International Journal of Pharmacology

ISSN 1811-7775

International Journal of Pharmacology 3 (2): 160-164, 2007
ISSN 1811-7775

Repeatedly Heated Frying Oil and High Cholesterol Diet are Detrimental to the Bone Structure of Ovariectomised Rats

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Abstract: Oxidative stress and hypercholesterolemia have been shown to contribute to post-menopausal osteoporosis. In post-menopausal women, the use of repeatedly heated frying oils may aggravate oxidative stress condition while intake of high cholesterol diet may worsen hypercholesterolaemia. The aim of this study is to determine the combined effects of repeatedly heated (five times) frying oils (palm or soy oil) and high cholesterol diet on the bone structure of ovariectomised rats, an animal model of post-menopausal osteoporosis, by using bone histomorphometry. It was found that the addition of fresh or once-heated palm or soy oil into high cholesterol diet was able to protect ovariectomised rats bone from structural deterioration. However, when these oils were repeatedly heated, the protective effects were lost and the bone structures of the ovariectomised rats were either maintained by palm oil or worsen by soy oil.

Key words: Post-menopausal, osteoporosis, heated oils, ovariectomy rats

INTRODUCTION

Osteoporosis is defined as a bone density T scores at or below 2.5 standard deviations (T score) below normal peak values for young adults (WHO 2001). Post-menopausal osteoporosis is the commonest form of osteoporosis which occurs in women with estrogen deficiency. Several studies have shown that post-menopausal women were exposed to oxidative stress (Sontakke and Tare, 2002; Lean *et al*., 2003) which may increase osteoclast activity (Basu *et al*., 2001; Maggio *et al*., 2003).

Post-menopausal women have higher risk of hypercholesterolemia as the protective effects of estrogen on lipid profile were lost (Folsom *et al*., 1996). Cholesterol is required for osteoclast differentiation (Luegmayr *et al*., 2004) and may stimulate the release of interleukin-1, a cytokine that promote osteoclast activity (Sjögren *et al*., 2002). Based on these studies, the combination of oxidative stress and hypercholesterolemia may aggravate bone loss in post-menopausal women which may lead to osteoporosis.

Vegetable oil is widely used for frying foods in household or food industries. The commonly used frying oils in the world are palm oil and soy oil (Oil World, 2002). It is free of cholesterol but the practice of using repeatedly heated frying oils is unhealthy. Normally, frying oils are used repeatedly to fry foods to save cost until they have changed colour, smell, taste or consistency. During the process of frying, lipids especially Polyunsaturated Fatty Acids (PUFA) undergo oxidation, hydrolysis and polymerization which lead to generations of volatile and non-volatile degradation products (Cuesta *et al*., 1988; Dobarganes *et al*., 2000). The non-volatile products of degradation consisting of polymers and polar compounds remained in the oil (Dobarganes *et al*., 2000). There would also be an increase in the production of free-radicals and polar compounds and compositional changes of free-fatty acids content. As much as one-third of the dry weight of a deep-fried food can be made up of oil adsorbed during frying process (Mekhta and Swinburn, 2001). Therefore, the oil along with its degradation products can get into the systemic circulation when the fried foods are consumed (Grootveld *et al*., 1998a). Ingestion of these degradation products has been linked to pathophysiological effects associated with oxidative stress (Esterbauer *et al*., 1991). Ingestions of repeatedly heated vegetable oils have been

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shown to cause negative effects on the bone structure and bone cells of ovariectomised rats (Nazrun *et al*., 2006). It has also been linked to an increased risk of hypertension (Soriguer *et al*., 2003), disturbance of the endothelial function (Williams *et al*., 1999) and increased lipoprotein oxidation (Sutherland *et al*., 2002). We suggest the possibility that repeatedly heated frying oil may affect bone metabolism as the oxidative stress condition may activate osteoclastic bone resorption *in vivo* and *in vitro* (Garrett *et al*., 1990).

These studies have suggested the possibility that repeatedly heated frying oil and high cholesterol may further aggravate bone osteoporosis in postmenopausal women. So far we could not find any study on their combined effects on postmenopausal osteoporosis. Therefore, we have conducted a preliminary animal study to look at the combined effects of feeding frying oils (fresh, heated once or repeatedly heated) and high cholesterol diet (rat chow with 2% cholesterol) on the bone structure of ovariectomised rat, an animal model for post-menopausal osteoporosis (Kalu, 1991). We also compare the effects of using repeated heated palm oil and soy oil on bone histomorphometry structural parameters.

MATERIALS AND METHODS

Sixty four female Sprague-Dawley rats aged 3 months old (200-250 g) were divided randomly into 8 groups with 8 rats in each group. The rats were kept in cages in groups of three rats per cage at room temperature (27°C) with 12 h light and dark cycle. The first group, the Normal Control group (NC) was not ovariectomised and given normal rat chow (Gold Coin, Malaysia). The second group, the ovariectomised control group (OvxC+HC) was given high cholesterol diet (MP Biomedics, LLC, Germany), while the rest of the groups were ovariectomised and given high cholesterol diet mixed with fresh soy oil (SOF+HC), fresh palm oil (POF+HC), oils heated once (SO1+HC, PO1+HC) or oils heated five times (SO5+HC, PO5+HC). The rats were treated daily for six months. The composition of rat chow is shown in Table 1.

