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ORIGINAL RESEARCH

Role of phytoestrogenic oils in alleviating osteoporosis associated with ovariectomy in rats

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Abstract The objective of this study was to elucidate the effect of soybean oil (SbO) and sesame oil (SO) supplemented diets on bone biomarkers changes in OVX (ovariectomized) rats. The current data exhibited significant decrease in BMD (bone mineral density), accompanied with marked depletion in the level of Ca, P and Mg in both serum and bone of OVX rats. Also, serum estrogen, total protein, HDL-C (high density lipoprotein cholesterol), bone NO levels were decreased in OVX rats. However, a significant increase in the level of serum TL (total lipids), TC (total cholesterol), TG (triglycerides), LDL-C (low density lipoprotein cholesterol), VLDL-C (very low density lipoprotein cholesterol), urine minerals (Ca, P, Mg), as well as serum, bone and urine ALP (alkaline phosphatase) and ACP (acid phosphatase) activity were recorded in OVX rats. Further changes were also detected by the increased level of urine hydroxyproline, serum parathyroid hormone and osteocalcin, as well as urea and creatinine level in both serum and urine. On the other hand, when OVX rats were fed on SbO (soy bean oil) (15 % w/w) or SO (sesame oil) (10 % w/w) supplemented diets, the data recorded a significant improvement in all the above mentioned parameters. So, it can be concluded that consumption of SbO or SO supplemented diets might be considered as a functional food for retarding risks of osteoporosis associated with estrogen deficiency in OVX states.

Keywords Ovariectomyized rat · Estrogen deficiency · Soybean oil · Sesame oil osteoporosis

Abbreviations

NC	Normal control
SbO	Soy bean oil
SO	Sesame oil
OVX	Ovariectomized
BMD	Bone mineral density
TL	Total lipids
TC	Total cholesterol
TG	Triglycerides
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
VLDL-C	Very low density lipoprotein cholesterol
ALP	Alkaline phosphatase
ACP	Acid phosphatase
NO	Nitric oxide
Ca	Calcium
P	Phosphorus
Mg	Magnesium

Introduction

Ovariectomy is one of the most common surgical operations in women throughout the world (Li et al. 2010). Ovariectomy results in excess bone resorption

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over bone formation which in turn leads to osteoporosis (He et al. 2010). Osteoporosis is a growing health problem in the elderly populations, particularly in postmenopausal women (Gennari et al. 2002). It is a general metabolic bone disease, characterized by osteopenia and degeneration of bone microstructure. It results from a disturbance in the normal bone remodeling, tilting the balance to bone resorption over formation, leading to increased bone brittleness and tendency for bone fracture (Li et al. 2010).

Deficiency of female hormones is considered as the major factor of osteoporosis in postmenopausal and OVX-women (An and Freidman 1998; Namkung-Matthai et al. 2001). So, hormonal replacement therapy (HRT) should theoretically be the best choice for the prevention and treatment of postmenopausal and OVX osteoporosis. Unfortunately, due to many side effects of HRT, as well as cultural differences; acceptance of HRT is extremely low in women (Lau and Cooper 1996). While, non-hormonal therapy or alternative treatments may be more acceptable in women for the treatment and prevention of osteoporosis.

Currently, dietary phytoestrogens are considered as a valid alternative to estrogens in the treatment of osteoporosis. Phytoestrogens are naturally occurring compounds in many foods, defined as plant substances that are structurally or functionally similar to E2. They consist of a number of classes, mainly isoflavones, lignans, and coumestans (Messina et al. 1994). Phytoestrogens including Soy bean and Sesame are plant-derived compounds, being able to elicit estrogenic activity and to maintain bone health (Chiechi and Micheli 2005). They are divided into many classes, but for human use the best known are isoflavones (Knight and Eden 1996). Fresh SbO contains other micronutrients such phytoestrogens which are effective antioxidants (Ima-Nirwana et al. 2007). The phytoestrogens are numerous and nearly ubiquitous in the plant kingdom, having been found in almost all plants (Ososki and Kennelly 2003). The estrogenic activity of isoflavones is attributed to their structural similarity to 17β–estradiol and because they may modulate transcription of estrogen-responsive genes through the estrogen receptor (ER) (Lund et al. 2007).

Lignans, such as enterolactone and enterodiol, are mostly found in oil seed such as sesame oil (SO) and flaxeed oil, whole cereals, legumes, and berries and, to a lesser extent, in some fruits and vegetables. Limited quantitative data are available for the concentrations of isoflavones and lignans in plant foods (Potter and Steinmetz 1996).

Moreover, Soy bean oil is rich in PUFA and tocopherols (Ima-Nirwana et al. 2007). Previous studies have shown that alpha-tocopherol was effective in protecting rats against osteoporosis due to ovariectomy (Norazlina et al. 2000). Lignans are also among the widely distributed phytoestrogens. They are necessary compounds for the formation of lignin constituent of the plant cell wall (Chiechi and Micheli 2005). The main food source of lignans are flaxseed, whole grain, vegetable, tea, strawberries, cranberries and oil seeds such as sesame oil (Cornwell et al. 2004). Sesame oil has been found to contain considerable amounts of the sesame lignans (sesamin, episesamin, sesamolin), vitamin E (40 mg/100 g oil), 43 % of PUFA, and 40 % monounsaturated fatty acids. The lignans present in SO are thought to be responsible for many of its unique chemical and physiological properties, including its antioxidant and antihypertensive properties (Matsumura et al. 1995; Yamashita et al. 1995).

For humans, lignans are described to elicit antioxidant, antitumor (Sok et al. 2009) and antihypertensive properties and also to have protective effects against postmenopausal osteoporosis (Chen et al. 2004).

In the light of these findings, the present study was undertaken to evaluate weather intake of phytoesterogenic oils (SbO or SO) could play a positive role in reducing development of osteoporosis and bone loss associated with estrogen deficiency in OVX rats.

Materials and methods

Experimental animals

This study was performed on adult virgin female Wistar rats, weighing 170 ± 5 g. Rats were obtained from the Institute of Ophthalmic Disease Research, Cairo, Egypt. They were housed in stainless steel cages in automatically illuminated and thermally controlled room (22–25 °C and 12 h light/dark cycle) at the Animal House, Faculty of Science, Mansoura University, Mansoura, Egypt. Rats were permitted adequate standard diet (60 % ground corn meal, 15 % ground beans, 10 % bran, 10 % corn oil, 3 % casein, 1 % mineral mixture and 1 % vitamins mixture) purchased from Meladco Feed Company (Aubor City,

Cairo, Egypt), and given water *ad libitum* for 1 week of adaptation period prior to the experimental work.

