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Sesame seed oil and flaxseed oil affect plasma lipid levels and biomarkers of bone metabolism in ovariectomized Wistar rats

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Abstract

The purpose of this study was to investigate the efficacy of flaxseed oil (FO) and sesame seed oil (SO) on lowering cholesterol and biomarkers of bone metabolism in Wistar rats as an ovariectomized (OVX) rat model. Thirty-two 90-day-old female Wistar rats were randomly assigned to four groups: sham-operated (sham) +control diet, ovx + control diet, ovx + 10% (FO), and ovx + 10% (SO). After 4 weeks of feeding, rats were euthanized and tissues and blood were collected for analyses. The total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), glucose concentration, alkaline phosphatase activity (ALP), and tartrate resistant acid phosphatase activity (TRAP) were measured. Results showed that ovariectomy significantly increased serum total – and LDL-cholesterol, these two parameters decreased significantly upon treatment with FO and SO. Serum HDL-cholesterol, triglyceride concentrations, and liver total cholesterol concentrations were unaffected by either of the treatments. The TRAP and ALP activities increased significantly in OVX rats compared with the sham group. A significant decrease in ALP and TRAP activities was observed in both treated groups supplemented with FO and SO at 10% of fat ($p < 0.05$). The ovariectomy, FO, and SO diets did not affect glucose levels. The findings of this study showed that FO and SO are beneficial in reducing plasma cholesterol, LDL- (low-density lipoprotein) cholesterol, and bone biomarkers induced by ovarian hormone deficiency.

Keywords: Flaxseed oil; sesame seed oil; ovariectomy; serum lipids; biomarkers of bone metabolism.

Introduction

Postmenopausal coronary heart disease (CHD) has become a major cause of morbidity and mortality in women (Van der Schouw *et al.* 1996). After the onset of menopause, the risk of CHD in women increases dramatically because of hormone deficiency especially in estrogens (Rosenberg *et al.* 1981). Decreased ovarian function involved in increased plasma concentrations of total and LDL-cholesterol and in an increased LDL/HDL ratio are among the important risk factors for the development of CHD (Assmann 1993; Martin *et al.* 1986). Several lines of evidences indicate that estrogens are important regulators of lipid homeostasis (Mendelsohn and Karas 1999; Basdevant 1992).

Estrogen replacement therapy has been shown to play an important beneficial role in the improvement of CHD in postmenopausal women (Stampfer and Colditz 1991). However, compliance with long-term estrogen replacement therapy for menopausal women is poor because of side effects (Stampfer and Colditz 1991; Samaan and Crawford 1995; Bush and Barret-Connor 1985). In recent years, several health foods have been used to improve nutritional status and prevent CHD. Both sesame seed (*Sesamum indicum* L.) and flaxseed (*Linum usitatissimum*) diets have shown to influence plasma lipid levels by changing lipid metabolism (Lucas *et al.* 2004; Chena *et al.* 2005). They are very rich source of lignans, α-linolenic acid (ALA), and soluble fiber (Beroza and Kinman 1955; Cunnane 2003), all that may positively affect women's CHD risk profile.

In patients with hypercholesterolemia, the consumption of sesame seed and flaxseed (FS) regimen reduces serum total cholesterol and LDL-cholesterol concentrations (Arjmandi *et al.* 1998; Hirata *et al.* 1996). In these clinical studies, the effect of dietary sesame seed (40 g/day) supplementation resulted in a 6.4% lower serum total cholesterol and 9.5% lower LDL-cholesterol, whereas the consumption of approximately 40–50

g/day of (Flaxseed) resulted in a 5–9% reduction in total cholesterol (Hirata *et al.* 1996; Lucas *et al.* 2002).

In animal studies, sesame oil has been shown to be slightly more hypocholesterolemic than corn oil (Koh 1987). Koh (1987) observed that sesame oil tended to reduce the serum cholesterol levels of rats compared with corn oil, in spite of the comparable fatty acid composition of the two oils. Results are controversial regarding the hypocholesterolemic effects of flaxseed oil (FO), Lucas *et al*. (2011) showed that flaxseed but not flaxseed oil prevented the rise in serum cholesterol due to ovariectomy in the Golden Syrian Hamsters. Whereas, a number of studies confirm the hypocholesterolemic effects of flaxseed in various experimental models (Yang *et al.* 2005; Prasad 1997; Prasad *et al.* 1998).