Table 1: Rat chow diet composition (Gold Coin, Malaysia)

Composition	Amount/Percentage
Crude protein	21-23%
Crude fibre (max)	5.0%
Crude fat (min)	3.0%
Moisture (max)	3.0%
Calcium	0.8-1.2%
Phosphorus	0.6-1.0%
Nitrogen free extract	49.0%
Vitamin A	10 M.I.U.
Vitamin D_3	2.5 M.I.U.
Vitamin E	15 g
Vitamin K	trace
Vitamin B_{12}	trace
Thiamine	trace
Riboflavin	trace
Pantothenic acid	trace
Niacin	trace
Pyridoxine	trace
Choline	trace
Santoquin	trace
Microminerals	trace

Bilateral ovariectomies were performed after the rats were anesthetized with intraperitoneal injection of ketamine hydrochloride and xylazine at doses of 50 and 10 mg kg^{-1} body weight, respectively. There was no sham-operated group as the treatment period was long. 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 marked atrophy of the uterus. This study was approved by the University Research and Animal Ethics Committee.

The soy oil (VeSoya, Yee Lee Edible Oil Industries, Perak) or palm oil (Buruh, Lam Soon Industries, Selangor) were heated according to modified methods of Owu *et al*. (1998). Briefly, the oils were heated in a stainless steel pan for 10 min at 180°C and were left to cool for 5 h to prepare the once-heated soy oil or palm oil. The procedure was repeated four more times to prepare oils that were heated five times. Fresh or heated (once or five times) oils were mixed with high cholesterol diet (rat chow with 2% cholesterol) at weight ratio of 15:100 and were fed to the rats daily for six months. The oil: High cholesterol diet ratio represents the average amount of daily oil intake in human (Owu *et al*., 1998).

The distal portion of the femur samples were dehydrated and embedded in methyl methacrylate according to Difford (1974). The block was sectioned at 10 μm with a microtome (Leica, Wetzlar, Germany) and stained with the Von Kossa stain. The samples were used to measure the structural parameters with an image analyzer (Nikon Eclipse 80i, Japan). All histomorphometric parameter measurements were performed at the metaphyseal region, which is rich in trabecular bone.

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). All the parameters were measured according to the American Society of Bone Mineral Research Histomorphometry Nomenclature Committee 1987 (Parfitt *et al*., 1987).

The results were expressed as mean values±SD. Data analysis was performed using SPSS software. Statistical test used was ANOVA followed by Tukey's hsd for normally distributed data and Kruskal-Wallis and Mann-Whitney test for data that is not normally distributed.

RESULTS

There was no significant difference of food intake or body weight between the groups throughout the study (data not shown). After six months (Table 2) the femur of ovariectomised-control rats fed with high cholesterol diet (OvxC+HC) had significant reductions in trabecular volume (BV/TV) and trabecular thickness (TbTh) but there was no change in Trabecular Separation (TbSp) compared to the Normal Control group (NC) (Table 2).

Ovariectomised rats fed with combination of high cholesterol diet and fresh or once-heated oils (SOF+HC, POF+HC, SO1+HC, PO1+HC) have higher trabecular volume compared to NC and OvxC+HC groups. Both the five-times heated oils group (SO5+HC, PO5+HC) had lower bone volume compared to NC group, but the bone volume of SO5+HC group was also lower than OvxC+HC group. Almost similar pattern were seen for the trabecular thickness parameter but the important finding is that the trabecular thickness of PO5+HC group was significantly higher than the OvxC+HC group. This means that even after repeated heating, palm oil was still partially able to maintain the trabecular thickness from the negative effects of ovariectomy and high cholesterol diet.

As for the trabecular separation parameter, SOF+HC, SO1+HC, POF+HC and PO1+HC groups had lower TbSp than NC or OvxC+HC groups. It means that the addition of fresh or once-heated oils to high cholesterol diet were able to reduce the separation between the trabeculae. However, the positive effect was lost when the oils were repeatedly heated as shown by the elevated TbSp of SO5+HC and PO5+HC. In fact, the separation was significantly wider in SO5+HC group compared to NC and Ovx+HC groups.

Table 2: Effect of different heated oil in the structural parameters

	Parameter (mean±SD)		
Groups	BV/TV (%)	Tb.Th (mcm)	Tb.Sp (mcm)
NC	34.81±1.34a	131.3±3.2	244.3±11.1
OVXC+HC	30.89±1.58ab	99.2±7.7a	224.4±23.9
SOF+HC	39.68±2.29ab	107.4±9.5a	160.3±34.0ab
POF+HC	50.61±7.25ab	130.3±10.0b	126.1±36.0ab
SO1+HC	38.78±3.05ab	105.1±11.2a	163.5±29.3ab
PO1+HC	43.93±4.09ab	115.4±9.7a	144.2±20.0ab
SO5+HC	18.74±4.05ab	89.9±2.1a	385.1±14.0ab
PO5+HC	30.72±0.41a	118.7±7.0ab	254.6±21.3

