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Bone Reversal Effects of Plant Bioactive Compounds in Postmenopausal Women

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BONE REVERSAL EFFECTS OF PLANT BIOACTIVE COMPOUNDS IN
POSTMENOPAUSAL WOMEN

By

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This dissertation is dedicated to my father;
I wish he could be here to see this day, but I am sure he is still looking down upon me with pride.

I love and miss him very much.
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<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
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<tr>
<td>BALP</td>
<td>Bone-specific alkaline phosphatase</td>
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<td>BMC</td>
<td>Bone mineral content</td>
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<td>BMD</td>
<td>Bone mineral density</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BV</td>
<td>Bone volume</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>Dpd</td>
<td>Deoxypyridinoline</td>
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<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
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<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<td>HD</td>
<td>High dose</td>
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<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
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<td>IGF-I</td>
<td>Insulin like growth factor-I</td>
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<td>LD</td>
<td>Low dose</td>
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<td>MD</td>
<td>Medium dose</td>
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<tr>
<td>OC</td>
<td>Osteocalcin</td>
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<td>OPG</td>
<td>Osteoprotegrin</td>
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<tr>
<td>ORAC</td>
<td>Oxygen radical absorbance capacity</td>
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<td>ORX</td>
<td>Orchidectomized</td>
</tr>
<tr>
<td>OVX</td>
<td>Ovariectomized</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
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<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
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<tr>
<td>RANKL</td>
<td>Receptor activator of NFκ-β ligand</td>
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<tr>
<td>RUNX-2</td>
<td>Runt-related transcription factor</td>
</tr>
<tr>
<td>SMI</td>
<td>Structural modular index</td>
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<tr>
<td>TRAP</td>
<td>Tartarate resistant acid phosphatase</td>
</tr>
<tr>
<td>TV</td>
<td>Total volume</td>
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<tr>
<td>µCT</td>
<td>Microcomputed tomography</td>
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Osteoporosis is a debilitating disorder that affects both female and male, albeit to a greater extent in women than men. As the demographic shift to a more aged population continues, a growing number of men and women will be afflicted with osteoporosis. Therefore, search for potential non-pharmacological alternative therapies for osteoporosis is of prime interest. Aside from existing drug therapies, certain lifestyle and nutritional factors are known to reduce the risk of osteoporosis. Among nutritional factors, recent observations suggest that dried plum, or prunes (*Prunus domestica* L.) is the most effective fruit in both preventing and reversing bone loss. Several animal studies, a 3-month and a one-year long clinical trials conducted in our laboratories have shown that dried plum has positive effects on bone indices and bone mineral density (BMD).

The animal data indicate that dried plum not only prevents but more importantly reverses bone loss in two separate models of osteopenia. Our initial animal study indicated that dried plum prevented the ovariectomy-induced BMD reduction of the femur and lumbar vertebra. In another study rats were ovariectomized and allowed to lose bone before the initiation of treatment to mimic established osteoporosis. Dried plum as low as 5% (w/w) gram per kilogram diet restored BMD to the level of intact rats. Dried plum also reversed the loss of trabecular architectural properties such as trabecular number, connectivity density, and trabecular separation. We have also shown the effectiveness of dried plum in reversal of bone loss due to skeletal unloading. Microcomputed tomography (µCT) analyses revealed that dried plum enhances bone recovery during reambulation following skeletal unloading and has comparable effects to parathyroid hormone. In addition to the animal studies, our 3-month clinical trial indicated that the consumption of dried plum daily significantly modulated serum markers of bone turnover in postmenopausal women.

To confirm the findings of the animal studies and the 3-month clinical trial, we conducted a relatively long-term randomized comparative-controlled clinical trial. The principal objective of this study was to examine the extent to which dried plum reverses bone loss in osteopenic postmenopausal women. We recruited 236 women, 1 to 10 years postmenopausal, not on hormone replacement therapy or any other prescribed medication known to influence bone metabolism. One hundred and sixty qualified participants were randomly assigned to one of two treatment groups: dried plum (100 g/d) or dried apple (comparative control) for one year. All study participants received 500 mg elemental calcium plus 400 IU vitamin D daily. Bone mineral density of lumbar spine, forearm, hip, and whole body were assessed...
at baseline and the end of the study using dual-energy x-ray absorptiometry. Blood and 24-hr urine samples were collected at baseline, 3-, 6-, and 12-month to assess bone biomarkers and bone metabolism measurements. Physical activity recall and one-week food frequency questionnaire were obtained at baseline, 3-, 6-, and 12-month to examine physical activity and dietary confounders as potential covariates. Dried plum significantly increased BMD of ulna and spine in comparison to the dried apple group. In comparison with corresponding baseline values, only dried plum significantly decreased serum levels of bone turnover markers including bone-specific alkaline phosphatase and tartrate resistant acid phosphatase-5b.
CHAPTER 1

BACKGROUND AND SIGNIFICANCE

Significance

Osteoporosis is a debilitating disorder that affects both female and male, albeit to a greater extent in women than men. As the demographic shift to a more aged population continues, a growing number of men and women will be afflicted with osteoporosis and a search for potential non-pharmacological alternative therapies for osteoporosis is of prime importance. Aside from existing drug therapies, certain lifestyle and nutritional factors are known to reduce the risk of osteoporosis. Among nutritional factors, recent observations suggest that dried plum, or prunes (Prunus domestica L.) is the most effective fruit in both preventing and reversing bone loss. Animal studies (1-5) and a 3-month clinical trial (6) conducted in our laboratories have shown that dried plum has positive effects on bone indices. The animal data indicate that dried plum not only protects against but more importantly reverses bone loss in two separate models of osteopenia. Our initial animal study indicated that dried plum prevented the ovariectomy-induced reduction in bone mineral density (BMD) of the femur and lumbar vertebra (1). In another study (2), to mimic established osteoporosis, rats were ovariectomized and allowed to lose bone before the initiation of treatment. Dried plum as low as 5 (w/w) gram per kilogram restored BMD to the level of intact rats. More importantly, dried plum reversed the loss of trabecular architectural properties such as the trabecular number, connectivity density, and trabecular separation. We have also shown the effectiveness of dried plum in reversal of bone loss due to skeletal unloading (3). Analysis of BMD and trabecular bone structure by microcomputed tomography (μCT) revealed that dried plum enhanced bone recovery during re-ambulation following skeletal unloading and had comparable effects to parathyroid hormone (PTH) (3;4). In addition to the animal studies, our 3-month clinical trial indicated that the consumption of dried plum daily by postmenopausal women significantly increased serum markers of bone formation, total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), and insulin-like growth factor-I (IGF-I) by 12, 6, and 17%, respectively (6). Although the effects of dried plum on bone biomarkers are promising, a longer-term study in which BMD and bone mineral content (BMC) are assessed is needed to confirm the bone protective effects of dried plum in postmenopausal women. Hence, we hypothesize that:

Daily consumption of 100 g of dried plum will reverse bone loss in postmenopausal women.
To test this hypothesis we have the following Specific Aims:

**Aim 1**: Determine the efficacy of dried plum in reversal of bone loss by measuring BMD and BMC of lumbar spine as primary outcome and proximal femur and forearm as secondary outcome using dual energy x-ray absorptiometry (DXA) at baseline and the end of the study.

**Aim 2**: Elucidate the potential mechanisms (i.e. anti resorptive vs. anabolic) by which dried plum exerts beneficial effects on bone in postmenopausal women by assessing:
   a) biochemical markers of bone formation and resorption,
   b) urinary calcium and phosphorus excretion, and
   c) serum C-reactive protein (CRP), a marker of inflammation.

**Aim 3**: Understand the degree to which physical activity and dietary intake influence bone mineral density. This aim will be tested by assessing physical activity and dietary intake at baseline, three- six-month, and at the end of the study.

**Introduction**

Thirty six million Americans were 65 years or older in 2005 and by the year 2030, approximately 71.5 million people will be 65 years of age or older (7). Trends in population aging have been accompanied nationally by an increased concern for the health-related needs of older people. Age-related bone loss that results in osteoporosis is a significant health problem (8). Although the prevalence of bone loss is greater in women (9), number of men with osteoporotic fractures is also towards an increase (9-11). In the United States alone, a conservative estimate is that 8 million women have osteoporosis that results in more than one million fractures per year in women 45 years and older (9). Similarly, there are more than 10 million men with low bone mass which 2 million of them have been diagnosed with osteoporosis (9). The cost of treating osteoporosis and its fractures has been estimated over $18 billion dollars per year. Despite this cost and hospitalization, fractures particularly hip fractures continue to be the cause of more than 50,000 deaths in the United States annually (12).

Although there are a number of agents available for treatment and/or prevention of osteoporosis, some patients choose alternative therapies over conventional medicine, including dietary supplements and functional foods (13). The focus of this review of literature is to present evidence that dried plum to our knowledge is the most effective natural compound in both preventing and reversing bone loss in females and males animal models of osteoporosis (1-5). Based on our collective observations, the efficacy of dried plum surpasses that of other naturally occurring compounds as well as functional foods.
Appropriateness of Rat Models for Studying Human Osteoporosis

Rat model of postmenopausal bone loss allow us to study spontaneous or induced bone loss due to ovarian hormone deficiency. Kalu (14) is among the first to recognize the appropriateness of the rat model to study human bone loss. He showed that rats in one or more respects have the characteristics of bone loss experienced by postmenopausal women and in particular its sequel resembles those of women. In comparison to humans, the skeletal mass of rats remains stable for a prolonged period during their lifespan. Ovariectomized rats similar to postmenopausal women experience accelerated bone loss that occurs in women following menopause. Ovarian hormone deficiency-associated bone loss in the ovariectomized rat and postmenopausal women shares many similar characteristics including increased rate of bone turnover with resorption exceeding formation; initial rapid phase of bone loss followed by a much slower phase due to ovariectomy; greater loss of trabecular bone than cortical bone; decreased intestinal absorption of calcium; and similar skeletal response to bone protective or bone building agents such as estrogen, raloxifene, bisphosphonates, PTH, calcium, vitamin D, and exercise. These shared characteristics in terms of bone loss are reassuring in terms of the findings of the studies presented in this review of literature and most likely the consumption of dried plum can prevent or reverse bone loss in postmenopausal women similar to rats.

Biological Markers Associated with Bone

Biomarkers of bone turnover are used for the efficacy of short-term intervention. Dual x-ray absorptiometry, DXA, can be used to determine long-term interventions, which causes a major limitation in monitoring treatment by BMD quantification (15). Furthermore, it has been suggested that early detection of osteoporosis might be detected by measuring bone biomarkers such as ALP, OC, BALP, TRAP5b, and Dpd (16). These biomarkers are the products of osteoblast or osteoclast function that can be measured in serum or urinary specimen. Markers of bone turnover represented by osteoblast activity include OC and BALP, while growth hormone IGF-I is associated with increased osteoblast activity. Osteoclast activity can be measured by biomarkers of TRAP5b, Dpd, cross linked telopeptides, N-telopeptide, and helical peptide.

Alkaline phosphatase has been measured in clinical settings since 1972 and is among the first biochemical markers of bone turnover. Its role is not still precisely clear and it is found in plasma membrane of osteoblasts and in cells of liver, kidney, intestine, spleen and placenta. It is thought to play a role in osteoid formation and mineralization (17). Osteocalcin is a bone matrix protein found within
the hydroxyapatite produced by osteoblasts that is associated with BMD (18). Weisman and colleagues (16) suggest OC to be monitored for efficacy of osteoporosis related treatment since slight changes in this biomarker can be seen within a few weeks of treatment. Furthermore, early monitoring of OC can be beneficial to fracture risk determination (16). Because OC fragments are released from the bone matrix during resorption, assays for circulating osteocalcin and its fragments reflect both bone formation and resorption (19). Bone specific alkaline phosphatase is another specific marker of bone formation that is produced by osteoblast cells (20). Low levels of circulating BALP are predictive of vertebral fracture as well as osteoporotic fractures (21). As a predictor of bone loss, BALP has been shown to account for 5% of hip and 8% of lumbar spine bone loss respectively (21). Similar to OC, BALP concentrations can be monitored to detect the effects of treatment on bone formation. The activity level of this marker is measured by immunoassay. Insulin-like growth factor-I is an anabolic hormone that has been associated with bone formation. Studies have found positive correlations between IGF-I levels and BMD (6;18). The IGF-I receptor has been found to decrease with age; therefore the anabolic response of IGF-I to the receptor becomes weak and requires a higher dose in order to elicit a response (18). Osteoblast cells in mice were found to decrease from the six-week old mice to the 24-month-old mice by 65%. In vivo administration of IGF-I increased osteoblast activity in the six-month adult mice 3-fold while the 24-month-old mice increased only 1.9-fold. Similarly, in vitro of the same young, adult, and old mice reported proliferation to increase in all groups with blunted results for old age mice (18). Apoptosis of osteoblasts were also decreased across the various groups with blunted results in the old mice group. The proliferation and apoptosis of osteoblast cells are therefore affected by IGF-I; however, the older the age of the mice, the less the response elicited (18). Serum levels of IFG-I can be measured by either radioimmunoassay or by enzyme-linked immunosorbent assay (ELISA).

