activity, while in contrast, during winter bone density decrease (Rapuri et al., 2002). Thus, BMD status between seasons overall fluctuates in a cyclical manner, with the total bone density returning to or slightly below baseline during the entire year. Rapuri et al. (2002) measured blood serum

25OHD, calcium, osteocalcin, and alkaline phosphates, as well as bone density for an entire year. The BMD of the spine (4.2%), total body (5%), and mid-radius (4.5%) was significantly higher in summer (June, July, August, and September) compared to winter (December, January, February, and March) (Rapuri et al., 2002). The fluctuation in serum levels of 25OHD also significantly correlated with bone density changes from summer to winter. It is likely that the seasonal timing of the present study may reflect the generated data and thus a lower bone density in the post-scans.

Secondly, in our health history questionnaire, we did not ask the subjects' start of menopause age. It is well known that bone density will be lost at a higher rate in the first five years after menopause (Harris & Dawson-Hughes, 1992; Pouilles, Tremollieres, & Ribot, 1995). BMD rate of change 1-2 years after menopause can be as high as $-2.24\%/$ year, but this rate of loss slows after 6 or more years after menopause and the rate of loss is around 1% (Harris & Dawson-Hughes, 1992). In the current study, subjects were between the ages of 60 and 70. With the average of menopause at 51, some women may not have reached menopause until age 55-57 (Daan & Fauser, 2015). Therefore the possibility exists that subjects were still in the more rapid bone loss stage of the first few years immediately after menopause.

Lastly, certain medications affect BMD. High blood pressure seems to be associated with lower BMD in elderly white women (Cappuccio, Meilahn, Zmuda, & Cauley, 1999). This study found that between the first and fourth quartile hypertensive medication taken, bone density dropped between 0.34% and 0.59% over a three-year period. A significant correlation in bone density and diastolic blood pressure was also observed in women under 70 years of age (Cappuccio et al., 1999). Although we did not specifically measure blood pressure, six of the 19 subjects studied were taking prescription blood pressure medications. Also, selective serotonin reuptake inhibitors (SSRI's), such as Lexapro or Prozac, tend to associate with lower BMD in

postmenopausal women (Diem et al., 2007). Bone density dropped 0.47% in non-SSRI users while in women who took SSRIs it dropped an average of 0.82% per year in one study (Diem et al., 2007). Six women took various SSRI's in this study. All of these related medical factors might contribute to the large percentage decrease seen in total body bone density.

A twelve week bone density intervention is around the minimum time required to observe bone density changes (Gregov & Šalaj, 2014), which is why the current duration was selected in the present study. Although the present study was unable to demonstrate a difference in bone density between the M. Oleifera and the placebo groups during the 12-week time frame, it is likely that with a longer-term supplementation period, significant changes could be seen. Despite the lack of bone density changes there may have been hormonal or molecular changes in this time period. Without taking blood samples, however, it is impossible to conclude that any significant molecular or hormonal changes took place due to M. Oleifera.

Secondly, the amount of M. Oleifera might not have been high enough to have measurable effects on BMD in only 12 weeks. It is important to mention that this is the first systematic study that examined the effects of M. Oleifera on bone density and provided the exact dosage and duration in humans. The lack of well-established prior dosage recommendations did not make it easy in designing the present study and may in part explain the lack of difference of statistical significance between the two groups (Estrella et al., 2000; Singh et al., 2011). Animal studies have used a wide range of M. Oleifera dosage, which overall is between 4mg/kg and 300mg/kg (Chen et al., 2012; Jaiswal et al., 2009; Nandave et al., 2009). However, these studies mostly used an aqueous form of M. Oleifera, rather than the whole leaf powder used in the present study. Another article using an avian animal model (chicken) and looking at the effects of M. Oleifera on bone strength used up to 25g of M. Oleifera per kg of chicken feed (Nkukwana et al., 2014). However, these animal models were not similar enough to humans to draw any useful

insights in determining specific appropriate dosage for human studies. In the absence of meaningful animal models or human studies, we reasoned that a one gram dosage might be enough to elicit changes, while ensuring safety (Asare et al., 2012).

