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Preventive Effects of Flaxseed and Sesame Oil on Bone Loss in Ovariectomized Rats

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Abstract: A study was designed to examine the effects of dietary flaxseed oil (FO) and sesame oil (SO) which are rich successively in n-3 and (n-9 and n-6) on biochemical parameters and histological status of bone. Sixty-four 90-day-old female wistar rats were randomly assigned to 6 groups: sham-operated rat (sham)+ control diets, ovariectomized rat (OVX))+ control diets, OVX+ 7% FO, OVX+ 7% SO, OVX+ 10% FO, OVX+ 10% SO. After 4 weeks of treatments, rats were euthanized; blood and tissues were collected for analyses. Markers of bone formation which is alkaline phosphatase activity and markers of bone resorption which is tartrate resistant acid phosphatase activity were measured. Present results showed that OVX increased significantly ALP and TRAP activity and the examination of bone tissue showed disruptive and lytic bone trabeculae. Animals fed 10% FO and 10% SO of fat reduced these parameters and improved bone microarchitecture. Whereas, there was no improvement in biochemical and histological states in OVX rats that received 7% of PUFAs successively provided from FO and SO diets. In conclusion, our results are encouraging because they suggest that PUFAs intake may help to prevent osteoporosis associated with estrogens deficiency. However, further studies are needed to determine the mechanism by which a diet rich in n-3 or lignans modulate bone tissue.

Key words: Flaxseed oil, sesame oil, ovariectomy, biochemical markers, PUFAs

INTRODUCTION

There is increasing evidence that dietary long-chain polyunsaturated fatty acids (PUFAs) may influence bone health in healthy states as well as in osteoporotic states (Claassen *et al*., 1995; Kelly *et al*., 2003). Their effects are exerted by altering the biosynthesis of prostaglandins (PGs). Prostaglandins are locally produced in osteogenic cells and they are considered as a potent stimulator of both bone formation and bone resorption (Raisz, 1999). The dietary n-3 PUFA were reported to lower arachidonic acid in bone and cartilage and to depress *ex vivo* PGE2 production in bone organ culture (Watkins *et al*., 1996; Xu *et al*., 1994). An over production of PGE2 is associated with a high ratio of n-6/n-3 fatty acids impairing the bone formation by reducing the activity of osteoblast (Watkins *et al*., 1996). In rat fed with a n-6 PUFA diet, the level of urinary pyridinium cross-links (markers of bone resorption) was significantly higher compared with that in rat fed with a n-3 PUFA diet (Kelly *et al*., 2003). Additional studies indicated that feeding diets containing n-3 PUFA have a positive effects on bone mass and biomarkers of bone metabolism in healthy well nourished (Griel *et al*., 2007; Green *et al*., 2004; Shen *et al*., 2007; Watkins *et al*., 2000) or ovariectomized animals (Sun *et al*., 2003; Watkins *et al*., 2006). The n-3 PUFAs content in flaxseed (Wallace *et al*., 2003) have anti-inflammatory properties that are mediated by the production of proinflammatory eicosanoids, which in turn offset the production of proinflammatory eicosanoids through competitive inhibition within their common metabolic pathway. It has been reported that diets containing α-linoleic acid (ALA) significantly decreased PGE2 production in monkey plasma (Wu *et al*., 1996) and in rat bone (Weiler *et al*., 2002). In addition, Caughey *et al*. (1996) have reported that flaxseed oil (FO) decreased TNF-α and IL-1β production in human peripheral blood mononuclear cells. Similar to (FO), sesame oil (SO) would exert the same effects on bone, through its composition characterized by a low level of saturated fatty acids and the presence of antioxidants such as sesamin, sesamolin and sesamol (Suja *et al*., 2004). Taking into account the FO and SO properties, the aim of this study was to investigate, in ovariectomized rats, the effects of FO and SO intake on several biochemical parameters and on the bone histological status comparing their ability to reverse established osteopenia.