To the best of my knowledge, in few studies, the effect of both FO and sesame seed oil (SO) on hypercholesterolemia and biomarkers of bone metabolism in ovariectomized Wistar rats was examined. Therefore, the aim of this study was to evaluate whether the incorporation of SO and FO to the diet can produce a beneficial effects on lipid profile and consequently on the prevention of atherosclerosis and bone markers using an ovariectomized rats as a suitable model of postmenopausal hypercholesterolemia.

Materials and Methods

(a) Animals and diets

Three-month-old virgin female Wistar rats (Department of Biology, Kenitra, Morocco) initially weighing (194.5 ± 15.1) g were either OVX (n = 24) or sham-operated (sham, n = 8). The 16 OVX rats were randomly assigned to one of the two dietary treatments. Both sham rats and OVX (n = 8/group) were given the control diet (n = 8/group; Table 1). Diets were formulated following the AIN-93 M diet and varied from this in the source of fat. It contained 10% corn oil (CO), 10% FO and 10% SO. The total fat concentration in each diet was 100 g/kg. Food consumption was measured at each feeding and the body weight was recorded weekly. Experimental procedures are also examined and approved by The Internal Ethical Committee for Animal Welfare (see Tables 1 and 2).

(b) Sample collection

After 4 weeks of feeding, rats were anesthetized with chloral hydrate (0.5 ml/100 g, Sigma-Aldrich, Gmbh, Germany), and decapitated to collect blood. Blood samples were collected and plasma was separated by centrifugation at 1500 × *g* for 20 min at 4°C. Aliquots of plasma were frozen and kept at −20°C for later analyses. The liver was immediately removed, rinsed with ice-cold saline, weighed, kept in a sealed container and stored at −20°C until further analysis. The uterus was collected, blotted dry, and weighed.

(c) Serum triglycerides and total, and high-density lipoprotein cholesterol

Plasma lipid parameters such as total cholesterol (TC), triacylglycerol (TG), and high-density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic methods, using commercial kits from Diagnostique Biologique (Ainsebaa, Casablanca, Morocco). The low-density lipoprotein cholesterol (LDL-C) fraction and atherogenic index (AI) were determined according to the Friedewald equations (Friedewald *et al.* 1972): LDLC=TC−(Triglycerides/5+HDL-C), and AI=(TC)/HDL-C.

(d) Liver total lipids and cholesterol

Portions of livers were homogenized, and then extracted with 2:1 (v/v) chloroform:methanol mixture. After the addition of 0.12 mol/L of NaCl solution to the extraction solution and separation of phases, aliquots of the organic phase were analyzed for liver total cholesterol. Liver total cholesterol was determined using a color reagent of glacial acetic acid–$FeSO_4$–H_2SO_4. Total liver lipids were determined using the Folch gravimetric method. The remainder of the organic phase was evaporated, dried and weighed to measure the total liver lipids. Liver total cholesterol and lipids were calculated and reported per gram of liver.

Table 1: Composition of experimental diets[1].

Component	Amount (g/kg)		
	Corn oil	Flaxseed oil	Sesame oil
Egg whites	140	140	140
Corn starch	432.96	432.98	432.98
Dextrose	132	132	132
Sucrose	100	100	100
Cellulose	50	50	50
Corn oil	100	0	0
Flax oil	0	100	0
Sesame oil	0	0	100
T-butylhydroquinone	0	0.02	0.02
Mineral mix[2]	35	35	35
Vitamin mix[3]	10	10	10

[1]The three diets were modified AIN-93G standard rodent diet and contained 10% Corn oil, 10% Flax oil and 10% sesame oil.
[2]Mineral mix provided (mg/kg 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 provided (mg/ kg of diet): Niacin, 36; calcium pantothenate, 12; pyridoxine HCl, 4; Thiamine HCl, 2.8; Riboflavin, 3.2; Folic acid, 0.4; Biotine, 0.3; Vitamin E acetate (500 UI/g), 20; Vitamin B12, 0.002, Vitamin A palmitate (600 UI/g), 1.6; Vitamin D2, 15.001; Sucrose, 9904.697.

Table 2: The fatty acid composition of each diet.

