Original Article

Recycled palm oil is better than soy oil in maintaining bone properties in a menopausal syndrome model of ovariectomized rat

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Palm oil is shown to have antioxidant, anticancer and cholesterol lowering effects. It is resistant to oxidation when heated compared to other frying oils such as soy oil. When a frying oil is heated repeatedly, it forms toxic degradation products, such as aldehydes which when consumed, may be absorbed into the systemic circulation. We have studied the effects of taking soy or palm oil that were mixed with rat chow on the bone histomorphometric parameters of ovariectomised rats. Female Sprague-Dawley rats were divided into eight groups: (1) normal control group; (2) ovariectomised-control group; (3) ovariectomised and fresh soy oil; (4) ovariectomised and soy oil heated once; (5) ovariectomised and soy oil heated five times; (6) ovariectomised and fresh palm oil; (7) ovariectomised and palm oil heated once; (8) ovariectomised and palm oil heated five times. These oils were mixed with rat chow at weight ratio of 15:100 and were given to the rats daily for six months. Ovariectomy had caused negative effects on the bone histomorphometric parameters. Ingestion of both fresh and once-heated oils, were able to offer protections against the negative effects of ovariectomy, but these protections were lost when the oils were heated five times. Soy oil that was heated five times actually worsens the histomorphometric parameters of ovariectomised rats. Therefore, it may be better for postmenopausal who are at risk of osteoporosis to use palm oil as frying oil especially if they practice recycling of frying oils.

Key Words: palm oil, vitamin E, heated frying oils, ovariectomy, bone histomorphometry

INTRODUCTION

Palm oil is beneficial to health as it contains carotenoids, tocopherols and tocotrienols which have antioxidant, anticancer and cholesterol lowering effects.[1,2,3] Studies have shown that palm vitamin E were able to prevent bone loss from FeNTA toxicity,[4] hyperthyroidism,[5] ovariectomy[6] and orchidectomy.[7] Palm oil is derived from the flesh of the fruit of the oil palm species *Elaeis guineensis* which is originated from West Africa. It has good resistance to oxidation and heat at prolonged elevated temperatures.[8] Therefore, high percentages of palm oil are incorporated in frying oil blends for both performance and economic reasons. In many instances, palm oil has been used as replacement for the traditional hydrogenated seed oils such as soy oil.

Frying is the process of cooking food using heated oil as the heat transfer medium. Deep frying is a frying process where the food is completely immersed in the frying oil. It is a common practice in the household or in the commercial sector to use the same frying oil repeatedly to save cost. The oils would only be discarded if they produced smoke, foam and bad odour or if the colour had become darkened. These changes occur because during the process of frying, lipids especially polyunsaturated fatty acids (PUFA) undergo chemical reactions of oxidation, hydrolysis and polymerization. These complex series of chemical reactions lead to generation of degradation products, both volatile and non-volatile. The non-volatile products of degradation consisting of polymers and polar compounds remained in the oil.[9,10] When frying oils are heated at 70°C, polar compounds like hydroperoxides and aldehydes are formed but when they are heated at 150 °C, aldehydes are mainly formed.[11] Repeatedly heating of frying oils may also cause rancidity and destruction of vitamins and essential fatty acids.

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Fried foods may absorb some of the repeatedly heated frying oil along with their degradation products. When ingested, aldehydes may be absorbed by the gastrointestinal system into the systemic circulation.[12]

Aldehydes have been shown to be cytotoxic and are related to many degenerative illnesses. Increased cellular production of 4-hydroxynonenal and related aldehydes has been linked to pathophysiological effects associated with oxidative stress.[13] Thus good frying oil would be able to withstand repeated heating with less formation of degradation products.

The use of repeatedly heated frying oils is an unhealthy practice. It has been linked to an increased of risk of hypertension,[14] disturbance of endothelial function[15] and increased lipoprotein oxidation.[16] There has been no report on the effect of using repeatedly heated frying oils on postmenopausal osteoporosis. Postmenopausal osteoporosis occurs in women with estrogen deficiency as a result of excessive bone resorption. The increase in bone resorption activities has been associated with an increase in oxidative stress.[17,18] It is believed that the estrogen deficiency condition in postmenopausal women has lead to a decrease in thiol antioxidants, exposing the bone to oxidative damage.[19] This was supported by a study which showed that women with postmenopausal osteoporosis have increased malondialdehyde levels and decreased superoxide dismutase levels.[20]

This study concentrates on comparing the effects of repeatedly heated (recycled) palm and soy oil on bone histomorphometric parameters in an experimental model of postmenopausal osteoporosis (the ovariectomised rat).

MATERIALS AND METHODS

Animals

64 female Sprague-Dawley rats aged 3 months old (200-250g) were obtained from the University Animal House. The rats were divided randomly into 8 groups with 8 rats in each group. The first group was not ovariectomised and acted as the normal control group (NC). The second group is the ovariectomised control group (Ovx), while the rest of the groups were ovariectomised and given rat chow mixed with fresh soy oil (SOF), fresh palm oil (PO), oils heated once (SO1, PO1) or oils heated five times (SO5, PO5). The rats were treated daily for 6 months.

The rats were kept in cages in groups of three rats per cage at room temperature with 12 hour light and dark cycle. The rats were allowed to acclimatize for three days before they were given rat chow (Gold Coin, Selangor, Malaysia) mixed with fresh or heated soy or palm oil *ad libitum*. They have access to tap water *ad libitum*. All the rats except the first group were ovariectomised after anesthesized with intraperitoneal injection of ketamine hydrochloride and xylazine at doses of 50 and 10 mg/kg body weight respectively. Bilateral ovariectomies were performed from a dorsal approach. There was no sham-operated group as the treatment period was long. The rat bones were fluorescence-labeled by injecting the rats twice with calcein at nine and two days before sacrifice. At the end of the study, the rats were anesthetized with ether and killed by cervical dislocation before the femurs were harvested. Success of ovariectomy was confirmed at necropsy by failure to detect ovarian tissue and by observation of marked atrophy of the uterine horns. This study was approved by the University Research and Animal Ethics Committee.