Future studies exploring M. Oleifera's health effects could measure blood samples to determine the potential interaction between M. Oleifera and hormones, such as estrogen or progesterone. Secondly, a blood sample potentially could show other benefits of M. Oleifera, such as its anti-hypertensive, anti-dislipidemic, anti-oxidant, and cardio-protective properties. Also future studies can use the data found in this study to better predict the sample size needed to find statistical differences. Thirdly, additional use of a peripheral CT scanner, which is a more sensitive tool, could be used to determine more specific and subtle bone changes. Fourthly, we suggest that the length of the study in future should be further extended by at least six months in order to show whether M. Oleifera has any measurable effects in the long term. Lastly, the effects of combined exercise and nutrition (M. Oleifera) on bone density could be further explored by separating the subjects into more groups, such as a control group, an exercise group, an exercise plus M. Oleifera group, and a M. Oleifera only group. This could ensure that the benefits of M. Oleifera are delineated from those of exercise on bone density.

## Conclusion

In conclusion, no evidence exists in short term supplementation of M. Oleifera on BMD of post-menopausal women. The most significant finding was the overall loss in total body bone density in the complete subject group. This loss may be due to a variety of factors, such as normal seasonal changes, higher initial bone densities, intake of medications, or others. Future studies can use data from the present study as a base to further our understanding and expand our research knowledge about effective nutritional options that can help reduce bone density. Future studies should use a larger sample size, increase the length of the intervention, take blood samples to examine interaction with hormones and levels of blood vitamin levels, and control more for exercise, diet and medication interactions.

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# Appendix A

# Figures
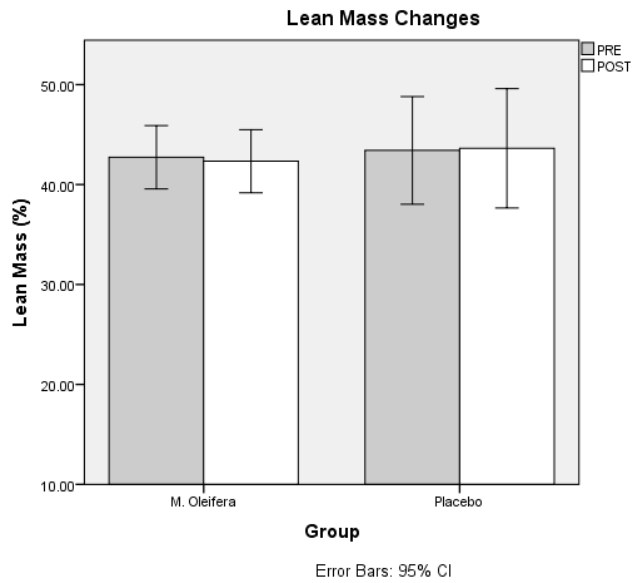


Figure 1. Post-menopausal women whole body mean lean mass percentage changes between the pre and post time periods for the M. Oleifera and Placebo groups. There were no significant differences in lean mass between the pre and post time periods between groups (p=.857).
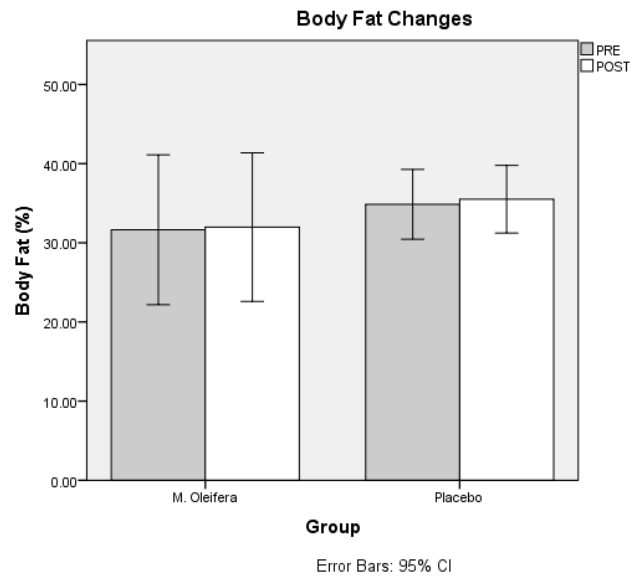


