Australian Journal of Basic and Applied Sciences, 3(2): 970-976, 2009
ISSN 1991-8178

The Protective Effect of Nondigestible Oligosaccharides from Chicory Roots and Phyto Soya Extract on Osteoporosis in Rats.

[1]Ibrahim H. Borai, [2]Hanaa A. Wafay, [1]Abdel-Rahman B. Abdel-Ghaffar, [2]Fatma S. Oraby, [2]Sherien M. El-Daly

[1]Department of Biochemistry, Faculty of Science, Ain Shams University
[2]Department of Medical Biochemistry, National Research Centre

Abstract: Osteoporosis is a serious disorder of the skeleton. Postmenopausal women are at increased risk of osteoporosis because of the decline in estrogen production that occurs following menopause. The objective of the present study was to determine the protective effect of nondigestible oligosaccharides (NDOs) from chicory roots and phyto soya extract rich with isoflavones whether using alone or in combination on the prevention of osteoporosis using ovariectomized (OVX) rats as model of postmenopausal women. Sixty female albino rats were subjected to either; Bilateral ovariectomized surgery (OVX, n=48), or sham operated surgery (Sham, n= 12), and then assigned to five groups of 12 rats each; {sham-operated control, OVX control, OVX supplemented with 5% Raftilose®Synergy1, OVX supplemented orally with a dose of 30.6 mg phyto soya extract/rat/day and OVX supplemented with both 5% Raftilose®Synergy1 plus 30.6 mg phyto soya extract/rat/day}. All rats fed a casein based diet (AIN-93M) for 12 weeks. At the end of the experiment urine, blood and femur were sampled to investigate; serum calcium, phosphorus and magnesium, bone turnover markers (serum osteocalcin, serum total alkaline phosphatase and urinary deoxypyridinoline) and femoral BMD. The results of the study revealed that the significant reduction in serum mineral concentrations observed in the OVX control group compared to sham group as a result of estrogen deficiency were improved by supplementing the rats with Raftilose®Synergy1 and/or phyto soya extract compared to OVX control rats. Ovariectomy was found to elevate the rate of bone turnover as indicated by the higher levels of bone turnover markers in OVX control group comparing to sham group and as a result femoral BMD was reduced, but in the three supplemented OVX groups the elevation in the levels of bone turnover markers were significantly reduced comparing to OVX control group and this was followed by an improvement in the femoral BMD. The reduction of bone turnover markers and the improvement in femoral BMD was found to be higher in the OVX group received a combination of the two supplements than using each supplement alone. These results suggest that supplementation with NDOs or phyto soya extract rich with isoflavone prevent the bone loss which might occur as a result of the decline in estrogen production that occurs following menopause. The results may also suggest that a combination of these supplements may have an additive and cooperative effect on the prevention of bone loss.

Key words: osteoporosis, ovariectomized rats, nondigestible oligosaccharides, phyto soya extract, isoflavones.

INTRODUCTION

Osteoporosis is a major worldwide public health problem. Osteoporosis has been defined as a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to bone fragility and an increased susceptibility to fractures especially of the hip, spine and wrist (Kanis *et al.,* 2008).

Evidence from clinical trials suggests that an increase in minerals balance -especially calcium- will positively affect bone mass but unfortunately, the solution is not as straightforward as simply consuming more calcium because the percentage absorption is inversely related to intake so that increasing calcium intake may be partially negated by a corresponding decrease in the efficiency of calcium absorption (Roberfroid, 2005). An important mechanism to increase fractional calcium absorption may be through the consumption of

Corresponding Author: Hanaa A. Wafay, Department of Medical Biochemistry, National Research Centre, Cairo Egypt

nondigestible oligosaccharides (NDOs) such as the oligofructose- enriched inulin (Raftilose®Synergy1). The nondigestible oligosaccharides, inulin and its hydrolysate oligofructose, are present in many plants and are extracted commercially from chicory roots (Griffin *et al.,* 2003). The fermentation of NDOs in the large intestine enhance mineral absorption so, when NDOs added to a diet they cause an increase whole body mineral retention and bone mineral accumulation, this in turn is thought to be preferable in preventing osteoporosis (Bosscher *et al.,* 2006).