Value is expressed as mean±SD; a: Significantly different to Nc; b: Significantly different to OvxC+Hc ($p<0.05$)
NC : Normal control
OvxC+ HC: Ovariectomy control + high cholesterol diet
SOF+ HC : Ovariectomy + high cholesterol diet + fresh soy oil
SO1+ HC : Ovariectomy + high cholesterol diet + soy oil heated once
SO5+ HC : Ovariectomy + high cholesterol diet + soy oil heated 5 times
POF+ HC : Ovariectomy + high cholesterol diet + fresh palm oil
PO1+ HC : Ovariectomy + high cholesterol diet + palm oil heated once
PO5+ HC : Ovariectomy + high cholesterol diet + palm oil heated 5 times

When comparisons were made between the oils, palm oil seems to offer more protection to the bone structures than soy oil.

DISCUSSION

Postmenopausal women are at risk of osteoporosis and hypercholesterolemia due to estrogen deficiency. Post-menopausal women with high cholesterol were found to have a more significant bone loss compared to post-menopausal women with normal cholesterol level (Tanko *et al.*, 2003). Studies have shown that bone density can be increased by lowering the cholesterol with simvastatin (Oxlund *et al.*, 2001; Lupattelli *et al.*, 2004). The negative effects of hypercholesterolemia on bone were seen in our study as shown by the deterioration of bone structures in ovariectomised rats given high cholesterol diet.

It was also found that the addition of fresh or heated-once palm or soy oil to the high cholesterol diet was able to protect bone from structural deterioration. We believe that the vitamin E content in frying oils may offer the protective effect on bone in ovariectomised rat given high cholesterol diet. Soy oil is rich in tocopherol while palm oil is rich in tocotrienols (Goh *et al.*, 1985). Several studies have shown the beneficial effects of vitamin E on bone. Vitamin E prevented the deleterious effects of skeletal unloading on bone mass and strength (Smith *et al.*, 2005). Vitamin E also improved material and structural bone properties in aged rats (Arjmandi *et al.*, 2002). Our studies have shown that palm vitamin E were able to prevent bone loss from FeNTA toxicity (Ahmad *et al.*, 2005), hyperthyroidism (Ima-Nirwana *et al.*, 1999), ovariectomy (Norazlina *et al.*, 2000) and orchidectomy (Ima Nirwana *et al.*, 1998).

Fresh or once-heated frying oils may be beneficial to the bone structure, but when they are repeatedly heated, the protective effects on bone structure are lost. In fact, the bone structure of the five-time heated soy oil group was worse than the ovariectomised-control group given high cholesterol diet. This shows that the combination of repeatedly heated frying oils and high cholesterol diet can cause further damage to the bone. Studies have found the association between oxidative stress and osteoporosis (Basu *et al.*, 2001; Maggio *et al.*, 2003). Postmenopausal women with osteoporosis were found to be under oxidative stress condition as shown by increased malondialdehyde levels and decreased superoxide dismutase levels (Sontakke and Tare, 2002). This oxidative stress condition exposes the bone of postmenopausal women to oxidative damage (Lean *et al.*, 2003). Intake of repeated heated (recycled) frying oils may further increase oxidative stress in postmenopausal women as these oils contain lipid peroxidation products (Kubow, 1992). Furthermore, vitamin E, which effectively protects fatty acids in the oil from oxidation deteriorate after each frying episode (Andrikopoulus *et al*., 2002). Therefore, repeated heating of frying oils destroys the vitamin E content and exposes the fatty acids to oxidation. Vitamin E plays an important role in the ability of frying oil to withstand thermal oxidative changes. Addition of vitamin E to frying oil was found to render polyunsaturated fatty acids more resistant to oxidation (Grootveld *et al*., 1998b). Rats fed on diet containing 15% oxidized frying oil had significantly lower α-tocopherol concentrations in plasma and most tissues than rats fed on diet containing similar level of fresh soybean oil (Liu and Huang, 1995). Supplementation of vitamin E to olive oil was found to increase the stability of this oil under pro-oxidant conditions and its intake was found to decrease the oxidative damage generated by adriamycin in rats (Quiles *et al*., 1999).

Another important finding in this study is that the bone structure was maintained in ovariectomised rat fed with repeatedly heated palm oil whereas the bone structural deterioration was worse with repeatedly heated soy oil. Palm oil may be better frying oil as it has good resistance to oxidation under intense and prolonged heat (Four, 1975). Therefore, palm oil may be a better choice if the frying oil needs to be used repeatedly. Histomorphometric analyses of the bone structure in this study have shown that the addition of fresh frying oil to high cholesterol diet may be beneficial to bone. However, addition of repeatedly heated frying oil may cause bone damage.

ACKNOWLEDGMENTS

We thank Puan Azizah Osman, Encik Muhammad Arizi Aziz and Encik Faisal Ariffin for their technical assistance. We are grateful to the UKM Dental Faculty for allowing us to use their equipments. This study was supported by UKM Medical Faculty Research grant (FF-148-2004).

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