Osteoprotegerin (OPG) is produced due to estrogen favoring bone formation through the action of OPG on osteoclast differentiation. The decoy receptor OPG binds to RANK ligand (RANKL) and prevents the binding of RANKL to the RANK receptor located on the surface of osteoclasts. This inhibits osteoclast differentiation and bone resorption without affecting osteoblasts (22). Therefore, OPG now decreases the activity of mature osteoclasts and induces osteoclastic apoptosis (23). Samelson and colleagues (23) found a positive association between OPG concentrations and BMD in postmenopausal women. This clinical trial also found an increase in the geometric thickness of the femoral neck in male participants (23). Marini and colleagues (24) found that genistein treatments significantly increased OPG
production by 3.712 pg/mL in the first year of treatment and 4.762 pg/mL in the second year of treatment when compared to baseline values. Total OPG concentrations can be determined by ELISA.

Receptor activator of nuclear factor-κB and RANKL are important factors for osteoclastogenesis. Osteoblasts produce RANKL that bind and activate RANK on the surface of osteoclasts. This activation induces osteoclast differentiation, producing more osteoclast cells and thus increasing bone resorption (24). Serum levels of RANKL are measured to detect unbound RANKL by method of ELISA.

Tartrate-resistant acid phosphatase (TRAP) is a nonspecific marker of bone resorption that is expressed by both osteoclasts and activated macrophages. Specifically, TRAP5b can be measured to determine osteoclast activity directly (25). Increased levels of TRAP5b occur during bone resorption. It is a useful indicator of antiresorptive treatment for osteoporosis as well as an early detection marker for risk of osteoporosis (25). The concentration of this TRAP5b can be detected in serum by method of ELISA.

Cross linked telopeptides of type I collagen are proteins found in both serum and urinary specimens that indicate bone resorption due to collagen degradation. Type I collagen is an important component of bone and that attributes to bone elasticity and resistance to fracture. As bone is resorbed, cross linked telopeptides of type I collagen such as amino terminal cross linked telopeptide, detectable in urine, and carboxy terminal cross linked telopeptide, found in both urine and serum, are released. Garnero and Delmas (26) suggest helical peptides (HP), which are bone resorption peptides cleaved at the helicoidal region of type-I collagen, to potentially be better determinants of osteoporotic treatments. These peptides can be measured by ELISA method.

Deoxypyridinoline (Dpd) is another type I collagen degradation product and is a consistent marker of bone resorption. Unlike TRAP5b that is a direct product of osteoclast activity, Dpd is the byproduct of collagen degradation excreted through urine during bone remodeling (20). Several fractures including the hip have been linked to increased urinary Dpd levels (16). As a reliable marker of bone resorption, urinary Dpd can be measured by ELISA method.

Evidence of Bone Protective Effects of Dried Plum in Female Rat Models of Osteoporosis

Animal studies (1-5) conducted in our laboratories have shown that dried plum has positive effects on bone and bone biomarkers. The animal data indicate that dried plum protects (1;5) and even
reverses bone loss in two separate models of osteopenia (2-4). The findings of an initial study by our group indicated that dried plum prevented the ovariectomy-induced reduction in BMD of the femur and lumbar vertebra. Serum IGF-I, which is associated with enhanced bone formation was elevated by the dried plum treatment (1). In that study, female Sprague-Dawley rats were divided into four groups: sham-operated (sham), ovariectomized (ovx), ovx + 5% (low-dose, LD), and ovx + 25% dried plum (high-dose, HD). Treatments were started immediately after surgery and continued for 45 days. Ovariectomy significantly reduced the BMD of the 4th lumbar vertebra and femur. HD-dried plum diet completely prevented this ovx-induced bone loss. Furthermore, the dried plum diets dose-dependently elevated circulating IGF-I levels which is known to stimulate bone formation, without altering the ovx-induced rise in serum tartrate-resistant acid phosphatase (TRAP) activity, a marker of bone resorption. The finding of that study suggested that the bone protective effects of dried plum, in part, may be explained by an increase in the rate of bone formation rather than suppression of the rate of bone resorption (1).

In a follow-up study, 90-day old rats (one sham group and five ovx groups) were allowed to loose bone for 40 days, thereafter they were placed on various doses of dried plum for 60 days. Dried plum, as low as 5%, was effective in restoring femoral and tibial bone density. Dried plum increased lumbar bone density as well, with HD achieving a statistical significance. The increase in femoral bone density of dried plum-fed rats resulted in improved bone quality as indicated by 6.9% and 6.0% improvement in overall yield and ultimate force, respectively. Various doses of dried plum were also able to significantly improve trabecular micro-architectural properties in comparison to ovx controls (2). The overall findings of this study indicated that the improvement in biomechanical properties of long bones as a result of the dried plum feeding, in part, was due to improvement in trabecular bone structural properties as evident by higher tibial bone volume and connectivity. These observations are rather unique because it is generally believed that once bone volume and connectivity are lost they cannot be brought back to normal (27).

We have also shown the effectiveness of dried plum in reversal of bone loss in female rats due to skeletal unloading (3). In a study by Smith et al., (3), six-month old female Sprague Dawley rats were either hind limb unloaded (HLU) or remained ambulatory for twenty-one days to induce osteopenia. After confirming bone loss in HLU, rats were treated with one of the three levels of dried plum (5, 15, or 25% w/w), or injected with PTH (80 μg/kg bw) for 90 days. Analysis of BMD and trabecular bone structure by μCT revealed that dried plum enhanced bone recovery during re-ambulation following
skeletal unloading and had comparable effects to PTH. In addition to bone density, architectural
arrangement is also known to affect bone strength (28;29). These animal findings along with significant
increases in serum ALP and IGF-I strongly suggest that dried plum acts to enhance bone formation.

Evidence of Bone Protective Effects of Dried Plum in Male Rat Model of Osteoporosis

Emerging data also indicate that dried plum is very effective in preventing bone loss in various
models of male osteoporosis. Muhlbauer et al. (30) examined the effects of a number of fruits and
vegetables on bone resorption by assessing the urinary excretion of tritium released from bone in nine
weeks old male Wistar rats. Rats were prelabeled with tritiated tetracycline in order to measure bone
resorption and were provided food containing dried fruits and vegetables. All diets were formulated to
be isocaloric and isonitrogenous with similar amounts of calcium and phosphorus. They showed that dried
plum has a strong bone resorption effect. To our knowledge, this was the first study that showed prunes
(dried plum) can benefit male osteoporosis by inhibiting bone resorption.

In a study by Franklin et al. (5), the preventive effects of dried plum was investigated using sixty
6-month-old orchidectomized (ORX) Sprague-Dawley rats. In that study, rats were either sham-operated
(sham) or ORX with 12 rats per treatment group. The four ORX groups were treated as follows: 1) ORX
control; ORX+5, 15, or 25% dried plum (LD, MD, HD, respectively). After 90 days rats were
anesthetized, scanned using DXA and bled via abdominal aorta. The results of this study indicated that
the loss of whole body BMD was completely prevented by MD and HD dried plum. However, all three
doses of dried plum were effective in preventing BMD of 4th lumbar vertebrae and femurs. In the same
study, the investigators assessed microarchitectural properties of both the distal femur and 4th lumbar
vertebra. MD and HD of dried plum were effective in preventing the ORX-induced loss of bone volume
(BV)/total volume (TV) of both distal femur and 4th lumbar vertebra, albeit only HD was able to
completely prevent this loss. Similar observations were made in trabecular number where both MD and
HD had preventive effects in the loss of trabecular number as a result of ORX. Dried plum was able to
prevent the trabecular separation dose-dependently in both distal femur and 4th lumbar and interestingly
HD dried plum decreased trabecular separation of 4th lumbar significantly below of the sham level. The
authors concluded that the bone protective mechanism of dried plum in part may be due to the ability of
dried plum to increase serum IGF-I. The results showed that serum IGF-I was increased as a result of
dried plum feeding with HD reaching a significant level. These observations were analogous to our
earlier findings in postmenopausal women that we showed dried plum elevated serum IGF-I levels (6).
In agreement with the findings of Muhlbauer and colleagues (30), that reported dried plum’s ability in reducing bone resorption, Franklin et al. (5) also showed that dried plum dose-dependently reduced urinary deoxypyridinoline (Dpd). To further explain these strong effects of dried plum in preventing bone loss, the investigators assessed bone mRNA levels of osteoprotegerin (OPG) and receptor activator of NFκ-β ligand (RANKL). It is generally believed that when the ratio of OPG to RANKL is increased bone formation is higher (31); however, in the study by Franklin et al. (5) the ratio of OPG and RANKL was not altered. These findings are in agreement with Carlo et al. (32), which have shown that serum ratio of RANKL/OPG was not correlated with bone protective effect of estrogen-progestin therapy.

In a follow up study by the same group (4), the bone reversal properties of dried plum was investigated in male rat model of osteoporosis. In that study, 50 six-month old Sprague Dawley rats were divided into 5 groups (1 sham and 4 ORX) and maintained on AIN-93 M diet for 90 days to establish bone loss. After 90 days, animals in the sham and one ORX were killed to measure baseline BMD and bone structure. As expected, whole body BMD was significantly decreased due to ORX. Thereafter, the remaining three ORX group were assigned to treatments as follows: 1) ORX-control, 2) ORX+ 25% dried plum (ORX-DP) and 3) ORX + PTH (80 µg/kg body weight 3X per week; ORX-PTH). After 90 days of treatment, both DP and PTH were able to restore BMD of ORX rats to the sham level at both whole body and regional sites. The authors also assessed the ability of dried plum in reversal of microstructural properties of distal femurs and vertebrae. Dried plum was able to reverse the loss of BV/TV, increase trabecular number and decrease trabecular separation without affecting trabecular thickness. The interesting part of this study was that the investigators showed that dried plum similar to PTH was able to reconstructs some of the bone structural elements lost as a result of ORX. Additionally, it was shown that dried plum was able to significantly decrease structural modular index (SMI), indicative of more favorable plate-like trabeculae structure, in distal femur. Similar trends were also observed in vertebrae. The investigators suggested that the favorable effect of dried plum in reversal of bone loss in the male model of osteoporosis is due in part to its ability to suppress bone resorption while at the same time enhancing bone formation similar to that of PTH which was used in their study as a positive control. Furthermore, they indicated that since there were no changes in trabecular thickness, the decrease in trabecular separation might have been due to the formation of new trabecular rather than increase in their size. However, these observations are in contrast to female rat model of osteoporosis which the opposite was true (1). Therefore, dried plum though effective in preventing and/or reversal of
bone loss in both female and male rat models of osteoporosis, its mechanism of action may be gender specific.

Implication of Dried Plum Bone Protective Effects in Human

In addition to the animal studies, the findings of our short-term clinical trial (6) indicated that the consumption of dried plum (100g per day) by postmenopausal women significantly increased markers of bone formation including serum total ALP activity, BALP activity, and IGF-I. IGF-I is also reported to correlate with bone formation and bone mass in men. Clinically relevant doses of potential bone forming agents such as PTH take several weeks to increase serum levels of BALP. Therefore, our observation of 6% increase in BALP and 17% increase in serum IGF-I after three months of consumption of dried plum supports the notion that dried plum will significantly improve bone mass in a longer treatment period. Although the effects of dried plum on bone biomarkers are promising, a longer-term study in which BMD and BMC are assessed is needed to confirm the bone protective effects of dried plum in postmenopausal women. Hence, we are conducting a one-year comparative control randomized study to examine the effects of the daily consumption of 100 g dried plum on BMD of osteopenic postmenopausal women not on hormone replacement therapy or any other agents known to significantly influence bone metabolism. In summary, the significance of the proposed study is three-fold. This study will: 1) determine whether dried plum increases bone mass or prevents further bone loss in osteopenic postmenopausal women; 2) confirm whether long-term consumption of dried plum exerts beneficial effects on markers of bone turnover; and 3) explore whether changes in BMD and BMC correlate with increased antioxidant defense. If our hypothesis is substantiated, this study will provide a safe, practical and effective approach in increasing bone mass in a very vulnerable portion of the population.