Preparation of heated oils

The soy oil or palm oil were not heated (SOF, POF) or heated according to modified methods of Owu *et al*., 1998.[21] Briefly, the oils were used to fry fish crackers which were obtained from the same supplier, in a stainless steel pan for 10 minutes at 180°C. The oils were left to cool for five hours. This would be the once-heated soy oil or palm oil (SO1, PO1). To prepare oils that were heated five times, the procedure was repeated four more times. The oils were mixed with rat chow at weight ratio of 15:100 and were fed to the rats daily for six months. The oil: rat chow ratio represents the average amount of daily oil intake in human.[21]

Bone histomorphometry

The distal portion of the femur samples were kept in 70% alcohol. For undecalcified samples, they were dehydrated and embedded in methyl methacrylate according to Difford, 1974.[22] The block was sectioned at 10 μm with a microtome (Leica, Wetzlar, Germany) and stained with the Von Kossa stain. Bone samples were also decalcified with EDTA for 4 weeks and embedded in paraffin wax. The decalcified samples were sectioned at 8 μm with a microtome (Leica, Wetzlar, Germany) and stained with hematoxylin and eosin. The undecalcified samples were used to measure the structural parameters with an image analyzer (Nikon Eclipse 80i, Japan) while the decalcified samples were used to measure static parameters by using the Weibel Technique, a quantitative stereological technique for histological sections.[23] The bones were decalcified for static parameter measurements to provide a clear background during staining so that the bone cells will be visualized easily. The bones labeled with the fluorescence marker calcein were examined with a fluorescence microscope and an image analyzer (Nikon Eclipse 80i, Japan) to measure dynamic parameters. All histomorphometric parameter measurements were performed at the metaphyseal region, which is located 3 to 7 mm from the lowest point of the growth plate and 1 mm from the lateral cortex. This secondary spongiosa area is rich in trabecular bone.

Parameters

Food intake and body weight were measured daily and weekly respectively. The structural parameters measured were bone volume per tissue volume (BV/TV %), trabecular thickness (TbTh, μm) and trabecular separation (TbSp, μm). The static parameter measured were osteoclast surface per bone surface (OcS/BS, %), osteoblast surface per bone surface (ObS/BS, %), eroded surface per bone surface (ES/BS, %). The dynamic parameters measured were single-labeled surface per bone surface (sLS/BS, %), double-labeled surface per bone surface (dLS/BS, %), mineralizing surface per bone surface (MS/BS, %) and bone formation rate per bone surface (BFR/BS, $\mu m^3/\mu m^2$/day). All the parameters were measured according to the American Society of Bone Mineral Research Histomorphometry Nomenclature Committee 1987.[24]

Statistical analysis

The results were expressed as mean values ± SD. Data analysis was performed using SPSS for Windows software (SPSS Inc., version 12.0.1). Statistical test used was ANOVA followed by Tukey's HSD (Honestly Significantly Different) for normally distributed data and Kruskal-Wallis and Mann-Whitney test for data that is not normally distributed. Changes were considered significance for p values less than 0.05.

RESULTS

There was no significant difference of food intake or body weight between the groups throughout the study ($p > 0.05$) (data not shown). In Figure 1 and 2, the density of bone trabecular at the secondary spongiosa which were stained black with Von Kossa for each group was

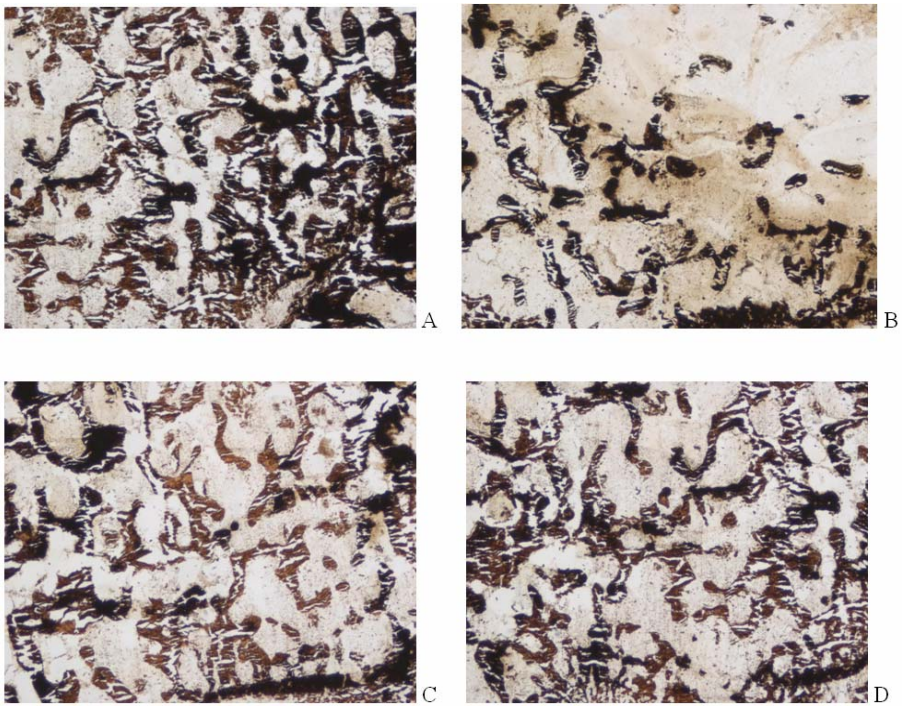

Figure 1. Bone trabeculae from rat femur were stained black with Von Kossa seen under the microscope (5X). A: normal control group; B: ovariectomised control group-reduction in the amount of bone trabeculae due to ovariectomy; C & D: the ovariectomised groups fed with fresh soy and palm oil – fresh oils restore the amount of bone trabeculae of the ovariectomised rats.