MATERIELS AND METHODS

Animals and diets: Three-month-old virgin female wistar rats (colony room of Biology Department, Faculty of

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Table 1: Composition of experimental diets[1]

	Amount (g kg^{-1})					
	CO		FO		SO	
Components	7%	10%	7%	10%	7%	10%
Egg whites	140.00	140.00	140.00	140.00	140.00	140.00
Cornstarch	462.98	432.98	462.98	432.98	462.98	432.98
Dyestrose	132.00	132.00	132.00	132.00	132.00	132.00
Sucrose	100.00	100.00	100.00	100.00	100.00	100.00
Cellulose	50.00	50.00	50.00	50.00	50.00	50.00
Corn oil	70.00	100.00	-	-	-	-
Flax oil	-	-	70.00	100.00	-	-
Sesame oil	-	-	-	-	70.00	100.00
T butylhydroquinone	0.02	0.02	0.02	0.02	0.02	0.02
Mineral mix[2]	35.00	35.00	35.00	35.00	35.00	35.00
Vitamin mix[3]	10.00	10.00	10.00	10.00	10.00	10.00

[1]The AIN-93G standard rodent diet was modified and contained respectively 7 and 10% of Corn oil (CO), Flax oil (FO) and sesame oil (SO).
[2]Mineral mix (mg kg^{-1} of diet): Na_2SO_4-$10H_2O$, 0.359; KI, 0.35; KH_2PO_4, 7112.6; NaCl, 3000; $CuSO_4$, 11.5; $MgCl_2$-$6H_2O$, 930; $CaCO_3$, 14500; $MnSO_4$-H_2O, 24.05; KCl, 2040; $FeSO_4$-nH_2O, 217.7; Molybdate, 0.1; Chrome, 0.005; Selenium, 0.1; Zinc,15; NH_4VO_3, 0.231; Sucrose, 7148.005.
[3]Vitamin mix (mg kg^{-1} of diet): Niacin, 36; Calcium pantothenate, 12; Pyridoxine HCl, 4; Thiamine HCl, 2.8; Riboflavin, 3.2; Folic acid, 0.4; Biotin, 0.3; Vitamin E acetate(500 UI g^{-1}), 20; Vitamin B12, 0.002, Vitamin A palmitate (600 UI g^{-1}), 1.6; Vitamin D2, 15.001; Sucrose, 9904.697

Table 2: Fatty acids composition of corn oil, flaxseed oil and sesame oil

Fatty acids	CO	FO	SO
16:0	11.61	6.33	9.06
16:1 (n-7)	0.22	0.19	0.19
18:0	1.47	4.27	4.30
18:1 (n-7)	0.35	0.17	0.53
18:1 (n-9)	29.80	25.53	43.33
18:2 (n-6)	54.94	17.44	41.46
18:3 (n-3)	1.00	45.77	0.34
20:0	0.39	0.18	0.64

Results are expressed as mean of triplicate analysis. Common abbreviations and names for fatty acids; 16:0 (palmitic acid), 16:1 (n-7) (palmitoleic acid), 18:0 (stearic acid), 18:1 (n-7) (vaccenic acid), 18:1 (n-9) (oleic acid), 18:2 (n-6) (linoleic acid), 18:3 (n-3) (-linolenic acid), 20:0 (arachidic acid)

Sciences, Kenitra, Morocco) initially weighing between (143.1±4.8) and (194.5±15.1) g were either subjected to bilateral ovariectomy OVX (n = 48) or sham operated (sham, n = 16). The animals were divided randomly into eight groups. After sham or OVX surgery, the rats were fed with an experimental diet for four weeks (Table 1). The sham group and one OVX control group (OVX) received the basal diet; it was formulated following the AIN-93 M diet and differed from this one in the source of fat. The other two OVX groups received either flaxseed oil (FO) or sesame oil (SO) at the same concentration as basal diet; the total fat concentration in each diet was successively 70 and 100 g kg^{-1}. Fatty acid compositions of diets are given in (Table 2). The rats were housed in propylene cages under standards conditions (20°C, 50-70% humidity and 12L: 12D cycle). They were given Food and water *ad libitum*. Food consumption was measured at each feeding. Body weight was recorded weekly.

Sample collection: After 4 weeks of feeding, rats were anesthetized with chloral hydrate (0.5 mL/100 g, Sigma-Aldrich, laborchemikalien Gmbh, Germany) and blood and tissues were collected for analysis. Blood samples were collected and plasma was separated by centrifugation at 1500 x g for 20 min at 4°C. Aliquots of plasma were frozen and kept at -20°C for further analyses.

The liver was immediately removed, rinsed with ice-cold saline serum, weighed, kept in a sealed container and maintained at -20°C for further analysis. Uterus was collected, blotted and weighed.

Measurements of serum ALP and TRAP: Serum samples were analyzed for serum alkaline phosphatase (ALP) activity, which is an index of bone formation and tartrate resistant acid phosphatase activity (TRAP) an index of bone resorption using BioSystems BTS 310 photocolorimeter and Standard BioSystems reagents (Biolab, Casablanca, Morocco).