Mechanisms of Action

Several reports have indicated that naturally occurring bioactive phenolic compounds such as isoflavones and lignans, positively influence bone health (33-36). Dried plum is rich in phenolic compounds such as neochlorogenic acid and chlorogenic acid, which act as antioxidants (37;38). Antioxidants scavenge potentially damaging free radicals and have been shown to inhibit bone resorption and stimulate bone formation (39;40). Dried plum is of particular interest because it has the highest oxygen radical absorbance capacity (ORAC) ranking among the most commonly consumed fruits and vegetables (37). Therefore, the beneficial effects of dried plum on bone may be mediated, in
part, through dried plum’s antioxidant properties. Dried plum is also a good source of other nutrients such as boron, potassium, and vitamin K (41;42) all of which have been reported to influence bone (see Table 1 for its compositions). Tucker and colleagues (43) investigated both cross sectional and longitudinal relationships between potassium and BMD using a Framingham Heart Study database and concluded that potassium contributes to the maintenance of BMD in men and women. Additionally, higher intakes of potassium have been shown to reduce bone resorption, particularly in the face of high protein intake (44). To our knowledge, there are no studies that have examined the effectiveness of dried plum in preventing bone loss to those of vitamins and minerals known to influence bone metabolism such as calcium and vitamin D. Studies similar to our studies using rat model under similar conditions to explore the effectiveness of vitamin and mineral in preventing and/or reversing bone loss is limited. Therefore, we cannot compare the effectiveness of dried plum to those of calcium and vitamin D. Nonetheless, a study similar to our studies in terms of rat model, age, use of similar model of DXA and other end points by Gala et al. (45), which investigated the effects of calcium on bone mineral density using three-month old rats reported that 15 g/Kg diet calcium was able to increase femoral BMD by 2.1% after three months of treatment. In comparison, dried plum in one of our studies (1) was able to increase femoral BMD by 2.0% in only 45 days.

The above discussion suggests that though polyphenols may be the major bone protective agents in dried plum, other components also to some extent contribute to the beneficial effects of dried plum on bone. The components of dried plum responsible for its osteoprotective effect are unknown, and require further investigation. However, it has to be emphasized that the bone protective effects of dried plum cannot be solely attributed to any one of its components at this time and it is imperative to evaluate the beneficial effects of whole dried plum rather than its isolated components. Messina et al. (46) has indicated that using a single chemical, such as an individual polyphenolic compound or a combination of a few, may hinder, rather than facilitate our understanding of the role of whole food or plant in bone health.
Table 1. Major components of dried plum powder

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount per 100 gram dried plum powder</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate analyses (g)</strong></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>3.0</td>
</tr>
<tr>
<td>Fat</td>
<td>0.5</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>80.0</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>9.0</td>
</tr>
<tr>
<td><strong>Minerals (mg)</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>72.0</td>
</tr>
<tr>
<td>Iron</td>
<td>3.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,050.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>5.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>108.0</td>
</tr>
<tr>
<td>Boron</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Anti-oxidants (mg)</strong></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.63</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.60</td>
</tr>
<tr>
<td>Phenolic and flavonoid compounds</td>
<td>22.4</td>
</tr>
<tr>
<td>Quinones</td>
<td>0.058</td>
</tr>
<tr>
<td><strong>ORAC</strong></td>
<td>5,770</td>
</tr>
</tbody>
</table>

1 These values are reported by the California Dried Plum Board (Pleasanton, CA) for 100 grams of dried plums and can slightly vary from batch to batch. 2 ORAC; oxygen radical absorbance capacity.

Potential Bone Protective Mechanism of Action of Dried Plum

Our findings indicate that dried plum increases serum levels of IGF-I both in a rat model of osteoporosis (1) and in postmenopausal women (6). This increase in IGF-I production, has also been demonstrated in MC3T3-1 (osteoblast-like cells) treated with dried plum extract (47). Dried plum extract was able to increase IGF-I significantly in dose of 1000 µg/ml in days of 12 and 15 compare to the control group. This IGF-I increase in part, may mediate the positive effects of dried plum on bone. The increase in IGF-I production by osteoblasts has also been shown in a study by Bu et al., (48) in which dried plum polyphenols was able to increase IGF-I mRNA levels at dose of 5µg/mL. However, the differences between our study and Bu et al., findings have to be further investigated. Nonetheless, in our hands these lower doses of dried plum (48;49) have not been found efficacious in modulating any of the
parameters including, nodule formation and nitric oxide production (50;51). The effects of growth hormone on bone are likely mediated locally through IGFs (52). At the local level bone cells synthesize IGF-I and IGF-II, but IGF-I is more potent in stimulating osteoblasts (53), increasing collagen synthesis, and enhancing matrix apposition (54). Serum IGF-I declines with age in both sexes (55), but also declines immediately after menopause (56;57). IGF-I administration stimulates bone turnover (58), similar to growth hormone (59), and plays a role in regulating bone remodeling (60). Also, IGF-I concentrations are correlated positively with bone mass in pre- (57), peri- (61), and post- (60) menopausal women, but are lower in osteoporotic individuals (62). Whether this decline is due to estrogen deficiency (63) or aging per se has not been resolved, but sex steroids may be involved in the regulation of serum IGF-I concentrations (64).

In an attempt to understand the mechanism of action of dried plum, Dr. Smith’s laboratory (48) showed that dried plum enhances osteoblast ALP activity, calcified nodule formation and type I collagen cross-linking in vitro. These alterations were suggested to be mediated by upregulation of transcription and growth factors such as runt-related transcription factor (RUNX2), osterix and IGF-I. In addition to stimulating bone formation, dried plum polyphenols decreased bone resorption by down regulating RANKL expression in osteoblasts (5;48). Dried plum polyphenols suppressed osteoclast differentiation and activity under normal, oxidative stress, and inflammatory conditions in vitro (49). Although, antiresorptive properties of dried plum observed in vivo are in part mediated by polyphenols and their effects on osteoclast precursors and osteoblast-mediated signaling for osteoclastogeneisis, the whole credit for bone protective effects of dried plum yet cannot be given to polyphenols as other components of dried plum may also contribute to these positive effects.

Summary

The findings of our animal studies strongly suggest that dried plum has a pronounce effects on bone in terms of both prevention and reversal as evident by higher bone densities, mineral contents, percent trabecular bone area, and the tendency to reduce marrow space in long bone. Although these results are mostly obtained from rat studies, we anticipated that the findings of these animal studies will be confirmed in human studies. Currently, we have finished a one-year long study in which 160 women received either 100 grams of dried plum or equivalent amount of dried apple. If the results of this study show the same magnitude improvement in bone density of postmenopausal women as has been the case in the rat model, dried plum would be considered the most effective functional food in terms of bone
health. From a mechanistic point of view, we have limited data to offer an explanation for the mode of action of dried plum; however, it appears that dried plum does not suppress the ovariectomy-induced elevated rate of bone formation. Rather, dried plum may further elevate the rate of bone formation as suggested by a dose-dependent rise in serum IGF-I levels. Higher serum IGF-I concentrations are considered to be reflective of the elevated rate of bone formation (65). Also evidence suggests (1;30) that some components of dried plum at higher concentrations may begin to suppress the ovariectomy-induced rate of bone resorption.
CHAPTER 2

COMPARATIVE EFFECTS OF TWO BIOACTIVE-RICH DRIED FRUITS ON BONE

Introduction

Osteoporosis is a major public health problem in postmenopausal women. In the United States alone, 8 million women have osteoporosis that results in more than one million fractures per year in women 45 years and older (66). The cost of treating osteoporosis and its fractures has been estimated over $19 billion dollars per year. Although there are a number of agents available for the treatment and/or prevention of osteoporosis, some patients prefer alternative therapies including dietary supplements and functional foods (13). Studies have consistently shown that higher fruits and vegetables intake has positive effects on bone mineral density (67-71). Muhlbauer et al. (30) examined the effects of a number of fruits and vegetables on bone resorption by assessing the urinary excretion of tritium released from bone. Results of Muhlbauer’s studies showed that dried plum (30) among fruits and onion among vegetables were the most effective functional foods with bone modulating effects. To our knowledge, Muhlbauer and colleagues (30) were the first group who showed prunes (dried plums) have the ability to prevent osteoporosis by inhibiting bone resorption. To follow up on Muhlbauer’s findings, our laboratory conducted several animal studies and a 3-month clinical trial. The results of these studies have shown that dried plum has positive effects on bone indices. The results from animal studies indicated that dried plum not only prevents (5), but more importantly reverses bone loss in two separate models of osteopenia (2-4). Our initial animal study (1) indicated that dried plum prevented the ovariectomy-induced BMD reduction of the femur and lumbar vertebra. In another study rats were ovariectomized and allowed to lose bone before the initiation of treatment to mimic established osteoporosis. Dried plum as low as 5% (w/w), restored BMD to the level of intact rats (2). More importantly, dried plum reversed the loss of trabecular architectural properties such as trabecular number, connectivity density, and trabecular separation which to our knowledge is unique to dried plums in comparison with soy or its isoflavones (34), flaxseed (72;73), apples (6), blueberries, and strawberries (unpublished data). According to Lane et al. (27), once trabecular bone is lost, it would be difficult to
restore it. The efficacy of dried plum in reversal of bone loss in rat models of established osteoporosis (3-5) exceeds many of the agents with bone forming ability such as growth hormone and insulin growth factor (74). In addition to the animal studies, the findings of our short-term clinical trial (6) indicated that the consumption of dried plum (100 g per day) by postmenopausal women significantly improved markers of bone turnover. Our observations of serum bone markers after three months of dried plum consumption supports the notion that dried plum significantly improves bone mass in a longer treatment period. Although the effects of dried plum on bone biomarkers are promising, a longer-term study with BMD and BMC assessments is needed to confirm the bone protective effects of dried plum in postmenopausal women. Hence, we proposed a one-year comparative-control randomized study to examine the effects of daily consumption of 100 g dried plum on BMD in osteopenic postmenopausal women not on HRT or any other agents known to significantly influence bone metabolism. The significance of the proposed study under our hypothesis was to determine the extent to which dried plum reverses bone mass in osteopenic postmenopausal women and to explore the mechanisms by which dried plum increases BMD. In summary, this study will: 1) determines whether dried plum increases bone mass or prevent further bone loss in osteopenic postmenopausal women, and 2) confirm whether long-term consumption of dried plum exerts beneficial effects on bone turnover markers.

**Methods**

**Subjects**

Two hundred and thirty-six (236) women, one to ten years postmenopausal not on HRT for at least three months prior to initiation of the study were recruited. Women whose BMD t-score at any site fell below 2.5 SD of the mean were excluded from the study and referred to their primary care physician. Furthermore, subjects treated with calcitonin, bisphosphonates, raloxifene and/or anabolic agents such as PTH and growth hormone, or steroids within three months prior to the start of the study were excluded. In addition, subjects with metabolic bone disease, renal disease, cancer, cardiovascular disease, diabetes mellitus, respiratory disease, gastrointestinal disease, liver disease, or other chronic diseases, heavy smoking (more than 20 cigarettes per day), and current use of any prescription medications known to alter bone and calcium metabolism were excluded. Women who regularly consumed dried plum or prune juice were not accepted into the study. The study protocol was approved by the Institutional Review Board at The Florida State University (FSU). Subjects signed a consent form after being provided with oral and written descriptions of the study. A complete medical and nutrition history was obtained from...
all subjects before initiating the treatments. Subjects were advised to maintain their usual physical activity and diet pattern.