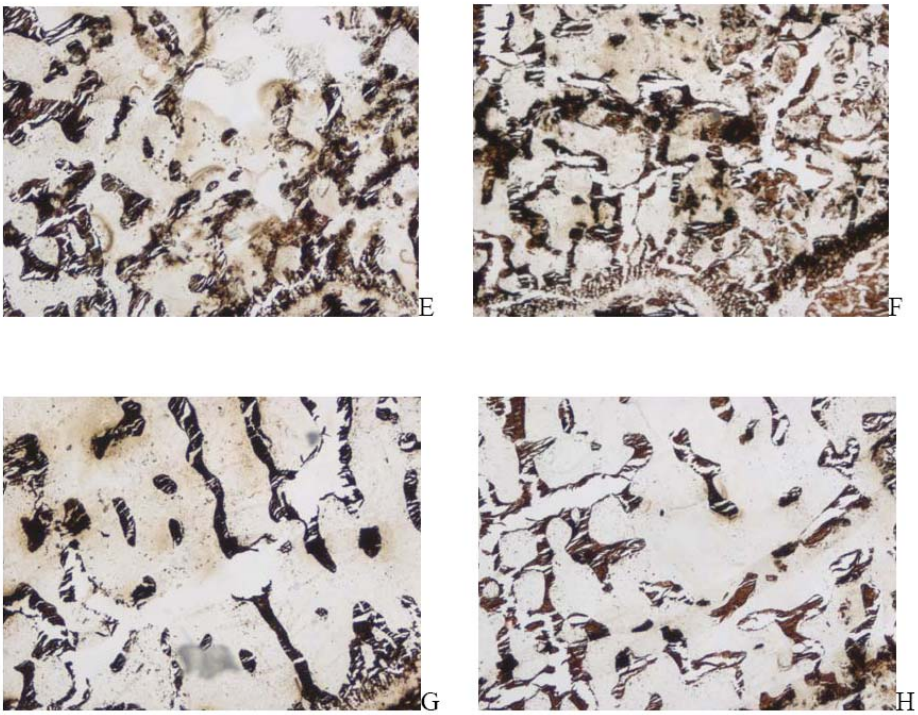

Figure 2. Bone trabeculae from rat femur were stained black with Von Kossa seen under the microscope (5X). E & F: ovariectomised groups fed with soy and palm oil heated once - partial restoration of the amount of bone trabeculae from the effects of ovariectomy; G & H : ovariectomised groups fed with soy and palm oil heated five times - the amount of bone trabeculae were similar or further reduced compared to ovariectomised control group (A).

Table 1. Effects of heated oils on structural parameters of ovariectomised rats

Groups	BV/TV (%)	Tb.Th (μm)	Tb.Sp (μm)
NC	33.8 ± 2.67	27.2 ± 1.81	53.5 ± 5.64
OVX C	16.1 ± 1.59	14.2 ± 0.95	74.6 ± 10.7
SOF	45.8 ± 0.83	33.3 ± 2.48	39.3 ± 2.92
POF	53.0 ± 4.04	36.7 ± 1.93	32.8 ± 3.60
SO1	32.3 ± 2.23	30.2 ± 1.99	63.6 ± 8.40
PO1	37.5 ± 2.66	31.7 ± 2.05	53.1 ± 5.12
SO5	9.02 ± 1.71	9.16 ± 1.20	94.1 ± 12.4
PO5	18.4 ± 3.15	16.7 ± 1.49	74.8 ± 11.3

Mean ± S.D. , n=8. NC: Normal control (not ovariectomised); OVX C: Ovariectomised control; SOF : OVX, fresh soy oil; SO1; OVX, soy oil heated once; SO5; OVX, soy oil heated five times; POF: OVX, fresh palm oil; PO1: OVX, palm oil heated once; PO5: OVX, palm oil heated five times; BV/TV: Bone volume per tissue volume; Tb.Th: Trabecularr thickness; Tb.Sp: Trabecular separation

Table 2. Effects of heated oils on static parameters of ovariectomised rats

Groups	Oc.S/BS (%)	Ob.S/BS (%)	ES/BS (%)
NC	16.4 ± 0.96	44.7 ± 2.66	29.3 ± 3.23
OVX C	26.5 ± 2.28	35.0 ± 1.44	44.6 ± 2.25
SOF	10.3 ± 1.64	66.8 ± 2.31	22.7 ± 2.25
POF	10.1 ± 1.84	78.8 ± 2.40	21.0 ± 2.89
SO1	19.9 ± 1.80	51.8 ± 3.18	39.0 ± 1.58
PO1	20.4 ± 2.02	54.5 ± 3.68	38.4 ± 2.46
SO5	33.0 ± 2.22	19.3 ± 4.44	64.2 ± 2.85
PO5	27.8 ± 2.39	35.5 ± 2.40	52.0 ± 2.55

Mean ± S.D. , n=8. NC: Normal control (not ovariectomised); OVX C: Ovariectomised control; SOF: OVX, fresh soy oil; SO1: OVX, soy oil heated once; SO5: OVX, soy oil heated five times; POF: OVX, fresh palm oil; PO1: OVX, palm oil heated once; PO5: OVX, palm oil heated five times; Oc.S/BS: Osteoclast surface per bone surface; Ob.S/BS: Osteoblast surface per bone surface; ES/BS: Eroded surface per bone surface

Table 3. Effects of heated oils on dynamic parameters of ovariectomised rats

Groups	sLS/BS (%)	dLS/BS (%)	MS/BS (%)	BFR/BS ($\mu m^3/\mu m^2$/day)
NC	45.4 ± 4.48	46.9± 2.45	69.6 ± 2.36	3.91 ± 0.18
OVX C	65.0 ± 2.13	22.0 ± 3.21	54.5 ± 3.00	3.14 ± 0.15
SOF	43.1 ± 3.60	60.1 ± 1.36	81.7 ± 1.47	5.67 ± 0.72
POF	40.7 ± 5.18	63.0 ± 5.13	83.4 ± 5.20	6.25 ± 0.22
SO1	46.3 ± 3.10	53.7 ± 3.10	76.9 ± 1.55	4.32 ± 0.35
PO1	45.7 ± 5.11	59.3 ± 4.36	82.2 ± 5.14	4.71 ± 0.27
SO5	91.8 ± 7.10	10.4 ± 1.04	56.3 ± 4.25	1.14 ± 0.21
PO5	64.9 ± 1.60	26.0 ± 3.35	58.5 ± 2.64	3.21 ± 0.24

Mean ± S.D. , n=8. NC: Normal control (not ovariectomised); OVX C: Ovariectomised control; SOF: OVX, fresh soy oil; SO1: OVX, soy oil heated once; SO5: OVX, soy oil heated five times; POF: OVX, fresh palm oil; PO1: OVX, palm oil heated once; PO5: OVX, palm oil heated five times; sLS/BS: Single- labeled surface per bone surface; dLS/BS: Double-labeled surface per bone surface; MS/BS: Mineralizing surface per bone surface; BFR/BS: Bone formation rate per bone surface

compared. Grossly, trabecular density of ovariectomised group was reduced compared to the normal control group. Fresh palm and soy oil restored the trabecular density but when they were heated once and five times, the trabecular density dwindles back to the pattern seen in ovariectomised group.