Figure 2. Post-menopausal women whole body fat percentage changes between the pre and post time periods for the M. Oleifera and Placebo groups. There were no significant differences in body fat percentages between the two groups frompre to post.  (p=.620)

**Pre and Post Total Body BMD**



Error Bars: 95% CI
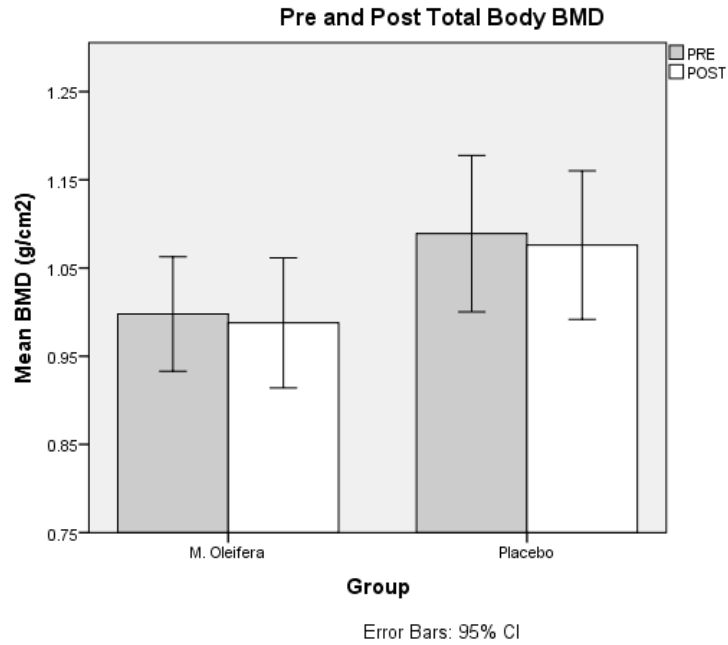
Figure 3. Post-menopausal women whole body mean total body BMD changes between the pre and post time periods for the M. Oleifera and Placebo groups. There were no significant differences between groups when looking at the pre to post differences of total body BMD (p=.620).

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**Pre and Post Total Hip BMD**



Error Bars: 95% CI
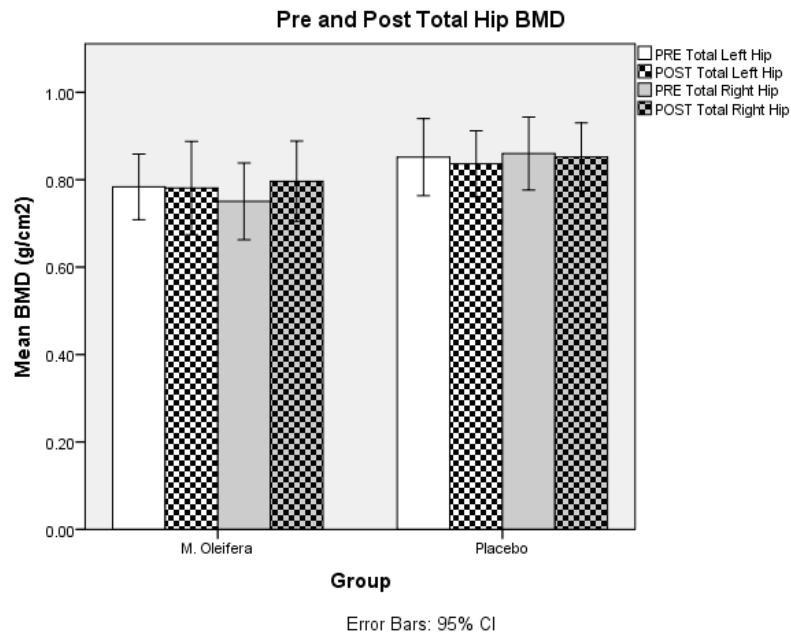
Figure 4. Post-menopausal women left and Right hip BMD changes between the pre and post time periods for the M. Oleifera and Placebo groups. There were no significant changes in left or right hip BMD within or between groups (p=.168 for Right Hip; p=.674 for Left Hip).