**Study Design**

One hundred and sixty eligible postmenopausal women out of 236 screened volunteers were randomly assigned to one of the two dietary treatment groups: dried plum (100 g) or dried apple (75 g). The amount of dried apple was chosen based on comparable amount of calories, carbohydrates, fat, and fiber that would be obtained from 100 g dried plum (6). The amount of dried plum was based on the findings of our short-term clinical trial (6) which indicated the consumption of 100 g dried plum per day for 90 days significantly modulated bone markers in postmenopausal women. Because of the known laxative properties of dried plum, study participants were asked to gradually incorporate dried plum into their daily diets so that they were consuming 100 g (8 to 10 dried plums) daily after one week of adaptation. Dried fruits were distributed to the subjects monthly in individually sealed and vacuum-packed daily portions for a period of one year. Subjects were asked to return any unused dried fruit as part of monitoring compliance. All participants received 500 mg calcium plus 400 IU vitamin D daily (regardless of treatment). The participants were also provided with customized calendar to record the amount of dried fruit and supplement consumed. The study participants were advised by registered dietitian to make appropriate adjustment in their daily food consumption to account for the additional energy, fat, protein, and carbohydrate intakes provided by the dried fruits. The calculated and actual analytical values for the nutrients compositions of the dried fruit regimens are presented in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Dried Apple (per 75 g)</th>
<th>Dried Plum (Per 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated(^a)</td>
<td>Actual(^b)</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>240</td>
<td>219</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.43</td>
<td>0.37</td>
</tr>
<tr>
<td>Total Carbohydrates (g)</td>
<td>58.5</td>
<td>70.5</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>6</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.5</td>
<td>0.83</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>11.25</td>
<td>15.0</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>14.2</td>
<td>30.6</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.05</td>
<td>--</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>30</td>
<td>--</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>2.25</td>
<td>--</td>
</tr>
</tbody>
</table>

\(^a\) Results obtained from Food Processor version 7.50 (ESHA Research, Salem, OR).

\(^b\) Gross energy analyzed by bomb calorimetry (Parr 1261 Calorimeter, Parr Inst. Co., Moline, IL), crude protein by the AOAC Kjeldahl method, fat by either extraction, and calcium and phosphorus content by atomic absorption spectrophotometer (Perkin-Elmer Atomic Absorption Spectrophotometer, model 5100PC, Perkin-Elmer, Norwalk, CT).
**Dietary and Physical Activity Assessment and Anthropometric Measurements**

For each subject, medical and nutrition histories were obtained at the beginning of the study. Seven-day food frequency questionnaires were completed via interview at the baseline, three-, six-, and 12-month. Nutrient analysis was performed using food analysis software (Food Processor version 7.50, ESHA Research, Salem, OR). Similarly, physical activity patterns were assessed at baseline, three-, six-, and 12-month. The Five-City Project Physical Activity Recall was used to assess current physical activity, sleep and activity patterns (75). This questionnaire assesses leisure, occupational, and home activities and classifies them based on intensity. This seven-day activity recall has been used in cross-sectional designs and community health surveys (76) and has been validated by Taylor et al. (77). Physical activity data were analyzed to determine usual activity level, consistency over time and deviations from baseline. Anthropometric data were also collected at the baseline, three-, six-, and 12-month and height and weight were used to calculate body mass index (BMI; kg/cm$^2$).

**Bone Density Assessments**

Bone density was assessed at the beginning and at the end of treatment using dual energy x-ray absorptiometry (iDXA; GE Healthcare Lunar, Madison, WI) equipped with appropriate software for whole body, lumbar spine, hip, and forearm BMD and BMC. Densitometer stability was evaluated by performance of phantom scans on the dates of all data acquisition.

**Bone Marker Measurements**

Venous blood samples were obtained after an overnight fast from each subject at the baseline, three-, six-, and 12-month of the study for various analyses. Blood samples were centrifuged at 3500 × g for 15 min at 4°C, serum samples were separated, aliquoted, and stored at -80°C until analyses. Each study participant collected a 24-h urine specimen, excluding the first void, at baseline, three-, six-, and 12-month of the study. For each subject, urine volume was recorded and aliquots were stored at -80°C for later analyses. BALP and OC, markers of bone turnover, were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quidel Biosystems, Mountain View, CA). TRAP5b, a marker of bone resorption in blood and Dpd, a marker of bone resorption in urine, were measured using ELISA kits (Quidel Biosystems). In order to determine the potential anti-inflammatory role of dried plum in bone modulating, serum CRP was assessed using SIRRUS clinical chemistry analyzer (Stanbio, Laboratory, Boerne, TX) at baseline, three-, six-, and 12- months. Blood and urinary levels of calcium, phosphorus, magnesium, and creatinine as well as blood alkaline phosphatase (ALP),
a nonspecific marker of bone turnover (78), were determined using SIRRUS clinical chemistry analyzer at baseline, three-, six-, and 12-month.

**Statistical Analyses**

Data were analyzed using analysis of variance methods with PROC MIXED in PC SAS (Version 9.1, SAS Institute, Cary, NC) analyzing the main and interaction effects of the two factors, treatment (dried apple or dried plum), and time (baseline or after treatment). Since each subject was measured at baseline and various time intervals, a split plot (repeated measures) model was utilized. The mean changes in each time point for the dried plum and dried apple treatment groups were compared by analyzing interaction effects of the two factors, treatment and time, using the SLICE option in an LSMEANS statement. Data are reported as least square mean ± standard error (SE); unless otherwise indicated, P < 0.05 was regarded as statistically significant.

**Results**

**Baseline Characteristic, Anthropometric Measurements, Dietary Intake and Physical Activity Assessments**

One-hundred women (55 on dried plum and 45 on dried apple) completed the study. As depicted by Figure 1, 160 women were randomly assigned to 75 g dried apple or 100 g dried plum daily. The attrition rates were not significantly different between the two treatment groups (37.5%). The most common reasons for attrition included non-compliance with the study protocol, claims of medical and health related conditions and personal reasons. Baseline characteristic data for women who completed the study are presented in Table 3.

<table>
<thead>
<tr>
<th>Table 3. Characteristics of study participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measures</strong></td>
</tr>
<tr>
<td>Age (yrs)</td>
</tr>
<tr>
<td>55.6±5.0</td>
</tr>
<tr>
<td>Time since menopause (yrs)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>T-Score (L1-L4)</td>
</tr>
</tbody>
</table>

Values are mean±SD, n= 45 for dried apple regimens and 55 for dried plum regimens.

*BMI: body mass index*
Figure 1: Flow chart of the study design and subject participation, GI; gastrointestinal; HRT; hormone replacement therapy.
Age, years since menopause, body weight and body mass index were similar at baseline between the treatment groups. The 100 participants who remained in the study adhered to the regimens, as indicated by self monitoring checklist provided to them on a monthly basis and by assessing 7-day food frequency questionnaire. Overall, the dried fruit regimens were well accepted and considered palatable, as stated by the subjects. Analysis of 7-day food frequency questionnaire (Table 4) indicated that the participant’s food intakes were not significantly different from their corresponding baseline values between the two treatment groups throughout the study period.

Physical activity levels were assessed at baseline, 3-, 6-, and 12-month and as expected there were no significant differences in their activity levels throughout the study (Table 5).
Table 4. Daily nutrient intake calculated from 7-day food frequency questionnaire of women at baseline, 3-, 6-, and 12-month supplementation with 100 g dried plum or 75 g dried apple daily.

<table>
<thead>
<tr>
<th>Measured</th>
<th>Dried apple</th>
<th>Dried plum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures</td>
<td>Baseline 1680±56</td>
<td>1847±77</td>
</tr>
<tr>
<td>Macronutrients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>72.5±3.0</td>
<td>72.5±3.2</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>212±8</td>
<td>214±5.5</td>
</tr>
<tr>
<td>Dietary Fiber (g)</td>
<td>21.2±1.2</td>
<td>21.5±1.1</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>61.1±3.2</td>
<td>60.9±3.0</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>18.8±1.0</td>
<td>20.6±2.5</td>
</tr>
<tr>
<td>Mono Fat (g)</td>
<td>21.4±1.5</td>
<td>19.4±1.4</td>
</tr>
<tr>
<td>Poly Fat (g)</td>
<td>11.6±0.9</td>
<td>11.0±0.8</td>
</tr>
<tr>
<td>Tran Fat (g)</td>
<td>0.5±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Total Chol (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>11533±876</td>
<td>196.5±14.5</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>107±8</td>
<td>95.9±15.8</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>103±11</td>
<td>227.2±18.0</td>
</tr>
<tr>
<td>Vitamin E (IU)</td>
<td>5.6±0.5</td>
<td>221.4±14.0</td>
</tr>
<tr>
<td>Vitamin K (µg)</td>
<td>115.6±11.6</td>
<td>202.5±12.5</td>
</tr>
<tr>
<td>Minerals (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>808±47</td>
<td>8261±216</td>
</tr>
<tr>
<td>Iron</td>
<td>14.7±1.2</td>
<td>7.5±0.8</td>
</tr>
<tr>
<td>Magnesium</td>
<td>245±10</td>
<td>2643±129</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>997±42</td>
<td>2916±121</td>
</tr>
<tr>
<td>Potassium</td>
<td>2661±97</td>
<td>2957±131</td>
</tr>
<tr>
<td>Zinc</td>
<td>7.1±0.3</td>
<td>7.8±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SE, n=45 in dried apple regimens and 55 in dried plum regimens. Analyses do not include nutrients, calcium and vitamin D from the supplements used by participants. There were no statistical significant differences observed between baseline values of two treatment groups and between baseline, 3-, 6-, and 12- months corresponding values.

Table 5: Physical activity patterns assessed at baseline, 3-, 6-, and 12-month.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Dried apple</th>
<th>Dried plum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures</td>
<td>Baseline 919.5±52.5</td>
<td>851.1±56.6</td>
</tr>
<tr>
<td>PA (Kcal/d)*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SE, n=45 in dried apple regimens and 55 in dried plum regimens. The Five-City Project Physical Activity Recall was used to assess current physical activity, sleep, and activity pattern including leisure, occupational, and home activities. There were no statistical significant differences observed between baseline values of two treatment groups and between baseline, 3-, 6-, and 12- months corresponding values.

* PA, Physical activity
Bone Mineral Density

Both dried fruit regimens had bone protective effects as it is indicated by positive changes from baseline in ulna, spine, femoral neck, total hip, and whole body BMD (Figure 2). It is important to note that dried plum had more pronounced effects on BMDs of ulna and spine as the increases were significantly different between the two treatments.

Figure 2: Bone mineral density (BMD) changes from baseline of ulna, spine, neck of femur, total hip, and total body after one year consumption of dried apple (green bars) or dried plum (purple bars). Bar represent mean±SD.★Significantly different at p<0.05 between treatment groups.
**Serum and Urinary Parameters of Relevance to Bone Metabolism**

Dried plum consumption resulted in time-dependent reduction in serum BALP levels of postmenopausal women at three-, six-, and 12-month time intervals and this reduction became significant at 12 months compared to baseline (Figure 3). In the dried apple group, serum BALP levels increased significantly in the first six months of the study and decreased numerically at 12 month. When comparing dried plum and dried apple groups, BALP levels were significantly lower in dried plum group at 6 months.

**Figure 3:** Mean serum bone specific alkaline phosphatase (BALP) at baseline, 3-, 6-, and 12-month in postmenopausal women consuming dried apple (green bars) or dried plum (purple bars). Bar represents mean±SE. ★ Significantly different at p<0.05 between treatment groups; # Significantly different at p<0.05 beween baseline value and corresponding values at 3-, 6-, and 12- months after treatment.

Osteocalcin (OC), a marker of bone turnover, numerically decreased in a time dependent manner in the dried plum group while it increased in the dried apple group over time and this increase reached a
significant level at six months (Figure 4). Serum OC levels were significantly lower in the dried plum group compared to the dried apple at 3- and 12-month time points.

![Figure 4: Mean serum osteocalcin (OC) at baseline, 3-, 6-, and 12-month in postmenopausal women consuming dried apple (green bars) or dried plum (purple bars). Bar represents mean±SE. ★Significantly different at p<0.05 between treatment groups; #Significantly different at p<0.05 between baseline value and corresponding values at 3-, 6-, and 12- months after treatment.]

TRAP5b, a specific marker of bone resorption, decreased significantly in the dried plum group at 3 months and stayed in the same level at six- and 12- month time points (Figure 5). TRAP5b did not change significantly in the dried apple group, however, it increased numerically at 12-month time point.

Serum CRP, a marker of inflammation, decreased numerically in the dried plum treatment group after 3 months and remained the same thereafter. On the other hand, CRP levels were the same after 3 months in the dried apple treatment group and decreased numerically thereafter. Serum CRP levels were significantly lower in dried plum group compared to dried apple group at 3 months (Figure 6).

There were no significant changes in urinary Dpd levels throughout the study in either dried plum or dried apple groups (Figure 7).
Figure 5: Mean serum tartrate resistant acid phosphatase-5b (TRAP5b) at baseline, 3-, 6-, and 12-month in postmenopausal women consuming dried apple (green bars) or dried plum (purple bars). Bar represents mean±SE. #Significantly different at p<0.05 between baseline value and corresponding values at 3-, 6-, and 12- months after treatment.