After six months, as expected, ovariectomy had caused negative effects on all the histomorphometric parameters (Table 1, Table 2 and Table 3). It reduced trabecular volume (BV/TV) (52.1%) and trabecular thickness (TbTh) (47.8%) but increased trabecular separation (TbSp) (39.2%) for the structural parameters (Fig 3). It increased osteoclast surface (OcS/BS) (61.7%) and eroded surface (ES/BS) (52.3%) but reduced osteoblast surface (ObS/BS) (21.6%) for the static parameters (Fig 4). It increased single-labeled surface (sLS/BS) (43.1%) and reduced double-labeled surface (dLS/BS) (53.2%), mineralizing surface (MS/BS) (21.8%) and bone formation rate (BFR) (19.7%) for the dynamic parameters (Fig 5).

DISCUSSION

The term repeatedly heated frying or cooking oil has been used interchangeably with thermally oxidized oils or recycled oils. The heating process has changed the properties of the frying oil in terms of quality, colour, smell and taste due to presence of polymers and polar compounds. The repeated heating has oxidized the lipid content to potentially toxic lipid peroxidation products.[25] Lipid hydroperoxides decomposed to highly cytotoxic products especially aldehydes [26] which are partly absorbed into the systemic circulation.[27] Peroxyl radicals and aldehydes caused severe damage to membrane proteins, inactivating

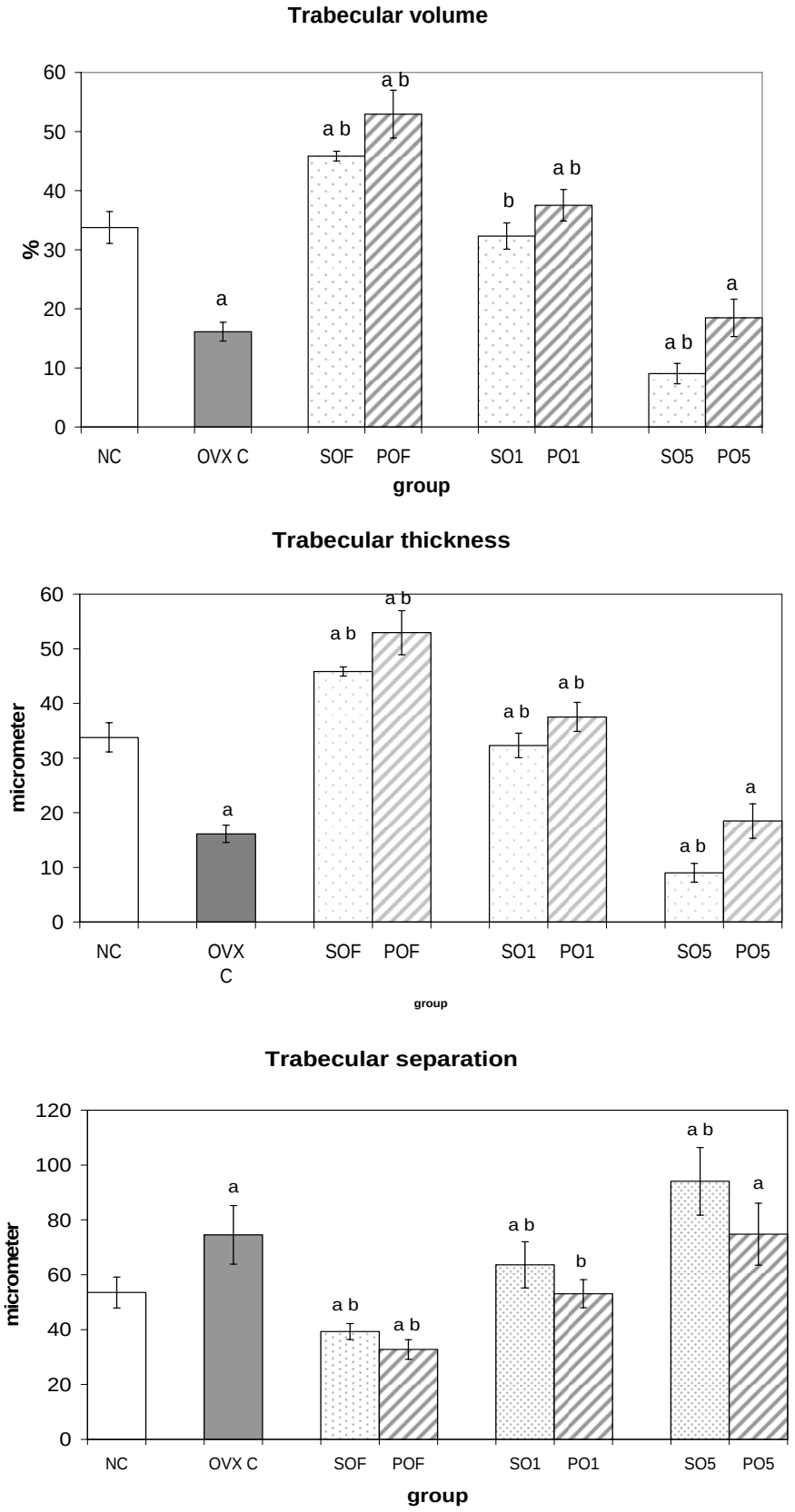

Figure 3. Effects of heated oils on structural parameters. a: significantly different to NC. b: significantly different to OVXC. (p<0.05). NC: Normal control; Ovx: Ovariectomised control; SOF: Ovariectomised + fresh soy oil; SO1: Ovariectomised + soy oil heated once; SO5: Ovariectomised + soy oil heated 5 times; POF: Ovariectomised + fresh palm oil; PO1: Ovariectomised + palm oil heated once; PO5: Ovariectomised + palm oil heated 5 times. Value is expressed as mean ± SD. p<0.05 is considered to be significance.