The consumption of plant-based foods rich in "phytoestrogens," more specifically the isoflavones and their derivatives, may provide an alternative to traditional hormone replacement therapy (HRT). Isoflavones are naturally occurring phytoestrogens that are found in soybeans and are structurally and functionally comparable to 17β-estradiol, exhibiting similar estrogenic action by binding to the estrogen receptors (Brouns, 2002). Soy isoflavones (genistein, daidzein and glycitein) have been characterized as naturally occurring selective estrogen receptor modulators (SERMs) as they can exert the beneficial effects of estrogen without its side effects (Setchell, 2001). Epidemiological studies indicate a lower incidence of osteoporosis in postmenopausal women who consume diets rich in isoflavones than those not consuming isoflavones (Scheiber *et al.,* 2001).

After ingestion, soy isoflavone glycosides are hydrolyzed to their absorbed form aglycones by the bacterial intestinal glucosidases (bifidobacteria and lactobacilli). Fermentation of nondigestible oligosaccharides in the large intestine stimulates the growth of these bacteria thus, it can be concluded that NDOs and soy isoflavones have cooperative effect in the prevention of bone loss in OVX rats (Mathey *et al.,* 2004). Uehara *et al.,* (2001) reported that NDOs improve the bioavailability of genistein and daidzein in rats given isoflavones conjugates. So the combination of dietary NDOs and soy isoflavones may be more efficient than either alone in the prevention of bone loss in osteoporosis (Ohta *et al.,* 2002). Therefore, the present study was designed to evaluate the protective effect of the NDO (Raftilose®Synergy1) from chicory roots and phyto soya extract rich with isoflavones, whether using alone or in combination, on the prevention of osteoporosis.

MATERIAL AND METHODS

Animals and Diets:

Sixty (60) adult female albino rats weight (160 g – 194 g) were purchased from the animal Laboratory of The National Research Centre, Giza, Egypt. Rats were housed individually at 21°C in metallic cages with free access to water. All Rats were anesthetized by exposure to diethyl ether and subjected to either; Bilateral ovariectomized surgery (OVX, n=48), or sham operated surgery (Sham, n= 12), and then assigned to five groups of 12 rats each; {sham-operated control, OVX control, OVX supplemented with 5% NDO (Raftilose® Synergy1) in the diet, OVX supplemented orally with a dose of 30.6 mg phyto soya extract/rat/day and OVX supplemented with both 5% Raftilose® Synergy1 plus 30.6 mg phyto soya extract/rat/day}. Rats were fed a casein based diet prepared according to the (AIN-93M) diet (Reeves *et al.,* 1993) for 12 weeks.

Table 1: Composition of casein based diet (AIN-93M diet)

Ingredients	Casein based diet (g/kg diet)
Cornstarch	620.692
Casein (≥85% protein)	140
Sucrose	100
*Corn oil	40
Fiber	50
**Mineral mixture (AIN-93M-MX)	35
**Vitamin mixture (AIN-93M-VX)	10
L-cystine	1.8
Choline chloride	2.5
Ter-bytylhydroquinone	0.008

*Corn oil was used instead of soybean oil to eliminate any possible interference with isoflavones in soybean oil. **Mineral mixture and vitamin mixture were prepared according to AIN-93M formula (Reeves *et al.,* 1993).

The NDO (Raftilose®Synergy1) used in this study is an oligofructose-enriched inulin, it is a combination of long chains chicory inulin molecules, enriched by a specific fraction of short chains oligofructose produced by partial enzymatic hydrolysis of chicory inulin. It was purchased from Orafti Active Food Ingredients, Tienen, Belgium. The nondigestible oligosaccharide (Raftilose® Synergy1) was added at 50 g/kg diet by replacing an equal amount of cornstarch in the casein based diet (AIN-93M) (Takahara *et al.,* 2000 and Zafar *et al.,* 2004).

Natural Phyto soya extract was purchased from Arkopharma, Laboratories Pharmaceutiques, France. This natural phyto soya extract is rich with isoflavones (daidzein and genistein) 41.08 mg/gm soya extract. The chosen dose used in this experiment for each rat was 30.6 mg/day. This dose was calculated according to the human dose recommended.

Analysis:

Urine sampling: At the end of the 12 weeks of dietary feeding and on the day before necropsy, 24 hours urine sample were collected from each rat and then centrifuged for the determination of:

- Urinary deoxypyridinoline (DPD), as a marker of bone resorption, by a competitive enzyme immunoassay in a microtiter stripwell plate utilizing a monoclonal anti-DPD antibody coated on the strip to capture DPD (Metra DPD, Quidel Corporation, San Diego, USA).
- Urinary creatinine which was measured colorimetrically using commercially available kit (Stanbio Creatinine Procedure No. 0400, Stanbio Laboratory, Boerne, TX, USA) to adjust the DPD values, this was done to eliminate errors due to the difference in renal function of individual rats (Mathey *et al*., 2004).