	FO	S0	C0
16:00	6.33	9.1	11.61
16:01	0.19	0.19	0.22
18:00	4.3	4.3	1.47
18:1n-9	25.53	45	27.33
18:2n-6	17.44	41	55.3
18:3n-3	45.77	0.34	3

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-9 (oleic acid), 18: 2 n-6 (linoleic acid), 18: 3n-3 (linolenic acid).

(e) Analytical procedures

Lipids from the diet and rat tissues were saponified, and fatty acid methyl esters (FAME) prepared by esterification using boron trifluoride (BF3) in methanol (14%, wt/wt, Supelco Bellefonte, PA, USA). FAME were analyzed using a gas chromatograph (GC HP 5890 series II, autosampler 7673, HP 3365 ChemStation; Hewlett-Packard, Avondale, PA, USA) equipped with a DB 23 column (30 m, 0.53 mm i.d., 0.5 µm film thickness; J&W Scientific, Folsom, CA, USA). The GC was operated at 140°C for 2 min; temperature programmed 1.5°C/min to 198°C and held for 7 min. The injector and flame-ionization detector temperatures were 225 and 250°C, respectively. FAME were identified by comparison of retention times with authentic standards (GLC-422, GLC-87, GLC-68A, Nu-Chek-Prep, Elysian, MN, USA) and FAME prepared from menhaden oil (Matreya, Pleasant Gap, PA, USA).

(f) Measurements of glucose, ALP and TRAP

Plasma glucose levels were assayed by enzymatic methods, using commercial reagent kits purchased from Diagnostique Biologique (Ainsebaa, Casablanca, Morocco). Serum samples were analyzed for serum alkaline phosphatase (ALP) activity, which is an index of bone formation and tartarate resistant acid phosphatase activity (TRAP) an index of bone resorption using BioSystems BTS 310 photocolorimeter and Standard BioSystems reagents (Biolab, Casablanca, Morocco).

(g) Statistical analyses

Results are expressed as mean ± SE. Repeated measure analysis of variance (ANOVA) was used for statistical analysis of blood lipid data and body weight. Mann–Whitney U-test was used to determine the significance of differences

between any two groups. A p-value of less than 0.05 was considered significant.

Results

(i) Food intake, body and organ weights

There were no significant differences in mean daily food intake, initial and final body weights among the groups. Ovariectomy caused atrophy of uterine tissue, indicating the success of the surgical procedure. There was no difference in liver weights among any of the experimented groups (Table 3).

Table 3: Effects of ovariectomy OVX; enriched diet of flaxseed oil (FO) and enriched diet of sesame oil SO on body weight and organ weights in rats.

Measures	Sham	OVX	FO	SO
Feed intake (g/day)	9.44 ± 0.4	9.42 ± 0.4	8.27 ± 0.4	9.84 ± 0.4
Body weight (g)				
Initial	194.8 ± 5.8	194.3 ±5.4	194.5 ±5.4	194.3 ± 5.7
Final	229.8 ±11.7	235.02±19.4	235.2 ±15.9	230.1 ±12.8
Organ weights (g/100 g body wt.)				
Uterus	0.24 ± 0.05[a]	0.12 ± 0.02[b]	0.12 ± 0.07[b]	0.11 ± 0.03[b]
Liver	2.4 ± 0.38	2.32 ± 0.43	2.34 ± 0.30	2.39 ± 0.56

Values are least square means± SE, n = 8 in each group. Values that do not share the same letters (a, b) are significantly ($p < 0.05$) different from each other.

(ii) Serum total, HDL, and non-HDL cholesterol and triglycerides and Liver total cholesterol and total lipids

Ovariectomy resulted in significant increases of serum total cholesterol and non-HDL concentrations and in the AI compared with sham animals. These parameters decreased significantly under both FO and SO treatment into levels similar to those of sham group. Moreover, the percentage of HTR was significantly decreased in ovariectomized groups compared with the sham and both FO and SO groups. The decrease in serum total and non-HDL cholesterol did not differ significantly between the two treatments. Serum HDL cholesterol and triglycerides were not altered by either regimen. Liver total cholesterol decreased significantly upon the FO and SO diets, whereas the total lipid levels were not affected by any of the used treatments and ovariectomy (Table 4).

Table 4: Effects of ovariectomy OVX; enriched diet of flaxseed oil (FO) and enriched diet of sesame oil SO on serum and liver parameters.