receptors and membrane-bound enzymes.[28] A great deal of attention on polar compounds has been focused on malondialdehyde, but there are other more noxious polar compounds such as 4-hydroxynonenal.[29] Ideally, frying oils for human consumption must be discarded when the polar compounds concentration reaches more than 25

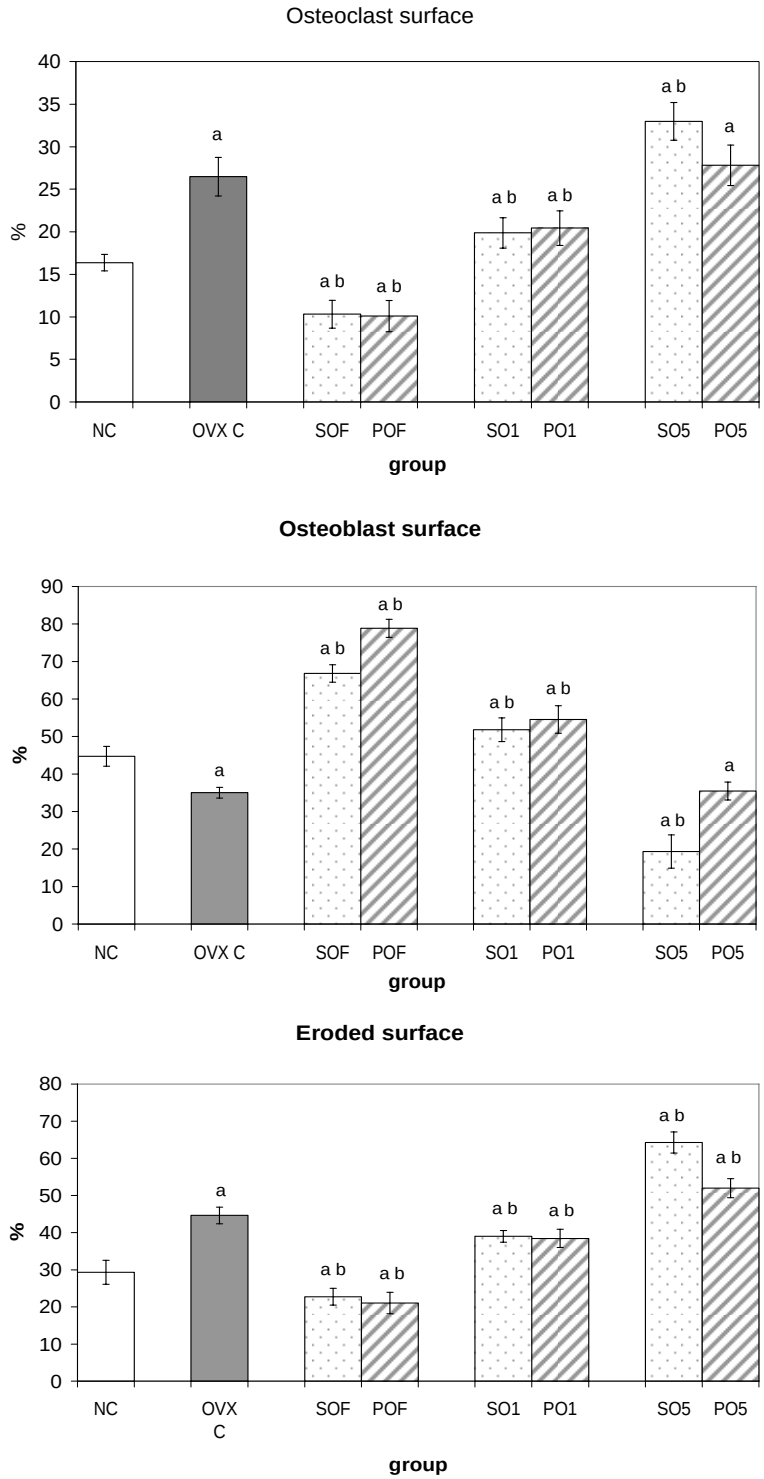

Figure 4. Effects of heated oils on static parameters. a: significantly different to NC. b: significantly different to OVXC. (p<0.05). NC: Normal control; Ovx: Ovariectomised control; SOF: Ovariectomised + fresh soy oil; SO1: Ovariectomised + soy oil heated once; SO5: Ovariectomised + soy oil heated 5 times; POF: Ovariectomised + fresh palm oil; PO1: Ovariectomised + palm oil heated once; PO5: Ovariectomised + palm oil heated 5 times. Value is expressed as mean ± SD. p<0.05 is considered to be significance.

percent.[30]

In this study, we have compared the effects of ingestion of the two main vegetable oils used in the world, palm oil and soy oil, on bone histomorphometric parameters in ovariectomised rats. These oils were used to fry fish crackers, a well known delicacy in Malaysia. It was selected because fish is usually part of the daily meals of most households in Malaysia. Usually, the same oil was used several times to fry fish before it is discarded.

There may be questions raised on the probability that fish oil might get into the frying oil and affect the result of this study. Some studies have suggested the benefit of fish oil consumption on cardiovascular system [31, 32, 33] and estrogen-deficiency bone loss.[34,35] However, all the treated groups in our study had received frying oils that were used to fry fish cracker and therefore would be exposed to the same effects of fish oil, if there is any. Chicken or other food may be used instead, but fish was