Figure 6: Mean serum C-reactive protein (CRP) at baseline, 3-, 6-, and 12-month in postmenopausal women consuming dried apple (green bars) or dried plum (purple bars). Bar represents mean±SE. ★Significantly different at p<0.05 between treatment groups.
Both dried fruit regimens significantly decreased ALP activity, a non specific marker of bone formation over time (Table 6). Mean blood calcium level decreased significantly after six months compared to baseline in the dried plum group. Blood phosphorus levels decreased significantly in dried apple and plum groups after three and six months, respectively. Blood magnesium levels increased significantly in dried apple and dried plum after 12 and six months, respectively. Urinary calcium excretion increased significantly in dried apple group at the end of the study but in the dried plum group urine phosphorus but not calcium excretion increased after three months. Urinary magnesium excretion was elevated at six-month time point in both treatment groups but the values were similar to the baseline values at the end of the study.

Discussion

In a recent review (79), we summarized the bone protective effects of dried plum and speculated the mechanisms of action while presenting all the evidence prior to the findings of the present study.
Earlier findings strongly suggest that dried plum has a potent effect on bone both in terms of prevention and reversal as is evident by higher bone densities, mineral contents, percent trabecular bone area, and tendency to reduce marrow space in long bone of rats (1-5). The findings of the present study confirmed the ability of dried plum in improving bone mineral density in postmenopausal women. Postmenopausal women in this age range (see Table 3) are prone to accelerated bone loss. Women potentially lose up to 50% of their trabecular bone and 30% of their cortical bone after reaching peak bone mass in which half of it occurs during the first 10 years from the onset of menopause (80). To our knowledge, this is the first study which clearly indicates that postmenopausal women benefit from consuming dried plum in terms of bone mineral density.

Table 6. Effect of one year supplementation with dried apple or dried plum on serum and urine parameters of bone metabolism at baseline, 3-, 6-, and 12-month.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Baseline</th>
<th>3-month</th>
<th>6-month</th>
<th>12-month</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried apple</td>
<td>80.10±2.59</td>
<td>75.74±3.5</td>
<td>70.67±3.16</td>
<td>74.68±3.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dried Plum</td>
<td>81.92±2.66</td>
<td>78.03±3.53</td>
<td>68.90±2.86</td>
<td>73.03±2.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried apple</td>
<td>2.52±0.04</td>
<td>2.50±0.08</td>
<td>2.50±0.08</td>
<td>2.47±0.06</td>
<td>0.3033</td>
</tr>
<tr>
<td>Dried Plum</td>
<td>2.54±0.06</td>
<td>2.51±0.08</td>
<td>2.48±0.05</td>
<td>2.44±0.06</td>
<td>0.0002</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried apple</td>
<td>1.28±0.05</td>
<td>1.24±0.08</td>
<td>1.24±0.09</td>
<td>1.22±0.07</td>
<td>0.0554</td>
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<tr>
<td>Dried Plum</td>
<td>1.29±0.04</td>
<td>1.26±0.08</td>
<td>1.23±0.07</td>
<td>1.20±0.06</td>
<td>0.0006</td>
</tr>
<tr>
<td>Magnesium (mEq/L)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dried apple</td>
<td>1.07±0.05</td>
<td>0.99±0.06</td>
<td>1.07±0.08</td>
<td>1.18±0.06</td>
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<tr>
<td>Dried Plum</td>
<td>1.05±0.05</td>
<td>1.01±0.06</td>
<td>1.15±0.05</td>
<td>1.22±0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mmol/mmol creatinine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried apple</td>
<td>0.45±0.01</td>
<td>0.52±0.01</td>
<td>0.54±0.01</td>
<td>0.56±0.01</td>
<td>0.1103</td>
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<tr>
<td>Dried Plum</td>
<td>0.60±0.01</td>
<td>0.65±0.01</td>
<td>0.66±0.01</td>
<td>0.68±0.01</td>
<td>0.8225</td>
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<tr>
<td>Phosphorus (mmol/mmol creatinine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried apple</td>
<td>2.12±0.02</td>
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<td>2.10±0.03</td>
<td>2.37±0.03</td>
<td>0.9242</td>
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<td>Dried Plum</td>
<td>2.19±0.02</td>
<td>2.51±0.03</td>
<td>2.35±0.02</td>
<td>2.59±0.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Magnesium (mmol/mmol creatinine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried apple</td>
<td>0.59±0.009</td>
<td>0.58±0.009</td>
<td>0.77±0.01</td>
<td>0.47±0.008</td>
<td>0.0014</td>
</tr>
<tr>
<td>Dried Plum</td>
<td>0.55±0.007</td>
<td>0.65±0.009</td>
<td>0.85±0.01</td>
<td>0.64±0.009</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Values are mean ±SE, n=45 for dried apple regimens and 55 for dried plum regimens. Asterisks denote a significant difference at P<0.05 in comparison between baseline value and corresponding values at 3-, 6-, and 12- months after treatment. ALP, alkaline phosphatase.

As expected, both groups started with similar baseline characteristics including age, time since menopause, weight, height, BMI, and lumbar t-score, indicative of complete randomization. Although the women on both regimens consumed 240 more kcal/day (albeit not significantly different from
baseline energy consumption in both regimens), neither their weight, nor BMI were significantly affected by the excess energy consumption. This may be due to the high fiber contents of the dried plum and dried apple. Other investigators have reported lack of weight gain despite higher caloric intakes associated with higher fiber content, such as flaxseed (81-83). These findings are also in accordance with our earlier findings (6; 82) in which women did not gain weight by consuming similar regimens for three months. Therefore, it can be concluded that the general public should not be concerned with few hundred extra calories and miss the opportunity to incorporate fruits such as plum and apple in their diet.

Figure 2 represents the mean BMD values for both treatment groups. Data indicate that postmenopausal women on both dried plum and dried apple regimens did not lose bone. However, the gain in BMD for ulna and spine were significantly higher in the dried plum group than dried apple. These results support the findings that in general, consumption of fruits and vegetables are beneficial to bone health (67;69-71). However, the majority of studies that have linked fruits and vegetables consumption to better bone, speculated that the beneficial effects of fruits and vegetables on bone were due to a shift in acid-base balance (70). Nonetheless, more recent studies suggest that the beneficial effects of fruits and vegetables consumption on bone may be due to their contents such as vitamin C, vitamin K, potassium, and phytochemicals. In the case of the present study, although the exact osteoprotective component(s) of dried plum is unknown, dried plums are rich in phenolic compounds such as neochlorogenic acid and chlorogenic acid, which act as antioxidants (37;38). Antioxidants that scavenge potentially damaging free radicals have been shown to prevent bone loss (39;40). Another factor in dried plum that exerts bone beneficial effect is its boron content. Dried plum contains higher amount of boron than most fruits. Boron has been shown to modulate bone and calcium metabolism (84), and play an important role in preserving BMD (85).

In terms of vitamins, dried plum contains high amounts of vitamin K among commonly consumed foods (41). Vitamin K influences bone health by improving calcium balance (86) and is also a co-factor needed for γ-carboxylation of osteocalcin. γ-Carboxylated osteocalcin promotes normal bone mineralization by regulating the growth of hydroxyapatite crystals (87). However, it should be emphasized that the bone protective effects of fruits and vegetables including dried plum should not be solely attributed to any one of their components and consumption of whole fruits and vegetables should be promoted.

From a mechanistic point of view, we measured both markers of bone formation and bone resorption including serum levels of BALP, OC, TRAP5b, and urinary Dpd concentrations. In earlier
studies BALP and OC were considered markers of bone formation; however, more recent studies (87-90) suggest that both markers are to be considered markers of bone turnover rather than bone formation. Therefore, our overall findings suggest that dried plum improves bone mass by slowing down the rate of bone turnover. Nonetheless, it should be noted that in order for dried plum to have bone reversal effects it must suppress the rate of bone resorption more so than the rate of bone formation. Though this is speculative, in order to examine this notion one must use bone biopsies to assess static and dynamic histomorphometry. Particularly, quantitative histomorphometry is necessary to assess the effects of dried plum on the appearance of the cellular components, the presence or absence of woven bone, and marrow fibrosis. Another explanation for the bone reversal property of dried plum may be through suppression of chronic inflammation as dried plum was able to significantly lower serum CRP level after three months and reached plateau thereafter. CRP is known to be linked to a number of chronic diseases including osteoporosis (90-93). Therefore, the efficacy of dried plum in lowering serum CRP levels may have far greater implications than just influencing bone turnover. In brief, the findings of the present study suggest that the ability of dried plum to increase BMD in postmenopausal women, in part, is due to suppressing the rate of bone turnover and perhaps through lowering CRP levels.
CHAPTER 3

CONCLUSION

Overall, the findings of the present study suggest that long-term consumption of dried plum suppresses the rate of bone turnover and perhaps the rate of bone resorption more than formation. This statement is supported by our observations that dried plum lowered OC, TRAP 5b, and BALP. It should be noted that BALP used to be considered as a sole marker of bone formation; however, this notion has been challenged by the findings of studies in last few years. Recently, both OC, BALP have been considered markers of bone turnover. In the face of increased BMD values as a result of dried plum consumption, one should hypothesize that the main reason for this increased BMD is because the rate of bone resorption is suppressed more so than the rate of bone formation. This notion is supported by the findings of our animal studies as well as other investigators. Another novel aspect of this study is the effect of dried plum consumption on CRP which was measured at baseline, 3-, 6-, and 12-month. Dried plum was able to significantly reduce CRP level after three months which stayed at the same level for the remainder of the study. Elevated levels of CRP have been implicated to be the culprit of most chronic diseases including osteoporosis.

Irrespective of the mechanism of action, our findings strongly suggest that postmenopausal women can regain their BMD by regular consumption of dried plum. The question remains as to what components of dried plum exert these bone protective effects. Dried plum like many other fruits contains numerous compounds that make it difficult to discriminate the effect of one compound versus another. In addition to being a good source of nutrients such as certain vitamins and minerals that can influence bone health, dried plum is a rich source of phenolic compounds that act as antioxidants. Hence, dried plum or its polyphenols may exert positive effects on bone, in part, through their antioxidative capabilities. The identification of the active components in dried plum, e.g. trace elements such as boron and selenium, and the exploration of their mechanisms of action is necessary for understanding the bone modulating effects of dried plum in ovarian hormone deficiency. The fact that dried plum had no uterotrophic activity in animal studies implies that the action of dried plum, or its components, on bone differ from those of estrogens. This observation along with the observed bone-protective effects of dried plum should generate enthusiasm in further exploring dried plum as a natural food source that is beneficial to skeletal health.
APPENDIX A

IRB APPROVAL LETTRES & INFORMED CONSENT FORM

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8633  FAX (850) 644-4392

REAPPROVAL MEMORANDUM

Date: 6/20/2008

To:
Bahram Arjmandi
436 Sandeis Building, FSU
Tallahassee, Florida  32306

Dept.:  NUTRITION FOOD AND MOVEMENT SCIENCES

From: Thomas L. Jacobson, Chair

Re:  Reapproval of Use of Human subjects in Research:
      Dried Plum Reverses Bone Loss in Postmenopausal Women

Your request to continue the research project listed above involving human subjects has been approved by the Human Subjects Committee. If your project has not been completed by 4/9/2009 please request renewed approval.

You are reminded that a change in protocol in this project must be approved by resubmission of the project to the Committee for approval. Also, the principal investigator must report to the Chair promptly, and in writing, any unanticipated problems involving risks to subjects or others.

By copy of this memorandum, the Chairman of your department and/or your major professor are reminded of their responsibility for being informed concerning research projects involving human subjects in their department. They are advised to review the protocols of such investigations as often as necessary to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

Cc:
HSC No. 2008.0311-R
Effects of Dried Fruits on reversing Bone Loss in Postmenopausal Women

FLORIDA STATE UNIVERSITY
Individual's Consent to Voluntary Participation in a Research Project

Effects of Dried Fruits on Reversing Bone Loss in Postmenopausal Women

INTRODUCTION
You are being invited to participate in a research study that is directed by Dr. Bahram H. Arjmandi in the Department of Nutrition, Food & Exercise Sciences at Florida State University (FSU). Dr. Arjmandi is the Chair and Professor of Nutrition at FSU. He is also a registered dietician and has conducted several clinical trials investigating the role of foods in improving bone and heart health. You are informed that while the research study will be supervised by him, other professionals who work with him may assist or act on his behalf.