Measures	Sham	OVX	FO	SO
Total cholesterol (mmol/L)	4.33 ± 0.5[a]	7.26 ±0.63[b]	4.22 ± 0.70[a]	4.9 ± 0.94[a]
HDLc (mmol/L)	0.99 ± 0.65	1.08 ± 0.65	1.03 ± 0.72	1.04 ± 0.68
LDLc (mmol/L)	2.77 ± 0.82 [a]	5.65 ± 0.84 [b]	2.56 ± 0.88 [a]	3.38 ± 0.84[a]
Triglycerides (mmol/L)	2.86 ± 1.14	2.92 ± 1.67	2.91 ± 1.54	2.6 ± 1.42
AI	3.33[a]	6.26[b]	3.22[a]	3.9[a]
HTR (%)	0.23[a]	0.15 [b]	0.24[a]	0 .21[a]
LDL/HDL ratio	2.80[a]	5.23[b]	2.49[a,c]	3.25[a]
Liver (mg/g)				
Total cholesterol	3.22 ± 0.17[a]	4.55 ± 0.52 [b]	3.52 ± 0.32 [a]	3.33 ± 0.15[a]
Total lipids	47.6 ± 0.14	48.9 ± 0.24	48.7 ± 0.24	47.2 ± 0.15

Values are least square means ± SE, n = 8 in each group. Values that do not share the same letters (a, b, c) are significantly ($P < 0.05$) different from each other.

A Non-HDL cholesterol = total cholesterol − HDL cholesterol- (triglycerides/5). The density lipoprotein cholesterol (Non-HDL) fraction was determined according to the Friedewald equations.

Atherogenic index (AI) = (TC-HDL)/HDL; HTR (%) = HDL−C/TC ratio. Values are given as means ± SD [mean of eight determinations].

(iii) Measurements of glucose, ALP and TRAP

The measurement of serum ALP and TRAP activities and plasma glucose levels are presented in Table 5. We observed that the TRAP and ALP activities increased significantly in OVX rats compared with the sham group. A

significant decrease in ALP and TRAP activities were observed in both treated groups supplemented with FO and SO at 10% of fat ($p < 0.05$). The ovariectomy, FO, and SO diets did not affect glucose levels.

Table 5: Effects of ovariectomy OVX; enriched diet of flaxseed oil (FO) and enriched diet of sesame oil (SO) on plasma glucose levels and bone biomarkers.

Measures	Sham	OVX	FO	SO
PAL (UI/L)	183.20 ± 11.8[a]	300 ± 49.1[b]	166.80 ± 17.9[a]	184 ± 32.0[a]
TRAP (UI/L)	0.48 ± 0.08[a]	0.80 ± 0.08[b]	0.37 ± 0.08[a]	0.44 ± 0.08[a]
Glucose (mg/dl)	423 ± 15.8	480 ± 4.2	450 ± 2.7	463 ± 4.5

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, PAL: alkaline phosphatase,TRAP: tartarate resistant acid phosphatase activity.

(iv) Liver total lipids and cholesterol

In the liver of ovariectomized rats, the palmitic (16:0), the stearic (18:00) and the arachidonic (20:4n–6) acids were significantly elevated. The sum of saturated fatty acids was significantly increased. Furthermore, their liver contained less polyunsaturated fatty acids of the n–3 series, the ratio of 20:n–6/20: 3n–6, which is an indirect index of delta 5 desaturase activity was significantly higher and the index of delta 6 desaturase activity (20:3n–6/A8:2n–6) significantly decreased in the ovariectomized rats compared with the sham rats. The liver fatty acids composition of FO groups enclosed high levels of polyunsaturated fatty acids of the n–3 series, increased levels of eicosapentaeoic (20:5n–3) and docosahexaenoic (22:6n–3) compared with the ovariectomized rats. The indexes of delta 5 desaturase and delta 6 desaturase were decreased upon the diet into values similar to the sham groups. In the SO groups, the sum of polyunsaturated fatty acids of the n–6 series was elevated in this treated group compared into sham, OVX, and FO groups. Similar data were observed concerning the index of delta 6 desaturase which was significantly higher in comparison with the other groups, but the index of delta 5 desaturase remained nearly similar to those of sham and FO groups. Moreover, the ∑n–6/n–3 ratio was significantly increased in SO and ovariectomized rats compared with the Sham and FO groups.