Oil samples and experimental diets

Two natural vegetable oils [soy bean oil (SbO) and sesame oils (SO)] were selected in this study. Both of them were purchased from a local market in Mansoura, Egypt. SbO and SO were added each alone to the standard diet at a dose of 15 and 10 % (w/w), respectively, and used as two experimental diets according to Shuid et al. (2007) and Boulbaroud et al. (2008), respectively.

Experimental animals

Three-month-old Sprague Dawley (Rattus norvegicus) adult virgin female rats (220–240 g) were maintained on ad libitum water and standard laboratory diet purchased from Meladco Feed Company (Aubor City, Cairo, Egypt). Animals were housed in ventilated cages in an artificially illuminated and thermally controlled room (22–25 °C and 12-h light dark cycle) at the Animal House Lab., Faculty of Science, Mansoura University, Mansoura, Egypt. After an acclimation period of 2 weeks, adult virgin female rats were subjected to bilateral ovariectomy, removing both the ovaries under halothane anesthesia (Lien et al. 2009). All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Mansoura University, and the protocol conformed to the guidelines of the National Institutes of Health. After 1 week of recovery from the surgery, rats were divided into six groups (6 rats/each) and received their respective treatment for 2 months as follow: group 1: normal control (NC) group fed on standard diet without any supplementation; group 2, (SbO): normal rats fed on standard diet supplemented with 15 % SbO; group 3, (SO): normal rats fed on standard diet supplemented with 10 % SO, group 4, ovariectomized (OVX): OVX rats received standard diet without any supplementation, group 5, (OVX + SbO): OVX rats received standard diet supplemented with 15 % SbO and group 6, OVX + SO: OVX rats received standard diet supplemented with 10 % SO.

Samples collection

At the end of experimental period (2 months), rats were placed in separated metabolic cages for 24 h to collect urine in tightly capped bottles, containing few drops of HCl as preservative to avoid fermentation. Then all animals were fasted for 12 h, sacrificed by cervical dislocation and blood samples were collected. Blood was collected into chilled non-heparinized tubes, centrifuged at 860 Xg for 20 min at 4 °C and the separated sera were frozen at −20 °C for future biochemical analysis.

Left and right femurs were immediately removed; washed using chilled saline solution. Left femur was weighed, minced in ice-cold saline solution using a Potter-Elvehjem type homogenizer. The homogenates were centrifuged at 860 Xg for 20 min at 4 °C, and the resultant supernatants were frozen at −20 °C for further biochemical analysis. While the right femur was weighed and used for bone mineral density (BMD) according to Archimedes principle (Kalu 1991).

Biochemical analysis

Calcium, phosphorus and magnesium levels were quantified using kits supplied by Bio-diagnostic Co. (Cairo, Egypt). Serum estrogen was quantified using Immulite analyzer Kit (Diagnostic Products Corp., Los Angeles, CA, USA). Parathyroid hormone, osteocalcin and 4-hydroxyproline were quantified using ELISA Diagnostic Kit (Cairo, Egypt). ALP and ACP activity was quantified using ABC diagnostic kits (Cairo, Egypt). While, nitric oxide (NO) level was determined by Minneapolis kit (New York, NY, USA). Total protein, urea and creatinine levels were quantified, using Diamond Diagnostic kits (Cairo, Egypt). Total lipids, total cholesterol, triglycerides and HDL-C levels were quantified using kits supplied by Spinreact S.A. (Sant Esteve de Bas, Spain). LDL-C and VLDL-C were calculated according to the following equations, respectively:

LDL-C ¼ TC-HDL-C-TG = 5 (Ahmedi et al. 2008).

VLDL-C ¼ TG = 5 (Satheesh and Pari 2008).

Statistical analysis

All data were statistically analyzed by one way analysis of variance test and post comparison was carried out with Waller-Duncan k-ratio (Waller and Duncan 1969) using the Statistical Package for Social

Table 1 Serum tested parameters in normal control and the different treated rat groups