Blood sampling: At necropsy, all rats were anesthetized by exposure to diethyl ether and blood samples were collected from the retro-orbital sinus. Serum was separated by centrifugation at 3000 rpm for 10 minutes at 25°C for the following analysis:

- Measuring serum calcium, phosphorus and magnesium colorimetrically using commercially available Kits (Fluitest Cat. # 1903, Elitech Cat. # 0600 and REF Cat. # MG358) respectively.
- Measuring serum osteocalcin (OC), as a marker of bone formation, by Sandwich Enzyme Linked Immunosorbent assay using rat ^{125}I-labelled OC, goat anti-rat OC antibody, and donkey anti-goat second antibody (Biomedical Technologies, Stoughton, MA, USA).
- Measuring serum total alkaline phosphatase, as marker of bone formation, colorimetrically using a colorimetric kit (BioMérieux Vitec, Missouri, USA)

Femur sampling: Directly after the blood collection, right Femurs were excised and cleaned from soft tissues, and then stored in saline water for measuring the bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) equipped.

Statistical Analysis:

Statistical analyses were performed using the SPSS program, version 9.05 and Microsoft Excel 2003. Data were expressed as mean ± standard deviation (SD). Independent samples T-test was performed to determine the specific differences between means. The results are considered to be significant when P value is less than 0.05 and highly significant when P value is less than 0.001.

RESULTS AND DISCUSSION

Serum Calcium, Phosphorus and Magnesium:

Mean concentrations of the serum minerals calcium, phosphorus and magnesium were significantly decreased in the OVX control group compared to sham group. On the other hand, Supplementing the diet of the OVX rats with the NDO (Raftilose®Synergy1) alone had a significant positive effect in increasing the mean of serum calcium and magnesium, but not phosphorus, comparing to the OVX control rats. The mean concentrations of serum phosphorus and magnesium, but not calcium, were significantly influenced in OVX rats supplemented with the phyto soya extract alone comparing to the OVX control rats. On the other hand the combination group showed significant increase in the mean concentrations of the three minerals compared to the OVX control group (table 2).

Bone Turnover Markers:

In the present study, ovariectomy elevated the rate of bone turnover and this was reported by the significant increase in the levels of bone turnover markers (osteocalcin, total alkaline phosphatase and deoxypyridinoline) in OVX control group compaed to sham group, but this elevation was significantly reduced in the three supplemented OVX groups (NDO group, phyto soya extract group and NDO + phyto soya extract group) compared to the OVX control group and this reduction was much noticed in the combination group (table 3).

Bone Mineral Density (BMD):

Ovariectomy induced a decrease in femoral BMD in OVX control rats compared to sham group. But this reduction was successfully improved by the supplementations since it was found that the three supplemented OVX groups have significantly higher femoral BMD than the OVX control group, in addition this improvement was exacerbated by the combination of the two supplements (Figure 1).

Table 2: Serum calcium, phosphorus and magnesium concentrations in the different studied groups.

	Sham	OVX control	Supplemented OVX groups		
			NDO	Phyto soya extract	{NDO +Phyto soya extract}
Calcium (mg/dl)	9.7 ± 0.54	8.42 ± 0.34##	8.92 ± 0.45	8.59 ± 0.4	9.03 ± 0.25**
Phosphorus (mg/dl)	5.33 ± 0.8	4.39 ± 0.94#	5.01 ± 0.43	6.89 ± 0.74**	5.89 ± 0.89*
Magnesium (mg/dl)	2.11 ± 0.07	1.94 ± 0.03##	2.06 ± 0.05**	2.05 ± 0.04**	2.04 ± 0.04**

Values are means ± SD, n = 12.
= Significant at P< 0.05, # # = Significant at P< 0.001 comparing with sham group.
* = Significant at P< 0.05, ** = Significant at P< 0.001 comparing with OVX control group.

Table 3: Serum osteocalcin , Serum total alkaline phosphatase and urinary deoxypyridinoline levels in the different studied groups.