50

**Effect of Exercise on Total Body BMD Change**

Error Bars: 95% CI
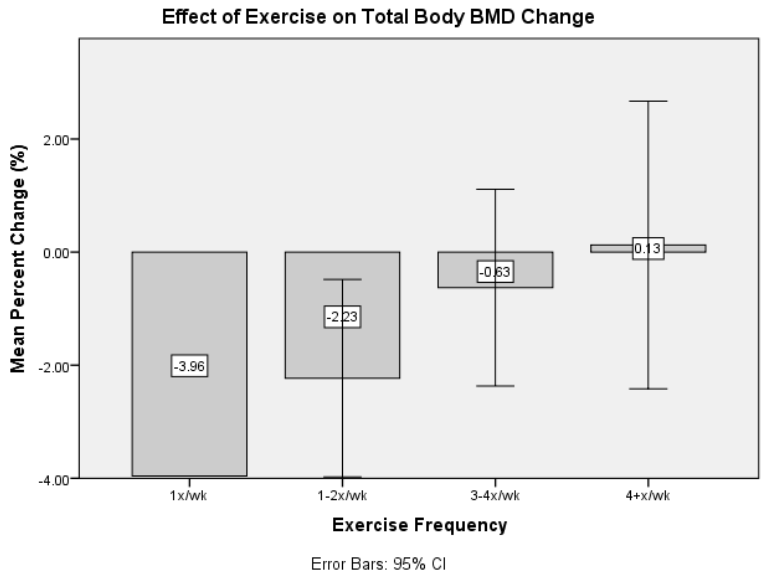
Figure 5. Post-menopausal whole body total BMD mean percentage change total for the M. Oleifera and Placebo groups. There was a significant correlation between number of times exercised per week and whole body BMD percent changes between the pre and post time periods (p=.030).



**Effect of Sodium on Total Body BMD Change**
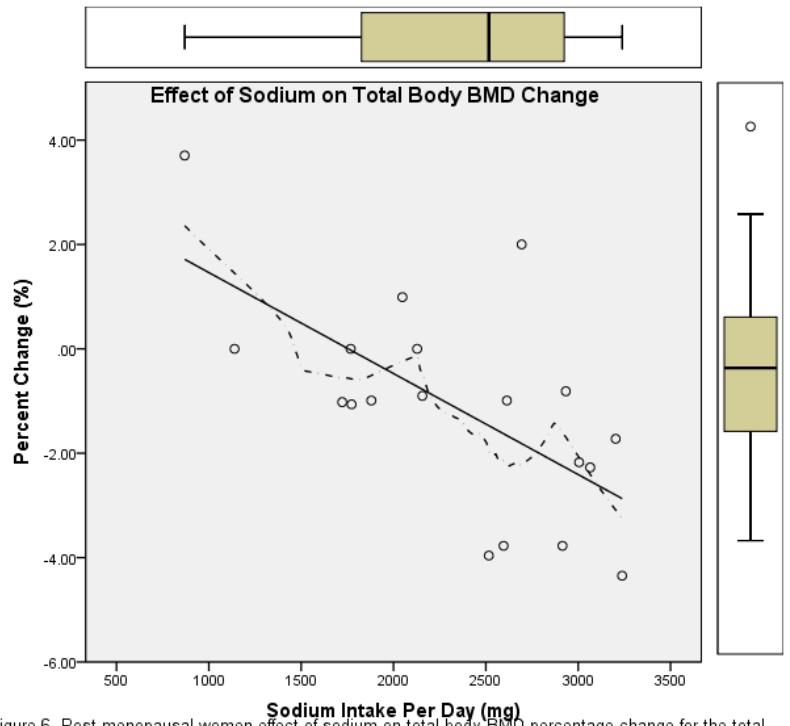
Figure 6. Post-menopausal women effect of sodium on total body BMD percentage change for the total subject population. There was a significant correlation betweem amount of sodium in the diet and total body BMD percent change from the pre to post time period (p=.003).