Histopathological studies: The histopathological studies were carried out on sections of decalcified right femur (fixed in faormalin). The bones were dehydrated in an ethanol series and embedded in paraffin and cut into longitudinal sections of 5 μm thickness. The sections were stained with hematoxylin phloxine saffron.

Statistical analyses: Data obtained are expressed as Mean±SEM. To evaluate the differences between groups we performed overall comparisons using non-parametric ANOVA (Kruskal-Wallis test). In groups that presented a statistically significant difference, the Mann-Whitney U-test was used to determine specific differences. We adopt a 5% significance level for all statistical tests.

RESULTS

Effects of ovariectomy, FO and SO on food intake, body weight and relative organ weigh: Data on food and body and organ weights are shown in Table 3. The average of

Table 3: Effects of ovariectomy, FO and SO on food intake, body weight and relative organs weight

Parameters		Sham	OVX	FO	SO
Food intake (g/day/rat)					
Diet 7%		26.70±4.21	26.75±4.21	26.74±4.21	26.71±4.21
Diet 10%		9.44±0.40	9.42±0.40	8.27±0.40	9.84±0.40
Body weight (g)					
Initial	Diet 7%	143.10±4.80	143.10±4.45	1430.10±4.28	143.10±4.28
	Diet 10%	194.80±5.80	194.30±5.40	194.50±5.40	194.30±5.70
Final	Diet 7%	151.40±13.2	169.20±14.5	151.20±14.5	151.20±14.5
	Diet 10%	229.80±10.7	235.02±19.4	235.20±15.9	230.10±12.8
Organs weight (g/100 g b.wt.)					
Uterus	Diet 7%	0.28±0.03^{a}	0.13±0.03^{b}	0.13±0.03^{b}	0.14±0.03^{b}
	Diet 10%	0.24±0.05^{a}	0.12±0.02^{b}	0.12±0.07^{b}	0.11±0.03^{b}
Liver	Diet 7%	3.11±0.40	3.10±0.40	3.016±0.4	3.06±0.40
	Diet 10%	2.40±0.38	2.32±0.43	2.34±0.30	2.39±0.56

Values are Mean±SEM; n = 6. Values with different superscript letter(s) are significantly different ($p<0.05$). Sham: Sham-operated; OVX: Ovariectomized rats; FO: Rat received a diet containing flaxseed oil; SO: Rat received a diet containing sesame oil

Table 4: Effects of ovariectomy, FO and SO on biochemical markers

	Treated groups			
Biochemical markers (UI L^{-1})	Sham	OVX	FO	SO
Diet 7%				
PAL	114.10±12.8^{a}	169.00±22.1^{b}	150.00±33.4^{b}	133.50±13.1^{b}
TRAP	0.60±0.06^{a}	0.79±0.35^{b}	0.67±0.23^{a,b}	0.72±0.15^{a,b}
Diet 10%				
PAL	183.20±11.8^{a}	300.00±49.1^{b}	166.80±17.9^{a}	184.00±32.0^{a}
TRAP	0.48±0.08^{a}	0.80±0.08^{b}	0.37±0.08^{a}	0.44±0.08^{a}

Values are Mean±SEM. Values with different superscript letter(s) are significantly different ($p<0.05$). Sham: Sham-operated; OVX: Ovariectomized rats; FO: Rat received a diet containing flaxseed oil; SO: Rat received a diet containing sesame oil

food intakes of OVX groups did not differ significantly from sham and other treated groups 6 with FO and SO supplemented diets (7 or 10% of fat). The treatment group that received supplemented diets with 7 or 10% of fat started with similar mean body weight; 143 and 194 g respectively. All rats gained weight during the study; however there were no significant differences between studied groups. Uterine weight of OVX, FO and SO greatly decreased by 50% in comparison with the sham rats ($p<0.05$). The liver weight (g/100 g body weight) did not show any significant differences among treatment groups in both FO and SO supplemented diets (7 or 10% of fat).

Effects of ovariectomy, FO and SO on biochemical markers: The measurement of activities of serum ALP and TRAP are shown in Table 4. We have observed that OVX rats presented an increase of TRAP and ALP activities compared with that of sham group. A significant decrease in ALP activity was shown in both treated groups supplemented with FO and SO at 10% of fat ($p<0.05$). No significant decrease was observed in groups that received 7% of fat. The OVX-induced TRAP activity increase was significantly lowered by intake of FO and SO diets at 10% but not at 7%. We didn't found any significant differences between FO and SO treated groups regarding the studied biochemical markers.