	Sham	OVX control	Supplemented OVX groups		
			NDO	Phyto soya extract	{NDO +Phyto soya extract}
Serum osteocalcin (ng/dl)	14.58 ± 1.24	19.77 ± 2.08##	14.74 ± 0.39**	12.93 ± 1.13**	11.33 ± 1.04**
Serum total alkaline phosphatase (U/l)	118.89 ± 7.12	248.39 ± 5.98##	116.65 ± 5.64**	106.7 ± 7.83**	98.32 ± 9.85**
Urinary deoxypyridinoline (nmol DPD/ mmol creatinine)	79.34 ± 6.86	313.88 ± 16.4##	142.64 ± 13.3**	148.97 ± 5.01**	132.24 ± 6.79**

Values are means ± SD, n = 12.
= Significant at P< 0.05, # # = Significant at P< 0.001 comparing with sham group.
* = Significant at P< 0.05, ** = Significant at P< 0.001 comparing with OVX control group

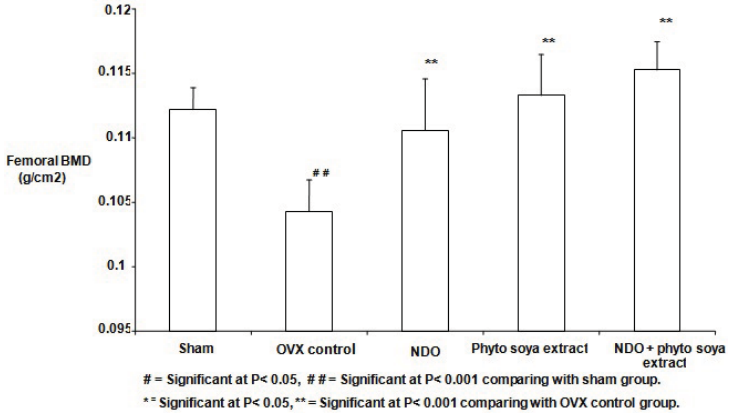

Fig. 1: Comparison of mean values of Femoral BMD between the different studied groups.

Discussion:

The results of the present study revealed significant decreases in mean serum calcium, phosphorus and magnesium in the OVX control rats compared to sham rats. This finding is in agreement with Draper *et al.,* (1999) who reported that osteoporotic women have decreased mineral absorption as a result of the reduction in serum 1,25-dihydroxyvitamin D [1,25-(OH)2D] which is known to be a regulator of bone mineral homeostasis. Estrogen plays an important role in the regulation of 1,25-(OH)2D levels, presumably through increasing its renal production, therefore estrogen deficiency is associated with a significant decline in serum mineral concentrations. Therefore, postmenopausal women could benefit the most from any agent that increases mineral absorption. Supplementing the diet of the OVX rats in this experiment with the NDO (Raftilose®Synergy1) alone had a significant positive effect in increasing the mean concentrations of serum calcium and magnesium when compared to the OVX control rats. These results are in agreement with many studies examined the beneficial effect of NDOs on mineral absorption in animals (Younes *et al.,* 2001 and Zafar *et al.,* 2004) and human subjects (Tahiri *et al.,* 2001 and Holloway *et al.,* 2007). Basically it has been speculated that the stimulatory effect of NDO on mineral absorption is mainly due to their prebiotic character (Scholz-Ahrens *et al.,* 2001). Their colonic fermentation produces SCFAs (mainly acetate, propionate, and butyrate) and other organic acids (e.g. lactate) that contribute to a significant reduction in caecal lumen pH. This reduction in caecal pH leads to greater solubilization of calcium and magnesium so that the biologically available concentration of these minerals is increased (Coxam, 2005). In addition both low pH and SCFAs induces caecal development and caecal weight rise at least 2-fold greater than normal, resulting in a greater exchange surface area in the caecum and thus enhanced mineral absorption (Scholz-Ahrens and Schrezenmeir, 2007).

In the present work, concerning the effect of phyto soya extract rich with isoflavones on serum mineral concentrations, mean concentrations of serum phosphorus and magnesium were significantly influenced in OVX rats supplemented with the phyto soya extract compared to OVX control rats, demonstrating that soy isoflavones have an estrogen-like activity and thus have the ability to influence mineral absorption (Brouns, 2002).

The OVX group supplemented with both NDO and phyto soya extract showed significant increases in the mean concentrations of the three minerals (calcium, phosphorus and magnesium) comparing to the OVX control group indicating an additive effect observed in the combination of the two supplements.