Parameters	Animal groups					
	NC	SbO	SO	OVX	OVX + SbO	OVXSO
Ca (mg/dl)	8.84 ± 0.05[a]	8.97 ± 0.05[a]	8.84 ± 0.13[a]	7.06 ± 0.05[b]	7.93 ± 0.02[c]	7.91 ± 0.11[c]
		1.47	0.00	−20.13	−10.29	−10.52
P (mg/dl)	3.77 ± 0.04[a]	3.79 ± 0.03[a]	3.82 ± 0.03[a]	3.10 ± 0.05[b]	3.48 ± 0.03[c]	3.47 ± 0.02[c]
		0.53	1.32	−17.77	−7.69	−7.95
Mg (mg/dl)	2.36 ± 0.03[a]	2.36 ± 0.03[a]	2.37 ± 0.04[a]	1.92 ± 0.02[b]	2.05 ± 0.02[c]	2.05 ± 0.02[c]
		0.00	0.42	−18.64	−13.13	−13.13
Estrogen	34.60 ± 0.92[a]	34.71 ± 0.84[a]	34.62 ± 0.56[a]	17.91 ± 0.57[b]	25.83 ± 0.71[c]	24.83 ± 0.71[c]
(pg/ml)		0.31	0.05	−48.23	−25.34	−28.23
PTH (pg/ml)	23.84 ± 0.11[a]	23.92 ± 0.14[a]	23.87 ± 0.16[a]	38.75 ± 0.35[b]	26.67 ± 0.34[c]	27.02 ± 0.31[d]
		0.33	0.12	62.54	11.87	13.33
Osteocalcine (pg/ml)	4.52 ± 0.14[a]	4.50 ± 0.11[a]	4.51 ± 0.09[a]	6.90 ± 0.13[b]	5.37 ± 0.15[c]	5.57 ± 0.13[c]
		−0.44	−0.22	52.65	18.80	23.23
ALP (K.A.U/dl)	7.08 ± 0.06a	6.83 ± 0.14[a]	6.89 ± 0.04[a]	9.17 ± 0.15[b]	8.13 ± 0.08[c]	8.25 ± 0.08[c]
		−3.53	−2.68	29.52	14.83	16.52
ACP	4.21 ± 0.07[a]	4.21 ± 0.06[a]	4.20 ± 0.06[a]	11.29 ± 0.06[b]	8.12 ± 0.07[c]	8.35 ± 0.09[d]
(K.A.U/d)		0.00	−0.23	168.17	92.87	98.33
T. Lipid (g/dl)	395.94 ± 3.07[a]	395.52 ± 5.86[a]	394.37 ± 2.73[a]	784.54 ± 8.54[b]	584.37 ± 6.16[c]	584.25 ± 1.69[c]
		−0.10	−0.39	98.14	47.59	47.56
T.Cholesterol (g/dl)	109.58 ± 0.63[a]	109.34 ± 1.01[a]	109.58 ± 0.35[a]	306.86 ± 4.78[b]	220.21 ± 1.74[c]	226.77 ± 0.62[d]
		−0.21	0.00	180.03	100.95	106.94
Triglycrides (g/dl)	43.64 ± 1.08[a]	43.33 ± 0.49[a]	43.55 ± 0.35[a]	134.04 ± 1.15[b]	87.55 ± 1.12[c]	95.82 ± 1.51[d]
		−0.71	−0.20	207.14	100.61	119.56
HDL-C (g/dl)	42.65 ± 0.89[a]	42.75 ± 0.91[a]	42.69 ± 0.90[a]	14.27 ± 1.52[b]	32.92 ± 1.52[c]	31.43 ± 1.32[c]
		0.23	0.09	−66.54	−22.81	−26.30
LDL-C (g/dl)	58.20 ± 0.97[a]	57.94 ± 1.84[a]	58.17 ± 0.96[a]	265.78 ± 5.28[b]	169.78 ± 0.95[c]	176.18 ± 1.32[c]
		−0.44	−0.05	356.66	191.7	202.71
V-LDL-C (g/dl)	8.72 ± 0.21[a]	8.64 ± 0.08[a]	8.71 ± 0.07[a]	26.80 ± 0.23[b]	17.50 ± 0.22[c]	19.15 ± 0.30[d]
		−0.91	−0.11	207.33	100.68	119.61
Total Protein (g/dl)	8.72 ± 0.21[a]	8.76 ± 0.24[a]	8.74 ± 0.22[a]	5.40 ± 0.18[b]	6.87 ± 0.03[c]	6.72 ± 0.12[c]
		0.45	0.22	−33.07	−21.21	−22.93
Urea (g/dl)	25.83 ± 0.70[a]	25.50 ± 0.95[a]	25.50 ± 0.76[a]	76.16 ± 1.57[b]	53.00 ± 1.15[c]	55.16 ± 1.13[c]
		−1.27	−1.27	194.85	105.18	113.55
Cretinine (g/dl)	0.92 ± 0.02a	0.92 ± 0.01[a]	0.92 ± 0.02[a]	2.67 ± 0.07[b]	1.23 ± 0.01[c]	1.26 ± 0.01[c]
		0.00	0.00	190.21	33.69	36.95

Data are mean ± SE of six determinations & % of change in comparison to NC group. Within each row, value superscripts with different letters (a–c) are significantly different ($P \leq 0.05$)

NC Normal control, *SbO* Soybean oil, *SO* Sesame oil, *OVX* Ovariectomized

Sciences (SPSS, version 15.0). The results were expressed as mean ± standard error (SE) and the values of $P \leq 0.05$ were considered statistically significant based on Least Significant Difference (LSD) probability.

Results

As shown in Tables 1, 2 and 3, the recorded data did not show any significant changes between the normal control (NC) rats group and normal rats fed on SbO

Table 2 Bone tested parameters in normal control and the different treated rat groups

Animal groups						
Parameters	NC	SbO	SO	OVX	OVX + SbO	OVX + SO
BMD (g/cm^3)	3.05 ± 0.1^a	3.07 ± 0.1^a	3.16 ± 0.1^a	2.05 ± 0.0^b	2.72 ± 0.11^c	2.55 ± 0.03^c
		0.65	3.60	−32.78	−10.82	−16.39
Ca (mg/mg)	153.33 ± 2.75^a	154.00 ± 2.75^a	153.83 ± 2.92^a	91.08 ± 6.82^b	119.16 ± 3.18^c	113.83 ± 5.34^d
		0.43	0.32	−40.59	−22.28	−25.76
P (mg/mg)	77.24 ± 1.64^a	78.39 ± 1.08^a	77.98 ± 1.13^a	50.90 ± 1.36^b	62.68 ± 1.06^c	63.69 ± 0.72^c
		1.48	0.95	−34.10	−18.85	−17.54
Mg (mg/mg)	62.25 ± 1.64^a	63.46 ± 1.33^a	63.98 ± 1.28^a	35.90 ± 1.36^b	47.68 ± 1.06^c	48.69 ± 0.72^c
		1.94	2.77	−42.23	−23.40	−21.78
NO (μ mol/g)	20.99 ± 0.37^a	21.32 ± 0.22^a	21.38 ± 0.63^a	13.83 ± 0.30^b	17.55 ± 0.52^c	16.99 ± 0.50^c
		1.57	1.85	61.50	31.01	28.63
ALP (K.A.U/g)	2.75 ± 0.21^a	2.83 ± 0.20^a	2.80 ± 0.14^a	4.84 ± 0.06^b	3.12 ± 0.13^c	3.14 ± 0.07^c
		2.90	1.81	76.00	13.45	14.18
ACP (K.A.U/g)	0.17 ± 0.004^a	0.17 ± 0.006^a	0.17 ± 0.006^a	0.36 ± 0.006^b	0.27 ± 0.007^c	0.29 ± 0.006^c
		0.00	0.00	111.76	58.82	70.58
Total Protein (g/dl)	2.69 ± 0.83^a	2.69 ± 0.44^a	2.69 ± 0.75^a	0.87 ± 0.58^b	1.25 ± 0.07^c	1.12 ± 0.9^d
		0.00	0.00	−67.65	−53.53	−58.36

Data are mean ± SE of six determinations & % of change in comparison to NC group. Within each row, value superscripts with different letters (a–c) are significantly different ($P \leq 0.05$)

NC Normal control, *SbO* Soybean oil, *SO* Sesame oil, *OVX* Ovariectomized

or SO supplemented diets. However, obtained serum data (Table 1) recorded a significant decrease in serum minerals (Ca, P, Mg), estrogen and HDL-C, accompanied with a significant increase in serum lipid profile (TL, TC, TG, LDL-C and VLDL-C), enzymes (ALP and ACP), parathyroid hormone and osteocalcin, as well as total protein, urea and creatinine in OVX rats if compared to NC rats group. Moreover, bone results in Table 2 showed a significant decrease in BMD, bone minerals (Ca, P and Mg), NO, total protein, accompanied with a significant increase in bone ALP and ACP activity of OVX rats group, comparing to NC rats. Additionally, the current urinary data recorded a significant increase in the level of urine minerals, hydroxyproline, total protein, urea and creatinine, as well as enzyme activities were recorded in OVX rats compared to NC group. Meanwhile, feeding OVX rats on SbO or SO supplemented diets for 2 months caused a significant improvement in all the above mentioned parameters when comparing to OVX rats group, indicating its anti-osteoporetic effects.