Effects of OVX, FO and SO on histological tissues: Microscopic examination of the femurs bone showed in sham-operated group (Fig. 1A, E); normal compact trabeculae with intertrabecular spaces (A and E). In ovariectomized group (Fig. 1B, F) and ovariectomized rats fed with 7% of fat provided from flaxseed oil (Fig. 1C) and sesame oil (Fig. 1D), sparse and thining trabeculae with tendency for disappearance, loss of connectivity and presence of adiposity cells. However, the ovariectomized rats fed with 10% of fat provided from flaxseed oil (Fig. 1G) and sesame oil (Fig. 1H) showed elongated trabeculae moderately tick and trabeculae's bone connectivity less lytic and disruptive than ovariectomized group.

DISCUSSION

In this study, the positive effect on bone metabolism and histology were observed when OVX rats were given 10% of FO and SO diets that are rich successively in (n-3) and (n-6 and n-9). We found that high PUFA diets reduced serum concentrations of TRAP and ALP activity and maintained their levels relatively similar to those of sham rats after 4 weeks of feeding. Whereas, there was no improvement in biochemical and histological states in OVX rats that received 7% of PUFAs successively provided from FO and SO diets.

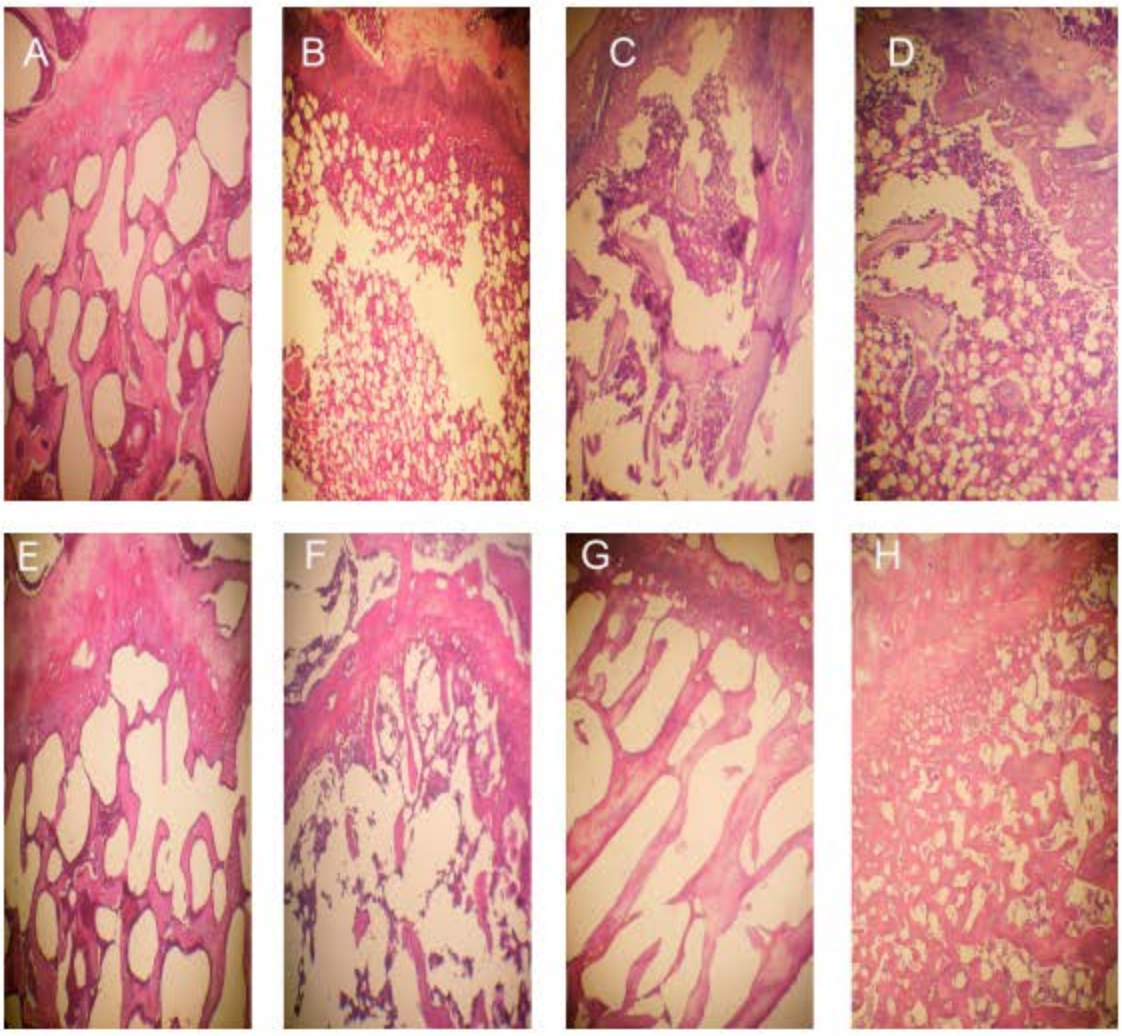

Fig. 1: Histological section [stained with hematoxylin-phloxin-safron (x120)] in longitudinal plane of the distal femur at the epiphyseal growth plate and metaphyseal area collected from sham-operated rats (A and E); ovariectomized rats (B and F) and ovariectomized rats fed 7 and 10% of fat provided from flaxseed oil (C and G) and sesame oil (D and H) successively (A and E). Epiphyseal region showing normal compact trabeculae with intertrabecular spaces in sham-operated group (B, F, C and D). Epiphyseal region showing sparse and thining of trabeculae with tendency for disappearance, loss of connectivity and presence of adiposity cells in ovariectomized group (B and F) and ovariectomized rats fed with 7% of fat provided from flaxseed oil (C) and sesame oil (D) (G and H). Epiphyseal region showing moderately tick elongated trabeculae and narrowed inter-trabecular spaces in ovariectomized rats fed with 10% of fat provided from flaxseed oil (G) and sesame oil (H)

Our results showed that, the biochemical parameters were characterized by an increase in ALP and TRAP activities in OVX rats. This indicates an increase in the osteoblastic and osteoclastic activity respectively, resulting in an overall net loss of bone. The histopathological examination of the femur also showed an increase in bone turnover and enhanced bone fragility, with disruptive and lytic changes in the bone architecture conduced to an osteodystrophy. All these changes showed that following OVX, there is a considerable increased in bone fragility indicating that estrogen deficiency causes osteoporosis. Interestingly, the benefits of dietary flaxseed oil and sesame oil were observed in this experiment by lowering the TRAP