Markers of bone turnover may be useful not only in identifying the rate of bone turnover, but also in following the response to treatment. In some disease states such as osteoporosis both events are coupled and change in the same direction (Riggs *et al.,* 2002). OVX-induced osteoporosis belongs to high conversion type where bone formation and bone resorption are all increased but bone resorption is more remarkable (Liu *et al.,* 2007). In the present study, ovariectomy induced the elevation of the bone turnover markers (serum osteocalcin, total serum ALP and urinary DPD) in OVX control group comparing to sham group. This result was in agreement with several experimental studies (Mathey *et al.,* 2004 and Liu *et al.,* 2007). The elevation in the rate of bone turnover after ovariectomy can be attributed to the absence of estrogens. Estrogens have the ability to decrease the differentiation of the bone resoprbing osteoclast progenitor cells (Sorensen *et al.,* 2006), inhibit the bone resorbing activity of terminally differentiated osteoclasts (Lerner, 2006) and regulate the life span of mature osteoclasts by inducing apoptosis. That is why estrogen deprivation can cause repeated activation of the bone remodeling mechanism and elevate the rate of bone turnover (Nakamura *et al.,* 2007). Several studies reported that the rate of bone turnover appears to play an important role as a determinant of bone mass (Giro *et al.,* 2008). This could also be noticed in the present experiment where the results revealed that the elevation in the rate of bone turnover as a result of ovariectomy process decreased BMD of the right femur in the OVX control rats comparing to sham rats.

Nondigestible oligosaccharides have the ability to reduce the elevation in the rate of bone turnover reported in this study. OVX rats supplemented with 5% Raftilose®Synergy1 in their diet showed a significant reduction in the mean concentrations of bone turnover markers. In agreement to this finding, several experimental studies (Mathey *et al.,* 2004 and Zafar *et al.,* 2004) and clinical studies (Holloway *et al.,* 2007 and Coxam, 2007) reported the lowering effect of NDO on bone turnover markers. The reduction effect of NDO on the rate of bone turnover could be attributed to the stimulatory effect of NDO on the enhancement of calcium absorption which is followed by a suppression of PTH and consequently a reduction in the osteoclastic activity thus the rate of bone resorption decreases (Scopacasa *et al.,* 2002 and Jensen *et al.,* 2002). The suppression of bone resorption would be expected to result in suppression of the bone remodeling rate and a measurable increase in bone mass over time (Raschka and Daniel 2005) and this was obvious in the present experiment by measuring femoral BMD of the OVX rats supplemented with 5% Raftilose®Synergy1, where an improvement in femoral BMD was observed in this group comparing to OVX control group.

In the present experiment, Phyto soya extract rich with isoflavones also proved to be able to reduce the elevation in the rate of bone turnover induced by ovariectomy, and this was indicated by the significant decrease in bone turnover markers in OVX rats supplemented with phyto soya extract compared to OVX control rats. The mechanisms by which soy isoflavones positively affect bone turnover rate may be directly by interacting with estrogen receptors ER-α and ER-β or indirectly by suppressing the release of the proinflammatory cytokines from bone cells which are often elevated after ovariectomy. These cytokines are known to exert pleiotropic effects on bone under pathological conditions such as estrogen deficiency, leading to osteoporosis (Arjmandi and Smith, 2002).

In addition Hutabarat *et al.,* (2000) explained that soybeans contain not only the phytoestrogen isoflavones but also the phytoestrogens coumestans and lignans and it is possible that these phytoestrogens can also suppress bone resorption under estrogen deficient conditions.

The reduction effect of phytosya extract on the rate of bone turnover was followed by an improvement in femoral BMD. The femoral BMD was significantly greater in the OVX rats supplemented with phyto soya extract comparing to OVX control rats. Viereck *et al.,* (2002) reported that mechanism by which phytosya extract can affect bone turnover rate and thus improve BMD may be related to the presence of specific isoflavones, such as genistein, which inhibit osteoclast activity and osteoclast survival in femoral–diaphysial tissues of elderly female rats and ultimately prevent the loss of trabecular bone after ovariectomy.