Discussion

In the present study, OVX rats were found to have marked bone loss characterized by reduced femoral BMD and significant lowering of bone minerals (Ca, P and Mg). The observed deficiency of estrogen in OVX rats is more likely to explain the onset of reduced mineral and bone formations associated with the deficiency of estrogen associated with osteoporosis (Namkung-Matthai et al. 2001; Deyhima et al. 2003). The deficiency of estrogen associated with ovariectomy selectively stimulates B-lymphopoiesis, resulting in an accumulation of pre-B cells in mouse bone marrow (Adlercreutz et al. 1995), which stimulates osteoclastic bone resorption (Ishimi et al. 1990). Also, estrogen deficiency leads to a significant increase in the number of bone-resorbing osteoclasts (Zhang et al. 2009), making bone more susceptible to osteoporosis and it results also in an increase in life span of osteoclasts and decrease in life span of osteoblasts, with incomplete mineralization due to decreased time

Table 3 Urine tested parameters in normal control and the different treated rat groups

Animal groups						
Parameters	NC	SbO	SO	OVX	OVX + SbO	OVX + SO
Ca (mg/dl)	153.41 ± 0.78[a]	153.19 ± 0.82[a]	153.24 ± 0.81[a]	175.39 ± 0.93[b]	169.38 ± 0.39[c]	162.16 ± 0.01[c]
		−0.14	−0.11	14.32	10.41	5.70
P (mg/dl)	2.12 ± 0.08[a]	2.09 ± 0.06[a]	2.10 ± 0.05[a]	2.57 ± 0.02[b b]	2.35 ± 0.01[c]	2.37 ± 0.01[c]
		−1.41	−0.94	21.22	10.84	11.79
Mg (mg/dl)	2.12 ± 0.08[a]	2.13 ± 0.07[a]	2.13 ± 0.07[a]	2.57 ± 0.02[b]	2.35 ± 0.01[c]	2.37 ± 0.01[c]
		0.47	0.47	21.22	10.84	11.79
ALP (K.A.U/dl)	4.98 ± 0.05[a]	4.92 ± 0.03[a]	4.91 ± 0.03[a]	7.50 ± 0.08[b]	6.75 ± 0.08[c]	6.81 ± 0.07[c]
		−1.20	−1.40	50.60	35.54	36.74
ACP (K.A.U/dl)	2.30 ± 0.12[a]	2.38 ± 0.12[a]	2.41 ± 0.12[a]	3.83 ± 0.40[b]	2.47 ± 0.14[c]	2.45 ± 0.15[c]
		3.47	4.78	66.52	7.39	6.52
Hydroxyproline (mg/dl)	9.07 ± 0.28[a]	8.94 ± 0.24[a]	9.03 ± 0.23[a]	14.29 ± 0.30[b]	10.76 ± 0.20[c]	10.64 ± 0.18[c]
		−1.43	−0.44	57.55	18.63	17.30
T. Protein (g/dl)	0.04 ± 0.01[a]	0.04 ± 0.03[a]	0.04 ± 0.03[a]	2.73 ± 0.04[b]	2.22 ± 0.08[c]	2.23 ± 0.06[c]
		0.00	0.00	62.50	32.73	32.14
Urea (g/dl)	35.11 ± 0.55[a]	34.94 ± 0.71[a]	35.08 ± 0.45[a]	57.34 ± 1.14[b]	47.05 ± 0.38[c]	48.98 ± 0.46[c]
		−0.48	−0.08	63.31	34.00	39.50
Creatinine (g/dl)	8.55 ± 0.23[a]	8.28 ± 0.21[a]	8.19 ± 0.18[a]	12.37 ± 0.41[b]	10.52 ± 0.18[c]	10.74 ± 0.16[c]
		−3.15	−4.21	44.67	23.04	25.61

Data are mean ± SE of six determinations & % of change in comparison to NC group. Within each row, value superscripts with different letters (a–c) are significantly different ($P \leq 0.05$)

NC Normal control, *SbO* Soybean oil, *SO* Sesame oil, *OVX* Ovariectomized

between remodeling episodes (Johnson-Lynn et al. 2008).

Further evidence of osteoporosis resulting from ovariectomy was indicated by the present elevation of parathyroid hormone (PTH) level. The elevated level of PTH causes additional loss of BMD in estrogen deficient animals beyond the rapid bone loss associated with ovariectomy. As decreased estrogen level in females increased the sensitivity of bones to the action of PTH, leading to bone resorption with lower BMD (Krivosíková et al. 2010). Moreover, ovariectomy causes hyperparathyroidism which may cause calcium change associated with a compensatory rise in PTH resulting in calcium release from the skeleton thus causing bone loss (Guillemant et al. 1999).

Also, the rise of PTH may be related to NO production, where the increase of PTH causes inhibition of NO production, which in turn leads to enhanced bone resorption. NO, a signaling molecule synthesized from L-arginine by nitric oxide synthases (NOS), is an important factor in regulating bone metabolism (Van't Hof and Ralston 2007). The impact of NO on bone metabolism is two-way directional, affecting not only the function of osteoclasts, but also the differentiation and proliferation of osteoblasts (Van't Hof et al. 2004).

An occurred inhibition in NO production in OVX rats causes bone loss following ovariectomy what may be result from bone resorption (Mancini et al. 1998).