activities and improvement of microarchitectural bone status, these changes in bone resorption parameters (TRAP) and bone formation (ALP) in FO and SO might indicate a reduction of bone resorption and increases in their bone formation. However, contrary to our finding, feeding diets supplemented with flaxseed improves lipid profiles but has no effect on biomarkers of bone metabolism in postmenopausal women (Lucas *et al*., 2002).

FO is a rich source of n-3 fatty acid; α-linolenic acid which can attribute to modulation of osteoblast and osteoclast functions during estrogen deficiency. Iwami-Morimoto *et al.* (1999) studied alveolar bone resorption in 4-week-old rats given diets supplemented with 10% of either fish oil or corn oil for 6 weeks. Dietary fish oil reduced osteoclastic activity (osteoclast number was only 60% of control) and alveolar bone resorption (80% of control). As an alternative to fish oil, flaxseed oil (FO), which contains approximately 56% a-linolenic acid (ALA) (Caughey *et al*., 1996), a precursor to Eicosapentaenoic acid EPA, has generated interest as a potential anti-inflammatory agent due to the ability of ALA to be converted to EPA in humans and animals (Li *et al*., 2003; Kelley, 1995). Moreover, an EPA-enriched diet was effective in minimizing bone loss induced by estrogen deficiency, which prevented the loss of bone weight and strength in OVX rats (Sakaguchi *et al*., 1994). While it is known that dietary EPA is more efficient than dietary ALA in raising tissue EPA concentrations (Caughey *et al*., 1996), the extent to which ALA is converted to EPA is controversial.

In other hand, the beneficial effects in SO diets might attributed to a group of lignans in the non-fat portion of the sesame oil which contains sesamin, sesamolinol, sesamol and other lignans (Chavali *et al*., 1997). Various biochemical actions related to lipid metabolism have been attributed to these lignans. They include specific inhibition of delta-5 desaturation of (n-6) fatty acids (Shimizu *et al*., 1991) that interrupt the formation of proinflammatory prostaglandins (Utsunomiya *et al*., 2000). Chavali and Forse (1999) have shown that the consumption of safflower oil diets supplemented with sesamol inhibited the production of cytokines such as IL6 and PGE2. Thus, we suggest that lignans in sesame oil might contribute to the prevention of osteoporosis by modifying cytokines and prostaglandins production and in consequent bone resorption markers. In conclusion, our results are encouraging because they suggest that PUFAs intake may help to prevent osteoporosis associated with estrogens deficiency. However, further studies are needed to determine the mechanism by which a diet rich in PUFAs especially n-3 or lignans modulate bone tissue.

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