The combination of Raftilose®Synergy1 and phyto soya extract rich with isoflavones have been reported to have an additive effect on the prevention of post-ovariectomy bone loss (Mathey *et al.,* 2004), and this cooperative effect was noticed in the reduction of bone turnover markers and improving femoral BMD higher

than using each supplement alone. The greater protection in rats fed the combination of Raftilose®Synergy1 and phyto soya extract can be explained by the fact that fermentation of NDOs in the large intestine modify the colonic bacteria and increase cecal β-glucosidase activity leading to an enhancement of the large intestinal absorption of isoflavone. The modulation of isoflavones by NDOs not only occurs in the hydrolysis of the isoflavones glycosides into glycones and thus enhancing the absorption of isoflavones, but also in transformation of the isoflavone daidzein into its potent metabolite equol (Ohta *et al.,* 2002). Equol is reported to have more estrogenic activity than daidzein 10 to 1000 fold (Setchell *et al.,* 2005). Thus because a greater efficacy of the soy isoflavones daidzein can be expected if converted into equol by the intestinal microflora, it is important to promote or activate gut microflora that produce equol as a tool to target an increase in isoflavones bioavailabilty and hence obtain the maximal effects of isoflavones on prevention of bone loss when estrogen status is deficient (Setchell *et al.,* 2002). Thus the combination of dietary NDOs and phyto soya extract rich with isoflavones were more efficient than using each alone in the prevention of postmenopausal osteoporosis.

REFERENCES

Arjmandi, B.H., B.J. Smith, 2002. Soy isoflavones' osteoprotective role in postmenopausal women: mechanism of action. J Nutr Biochem. Mar., 13(3): 130-137.

Bosscher, D., J. Van Loo, A. Franck, 2006. Inulin and oligofructose as functional ingredients to improve bone mineralization . Int Dairy J., 16(9): 1092-1097.

Brouns, F., 2002. Soya isoflavones: a new and promising ingredient for the health food sector. Food Res Int., 35(2): 187-193.

Coxam, V., Current 2007. data with inulin-type fructans and calcium, targeting bone health in adults. J Nutr., 137(11 Suppl): 2527S-2533S.

Coxam, V., 2005. Inulin-type fructans and bone health: state of the art and perspectives in the management of osteoporosis. Br J Nutr., 93(1): 111-S123.

Draper, C.R., I.M. Dick, R.L. Prince, 1999. The effect of estrogen deficiency on calcium balance in mature rats. Calcif Tissue Int., 64(4): 325-328.

Giro, G., D. Gonçalves, C.E. Sakakura, R.M. Pereira, E. Marcantonio Júnior, S.R. Orrico, 2008. Influence of estrogen deficiency and its treatment with alendronate and estrogen on bone density around osseointegrated implants: radiographic study in female rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 105(2): 162-167.

Griffin, I.J., P.M. Hicks, R.P. Heaney, S.A. Abrams, 2003. Enriched chicory inulin increases Ca absorption mainly in girls with lower Ca absorption. Nutr Res., 23(7): 901-909.

Holloway, L., S. Moynihan, S.A. Abrams, K. Kent., A.R Hsu., A.L. Friedlander, 2007. Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. Br J Nutr., 97(2): 365-372.

Hutabarat, L.S., H. Greenfield, M. Mulholland, 2000. Quantitative determination of isoflavones and coumestrol in soybean by column liquid chromatography. J Chromatogr A., 21: 886(1-2): 55-63.

Jensen, C., L. Holloway, G. Block, G. Spiller, G. Gildengorin, E. Gunderson, G. Butterfield, R. Marcus, 2002. Long-term effects of nutrient intervention on markers of bone remodeling and calciotropic hormones in late-postmenopausal women. Am J Clin Nutr., 75(6): 1114-1120.

Kanis, J.A., E.V. McCloskey, H. Johansson, A. Oden, L.J. Melton, 2008. 3rd, Khaltaev N. A reference standard for the description of osteoporosis. Bone., 42(3): 467-475.

Lerner, U.H., 2006. Bone remodeling in post-menopausal osteoporosis. J Dent Res., 85(7): 584-95.

Liu, K., G. Ma, G. Lv, Y. Zou, W. Wang, L. Liu, P. Yan, Y. Liu, L. Jiang, Y. Liu, Z. Liu, 2007. Effects of soybean isoflavone dosage and exercise on the serum markers of bone metabolism in ovariectomized rats. Asia Pac J Clin Nutr., 16(1): 193-195.

Mathey, J., C. Puel, S. Kati-Coulibaly, C. Bennetau-Pelissero, M.J. Davicco, P. Lebecque, M.N. Horcajada, V. Coxam, 2004. Fructooligosaccharides maximize bone-sparing effects of soy isoflavone-enriched diet in the ovariectomized rat. Calcif Tissue Int., 75(2): 169-179.