Other etiologic factors for osteoporosis include kidney dysfunction, as recorded in the present study by the increased serum level of urea and creatinine and decreased protein, with increased urinary calcium loss, indicating defective calcium absorption mechanisms (Zung and Chalew 1997). The elevated blood urea is correlated with an increased protein catabolism in the mammalian body or more efficient conversion of ammonia to urea as a result of increased synthesis of enzymes involved in urea production (Rodwell 1979). Furthermore, the raised bone (ALP and ACP) activity occurring with ovariectomy could contribute to higher bone turnover rate, being characterized by an increase in both bone resorption and formation, but bone resorption excesses formation, leading to bone loss

(El-Wakf et al. 2003). Thus, indicating an increase in the osteoblastic and osteoclastic activity, respectively, resulting in an overall net loss of bone with an increase in excretion of urinary hydroxyproline, as an index of bone turnover (Wu et al. 2008). In particular the elevated osteocalcin, which is a marker for bone formation activity by osteoblasts may indicate an increase of the bone turnover level causing an imbalance between bone formation and resorption (He et al. 2010). Although serum osteocalcin is thought to be a bone formation marker, osteocalcin has been also a bone turnover marker, because the polyclonal antibodies used in the radioimmunoassay for detection of serum osteocalcin did not only recognize intact osteocalcin but also N-fragment osteocalcin, which is released into the blood during bone resorption (Kasugai et al. 1998). The lack of estrogen in OVX states increases bone resorption followed by bone loss leading to osteoporosis status (Lerner 2006). So, the increase in ALP and osteocalcin in the OVX group, indicates that bone-remodeling activity was increased (Keller et al. 2000).

Further investigations regarding lipid metabolic disorders were also recorded in OVX rats. These disorders are manifested by hyper-cholesterolemia, hyperlipidemia and hypertriglycridemia. Also, increased visceral fat accumulation, elevation of circulating levels of total cholesterol and low density lipoproteins (LDL-C), as well as decreased high density lipoproteins cholesterol (HDL-C) were recorded after surgical menopause ovariectomy in Golden Syrian Hamster (Deshaies et al. 1997). The increase in energy flux brought by OVX is accompanied by concomitant adaptations of peripheral lipid metabolism that include increased hepatic lipid production, elevated levels of circulating lipoproteins, and the induction of pathways involved in fat accumulation (Deshaies et al. 1997). A recent study indicated lipid metabolic disorders with hyperlipidemia and hypercholesterolemia as a distinct risk for osteoporosis in OVX rats (Hassan and Abdel-Wahhab 2012). A close relation between hyper-cholesterolaemia and osteoporosis was reported, where, high cholesterol diet increases the risk of osteoporosis, through inhibiting the differentiation and proliferation of osteoblasts (You et al. 2011).

On the other hand, the present study demonstrated that modification of diet through administration of selected phytoestrogens rich oils [soy bean oil (SbO) or sesame oil (SO)] was found to attenuate all above mentioned attributes of osteoporosis, indicating their anti-osteoporotic effect. Phytoestrogens in the diet may have a role in modulating hormone related diseases based on their structural similarity to 17β-estradiol the main female estrogen in human (Barnes, 2004). Phytoestrogens may act on osteoblasts and osteoclasts through similar mechanisms as estrogen (Reinwald and Weaver 2006). Specially, soy bean isoflavones stimulate differentiation and proliferation of osteoblasts, but also stimulate the release of paracine factors that affect the activity of osteoclasts. Receptor activation of nuclear factor-κβ ligand is an essential factor for osteoclast formation and activation that enhances bone resorption (Chilibeck and Cornish 2008).

The current positive effect of SbO on bone status could be attributed to its anabolic properties that can affect bone turnover rate and thus improve BMD, bone mineral content and reduce urinary excretion of bone resorption markers (Chen et al. 2008). This finding may be related to the presence of specific isoflavones (genistein, daidzein and glycitein) which have estrogen-like activity and thus have the ability to influence mineral absorption, and to prevent or treat bone metabolic disorders, particularly bone loss. Prevention of bone loss occurs mainly through inhibiting osteoclast activity and survival, as indicated in femoral–diaphysial tissues of elderly female rats, thus ultimately prevents loss of trabecular bone after ovariectomy (Brouns 2002). This effect may be attributed to the fact that isoflavone components stimulate the ability to bind both the traditional estrogen receptor alpha (ERα) and the more recently described estrogen receptor beta (ERβ), with higher affinity for ERβ (Kuiper et al. 1998), which in all, are present in adipose tissue (Anwar et al. 2001). Genistein binding to the estrogen receptors increases the expression of estrogen responsive genes inhibit the increase of osteoclast-like multinucleated cells stimulated by PTH in OVX states (Binbin and Shifeng 2003). Other mechanisms, including lowering of oxidative stress, stimulating estrogen receptor, modulating a number of inflammatory markers, and preventing the proliferation of osteoclast cells have been also proposed (Hassan and Abdel-Wahhab, 2012). In parallel, it was also indicated that sesame oil (SO) can exert multiple functions related to bone health, probably through their estrogenic active components, lignans (Wu 2007). Seasame lignans or their metabolites may induce beneficial effects on bone by binding to bone

estrogen receptors which promote bone strength perhaps through production of bone matrix proteins. (Sacco et al. 2007). Moreover, the current modulating effects of SbO or SO supplemented diet on hormonal changes in OVX rats may be attributed to other mechanisms. Phyto-estrogens are potent scavengers of ROS, which might prevent formation of the powerful oxidant peroxynitrite (OONO–), leading to an increase in NO level (Briante et al. 2001). In support, the present data showed increased NO level in OVX rats fed SbO or SO supplemented diets. This observation may be attributed to the fact that phytoestrogens in the diet may have a role in modulating hormone related disease based on their ability to elicit estrogenic activity (Sakai and Kogiso 2008). Estrogen increases the production of NO by enhancing endothelial NO synthase activity (Sasaki et al. 2002). Estradiol treatment induces vasodilation by inhibiting the production of endothelin-1, or by enhancing synthesis of NO, and by a direct Ca^{2+} antagonistic effect on vascular smooth muscles (Veille et al. 1996), which may attenuate bone arterial blood flow and thereby bone health. NO may modulate the anabolic effects of estrogen on bone homeostasis by restraining osteoclast-mediated bone resorption and stimulating osteoblast activity. Accordingly, NO donated by organic nitrates, including nitroglycerin, is thought to protect from bone loss associated with estrogen deficiency (Wimalawansa, 2008). Other beneficial effects of SbO and SO have been reported by Yamaguchi and Sugimoto (2000), indicating that phytoestrogen rich diets are able to stimulate osteoblasts to produce protein synthesis. The administration of SbO, reduces the role of kidney in the excretion of nitrogen causing decreased blood urea concentration. These changes may help to delay the progression of renal failure and prevent the consequences of uremia (Younes et al. 1998). However, isoflavones-drived genistein can maintain the normal kidney function through the attenuation of many detrimental OVX-induced effects (Choi and Song 2009).