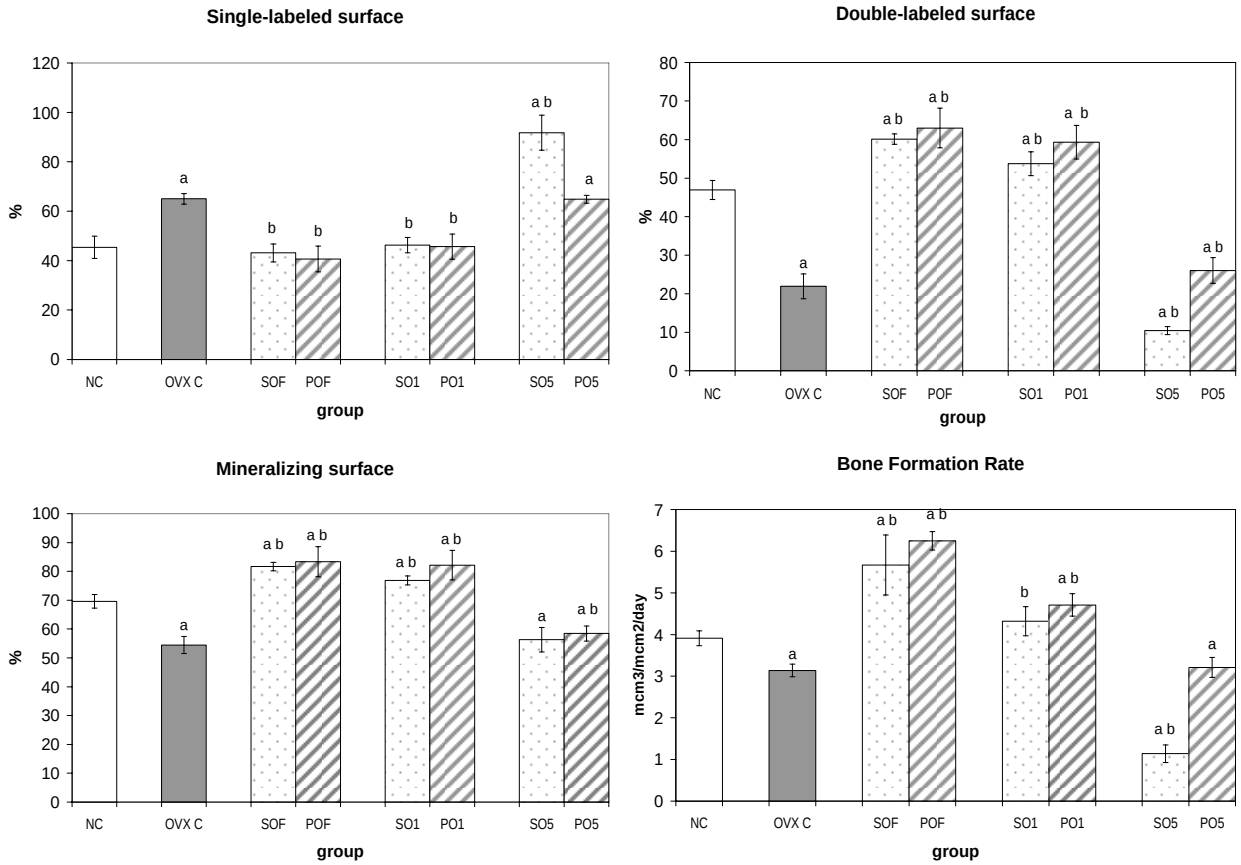

Figure 5. Effects of heated oils on dynamic parameters. a: significantly different to NC. b: significantly different to OVXC. (p<0.05). NC: Normal control; Ovx: Ovariectomised control; SOF: Ovariectomised + fresh soy oil; SO1: Ovariectomised + soy oil heated once; SO5: Ovariectomised + soy oil heated 5 times; POF: Ovariectomised + fresh palm oil; PO1: Ovariectomised + palm oil heated once; PO5: Ovariectomised + palm oil heated 5 times. Value is expressed as mean ± SD. p<0.05 is considered to be significance.

selected as it was more representative of the local setting. There was no sham-operated group in this study. The purpose of sham is to expose the rat to the same surgical stress as the ovariectomised groups but since the duration of the study is long, the surgical stress that may influence the result would have subsided. Therefore the sham-operated groups were not included due to ethical reasons.

In this study, the ovariectomised rats, which are accepted models for postmenopausal bone loss,[36,37] had shown deterioration in all the histomorphometric bone parameters. This was expected due to the increased bone resorption in estrogen deficiency. Estrogen deficiency has been shown to reduce the thiol antioxidants, exposing bone to free radical toxicity.[19] Postmenopausal osteoporosis had also been associated with conditions of oxidative stress.[20] In this study, we found that ingestion of heated oils further aggravate the negative effects of estrogen deficiency on bone.

Our findings have shown that soy or palm oil in the fresh form or heated once, when fed to ovariectomised rats, were able to prevent all the ovariectomy-induced changes on the histomorphometric parameters. Fresh and once-heated palm and soy oils had improved all the bone histomorphometric parameters except sLS/BS when compared to the normal control group. However when soy oil was heated five times, these protective effects were lost. In fact, it further deteriorates all the parameters in ovariectomised rats except for MS/BS by 24.6% to 63.7%.

Heating the soy oils five times had caused further increased in OcS and decreased in ObS in the ovariectomised rats. This could lead to higher bone resorption than bone formation and eventually the negative changes seen in the histomorphometric structural and dynamic parameters. However, this effect was not seen in the five times-heated palm oil group.

Palm oil seems to be able to withstand being heated five times better than soy oil. Although most of the protective effects on ovariectomy-induced changes were lost, it does not cause further deterioration of the histomorphometric parameters except for ES/BS. Some protective effects were still seen on dLS/BS and MS/BS. Therefore, palm oil may be a safer vegetable oil for postmenopausal women especially when the oil is repeatedly heated.

Previous studies have suggested that the unique composition of palm oil allows it to withstand heat better than soy oil. Firstly, it is rich in monounsaturated fatty acids (MUFA) but has low level of polyunsaturated fatty acids (PUFA) compared to soy oil.[38] PUFA is more easily oxidised compared to MUFA.[39] Repeated heating of vegetable oil high in PUFA results in formation of toxic compounds that increased the risk of hypertension.[14] Whereas, oils that are rich in MUFA (oleic acid) such as palm oil and olive oil can better withstand oxidation and formed less degradation products when they are heated. Repeated

heating of olive oil generate less aldehyde compared to other oils.[40,41]

Secondly, vitamin E may play an important role in the ability of frying oil to withstand thermal oxidative changes. Inclusion of α -tocopherol to frying oil was found to render PUFA more resistant to oxidation.[12] Rats fed on diet containing 15% oxidized frying oil (OFO) had significantly lower α-tocopherol concentrations in plasma and most tissues than rats fed on diet containing similar level of fresh soybean oil.[42] These OFO-fed rats also had accelerated body α-tocopherol catabolism in a radioisotope study.[43] Supplementation of vitamin E to olive oil was found to increase the stability of this oil under prooxidant conditions, and its intake was found to decrease the oxidative damage generated by adriamycin in rats.[44] Vitamin E, which effectively protects fatty acids in the oil from oxidation deteriorate after each frying episode.[45] Therefore, repeated heating of frying oils destroys the vitamin E content and exposes the fatty acids to oxidation. The vitamin E content of palm oil mainly consists of tocotrienols, while the main vitamin E in soy oil is tocopherols.[46] Tocotrienols have better antioxidant capacity than tocopherols[47,48] and this may contribute to the better resistance to oxidative changes due to repeated heating seen in this study.