PURPOSE
The goals of this study are 1) to determine the bone protective effects of dried fruits by measuring bone density (a measure of bone strength) of whole body, hip, and forearm, indicators of bone formation and bone breakdown; 2) to identify potential ways by which dried fruits increase bone density. Additionally, dietary intakes of calcium, magnesium, and boron, vitamins D and K, protein, and fiber as well as relevant medical history and lifestyle variables will be recorded.

SUPPLEMENT INFORMATION
You will be randomly assigned to one of the two treatment groups: dried plum (100 g; 10-12 nos) or equivalent amount of dried apple (75g; 10-12 slices of dried apples) daily for 1 year. Additionally, you will receive 500 mg elemental calcium plus 250 IU vitamin D daily for one year. Therefore your chance of receiving dried plum or dried apple is 1 in 2.

SCREENING AND STUDY ASSIGNMENT
For you to qualify for this study, you will require two visits to the study site (Department of Nutrition, Food & Exercise Sciences, Sandels Building, FSU). On the first visit, the study coordinator, Shirin Hooshmand, will provide you with a verbal and written explanation of the project and will answer any questions regarding the study. At this time, you will be assured your participation is completely voluntary. You will then be asked to sign an informed consent form. A copy of the signed consent form will be made available to you. Secondly, a detailed medical history will be taken by the study coordinator to confirm your prescreening findings and to assure that you do not have any of the conditions violating the inclusion/exclusion criteria (guide for including/excluding you in the study). If you have histories of chronic diseases such as liver, kidney or cancer, you will be excluded (not allowed) from participating in the study. If all the conditions are satisfied, the third step will involve the assessment and analysis of your regional and whole body bone mineral density (BMD; a measure of bone strength) including lumbar spine (L1-L4) BMD using dual energy absorptiometry (DXA; a standard instrument used to measure bone strength). At the same visit, you will be instructed as to the collection of a 24-hour urine sample a day before the second scheduled visit.

Updated 05/08/07

Patient Initial
Effects of Dried Fruits on reversing Bone Loss in Postmenopausal Women

On the second visit, between the hours of 8-10 a.m., you will be asked to come to the study center and 1) bring in your 24-hour urine sample; 2) provide a fasting blood sample (20 ml venous blood drawn by a trained personnel which is approximately 4 teaspoons; you will be asked not to eat or drink anything other than water for 12 hours before your visit); 3) and anthropometric measurements such as your height and weight will be obtained. After you meet all the inclusion criteria, you will be assigned to treatment groups by using a pre-generated randomization list (a method used to assure that the chance of you receiving either of the supplement is 1 to 2). Finally, your dietary history, physical activity level, and menopausal symptoms will be assessed using validated questionnaires (a form containing questions used to collect relevant information).

DESCRIPTION OF THE STUDY

You will be asked to take 100g dried fruits, dried plum (100 g; 10-12 nos) or equivalent amount of dried apple (75g; 10-12 slices of dried apples), daily for a period of one year. You may eat your dried fruit regimens in small portions throughout the day, cooked or uncooked. During the course of the study, you will be asked to fill out the diaries provided to you for consumption of dried fruit and calcium/vitamin D. You take the study regimens daily and return any unused regimens to the study staff on your next scheduled visit to Nutrition, Food & Exercise Sciences at FSU in Tallahassee. You will also complete certain questionnaires such as food frequency, medical and diet history and physical activity at the beginning of the study, 3- and 6-months and at the end of the study (one year). Additionally you will give 20 ml (approximately 4 teaspoons of blood) and 24-hr urine four times during the study.

You agree to answer questions about your medical, menopausal, diet, and physical activity histories. Throughout the study you will also be asked about any bad experiences with the study regimens.

You agree to have 20 ml, which is approximately 4 teaspoons of your blood drawn **four times** by trained personnel. You will give this amount of blood at the beginning, 3- and 6-months and at the end of the study. You know that you must not consume food for 12 hours before each blood collection, but you may drink water. You will be provided with a light breakfast after the blood collection.

COSTS

There will be no cost to you for the examinations performed in this research study. Travel and transportation costs such as bus or taxi fares, gasoline, and mileage to and from the study site will be your responsibility.

If you develop health problems related to the study, your problems will be discussed by Dr. Arjmandi with the consulting gynecologist on the study. Dr. Arjmandi will then decide whether to exclude you from the study in which case you will be referred to the physician of your choice at your own cost, or to continue with the study. It would be your responsibility to seek additional health-related advice/follow-up examinations.

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Patient Initial
RISKS

As dried plums and dried apples are natural food compounds recognized for human consumption, we expect no or minimal side effects. If there is any new and significant information which might change your decision to remain in the study, you will be made aware of this. To minimize the stomach problems such as bloating and diarrhea, you will be advised to slowly increase the amount of the supplements in about a week or 10 days. You should contact Dr. Arjmandi if you have any stomach problem such as bloating and diarrhea. To minimize bruising during the blood draw, a well trained and certified person (phlebotomist) will be strictly follow the procedure for blood draw. You understand that you are exposed to minimal radiation during bone scan and this amount of radiation has no health risk. By comparison, it is 60 times lower than the radiation that you are exposed to naturally (natural background radiation) per year. Additionally, the 24 hr collection of urine may cause embarrassment and may be inconvenient. You will be provided with a brown bag to carry your urine collection container to minimize embarrassment. The calcium and vitamin supplements provided in this study are half of the amount required for one day and are not known to cause any side effects. However, if you are taking any medications that are known to be affected by either calcium or vitamin D, you will take them in a way that will minimize the interaction.

BENEFITS OF PARTICIPATION

As a part of this research study, you will receive information regarding your bone status, blood profiles, and information on weight and height. Dried plum and apple have been shown to lower blood cholesterol. Subjects receiving dried plum may see an increase in bone mass. Additionally, the society at large will benefit from the findings of this study. It will provide some insights as to the beneficial effects of naturally occurring components in dried fruits in preserving or increasing bone mass. This study will confirm whether dried fruits exert a beneficial effect on bone density. If our results are positive, this study will provide a base for further exploring a safe, practical and effective alternative or supplement to prescription drugs in maintaining or increasing bone mass in a very vulnerable section of the population.

COMPENSATION AND INJURY

You will receive an honorarium and incentives worth $100 paid in installments ($25 per each visit). You will also receive daily supplements of 500 mg calcium and 250 IU vitamin D free of charge.

If you develop health problems during the course of the study, Florida State University will not provide compensation and will not provide medical treatment without charge for any medical charges as a result of this research investigation.

SUBJECT ASSURANCES

You are being informed that:

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Effects of Dried Fruits on reversing Bone Loss in Postmenopausal Women

- Your participation in this study is voluntary.
- You may withdraw from this study at any time without penalty.
- You can't participate in this study if you do not sign the informed consent.
- You may be removed from the study for medical reasons that change during the course of the study or for not taking your fruit regimen or completing the calendars.
- You may not be enrolled if values measured from the blood that was drawn fall out of the acceptable ranges established for inclusion in the study or you fail to meet other inclusion/exclusion criteria.
- Your treatment by and relations with the researchers involved in this research study will not be affected now or in the future if you decide not to participate, or if you start the study and decide later to withdraw.
- You will not be giving up any of your legal rights.

To formally withdraw your authorization you should notify Dr. Arjmandi, the Principal Investigator of this research study. However, your blood and urine samples and bone scan that have already been obtained may be included for analysis in the study. Contact information is listed on the first page of this form. The investigator will ask you to sign a document with the date you asked to withdraw.

Your records from this study will be kept confidential and that you will not be identifiable by name or description in any reports or publications about this study. All the records will be kept in a locked cabinet in a locked room, room 426, SAN building and the data will be destroyed by 2012. Only authorized personnel will have access to your records. For laboratory tests and any paperwork your name will be removed and the records will be assigned a number.

The records can be looked at by the investigators, the sponsor of the study, and the Institutional Review Board members. If requested, only the information collected during the study will be provided to the above mentioned people. You have the right to revoke the consent or authorization at any time during the study, but the information already collected will be used.

If you have questions about this study or need to report any bad experiences from the research procedures you should contact Dr. Bahram H. Arjmandi at (850) 645-7169. If you have questions about your legal rights as a research participant, you may contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President for Research at (850) 644-8633. An Institutional Review Board is a group of people that review research studies and protects the rights of individuals involved in research. If you have any medical questions, we recommend that you contact your primary care physician.

Authorization:

I have read this consent document. I understand its contents, and freely agree to participate in this study under the conditions described. I will receive a copy of this consent form.

Updated 05/08/07

Patient Initial _____

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Effects of Dried Fruits on reversing Bone Loss in Postmenopausal Women

Research Participant: ___________________________ Date: ______________________

I certify that I have personally explained all elements of this form to the subject before requesting the subject to sign it.

____________________________________________ Date: ______________________

(Project Director or authorized representative)
APPENDIX B

MEDICAL HISTORY QUESTIONNAIRE

Subject ID: ____________________
Interviewer: ____________________    Date______________

HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

Age________ Height_______ Weight________ BMI__________

I. Medical History

A. Skeletal Health

Personal history of skeletal disorders:
1. Not known ________________
2. Yes: uncontrolled ________________
3. Yes: Medications ________________
4. Yes: Exercise program ________________
5. Yes: Modified diet ________________
6. Yes: Surgery ________________
7. Yes: Combined program ________________
Give details ______________________________________________________
Type of Medication(s)_____________________________________________
Current dosage ________________ Years taken _________________________
How does this condition affect your activity?

Family history of skeletal disorders:
1. None _____
2. One parent _____
3. Both parents _____
4. One close relative _____
5. More than one close relative _____

Relative(s) ______________________________________________
Comments: ______________________________________________

B. Cardiovascular Function

Personal history of cardiovascular disease:
1. Not known ________________
2. Yes: uncontrolled ________________
3. Yes: Medications ________________
4. Yes: Exercise program ________________
5. Yes: Modified diet ________________
6. Yes: Surgery ________________
7. Yes: Combined program ________________
Give details _______________________________________________________
Type of Medication(s)_______________________________________________
Current dosage _____________________ Years taken _______________________
How does this condition affect your activity?

Family history of cardiovascular disease:
1. None ______
2. One parent _____
3. Both parents _____
4. One close relative _____
5. More than one close relative _____
Relative(s) _______________________________________________________
Comments: _______________________________________________________

C. Hypertension
1. None known ________________
2. Yes: uncontrolled ________________
3. Yes: Medications ________________
4. Yes: Exercise program ________________
5. Yes: Modified diet ________________
6. Yes: combined program ________________
7. Most recent blood pressure ________________
Explain _________________________________________________________
Type of Medication(s)_______________________________________________
Current dosage _____________________ Years taken _______________________
Ever taken thiazide diuretics? ________________

D. Diabetes
1. No record or indication _____
2. In past, but not now _____
3. Yes, well controlled _____
4. Yes, not controlled _____
Explain _________________________________________________________
Type of Medication(s)_______________________________________________
Current dosage _____________________ Years taken _______________________
How does this condition affect your activity? _________________________

E. Gastrointestinal/Digestive Problems
1. No record or indication _____
2. In past, but not now _____
3. Yes, well controlled _____
4. Yes, not controlled _____
   Explain ____________________________________________________
Type of Medication(s)_________________________________________
Current dosage _____________________Years taken________________
Ever taken steroids (i.e., prednisone)? __________________________
Currently taking antacids? _____________________________________
How does this condition affect your activity? ________________

F. Liver Disease/Problems
1. No record or indication _____
2. In past, but not now _____
3. Yes, well controlled _____
4. Yes, not controlled _____
   Explain ____________________________________________________
Type of Medication(s)_________________________________________
Current dosage _____________________Years taken________________
How does this condition affect your activity? __________________

G. Respiratory Problems
1. No record or indication _____
2. In past, but not now _____
3. Yes, well controlled _____
4. Yes, not controlled _____
   Explain ____________________________________________________
Type of Medication(s)_________________________________________
Current dosage _____________________Years taken________________
How does this condition affect your activity? __________________

H. Thyroid Disorder
1. No record or indication _____
2. In past, but not now _____
   Hyper? _____  Hypo? __
   Explain ____________________________________________________
Type of Medication(s)_________________________________________
Ever taken thyroid hormones (i.e., Synthroid)? _____

I. Reproductive history:  LMP__________, No. of children__________

J. Kidney Diseases
1. No record or indication _____
2. In past, but not now _____
3. Yes, well controlled _____
4. Yes, not controlled _____
   Explain ____________________________________________________
Type of Medication(s)_________________________________________
Current dosage _____________________Years taken________________
How does this condition affect your activity? ____________________
**K. Cancer**
1. No record or indication _____
2. In past, but not now _____
3. Yes, well controlled _____
4. Yes, not controlled _____