The positive role of both SbO and SO supplemented diets were also achieved by the observed improvement of bone metabolic markers (ALP, ACP and hydroxyproline). This observation may be related to reduced bone resorption biomarkers (ACP) and increased bone formation biomarkers (ALP), with increased BMD, (Chiechi et al. 2002; Boulbaroud et al. 2008). Moreover, soybean containing phytoestrogens - derived coumestrol was almost as potent as genistein and daidzein, in suppressing urinary excretion of hydroxyproline (Ya et al. 2003). Several clinical trials in postmenopausal women showed that a supplement of phytoestrogens, improves spine BMD and reduces urinary excretion of hydroxylproline content and increases bone formation (Setchell 2001; Ya et al. 2003). In addition, osteocalcin was found to be lowered after SbO or SO administration to rats. This suggests that they were able to suppress the increased bone turnover due to estrogen deficiency (Wu 2007).

Further protection was also demonstrated by improving lipid metabolism which occurs frequently in OVX rats following SbO administration. There are, theoretically, three mechanisms to control the expansion of the cholesterol pool; (1) decreased absorption of dietary cholesterol; (2) decreased synthesis of cholesterol; and (3) increased excretion of either or both cholesterol and its metabolites, the bile acids (Chiang 2003). The major mechanisms involved in lowering of LDL-C include interruption of enterohepatic circulation of bile acids and alterations in hepatic cholesterol and lipoprotein metabolism (Fernandez 1995). The biochemical effect of both SbO and SO on lipid metabolism indicates their protective roles against deleterious effects on lipid metabolism in experimental bilateral OVX rats. This observation may be related to soy isoflavones (genistein, daidzein, glycitein) that have a distinct effect on abdominal body fat distribution (Cynthia et al. 2008). Another proposed mechanism for soy hypo-cholesterolemic effect is through binding bile acids and preventing their reabsorption in the gastrointestinal tract (Torres et al. 2006). Because bile acids are synthesized from cholesterol, enhancing their excretion would enhance hepatic bile acid synthesis, triggering an up-regulation of LDL-C receptors, thus reducing the circulating concentrations of cholesterol carried by LDL-C and other atherogenic particles (Cho et al. 2008). SO-phytoestrogens may prevent bone loss through reducing the lipid profile, thereby increasing osteoblastic activity (Arjmandi et al. 1998). Additionally, the positive effects of the other selected phytoestrogen rich oil (SO) on the bone health were recorded as achieved by the decreased lipid profile. This may be attributed to a group of lignans in the non-fat portion of the SO which contains sesamin, sesamolinol, sesamol and other lignans (Boulbaroud et al. 2008). The hypochlesterolemic action of SO may also be due to the

high polyunsaturated fat content in the oil (Sowmya et al. 2009). Another mechanism of sesamin for the hypo-cholesterolemic effect is believed to be related to the inhibition of intestinal absorption of cholesterol, increased excretion of cholesterol into bile, and decreased activity of 3-hydroxy-3-methylglutaryl coenzyme-A reductase (Chen et al. 2005), which in all seemed of importance for maintaining bone health (Fernandez et al. 2002).

Conclusion

The present study indicated that estrogen deficiency in rats subjected to bilateral ovariectomy caused bone metabolic alterations. The study also showed the ability of both SbO and SO supplemented diets to have nearly the same positive and ameliorating effects on the above mentioned bone alterations. So they could be considered as useful natural anti-osteoporetic agents in experimentally bilateral OVX-rats. Therefore it can be said that phytoestrogens, especially as food supplements, help to prevent osteoporosis in women around the world who are involuntarily subjected to ovariectomy.

References

Adlercreutz H, Goldin BR, Gorbach SL, Hockerstedt KAV, Watanabe S, Hamalainen EK, Markkanen MH, Makela TH, Wahala KT, Hase TA, Fotsis T (1995) Soybean phytoestrogen intake and cancer risk. J Nutr 125:757–770

Ahmedi SA, BorouMand MA, Moghddam KG, Tajik P, Dibaj SM (2008) The impact of low serum triglyceride on LDL-cholesterol estimation. Arch Iran Med 11:318–321

An YH, Freidman RJ (1998) Animal models in orthopaedic. Research. Boca Raton: CRC press and bone health: a brief overview. J Ren Nutr 10:63–68

Anwar A, McTernan PG, Anderson LA, Askaa J, Moody CG, Barnett AH (2001) Site-specific regulation of oestrogen receptor-alpha and—beta by oestradiol in human adipose tissue. Diabetes Obes Metab 5:338–349

Arjmandi BH, Birnbaum R, Goyal NV, Getlinger MJ, Juma S, Alekel L, Hasler CM, Drum ML, Hollis BW, Kukreja SC (1998) Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. J Am Clin Nutr 68:1364–1368

Barnes S (2004) Supplement: fifth internat'l symposium on the role of soy in preventing and treating chronic disease soy isoflavones-phytoestrogens and what else? J Nutr 134:1225–1228

Binbin LI, Shifeng YU (2003) Genistein prevents bone resorption diseases by inhibiting bone resorption and stimulating bone formation. Biol Pharm Bull 26:780–786

Boulbaroud S, Mesfioui A, Arfaoui A, Ouichou A, El- Hessni A (2008) Preventive effects of flaxseed and sesame oil on bone loss in ovariectomized rats. J Pak Biol Sci 11:1696–1701

Briante R, La Cara F, Tonziello MP, Febbraio F, Nucci R (2001) Antioxidant activity of the main bioactive derivatives from oleuropein hydrolysis by hyperthermophilic beta-glycosidase. J Agric Food Chem 49:3198–3203

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

Chen J, Hui E, Ip T, Thompson LU (2004) Dietary flaxseed enhances the inhibitory effect of tamoxifen on the growth of estrogen dependent human breast cancer (MCF-7) in nude mice. Clin Cancer Res 10:7703–7711