Table 6: Liver fatty acid composition of the experimental diet.

	Sham CO	OVX CO	FO	SO
16:00	11.45 ± 1.2[a]	28.4 ± 1.02[b]	17.2 ± 1.03[a]	15.2 ± 1.57[a]
18:00	14.5 ± 1.16[a]	20.9 ± 1.71[b]	12.3 ± 1.33[a]	19.5 ± 1.02[b]
18:1n-9	16 ± 0.05[a]	4.45 ± 0.03[b]	10.2 ± 0.04[c]	9.4 ± 0.05[c]
18:2n-6	23.7 ± 0.63[a]	10.65 ± 1.22[b]	17 ± 1.17[a]	11.9 ± 1.40[b]
18:3n-6	0.04 ± 0.02[a]	0.6 ± 0.04[b]	0.3 ± 0.04[b]	0.5 ± 0.12[b]
20:3n-6	0.93 ± 0.02[a]	0.13 ± 0.07[b]	0.9 ± 0.02[a]	2.4 ± 0.13[c]
20: 4n-6	13.45 ± 0.73[a]	25.2 ± 0.70[b]	13.45 ± 1.53[a]	29.1 ± 1.44[b]
22:4n-6	1.79 ± 0.32[a]	0.8 ± 0.27[b]	1.2 ± 0.28[a]	4.35 ± 0.20[c]
20:4n-3	9.25 ± 2.08[a]	3.27 ± 2.5[b]	5.8 ± 1.8[a]	1.55 ± 0.74[c]
20:5n-3	2.63 ± 0.63[a]	1.75 ± 0.86[a]	12.65 ± 0.58[c]	1.5 ± 0.78[a]
22:6n-3	6.25 ± 0.50[a]	3.85 ± 0.70[b]	9 ± 0.62[c]	4.5 ± 0.45[b]
∑SFA	25.95 ± 1.2[a]	49.3 ± 0.96[b]	29.5 ± 1.02[a]	34.7 ± 1.25[a]
∑MUFAS	16 ± 0.55[a]	4.45 ± 0.65[b]	10.2 ± 0.79[c]	9.4 ± 0.77[c]
n-6 PUFAs	39.91 ± 0.43[a]	37.38 ± 0.62[a]	32.85 ± 1.52[a]	48.25 ± 0.98[b]
∑n-3 PUFAs	18.13 ± 1.14[a]	8.87 ± 0.48[b]	27.45 ± 1.2[c]	7.55 ± 1.2[b]
∑n-6/n-3	2.20 ± 0.2[a]	4.21 ± 0.2[b]	1.20 ± 1.00[a]	6.39 ± 0.8[b]
D6D	0.04 ± 0.08[a]	0.01 ± 0.12[b]	0.05 ± 0.16[a]	0.20 ± 0.28[c]
D5D	14.46 ± 0.96[a]	193.85 ± 0.81[b]	14.94 ± 0.77[a]	12.13 ± 0.68[c]

Values are area per cent (mean ± SD of 8 rat/group); Values with different superscript letter(s) are significantly different ($p < 0.05$), Abbreviations: PUFA-polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA:saturated fatty acid; D6D-delta-6 desaturase index(20:3n-6/18: 2n-6); D5D:-delta-5 desaturase index (20:4n-6/20: 3n-6).

Discussion

In this study, ovariectomy increased serum total cholesterol and LDL-cholesterol; this enhancement might bring information about accumulation and deposition of LDL and cholesterol (within the arterial wall, which might lead to the initiation and promotion of atherosclerotic process (Persy and D'Haese 2009; Gardner *et al.* 1999; Westerveld and Erkelens 1998). Our results showed that the ratio of total cholesterol to HDL did not increase for ovariectomy and the AI was higher in ovariectomized rats compared with the sham rats, these data indicate that ovariectomy tend to increase LDL levels and may be the progression of atherosclerosis and especially hypercholesterolemia as a risk factor for atherosclerosis, however, the exact mechanism by which ovariectomy causes hypercholesterolemia is unknown.