| Subject: | Group | Age | Height (cm) | Weight (kg) | BMI | % Body Fat |
|----------|-------|-----|-------------|-------------|-----|------------|
| 1 | A | 63 | 179.07 | 72.12 | 22.51 | 29.9 |
| 2 | A | 66 | 170.18 | 52.16 | 18 | 15.9 |
| 3 | B | 63 | 152.4 | 50.35 | 21.64 | 30.3 |
| 4 | B | 65 | 163.83 | 90.72 | 33.46 | 45.9 |
| 5 | A | 64 | 170.18 | 72.57 | 25.09 | 34.9 |
| 6 | B | 62 | 158.75 | 57.15 | 22.55 | 31.2 |
| 7 | A | 63 | 170.18 | 60.33 | 20.88 | 32.5 |
| 8 | A | 67 | 176.53 | 90.72 | 29.29 | 46.6 |
| 9 | B | 60 | 172.72 | 65.77 | 22 | 32.6 |
| 10 | A | 60 | 165.1 | 71.21 | 26.16 | 39.1 |
| 11 | A | 64 | 165.1 | 70.76 | 25.99 | 40.5 |
| 12 | B | 66 | 157.48 | 61.24 | 24.84 | 37.3 |
| 13 | B | 68 | 167.64 | 92.87 | 33 | 39.9 |
| 14 | B | 67 | 167.64 | 63.5 | 22.5 | 32.9 |
| 15 | B | 63 | 175.56 | 108.86 | 35.27 | 42 |
| 16 | B | 68 | 158.75 | 56.69 | 22.86 | 26.2 |
| 17 | A | 64 | 167.64 | 46.72 | 16.6 | 7.96 |
| 18 | B | 64 | 165.1 | 68.04 | 24.98 | 30.3 |
| 19 | A | 63 | 160.02 | 72.57 | 28.35 | 42.2 |
| 20 | A | 68 | 165.1 | 61.68 | 22.77 | 37.4 |
| **Averages** | | 65.615789 | 164.23395 | 70.498105 | 26.042263 | 34.430737 |
| **Std. Deviations** | | 2.4365636 | 6.8098437 | 15.884186 | 4.9085351 | 9.4058658 |

Table 1. Post-menopausal women anthropometric data for subjects at the pre scan time period.