Nakamura, T., Y. Imai, T. Matsumoto, S. Sato, K. Takeuchi, K. Igarashi, Y. Harada, *et al.* 2007. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. Cell., 7; 130(5): 811-823.

Ohta, A., M. Uehara, K. Sakai, M. Takasaki, H. Adlercreutz, T. Morohashi, Y. Ishimi, 2002. A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. J Nutr., 132(7): 2048-2054.

Raschka, L., H. Daniel, 2005. Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. Bone., 37(5): 728-735.

Reeves, P.G., F.H. Nielsen, G.C. Fahey J.R., 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr., 123(11): 1939-1951.

Riggs, B.L., S. Khosla, L.J. Melton, 2002. 3rd. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev., 23(3): 279-302.

Roberfroid, M.B., 2005. Introducing inulin-type fructans. Br J Nutr., 93(1): 13-25.

Scheiber, M.D., J.H. Liu, M.T. Subbiah, R.W. Rebar, K.D. Setchell, 2001. Dietary inclusion of whole soy foods results in significant reductions in clinical risk factors for osteoporosis and cardiovascular disease in normal postmenopausal women. Menopause, 8(5): 384-392.

Scholz-Ahrens, K.E., G. Schaafsma, E.G. van den Heuvel, J. Schrezenmeir, 2001. Effects of prebiotics on mineral metabolism. Am J Clin Nutr., 73(2): 459S-464.

Scholz-Ahrens, K.E., J. Schrezenmeir, 2007. Inulin and oligofructose and mineral metabolism: the evidence from animal trials. J Nutr., 137(11): 2513S-2523S.

Scopacasa, F., A.G. Need, M. Horowitz, J.M. Wishart, H.A. Morris, B.E. Nordin, 2002. Effects of dose and timing of calcium supplementation on bone resorption in early menopausal women. Horm Metab Res., 34(1): 44-47.

Setchell, K.D., N.M. Brown, E. Lydeking-Olsen, 2002. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr., 132(12): 3577-3584.

Setchell, K.D., C. Clerici, E.D. Lephart, S.J. Cole, C. Heenan, D. Castellani, *et al.,* 2005. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. Am J Clin Nutr., 81(5): 1072-1079.

Setchell, K.D., 2001. Soy isoflavones-benefits and risks from nature's selective estrogen receptor modulators (SERMs). J Am Coll Nutr., 20(5): 354S-362S.

Sorensen, M.G., K. Henriksen, M.H. Dziegiel, L.B. Tankó, M.A. Karsdal, 2006. Estrogen directly attenuates human osteoclastogenesis, but has no effect on resorption by mature osteoclasts. DNA Cell Biol., 25(8): 475-483.

Tahiri, M., J.C. Tressol, J. Arnaud, F. Bornet, C. Bouteloup-Demange, C. Feillet-Coudray, V. Ducros, D. Pépin, F. Brouns, A.M. Rayssiguier, C. Coudray, 2001. Five-week intake of short-chain fructooligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. J Bone Miner Res., 16(11): 2152-2160.

Takahara, S., T. Morohashi, T. Sano, A. Ohta, S. Yamada, R. Sasa, 200. Fructooligosaccharide consumption enhances femoral bone volume and mineral concentrations in rats. J Nutr., 130(7): 1792-1795.

Uehara, M., A. Ohta, K. Sakai, K. Suzuki, S. Watanabe, H. Adlercreutz, 2001. Dietary fructooligosaccharides modify intestinal bioavailability of a single dose of genistein and daidzein and affect their urinary excretion and kinetics in blood of rats. J Nutr., 131(3): 787-795.

Viereck, V., C. Gründker, S. Blaschke, H. Siggelkow, G. Emons, L.C. Hofbauer, Phytoestrogen genistein stimulates the production of osteoprotegerin by human trabecular osteoblasts. J Cell Biochem, 84(4): 725-735.

Younes, H., C. Coudray, J. Bellanger, C. Demigné, Y. Rayssiguier, C. Rémésy, 2001. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. Br J Nutr., 86(4): 479-485.

Zafar, T.A., C.M. Weaver, Y. Zhao, B.R. Martin, M.E. Wastney, 2004. Non-digestible oligosaccharides increase calcium absorption and suppress bone resorption in ovariectomized rats. J Nutr., 134(2): 399-402.