Chen Z, Bassford T, Green SB, Cauley GA, Jackson RD, La Croix AZ (2005) Postmenopausal hormone therapy and body composition – a substudy of the estrogen plus progestin trial of the womens's health initiative. J Am Clin Nutr 82:651–656

Chen J, Singhal R, Lazarenko OP, Badger TM, Ronis MJ (2008) Promotion of bone growth by dietary soy protein isolate: comparision with dietary casein, whey hydrolysate and rice protein isolate in growing female rats. J FASEB 22:303–305

Chiang JY (2003) Bile acid regulation of hepatic physiology III. Bile acids and nuclear receptors. J Am Physiol Gastrointest Liver Physiol 284:349–356

Chiechi LM, Micheli L (2005) Utility of dietary phytoestrogens in preventing postmenopausal osteoporosis. Nutraceutic Res 3:15–28

Chiechi LM, Secreto G, Vimercati A, Greco P, Venturelli E, Pansini F, Fanelli M, Loizzi P, Selvaggi L (2002) The effects of a soy rich diet on serum lipids, the Menfis randomized trial. J Matur 41:97–104

Chilibeck PD, Cornish SM (2008) Effect of estrogenic compounds (estrogen or phytorsteogens) combined with exercise on bone and muscle mass in order individuals. Appl Physiol Nutr Metab 33:200–212

Cho SJ, Juillerat MA, Lee CH (2008) Identification of LDL-receptor transcription stimulating peptides from soybean hydrolysate in human hepatocytes. J Agric Food Chem 56:4372–4376

Choi JS, Song J (2009) Effect of genistein on insulin resistance, renal lipid metabolism, and antioxidative activities in ovariectomized rats. J Nutr 25:676–685

Cornwell T, Cohick W, Raskin I (2004) Dietary phytoestrogens and health. Phytoche 65:995–1016

Cynthia KS, Brian CC, Michael JT, Amalia G, Ali A, Stephen B (2008) Effect of a daily supplement of soy protein on body composition and insulin secretion in postmenopausal women. J Rev 88:1609–1617

Deshaies Y, Dagnault A, Lalonde J, Richard D (1997) Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. J Am Physiol 273:355–362

Deyhima F, Stoeckerb BJ, Brusewitzc GH, Arjmandib BH (2003) The effects of estrogen depletion and isoflavones on bone metabolism in rats. Nutr Res 23:123–130

El-Wakf AM, Ibrahim HA, El-Sawy MR, El-Said FG (2003) Aging-related bone changes in rats: role of antioxidants. Egypt J Zool 40:506

Fernandez ML (1995) Distinct mechanisms of plasma LDL lowering by dietary soluble fiber: specific effects of pectin, guar gum and psyllium. J Lipid Res 36:2394–2404

Fernandez N, Renedo M, Garcia-Rodriguez C, Sanchez CM (2002) Activation of monocytic cells through Fc receptors induces the expression of macrophage-inflammatory protein (MIP)-1alpha, MIP-beta, and RANTES. J Immunol 169:3321–3328

Gennari L, Becherini L, Falchetti A, Masi L, Massart F, Brandi ML (2002) Genetics of osteoporosis: role of steroid hormone receptor gene polymorphisms. J Steroid Biochem Biol 81:1–24

Guillemant J, Taupin P, Le H, Tarigh N, Allemandou A, Pérès G, Guillemant S (1999) Vitamin D status during puberty in French healthy male adolescents. Osteoporos Int 10: 222–225

Hassan HA, Abdel-Wahhab MA (2012) Effect of soybean oil on atherogenic metabolic risks associated with estrogen deficiency in ovariectomized rats. J Physiol Biochem 68: 247–253

He C, Hui R, Tezuka Y, Kadota S, Li J (2010) Osteoprotective effect of extract from achyranthes bidentata in ovariectomized rats. J Ethnopharmacol 127:229–234

Ima-Nirwana S, Ahmad NS, Yee LJ, Loh HC, Yew SF, Norazlina M, Abdul GMT, Kamsiah JS (2007) Reheating of soy oil is detrimental to bone metabolism in oestrogen deficient rats. J Med 48:200–206

Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, Yamaguchi A, Yoshiki S, Matsuda T, Hirano T, Kishimoto T, Suda T (1990) IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 145:3297–3303

Johnson-Lynn SE, Francis R, McCaskie A (2008) Osteoporosis treatments and their effect on fracture healing. Curr Orthopaed 22:336–340

Kalu DN (1991) The ovariectomized rat model of postmenopausal bone loss. Bone Miner 15:175–191

Kasugai Y, Ikegami A, Matsuo K, Ohashi M, Sukamoto T, Hosoi T, Ouchi Y, Orimo H (1998) Effects of tibolone (Org OD14) treatment for 3 months on ovariectomy-induced osteopenia in 8-month-old rats on a low-calcium diet: preventive testing for 3 months. Bone 22:119–124

Keller ET, Binkley NC, Stebler BA, Hall DM, Johnston GM, Zhang J, Ershler WB (2000) Ovariectomy does not induce osteopenia through interleukin-6 in rhesus monkeys (Macaca mulatta). Bone 26:55–62

Knight DC, Eden JA (1996) A review of the clinical effects of phytoestrogens. Obst Gynecol 87:897–904

Krivosíková Z, Krajcovicová-Kudlácková M, Spustová V, Stefíková K, Valachovicová M, Blazícek P, Němcová T (2010) The association between high plasma homocysteine levels and lower bone mineral density in Slovak women: the impact of vegetarian diet. J Eur Nutr 49:147–153

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van der Saag PT, Van der Burg B, Gustafsson JA (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. J Endocrinol 139:4252–4263

Lau EM, Cooper C (1996) The epidemiology of osteoporosis: the oriental perspective in a world context. Clin Orthop Relat Res 323:65–74

Lerner UH (2006) Bone remodeling in post-menopausal osteoporosis. Dent J Res 85:584–595

Li S, He H, Ding M, He C (2010) The correlation of osteoporosis to clinical features: a study of 4382 female cases of a hospital cohort with musculoskeletal symptoms in southwest China BMC. Musculoskeletal 11:1471–2474