Explain ____________________________________________________

Type of Medication(s) _______________________________________

Current dosage _____________________ Years taken________________

How does this condition affect your activity? _____________________

**II. Medication or Drug Use**

A. *Previous or Present Use of Any of the Following (Specify):*
   1. Anabolic steroids _____________
   2. Corticosteroids or glucocorticoids _____ prednisone _____
   3. Thiazide diuretics _____ Other diuretics _____
   4. Vitamin D
   5. Medications for bone:
      - Fosamax: __________________________
      - Evista (Roxlopholin): _______________
      - Miacalcin (Calcitonin): ______________
      - Teriparatide (Forteo):_______________
   6. Others:
      __________________________________
      __________________________________
      __________________________________

B. *Previous or Present Use of Alcoholic Beverages (Beer, wine, hard liquor).*
   Please indicate:
   - Frequency of intake (Times/week or times/month): _____
   - Number of servings at a sitting: _____
   - Number of years of use: _____

C. *Estrogen or Hormone Replacement (ERT or HRT)*
   1. Never ___ move along to section D.
   2. Yes, in past ___ at what age? ___ & How long _____

D. *Currently /previously a smoker?_____* If yes, number of cigarettes per day___________

**III. Physical Activity**

A. *Occupational Intensity (respond to 1, 2, 3, or 4):*
   1. Majority of time: Sitting ____ Standing ____ Walking ____
   2. Equal amount of time: Sitting and Standing ____
      Walking and Sitting ___
      Standing and Walking ___
   3. Combination: Sitting, Standing, and Walking ___
   4. Much of time: Lifting & Carrying ___
APPENDIX C

NUTRITION HISTORY QUESTIONNAIRE

Subject ID:_________________
Interviewer:_________________

Date:____________________

NUTRITION HISTORY

Modified/Specialized Diet(s) Followed:_____________________________________________________

Recommended By________________ Length of time Followed_____________________

Recent Changes in Appetite?________________ Due to_________________________________________

Foods Avoided?________________________ Due to_________________________________________

History of Problems Digesting Milk?________ When?_______ How Long?_____________________

Diagnosis of Lactose Intolerance?________ When?_______ By whom? __________

Intake of Milk/Dairy Products & Calcium-Containing Foods (No Times/Day, Wk or Month, Portion
Size):

Milk_______________ Yogurt______________ Frozen Yogurt____________________

Ice Cream/Milk_________ Pudding/Custard________ Hard Cheese_____________________

Mixed Dishes with Cheese_______ Soft Cheese_________ Grated Cheese_______________

Donuts/Cakes/Cookies_________ Eggs______________ Dark Breads___________________

How has this intake changed during that past three years?

Intake of High Fiber Foods (Specify Type, No Times/Day, Week or Month, Portion Size):

Whole Grain Products: Breads___________________ Bran (wheat, oat)__________________

41
Cereals___________ Crackers___________ Grains (i.e., popcorn)___________
Nuts_________________ Seeds_________________ Fruits_________________ Dried
Fruits (prunes/dried apples)__________ Vegetables__________________________ Beans/Legumes (i.e., chili)___________

How has this intake changed during the past three years?

**Nutritional Supplements** (Type, Dose, Times/Week, No Years):

Vitamin Supplements___________________________________________________________
Mineral Supplements___________________________________________________________
Vitamin/Mineral Supplements____________________________________________________
Other Supplements (e.g., cod liver oil, protein powder)________________________________

**DAILY INTAKE** *(Typical weekday)*

<table>
<thead>
<tr>
<th>Time</th>
<th>Food/Description</th>
<th>Serving Size</th>
<th>Food Code</th>
</tr>
</thead>
</table>

*Sodium Intake* (amount of salt used (tsp)/day): in cooking_________ at the table_________ Estimate of Na⁺ Intake/Day: Salt Shaker___________ High Na⁺ foods:_______________

**Beverages Consumed** (servings per day):

Coffee:  reg_________ decaf_________ Cocoa:_______ Tea:  reg_____ decaf_____
Soda:  reg (+caf)_______ diet(+caf)_______ reg (caf free)_______ diet (caf free)_____
Milk (oz/day):  reg (3.5%)_________ low fat (2%)___________ skim(1% or<1%)_________

During what period(s) of life have you been a milk drinker? As a(n):

___5. mid-life adult (40-59)  ____6. Older adult (60+)  ____7. Never

If you excluded milk during any part of your life, was (is) this due to digestive problems?

(Explain):_______________________________________________________________
Milk consumed/day: child_____ teenager_____ young adult (20-29)_____ adult (30-39)_____ mid-life adult (40-59)_____ older adult (60+)_____. Juice (type): ________________ Calcium fortified Juice: ________________ Other:___________

Alcoholic Beverages:________ If yes, please specify:

<table>
<thead>
<tr>
<th>Type</th>
<th>Times/week or Mo</th>
<th>Serving Size</th>
<th>No Servings</th>
<th>Total/Week or Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer:</td>
<td>Reg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine:</td>
<td>White</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Red</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rose (Blush)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed Drink (specify):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (specify):</td>
<td></td>
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</tr>
</tbody>
</table>

How did your food or beverage intake differ from the above during your younger years (teenager and young adulthood)?
APPENDIX D

FOOD FREQUENCY QUESTIONNAIRE

Subject Number_________    Date_________

Vitamin and Mineral Supplement

1. Do you take any vitamin or mineral supplement(s)? Yes ___No _____

2. If Yes, please, list all names of vitamin or mineral supplements, and how often do you take the supplement(s)?

   Name ______________________  How often ____ per day Or ____ per week
   Name ______________________  How often ____ per day Or ____ per week
   Name ______________________  How often ____ per day Or ____ per week
   Name ______________________  How often ____ per day Or ____ per week
   Name ______________________  How often ____ per day Or ____ per week
Seven Day Food Frequency Questionnaire

This questionnaire asks you about your consumption of foods and beverages over the past week, which includes the time from exactly one week ago until the last meal you had before you fill out this questionnaire. The “How Often” columns are for day, week, or rarely/never. We want you to think back over the past week and tell us how many times (per day, if you consume the item every day, or per week) you consumed each item. A medium serving is in parentheses.

EXAMPLES:
Ate 1/2 grapefruit about twice last week.
Ate 1 large hamburger four times last week.
Drank 2 cups of whole milk each day.

<table>
<thead>
<tr>
<th>Type of Food (Medium Serving)</th>
<th>How Often</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td>Grapefruit (1/2)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hamburger, regular (1 patty, 3 oz)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Whole milk (1 cup, 8 oz)</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of Food (Medium Serving)</th>
<th>How Often</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td>DAIRY FOODS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole milk (1 cup, 8 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% milk (1 cup, 8 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim milk (1 cup, 8 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream, whipped (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour cream (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee cream (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice cream (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat ice cream (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen yogurt (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yogurt (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat yogurt (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottage cheese (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream cheese (1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat cream cheese (1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other cheese (1 slice or 1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat cheese (1 slice or 1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine (1 tsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter (1 tsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced fat margarine (1 tsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of Food</td>
<td>How Often</td>
<td>Size</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td>FRUITS, FRUIT JUICES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raisins (1 oz or 1 sm box)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapes (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prunes (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bananas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cantaloupe (¼ melon)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watermelon (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apples, applesauce or pears (1 fresh, ½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried Apples (1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple juice (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oranges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapefruit (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapefruit juice (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other fruit juices (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberries—fresh, frozen, or canned (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blueberries—fresh, frozen, or canned (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEACHES (1 fresh, ½ cup canned)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APRICOTS (1 fresh, ½ cup canned)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLUMS (1 fresh, ½ cup canned)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HONEYDEW MELON (¼ melon)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGETABLES, VEGETABLE JUICE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato juice (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato sauce (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti sauce (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red chili sauce, taco sauce, or salsa (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOFU OR SOYBEANS (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STRING BEANS, GREEN BEANS (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BROCCOLI (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABBAGE (½ cup)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Type of Food</th>
<th>How Often</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Medium Serving)</td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td><strong>EGGS, MEAT, ETC.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken or turkey, roasted or broiled with skin (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken or turkey, roasted or broiled skinless (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken, fried with skin (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacon (2 slices)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot dogs (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat hot dogs (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sausage (2 patties or 2 links)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bologna (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other processed luncheon meat (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver, chicken or beef (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamburger, regular (1 patty, 3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamburger, lean (1 patty, 3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat loaf (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork, chops, roasts (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb (3-4 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Item</td>
<td>Portion Size</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>Beef, roast, steak</td>
<td>(3-4 oz)</td>
<td></td>
</tr>
<tr>
<td>Beef stew with vegetables</td>
<td>(1 cup)</td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td>(3-4 oz)</td>
<td></td>
</tr>
<tr>
<td>Tuna fish</td>
<td>(3-4 oz)</td>
<td></td>
</tr>
<tr>
<td>Tuna salad</td>
<td>(½ cup)</td>
<td></td>
</tr>
<tr>
<td>Fish, baked or broiled</td>
<td>(3-4 oz)</td>
<td></td>
</tr>
<tr>
<td>Fish, fried or fish sandwich</td>
<td>(3-4 oz)</td>
<td></td>
</tr>
<tr>
<td>Shrimp, Lobster, Scallops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pizza</td>
<td>(2 slices)</td>
<td></td>
</tr>
<tr>
<td>Mixed dishes with cheese</td>
<td>(1 cup)</td>
<td></td>
</tr>
<tr>
<td>Lasagna or meat pasta dishes</td>
<td>(1 cup)</td>
<td></td>
</tr>
<tr>
<td>Type of Food (Medium Serving)</td>
<td>How Often</td>
<td>Size</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td><strong>BREADS, CEREALS, STARCHES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold breakfast cereal (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold breakfast cereal—fortified (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked oatmeal (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other cooked breakfast cereal (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bread (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pita bread (1 piece)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark bread (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>English muffin (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bagel (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner roll (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamburger or hotdog bun (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muffin (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biscuit (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn bread, corn muffin (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown rice (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White rice (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti noodles (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni noodles (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other pasta noodles (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulgar, kasha, couscous (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancakes or waffles (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes, french fries or fried (½ cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes, baked or boiled (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mashed potatoes (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato chips or corn chips (small bag or 1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltine crackers (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltine crackers, low sodium (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltine crackers, fat free (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other crackers (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other crackers, low fat (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of Food (Medium Serving)</td>
<td>How Often</td>
<td>Size</td>
</tr>
<tr>
<td>------------------------------</td>
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<td>------</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td><strong>BEVERAGES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular soft drink (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet soft drink (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine free soft drink (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine free, Diet soft drink (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemonade or other non-carbonated drink (1 glass, bottle, or can)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decaffeinated coffee (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbal tea (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer (1 glass, bottle, or can)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red wine (4 oz glass)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White wine (4 oz glass)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whiskey, gin, or other liquor (1 drink or shot)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SWEETS, BAKED GOODS, MISC.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate (1 small bar or 1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candy bar (1 small bar)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candy without chocolate (1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cookies, home baked (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cookies, readymade (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brownies (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doughnuts (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cake, home baked (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cake, readymade (1 slice)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet roll, coffee cake, or other pastry readymade (1 serving)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet roll, coffee cake, or other pastry home baked (1 serving)</td>
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<td></td>
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<tr>
<td>Pie, homemade (1 slice)</td>
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<td></td>
</tr>
<tr>
<td>Pie, readymade (1 slice)</td>
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<td></td>
</tr>
<tr>
<td>Jam, jelly, preserves, syrup, or Honey (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut butter (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popcorn (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popcorn, air popped (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of Food</td>
<td>How Often</td>
<td>Size</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td></td>
<td>Day</td>
<td>Week</td>
</tr>
<tr>
<td>Nuts (small packet or 1 oz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bran, added to food (1 Tbsp)</td>
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<td></td>
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<tr>
<td>Wheat germ (1 Tbsp)</td>
<td></td>
<td></td>
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<tr>
<td>Chowder or cream soup (1 cup)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil and vinegar dressing (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayonnaise or other creamy salad dressing, Regular (1 Tbsp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayonnaise or other creamy salad dressing, Low Fat or Reduced Calorie, Lite (1 Tbsp)</td>
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</tr>
<tr>
<td>Mayonnaise or other creamy salad dressing, Fat Free (1 Tbsp)</td>
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<td></td>
</tr>
<tr>
<td>Mustard, dry or prepared (1 tsp)</td>
<td></td>
<td></td>
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<tr>
<td>Salt (1 shake)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepper (1 shake)</td>
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</tbody>
</table>

Can you think of any other food or drink that you had in the past week that was not on this form? If so, what was it? What was the amount? How many times did you have it this past week?