In conclusion, repeatedly heated frying oils especially soy oil, may further deteriorate the ovariectomy-induced bone changes in rats. Palm oil maybe more stable to heat than soy oil because of its unique composition of fatty acids and vitamin E. Therefore, it may be unhealthy for post-menopausal women to use repeatedly heated frying oils due to their negative effects on bone, but if this is not practical economically, then it may be better to use palm oil instead of soy oil.

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REFERENCES

1. Nesaretnam K., Stephan R, Dils S, Darbrey P. Tocotrienol inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. Lipids. 1988;33: 461-469.
2. Guthrie N, Gapor A, Chambers AF, Caroll KK. Inhibition of proliferation of estrogen-receptor –negative MDA-MB-435 and –positive MCF-7 human breast cancer cells by palm oil tocotrienols and tamoxifen, alone or in combination. J Nutr. 1997;127:5445-5485.
3. Ong ASH, Goh SH. Palm oil: A healthy food and cost effective dietary component. Food Nutr Bul. 2002;23:11-22.
4. Ahmad NS, Khalid BAK, DA Luke, Ima-Nirwana S. Tocotrienol Offers Better Protection Than Tocopherol From Free Radical-Induced Damage of Rat Bone. Clin Exp. Pharm Physiol. 2005;32:761-770.
5. Ima-Nirwana S., Kiftiah A., Sariza T., Gapor MT. & Khalid BAK. Palm vitamin E improves bone metabolism and survival rate in thyrotoxic rats. Gen Pharm. 1999;2:621-626.
6. Norazlina M., Ima-Nirwana S., Gapor MT. & Khalid BAK. Palm vitamin E is comparable to alpha-tocopherol in maintaining bone mineral density in ovariectomised female rats. Exp Clin Endocrinol Diab. 2000;108:305-310.
7. Ima Nirwana S., Norazlina M. & Khalid BAK.. Pattern of bone mineral density in growing male and female rats after gonadectomy. Jour Asean Fed Endoc Soc. 1998; 16:21-26.
8. Four L. Use of palm oil: comparative performance. Rev. Franc. Crops Gras 1975;22:77-83.
9. Cuesta C, Sánchez-Muñiz FJ, Varela G. Nutritive value of frying fats. In: Varela G, Bender AE, Morton ID, eds. Frying of food. Chichester, United Kingdom: Ellis Horwood Ltd, 1988;112-128.
10. Dobarganes MC, Marquez-Ruiz G, Velasco J. Interactions between fat and food during deep frying. Eur J Lip Sc Tech 2000;102:521-528.
11. Dobarganes C, Marquez-Ruiz G. Oxidized fats in foods. Curr Op Clin Nutr Met Care 2003;6:157-163.
12. Grootveld M, Atherton MD, Sheerin AN, Hawkes J, Blake DR, Richens TE, Silwood CJ, Lynch E, Claxson AW. In vivo absorption, metabolism, and urinary excretion of alpha,beta-unsaturated aldehydes in experimental animals. Relevance to the development of cardiovascular diseases by the dietary ingestion of thermally stressed polyunsaturate-rich culinary oils. J Clin Invest. 1998; 101:1210-1218.
13. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radic Biol Med. 1991;11:81-128
14. Soriguer F, Rojo-Martinez G, Dobarganes MC, Garcia-Almeida JM, Esteva I, Beltran M, Ruiz De Adana MS, Tinahones F, Gomez-Zumaquero JM, Garcia-Fuentes E, Gonzalez-Romero S.. Hypertension is related to the degradation of dietary frying oils. Am J Clin Nutr. 2003;78:1092-1097.
15. Williams MJ, Sutherland WH, McCormick MP, de Jong SA., Walker RJ, Wilkins GT. Impaired endothelial function following a meal rich in used cooking fat. J Am Coll Cardiol. 1999;33:1050-1055.
16. Sutherland WH, De Jong SA, Walker RJ, Williams SM.. Release of cholesterol from cell membranes to postprandial plasma from mildly hypercholesterolemic subjects: the effect of meals rich in olive and safflower oils. Metabolism. 2002;51:1306-1312.
17. Basu S, Michaelsson K, Olofsson H, Johansson S, Melhus H... Association between oxidative stress and bone mineral density. Biochem Biophy Res Comm. 2001; 288:275-279.
18. Maggio D, Barabani M, Pierandrei M, Polidori MC, Catani M, Mecocci P, Senin U, Pacifici R, Cherubini A.. Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. J Clin Endo Metab. 2003;88:1523-1527.
19. Lean JM, Davies JT, Fuller K, Jagger CJ, Kirstein B, Partington GA, Urry ZL, Chambers TJ.. A crucial role for thiol antioxidants in estrogen-deficiency bone loss. J Clin Invest. 2003;112:915-923.
20. Sontakke AN, Tare RS. A duality in the roles of reactive oxygen species with respect to bone metabolism. Clin Chim Act. 2002;318:145-148.
21. Owu DU, Osim EE, Ebong PE. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. Act Trop. 1998;69:65-73.