| Subject: | Group | Medications | Supplements |
|---|---|---|---|
| 1 | A | Depression (Buproprion), Pain (Aspirin, Naproxin) | Super B & Vit C (150mcg), Biotin (5000mg), D3 (5000IU) |
| 2 | A | Thyroid (levothyroxine) | Multivitamin, Vit D3 (1000 IU), Fish Oil 1200mg |
| 3 | B | Cholesterol (Simvastatin), Arthritis (Ibuprofen), Depression (Wellbutrin), Allergy | D3 (5000mg) |
| 4 | B | Blood Pressure (Lisinopril), Cholesterol (Simastatin), Diabetes (Metformin, Gupizide) | Multivitamin, D3 (5000IU), Magnesium Citrate (100mg), |
| 5 | A | Blood Pressure (Norvasc), Allergy (Flonase) | One A Day Womens, B Complex, Biotin (1000 mg), Co-Q-10 (100mg), Niacin (400mg), Magnesium (400mg), Calcium w/ Vit D (1200mg Ca, 1000mg D) |
| 6 | B | | One A Day Womens, D3 (100mg), B6 (2000IU), Fish Oil (1000mg), Throid Complex (Mg, Cu, Zn, B12 (100mcg), Tyrosine), Selenium (200mcg), Lutein (20mg) |
| 7 | A | | B Total Multivitamin, Opticon |
| 8 | A | Blood Pressure (Clonidine), Anxiety (Fluoxetine), Allergy (Fluticasone) | Vit D (2000mg) |
| 9 | B | Anxiety (Occasional Clonazapam), Hormone Based Chemotherapy (Anastrazole) | Multivitamin, Calcium, Magnesium (No mg given) |
| 10 | A | Blood Pressure (HTZ), Cholesterol (Atorvastatin), Depression (Escitalopram), Thyroid (Levothyroxine), Allergy (Hydroxyzine) | |
| 11 | A | | Multivitamin, Calcium, Magnesium (No mg given) |
| 12 | B | Depression (Lexapro), Allergy (Loratadine) | Calcium w/ Vit D (600mg) |
| 13 | B | Blood Pressure (Lisinopril), Cholesterol (Pravastatin), Depression (Fluoxentine), Throid (Levothyroxine), Allergy (Zertec) | Vit C (500mg), Zinc (50mg), Fish Oil (2000mg) |
| 14 | B | | |
| 15 | B | Blood Pressure (Diovan), Athritis (Naproxen), Weight Loss (contrave) | |
| 16 | B | ADD (Strattera), Pain (Aspirin) | Vit C (1000mg), Biotin, Citracal |
| 17 | A | Depression (Prozac), Anxiety (Buspar) | |
| 18 | B | Ulcer (Omeprazole) | Equate Womens Vitamin, D3 (250IU), Calcium Citrate (315mg), Fish Oil (1200mg), Glucosamine |
| 19 | A | Allergy (Cetirizine) | B-12 (100mg) |
| 20 | A | | Reliv Supplement (Vit D (400), Vit C (480mg), Vit A (2500IU), Calcium (1000mg), Iron (10mg) |

Table 2. Post-menopausal women medicines and supplements taken by individual as seen by Health History Questionnaire