Food________________________________________________________

Amount______________, How often per day______________, per week__________

Food________________________________________________________

Amount______________, How often per day______________, per week__________

Food________________________________________________________

Amount______________, How often per day______________, per week__________

Food________________________________________________________

Amount______________, How often per day______________, per week__________
APPENDIX E

PHYSICAL ACTIVITY QUESTIONNAIRE

Subject ID: ________________________    Interviewer: ________________
Date: ______________________________

Physical Activity Recall Items

First we would like to know about your physical activity during the past 7 days. But first, let me ask you about your sleep habits.

1. On the average, how many hours did you sleep each night during the last five weekday nights (Sunday-Thursday)? ______hours

2. On the average, how many hours did you sleep each night last Friday and Saturday nights? _____hours

Now I am going to ask you about your physical activity during the past 7 days, that is, the last 5 weekdays and last weekend, Saturday and Sunday.

We are not going to talk about light activities such as slow walking, light housework, or non-strenuous sports such as bowling, archery, or softball.

Please look at this list which shows some examples of what we consider moderate, hard, and very hard activities. (interviewer: hand subject the following card and allow time for the subject to read it over.)

People engage in many other types of activities, and if you are not sure where one of your activities fits, please ask me about it.

3. First, let’s consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them?

   Please tell me to the nearest half-hour. _____hours

4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do?

   (Probe: Can you think of any other sports, job, or household activities that would fit into this category?)
   _____hours
5. Now, let’s look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them?

Please tell me to the nearest half-hour. _____ hours

6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do?
(Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

_________ hours

7. Now, let’s look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them?

Please tell me to the nearest half-hour.

(Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

_________ hours

8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do?

(Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

_________ hours

9. Compared with your physical activity over the past 3 months, was last week’s physical activity more, or less, or about the same?

_____ 1. More

_____ 2. Less

_____ 3. About the same

Interviewer: Please list below any activities reported by the subject, which you don’t know how to classify. Flag this record for review and completion.

<table>
<thead>
<tr>
<th>Activity (brief description)</th>
<th>Hours: workday</th>
<th>Hours: weekend day</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
### EXAMPLES OF ACTIVITIES IN EACH CATEGORY

#### Moderate activity (3-5 mets)

**Occupational Tasks:**
1. Delivering mail or patrolling on foot
2. House painting
3. Truck driving (making deliveries, lifting/carrying light objects)

**Household Activities:**
1. Sweeping, mopping, cleaning windows
2. Mowing the lawn with a power mower
3. Raking the lawn and yardwork
4. Light carpentry

**Sports:**
1. Table tennis or Ping-Pong
2. Softball, baseball
3. Volleyball
4. Dancing: folk, square, aerobics (low impact & intensity)
5. Brisk walking (3 to 4 mile/hr; 15-20 min/mile)
6. Bicycling on level ground (10-15 mile/hr)
7. Golfing (walking and pulling/carrying own clubs)

#### Hard activity (5.1 – 6.9 METS)

**Occupational Tasks:**
1. Heavy carpentry
2. Construction work

**Household Tasks:**
1. Scrubbing floors
2. Shoveling snow
3. Moving (lifting furniture and boxes)

**Sports:**
1. Racket Sports: badminton, paddleball, tennis (double)
2. Basketball
3. Rowing or canoeing leisurely
4. Dancing: disco, jazz, aerobics (medium impact & intensity)
5. Power walking (>mile/hr; <15 min/mile) or hiking
6. Vigorous bicycling (16 – 20 mile/hr)
7. Jogging (≥5 mile/hr)
8. Swimming
9. Roller or ice skating
10. Stationary bicycling

#### Very hard activity (≥7.0 METS)

**Occupational Tasks:**
1. Digging or chopping with heavy tools
2. Carrying heavy loads, such as bricks or lumber

**Sports**
1. Racket Sports: handball, racketball, squash, tennis (singles)
2. Soccer
3. Snow skiing (down hill and cross country)
4. Dancing: aerobics (high impact & intensity)
5. Jumping rope
6. Vigorous bicycling on hills
7. Jogging or running (≥8 mile/hr)
### PHYSICAL ACTIVITY RECALL

1. _____ HOURS week-day sleep 
   Total (sum of #1 and #2) _____

2. _____ HOURS week-end sleep 

3. _____ HOURS week-day moderate/5 = ______

4. _____ HOURS week-end moderate/2 = ______ 
   Average

5. _____ HOURS week-day hard/5 = ______

6. _____ HOURS week-end hard/2 = ______ 
   Average

7. _____ HOURS week-day very hard/5 = ______

8. _____ HOURS week-end very hard/2 = ______ 
   Average

9. 1 _____ 2 _____ 3 _____

1. Total sleep hours _____ x 1 = ______

2. Average = hours moderate act. ______ x 4 = ______

3. Average = hours hard act. ______ x 6 = ______

4. Average = hours very hard act. ______ x 10 = ______

5. Sum of hours _____ - 24 _____ x 1.5 = ______

10. Sum of 1,2,3,4, & 5 

   [Box]

   [Box]
REFERENCES


15. Anastasilakis AD, Goulis DG, Polyzos SA et al. Serum osteoprotegerin and RANKL are not specifically altered in women with postmenopausal osteoporosis treated with teriparadate or risedronate: a randomized, controlled trial. Horm Metab Res 2008;40:281-5.


22. Lacey DL, Timms E, Tan HL et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165-76.


BIORGRAPHICAL SKETCH

EDUCATION

Present  
**PhD candidate** in Human Nutrition, Department of Nutrition, Food & Exercise Sciences, College of Human Sciences, Florida State University, FL.

2004-2006  
**Master’s of Science** in Human Nutrition, Department of Nutritional Sciences, College of Human Environmental Sciences, Oklahoma State University, OK.  
**Thesis Title:** Genistein Reduces Production of Proinflammatory Molecules in Human Chondrocytes

1999-2004  
**Bachelor’s of Sciences** in Nutritional Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.  
**Thesis Title:** Nutritional status of a randomized group in a nursing home in Tehran

1992-1999  
National Organization for Development of Exceptional Talents (NODET), Farzanegan High School and Middle School, Tehran, Iran. Diploma in Experimental Sciences

TEACHING EXPERIENCE

2008-2010  
**Instructor**, Metabolism II (HUN 3206); Department of Nutrition, Food & Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, FL

2006  
**Guest lecturer**, Sciences of Nutrition (HUN 1201); Topics covered: Metabolism of carbohydrate, protein, fat and alcohol, Energy balance and weight management. (5 sessions). Department of Nutrition, Food & Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, FL

2006  
**Guest lecturer**, Principle of Human Nutrition (NSCI 2114); Topics Covered: Water and body fluid balance, Nutrition and elderly (2 sessions). Department of Nutritional Sciences, College of Human Environmental Sciences; Oklahoma State University, Stillwater, OK

2006  
**Discussion instructor**, Principle of Human Nutrition (NSCI 2114); Department of Nutritional Sciences, College of Human Environmental Sciences; Oklahoma State University, Stillwater, OK

2005  
**Teaching Assistant**, Nutrition and Health Issue (NSCI 4023); Department of Nutritional Sciences, College of Human Environmental Sciences; Oklahoma State University, Stillwater, OK

1999-2004  
**Instructor**, Computer, Farzanegan Middle School, National Organization for Development of Exceptional Talents

RESEARCH EXPERIENCE

2006-2010  
**Graduate Research Assistant**, Department of Nutrition, Food & Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, FL
2007-2009  **Clinical Trial Study Coordinator**, Department of Nutrition, Food & Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, FL

2004-2006  **Graduate Research Assistant**, Department of Nutritional Sciences, College of Human Environmental Sciences, Oklahoma State University, Stillwater, OK.

**Undergraduate Students trained as Niblack, Science Prep Scholars and Honors in the Major:**

*Jenna Schmid*, as undergraduate mentee from the Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL (2009-2010)

*Gabreol Bryan*, as undergraduate mentee from the Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL (2006-2007)

*Mary Whiteneck*, an undergraduate advisee from the Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK (2005-2006)

**PROFESSIONAL SOCIETIES**

- American Society for Bone and Mineral Research
- American Society for Nutritional Sciences
- Kappa Omicron Nu Honor Society

**TRAINING**

- **Aug 2008**  International Training Program on Natural Products: Botanicals, Nutraceuticals and Medicinal and Aromatic, New Use Agriculture & Natural Plant Products Program, Rutgers University, New Jersey, NJ. (Dr. Simon)

- **Jan-March 2005**  Training in Flow Cytometry and 3-dimensional cell culture techniques (3 credit hours), Department of Chemical Engineering, Oklahoma State University, Stillwater, OK. (Dr. Madihally)

- **Jan 2005**  Training in MALDI-TOF Mass Spectrometry and Proteomics (1 credit hour), Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Ok. (Dr. Hartson)

- **Oct-Dec 2004**  Training in Molecular Biology techniques, Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK. (Dr. Soung)

**AWARD AND HONORS**

- **2010**  Margaret Rector Sandels Scholarship, College of Human Sciences, Florida State University, $300
- **2009**  Graduate Women in Science Fellowship, GWIS, $8,333
- **2009**  Graduate Research and Creativity Award, Florida State University, $500
- **2009**  Natholyn D. Harris Scholarship, College of Human Sciences, Florida State University, $500
- **2009**  First Place winner in oral presentation, Research and Creativity day, College of Human Sciences, Florida State University, $200
2009  Graduate Scholar Award, Phi Kappa Phi Honor Society, Florida State University, $750
2008  College of Human Sciences Dissertation Award, Florida State University, $500
2008  Winner of NIH Training Award for Botanical and Medicinal Plant. One of the four winners among the faculty, postdoctoral fellows and predoctoral students Nationwide, $1000
2008  Member of Glen Society, College of Human Sciences, Florida State University
2008  Florence Smith-McAllister Fellowship, College of Human Sciences, Florida State University, $5000
2008  Featured at FSU homepage (Student Profile)  http://www.fsu.edu/students/profiles/archive.html
2007  Winner of Young Investigators’ Travel Award, American Society of Bone and Mineral Research, $400
2007  Ann Marie Erdman Scholarship, Department of Nutrition, Food and Exercise Sciences, Florida State University, $1000
2006  Jean Shipman Scholarship, College of Human Environmental Sciences, Oklahoma State University, $1000

PRESENTATIONS

National

July 2007  Dried plum reverses bone loss in postmenopausal women, United States Department of Agriculture/ National Research Initiative, Washington D.C.

Local

March 2009  Dried plum reverses bone loss in postmenopausal women. Research and Creativity Day, Florida State University, Tallahassee, FL
Feb 2009   Is obesity a risk factor for osteoarthritis? Human Sciences Research and Creativity Day, Florida State University, Tallahassee, FL
Feb 2008   The role of estrogen and estrogen-like compounds in osteoarthritis, Human Sciences Research and Creativity Day, Florida State University, Tallahassee, FL
Feb 2007   The role of naturally occurring compounds in the treatment of osteoporosis, Invited Speaker, College of Human Sciences, Florida State University, Tallahassee, FL
Sept 2007  Osteoporosis: The role of functional food in preventing bone loss or rebuilding bone. Invited speaker, Department of Nutrition, Food & Exercise Sciences, Florida State University, Tallahassee, FL

Community

Jan 2007   Role of nutrition and exercise in lowering cholesterol. FECA, Tallahassee, FL.
Oct 2007   Equality of women and men. Tallahassee Community, Tallahassee, FL


ABSTRACTS


**SERVICE**

Oct 2010  
Dissertation Award Program Committee, College of Human Sciences, Florida State University, Tallahassee, FL.

Nov 2009  
Graduate Policy Committee, Florida State University, Tallahassee, FL.

Oct 2009  
Dissertation Award Program Committee, College of Human Sciences, Florida State University, Tallahassee, FL.

April 2008  
Dissertation Award Program Committee, College of Human Sciences, Florida State University, Tallahassee, FL.

Nov 2007  
Graduate Showcase panel member, College of Human Sciences, Florida State University, Tallahassee, FL.