Lien T, Hsu Y, Lo D, Chiou RY (2009) Supplementary health benefits of soy aglycons of isoflavone by improvement of serum biochemical attributes, enhancement of liver antioxidative capacities and protection of vaginal epithelium of ovariectomized rats. Nutr Metabol 6:6–15

Lund CO, Mortensen A, Nilas L, Breinholt VM, Larsen J (2007) Bent Ottesen estrogen and phytoestrogens: effect on eNOS expression and in vitro vasodilation in cerebral arteries in ovariectomized Watanabe heritable hyperlipidemic rabbits. Eur J Obst Gynecol Reprod Biol 130:84–92

Mancini L, Moradi-Bidhendi N, Brandi M, MacIntyre I (1998) Nitric oxide, superoxide and peroxynitrite modulate osteoclast activity. Biochem Biophys Res Commun 243: 785–790

Matsumura Y, Kita S, Morimoto S, Akimoto K, Furuya M, Oka N, Tanaka T (1995) Antihypertensive effect of sesamin: protection against deoxycorticosterone acetate-salt-induced hypertension and cardiovascular hypertrophy. Biol Pharm Bull 18:1016–1019

Messina MJ, Persky V, Setchell KDR, Barnes S (1994) Soy intake and cancer risk: a review of the in vitro and in vivo data. J Nutr Can 21:113–131

Namkung-Matthai H, Appleyard R, Jansen J, Hao LJ, Maastricht S, Swain M, Mason RS, Murrell GA, Diwan AD, Diamond T (2001) Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. Bone 28:80–86

Norazlina M, Ima-Nirwana S, Gapor MT, Khalid BA (2000) Palm vitamin E is comparable to alpha-tocopherol in maintaining bone mineral density in ovariectomised female rats. Exp Clin Endocrinol Diabetes 108:305–310

Ososki A, Kennelly EJ (2003) Phytoestrogens, a review of the present state of research. Phytother Res 17:845–869

Potter JD, Steinmetz K (1996) Vegetables, fruit and phytoestrogens as preventive agents. IARC Sci Publ 139:61–90

Reinwald S, Weaver CM (2006) Soy isoflavones and bone health: a double- edged sword? J Nat Prod 69:450–459

Rodwell EW (1979) A review of physiological chemistery. In: Harper HA, Rodwell EW, Mayes P (eds), 17th edn. Lange Medical Publications, California, pp 401–404

Sacco SM, Power KA, Chen J, Ward WE, Thompson LU (2007) Interaction of sesame seed and tamoxifen on tumor growth and bone health in athymic mice. Exp Biol Med 232: 754–761

Sakai T, Kogiso M (2008) Soy isoflavones and immunity. Med J Invest 55:167–173

Sasaki Y, Ono H, Seki J, Giddings JC, Yamamoto J (2002) Effect of estrogen on the microcirculation and thrombus formation un pial vessels of the rat. Int Cong 1235:413–418

Satheesh M, Pari L (2008) Effect of pterostilbene on lipids and lipid profiles in streptozotocin–nicotinamide induced type 2 diabetes mellitus. J Appl Biomed 6:31–37

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

Shuid AN, Chuan LH, Mohamed N, Jaarin K, Fong YS, Soelaiman IN (2007) Recycled palm oil is better than soy oil in maintaining bone properties in a menopausal syndrome

model of ovariectomized rat. J Asia Pac Clin Nutr 16:393–402

Sok D, Cui HS, Kim MR (2009) Isolation and boactivities of furfuran type lignan compounds from edible plants. Nutr Agric 1:87–95

Sowmya M, Jeyarani T, Indrani D (2009) Effect of replacement of fat with sesame oil and additives on rheological, microstructural, quality characteristics and fatty acid profile of cakes. Food Hydrocoll 23:1827–1836

Torres N, Torre-Villalvazo I, Tovar AR (2006) Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. J Nutr Biochem 17: 365–373

Van't Hof RJ, Ralston SH (2007) Genetics of osteoporosis. J Nutr Soc 66:158–165

Van't Hof RJ, Macphee J, Libouban H, Helfrich MH, Ralston SH (2004) Regulation of bone mass and bone turnover by neuronal nitric oxide synthase. Endocrinology 145:5068–5074

Veille JC, Li P, Eisenach JC (1996) Effect of estrogen on nitric oxide biosynthesis and vasorelaxant activity in sheep uterine arteries and renal arteries *in vitro*. J Am Obstet Gynecol 174:1043–1049

Waller RA, Duncan DB (1969) A Bayes rule for the symmetric multiple comparison problems. J Am Stat Assoc 64:1484–1503

Wimalawansa SJ (2008) Nitric oxide: novel therapy for osteoporosis. Exp Pharmacol 9:3025–3044

Wu W (2007) The contents of lignans in commercial sesame oils of Taiwan and their changes during heating. Food Chem 104:341–344

Wu T, Zhang Y, Yuan H, Wu G, Li B, Cui L (2008) Preventive effects of Salvianolic acid B on osteopenia of rats induced by ovariectomy. Bone 43:75–76

Ya S, Saga I, Ichimura I, Nagai T, Shinoda M, Matsuzki S (2003) Coumesterol as well as isoflavones in soybean extract prevent bone resorption in ovariectomized rats. Endocrin Regul 37:145–152

Yamaguchi M, Sugimoto E (2000) Stimulatory effect of genistein and daidzein on protein synthesis in osteoblastic MC3T3-E1 cells, activation of aminoacyl-t-RNA synthetase. Mol Cell Biochem 214:97–102

Yamashita K, Iizuka Y, Imai T, Namiki M (1995) Sesame seed and its lignans produce marked enhancement of vitamin E activity in rats fed a low alpha-tocopherol diet. J Lipids 30:1019–10028

You L, Sheng Z, Tang C, Chen L, Pan L, Chen J (2011) High cholesterol diet increases osteoporosis risk via inhibiting bone formation in rats. Acta Pharmacol Sinica 32:1498–1504

Younes H, Garleb KA, Behr SR, Demigné C, Rémésy C (1998) Dietary fiber stimulates the extra-renal route of nitrogen excretion in partially nephrectomized rats. J Nutr Biochem 9:613–620

Zhang Y, Dong X, Leung P, Wong M (2009) Differential mRNA expression profiles in proximal tibia of aged rats in response to ovariectomy and low-Ca diet. J Bone 44:46–52

Zung A, Chalew SA (1997) Effect of age on the response to parathyroid hormone. Metabol 46:1246–1251