| Subject: | Group: | Total Kcal | Protein (g) | Carb (g) | Fat (% of calories) | Vitamin C (mg) | Vitamin E (mg) | Vit D (ug) | Vit A (ug) | Selenium (ug) | Iron (mg) | Zinc (mg) | Copper (ug) | Magnesium (mg) | Sodium (mg) | Potassium (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1470 | 69 | 153 | 35% | 65 | 12 | 3 | 1250 | 87 | 16 | 10 | 1531 | 319 | 3203 | 2487 |
| 2 | A | 911 | 42 | 127 | 29% | 54 | 4 | 11 | 1064 | 48 | 11 | 4 | 652 | 186 | 1722 | 1902 |
| 3 | B | 1525 | 40 | 241 | 24% | 135 | 7 | 3 | 1474 | 73 | 12 | 6 | 1147 | 231 | 2128 | 2837 |
| 4 | B | 1292 | 89 | 104 | 42% | 94 | 7 | 4 | 900 | 127 | 9 | 10 | 1344 | 300 | 1139 | 2649 |
| 5 | A | 2190 | 94 | 350 | 21% | 135 | 50 | 4 | 847 | 61 | 17 | 20 | 3330 | 627 | 1880 | 3878 |
| 6 | B | 1790 | 81 | 163 | 38% | 85 | 13 | 3 | 503 | 109 | 9 | 109 | 1231 | 343 | 1768 | 2823 |
| 7 | A | 1842 | 64 | 242 | 34% | 111 | 6 | 1 | 2219 | 92 | 14 | 8 | 1435 | 235 | 3065 | 2795 |
| 8 | A | 1406 | 88 | 161 | 28% | 23 | 4 | 2 | 1151 | 115 | 12 | 9 | 973 | 258 | 2516 | 2146 |
| 9 | B | 1297 | 46 | 126 | 48% | 12 | 4 | 1 | 262 | 84 | 9 | 7 | 720 | 137 | 2614 | 1113 |
| 10 | A | 1678 | 78 | 192 | 31% | 116 | 4 | 2 | 592 | 75 | 17 | 11 | 1057 | 247 | 2694 | 2640 |
| 11 | A | 2000 | 69 | 249 | 37% | 40 | 8 | 1 | 232 | 96 | 15 | 9 | 1142 | 239 | 3238 | 2063 |
| 12 | B | 1718 | 98 | 205 | 31% | 153 | 9 | 13 | 545 | 105 | 9 | 11 | 1525 | 309 | 2048 | 2919 |
| 13 | B | 1501 | 57 | 179 | 38% | 40 | 3 | 1 | 720 | 93 | 14 | 8 | 1083 | 178 | 3005 | 1773 |
| 14 | B | 1314 | 52 | 174 | 33% | 77 | 10 | 2 | 940 | 61 | 13 | 9 | 1382 | 243 | 2596 | 2490 |
| 15 | B | 1243 | 82 | 78 | 50% | 205 | 5 | 2 | 626 | 86 | 9 | 10 | 781 | 198 | 2933 | 2319 |
| 16 | B | 2041 | 98 | 204 | 38% | 141 | 25 | 2 | 509 | 98 | 13 | 11 | 1572 | 481 | 2915 | 3012 |
| 17 | A | 1324 | 93 | 133 | 35% | 93 | 12 | 3 | 570 | 81 | 7 | 7 | 1051 | 297 | 869 | 3012 |
| 18 | B | 1535 | 70 | 165 | 37% | 94 | 8 | 8 | 1053 | 86 | 12 | 10 | 1144 | 290 | 2156 | 2895 |
| 20 | A | 1186 | 57 | 118 | 42% | 80 | 11 | 1 | 504 | 68 | 10 | 8 | 1430 | 377 | 1773 | 2046 |
| **Averages** | | 1540.1 | 71.9 | 177.1 | 35.3% | 92.3 | 10.6 | 3.5 | 840.1 | 86.6 | 12 | 14.6 | 1291.1 | 289.2 | 2329.6 | 2515.7 |
| **Std. Deviations** | | 319.7 | 18.6 | 61.6 | 0.07% | 47.4 | 10.6 | 3.4 | 458.8 | 19.3 | 2.9 | 22.5 | 548.7 | 110.3 | 669.2 | 583.2 |

Table 3. Post-menopausal women diet record three day averages for each individual as interpreted by USDA Super Tracker online.

| Group | Avg. % Change - Total Body | Standard Deviation | p value | Effect size | Power |
|---|---|---|---|---|---|
| M. Oleifera | -1.08 | 2.58 | 0.945 | 0.000 | 0.052 |
| Placebo | -1.14 | 1.62 | | | |
| | Avg. % Change - Total Left Hip | | | | |
| M. Oleifera | 0.00 | 0.12 | 0.674 | 0.011 | 0.069 |
| Placebo | -0.02 | 0.07 | | | |
| | Avg. % Change - Total Right Hip | | | | |
| M. Oleifera | 0.07 | 0.17 | 0.168 | 0.109 | 0.275 |
| Placebo | -0.01 | 0.04 | | | |

Table 4. Post-menopausal women percent changes for average Total Body, Left Hip, and Left hip BMD between the pre and post time periods. Result showing significance level, effect size and power of sample.

**Vita**

Jason Michael Brown was born in Hamilton, Ohio to Dr. Tim and Vivian Brown. He graduated from Cincinnati Christian High School in 2009. The next fall he entered Furman University and studied Health and Exercise Science. In August 2013 he was awarded the Bachelor of Science degree. In the fall of 2014 he accepted a research assistantship in Exercise Science at Appalachian State University and began study towards a Master of Science degree.