22. Difford J. A simplified method for the preparation of the methyl methacrylate embedding medium for undecalcified bone. Med Lab Tech. 1974;31:79-81.
23. Freere RH, Weibel ER. Sterologic techniques in microscopy. J Micros Soc. 1967;87:25-34.
24. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ. Bone histomorphometry: standardisation of nomenclature, symbols and units. Report of the ASMBR Histomorphometry Nomenclature Committee. J Bone Min Res. 1987;2:595-610.
25. Kubow S. Routes of formation and toxic consequences of lipid oxidation products in foods. Free Radic Biol Med. 1992;12:63-81.
26. Esterbauer H, Zollner H, Schaur RJ. Hydroxyalkenals: Cytotoxic products of lipid peroxidation. ISI At Sci Biochem. 1988;1:311-315.
27. Kanazawa K, Ashida H.. Dietary hydroperoxides of linoleic acid decompose to aldehydes in stomach before being absorbed into the body. Biochim Biophy Acta. 1998. 1393;2-3:349-361.
28. Dean RT, Thomas SM, Garner A. Free-radical-mediated fragmentation of monoamine oxidase in the mitochondrial membrane. Role of lipid radicals. Biochem J. 1986;240:489-94.
29. Curzio M. Interaction between neutrophils and 4-hydroxylkenals and consequences on neutrophil mobility. Free Radic Res Com. 1986;5:55-66.
30. Firestone D. Regulation of frying fats and oils. In: Perkins EG, Erickson MG, eds. Deep frying: chemistry, nutrition and practical applications. Champaign, IL: AOCS Press. 1996;323-334.
31. Shekelle RB, Missell L, Paul O. Fish consumption and mortality from coronary heart disease. N Eng J Med. 1985;313:820.
32. Kromhout D, Feskens EJ, Bowles CH. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int J Epid. 1995;24: 340-345.
33. Stone NJ. Fish consumption, fish oil, lipids, and coronary heart disease. Circulationx. 1996;94:2337-2340.
34. Sakaguchi K, Morita I, Murota S. Eicosapentaenoic acid inhibits bone loss due to ovariectomy in rats. Prostglandins Leukot Essnt Fatty Acids. 1994;50:81-84.
35. Fernandes G, Lawrence R, Sun D. Protective role of n-3 lipids and soy protein in osteoporosis**.** Prost Leuk Ess Fat Acids. 2003;68:361-372.
36. Kalu DN. The ovariectomized rat model of postmenopausal bone loss. Bone Min. 1991;15:175-192.
37. Wronski TJ, Yen CF. The ovariectomized rat as an animal model for postmenopausal bone loss. Cells Mat. 1991;Suppl 1:69-74.
38. Reeves JB, Weihrauch JL. Agriculture Handbook, No 8-4. Composition of Foods.. US Dept of Agriculture, Science and Education Administration. Washington DC. 1979;142 pp
39. Regnström J, Nilsson J, Tomvall P, Landou C, Hamsten A. Susceptibility to low density lipoprotein oxidation and coronary atherosclerosis in man. Lancet. 1992;339:1183-1186
40. Claxson AW, Hawkes GE, Richardson DP, Naughton DP, Haywood RM, Chander CL, Atherton M, Lynch EJ, Grootveld MC. Generation of lipid peroxidation products in culinary oils and fats during episodes of thermal stressing: a high field 1H NMR study. FEBS Letter. 1994; 21,355:81-90.
41. Haywood RM, Claxson AW, Hawkes GE, Richardson DP, Naughton DP, Coumbarides G, Hawkes J, Lynch EJ, Grootveld MC. Detection of aldehydes and their conjugated hydroperoxydiene precursors in thermally-stressed culinary oils and fats: investigations using high resolution proton NMR spectroscopy. Free Radic Res. 1995;22:441-482.
42. Liu JF, Huang CJ. Tissue αtocopherol retention in male rats is compromised by feeding diets containing oxidized frying oils. J Nutr. 1995;125:3071-3080.
43. Liu JF, Huang CJ. Dietary oxidized frying oil enhanced tissue α-tocopherol depletion and radioisotope tracer excretion in vitamin E-deficient rats. J Nutr. 1996;126: 2227-2235.
44. Quiles JL, Ramirez MC, Ibanez S, Alfonso J, Duthie CG, Huertas JR, Mataix J. Vitamin E supplementation increases the stability and the in vivo antioxidant capacity of refined olive oil. Free Radic Res. 1999;31:129-135.
45. Andrikopoulus NK, Dedoussis GV, Falirea A, Kalogeropoulos N, Hatzinikola HS. Deterioration of natural antioxidant species of the vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. Int JFood Sci Nutr. 2002;53:351-63.
46. Goh SH, Choo YM, Ong SH. Minor constituents of palm oil. J Am Oil Chem Soc. 1985;62:237-240.
47. Serbinova E, Kagan V, Han D, Packer L. Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha tocotrienol. Free Radic Biol Med. 1991;10:263-275.
48. Kamat JP, Sarma HD, Devasagayam TPA, Nesaretnam K, Basiron Y. Tocotrienols from palm oil as effective inhibitors of protein oxidation and lipis peroxidation in rat liver microsomes. Mol Cell Biochem. 1997;170:131-138.

Original Article

Recycled palm oil is better than soy oil in maintaining bone properties in a menopausal syndrome model of ovariectomized rat

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回鍋棕櫚油在維持有更年期症狀卵巢切除的大鼠之骨質狀況較黃豆油佳

棕櫚油具抗氧化作用、抗癌及降低膽固醇之效力。它比起其他如黃豆油等炸油，在加熱時抗氧化作用。當炸油重複地加熱，會生成毒性降解產物像是醛類，在被攝取後可能被吸收進入系統循環中。我們研究卵巢切除的大鼠攝取混合黃豆油或是棕櫚油的大鼠飼料，對骨質組織型態學參數之影響。將母的 Sprague-Dawley 大鼠分成八組：(1)正常控制組；(2)卵巢切除控制組；(3)卵巢切除及新鮮黃豆油；(4)卵巢切除及加熱一次黃豆油；(5)卵巢切除及加熱五次黃豆油；(6)卵巢切除及新鮮棕櫚油；(7)卵巢切除及加熱一次棕櫚油；(8)卵巢切除及加熱五次棕櫚油。這些油脂與大鼠飼料以大鼠體重比為 15：100 的比例混合，每天給予此食物共六個月。卵巢切除對骨質組織型態學參數有負面影響。攝取新鮮及加熱一次的油可以提供保護作用，對抗卵巢切除術的負面影響，但是這些保護作用在加熱五次的油則消失。大豆油加熱五次確實使卵巢切除大鼠的組織型態學參數變得較差。因此，對於有骨質疏鬆危險性的已停經者而言，最好使用棕櫚油當做炸油，尤其是習慣使用回鍋油者。

關鍵字：棕櫚油、維生素 E、加熱炸油、卵巢切除、骨質組織型態學。