The plasma cholesterol and LDL levels were decreased for rats consuming a diet supplemented with FO (10% oil) and sesame oil (10% oil) compared with the ovariectomized rats. The AI decreased significantly upon the used diets, this improvement is in accordance with other studies which shown that dietary supplementation of FO and SO reduce these parameters and improves lipid profiles (Hirose *et al.* 1991; Vijaimohan *et al.* 2006). Even both diets are given the same effects on lipid profile, but the SO diet effects it might not belong to its ALA contents (fair levels) if we compared with the FO diet (which is rich in ALA), whereas, we suggest the presence of other components in SO that play a role in the regulation of lipid profile such as the antioxidant contents.

The changes of fatty acids pattern are usually indicated by elevated 18:2n–6 and reduced 20:4n–6 levels (Kanzaki *et al.* 1987; Parinandi *et al.* 1990; Mimouni and Poisson 1992). In our study, the liver of ovariectomized rats showed an increased arachidonic acid levels, reduced D6D desaturase index and elevated D5D desaturase index, these changes might be accompanied by an impairment of desaturase activities, which consequently induced an unbalance increases in fatty acids profile. Thus, considering the increment in the 20:4n–6:18:2n–6 ratio observed in OVX groups compared with the control, we infer that there was an enhancement of D5D desaturase activity whose magnitude may have been high enough to cause the observed reduction in 18: 2n–6 despite the concomitant depression of D6D desaturase activity.

The liver total cholesterol concentrations were unaffected by either ovariectomy or any of dietary treatments. Previous studies have reported that FO and SO participate in the normal regulation of cholesterol metabolism in the liver (Ogawa *et al.* 1995; Vijaimohan *et al.* 2006), and ALA rich diet reduces hepatic lipid accumulation both by stimulating β-oxidation and by suppressing fatty acid synthesis (Murase *et al.* 2005). In current experiment, we did not note any changes in liver lipid profile, the explanation that we can provide is related to the duration time of experiment and the dose–response of FO and SO used was insufficient to induce the variation in liver lipid profile, other explanation is may be associated to the bile acid pool composition and metabolic response to changes in dietary cholesterol (Choi *et al.* 2001).

The estrogens effects on glucose metabolism are complex. Estrogen also decreases blood glucose and glycosylated hemoglobin, a marker of long-term vascular damage in diabetes (Brussaard *et al.* 1997; Andersson *et al.* 1997). In this study, the ovariectomy reduces ovarian hormone (atrophy of uterine tissue), and we did not shown any variation of glucose concentration in this group compared with the control one. Other measure and experiment are needed to ensure that the ovariectomy induces changes in glucose concentrations (for example, insulin concentration and hepatic glycogen concentration) and also to see whether the Wistar rat serves as a good model to study vascular damage, glucose metabolism, and their relationships.

The biochemical parameters of bone resorption (TRAP) and bone formation (ALP) were also evaluated in this study. In OVX group, the ALP and TRAP activities were increased compared with the control. This indicates an increment in the osteoblastic and osteoclastic activity respectively resulting in an overall net loss of bone (Kim *et al.* 2012; Boulbaroud *et al.* 2008). The administration of FO and SO improves these parameters, and might reduce bone resorption and increases the bone formation (Boulbaroud *et al.* 2008).

Many reviews focused on the mechanisms underlying the association of vascular tunica media (middle layer of artery wall) calcification with decreased bone density or disturbed bone turnover (Okuno *et al.* 2007; Persy and D'Haese 2009). Some studies also indicated that vascular calcification was associated with the risk of fragility fractures (Schulz *et al.* 2004; Bagger *et al.* 2006) and the induction of this process by a variety of stimuli has been reported, including elevated phosphate and calcium levels, alkaline phosphatase, BMP-2, oxidized low-density

lipoprotein (LDL) (Persy and D'Haese 2009; You *et al.* 2011). In our study, we noted that there was a bone turnover disturbance and enhancement in LDL and total cholesterol levels following ovariectomy, these changes were corrected upon the enriched diet with FO and SO at 10%. Unfortunately, we did not check the cross-section of arterial wall and its calcification, and also if there is a relationship between vascular calcification, bone mineral and bone turnover.

In summary, our data support the suggestion that FO and SO have a beneficial effect on hypercholesterolemia in ovariectomized rat, but their effect on the process of vascular calcification associated with disturbance of bone turnover needs further investigation.

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Ethical Approval

The study was approved by ethical committee of the Department of Biology, Faculty of Sciences, University Ibn Tofail.

Conflict of Interests

None declared.

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