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# Effects of Plant-derived Frying Oils on the Bone Structure of Normal Male Rats

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**Citation**

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**Abstract**

Vitamin E has been shown to have anabolic action on bone of normal male rats. The presence of vitamin E in palm and soy oil, the commonly used plant-derived frying oils have rendered them more resistant to oxidation when repeatedly heated. Previous studies have shown that repeatedly heated frying oils had caused bone loss in ovariectomised rats. This was believed to be contributed by the generation of free radicals in the heated frying oils. Our study aimed to evaluate the effects of repeatedly heated palm and soy oils on the bone of normal male rats. Methods: Forty-two male Sprague-Dawley rats were randomly assigned into seven groups according to their diet; normal control (NC), fresh palm oil (FPO), fresh soy oil (FSO), five times heated palm oil (5HPO), five times heated soy oil (5HSO), ten times heated palm oil (10HPO) and ten times heated soy oil (10HSO). The rats were fed for six months with rat chow mixed with the respective oils at 15% (w/w). Bone structural changes were assessed by performing histomorphometry on the rat femora. The parameters measured were Trabecular volume (BV/TV), Trabecular thickness (TbTh), Trabecular separation (TbSp) and Trabecular Number (TbN). Results: There were no significant differences in all the structural histomorphometric parameters of the repeatedly heated oils groups when compared to normal control and fresh oils groups. Conclusion: Repeatedly heated palm and soy oils are safe and not detrimental to the bone structure of normal male rats. The vitamin E content in the oils may be responsible for these bone protective effects.

## INTRODUCTION

Palm and soy oils are the commonly used plant-derived frying oils which are rich in vitamin E. Palm-oil derived vitamin E were shown to protect bones against osteoporosis in orchidectomised male rats (1). The vitamin E content of palm oil mainly consists of tocotrienols, while the main vitamin E in soy oil is tocopherols (2). Previous studies have also shown that palm oil-derived tocotrienols were comparable to -tocopherol in protecting rats against osteoporosis due to ovariectomy (3,4). Fresh palm and soy oils had improved the histomorphometric bone parameters of ovariectomised rats (5). The palm-oil derived vitamin E was also found to have anabolic actions on the bone of normal male rats (6).

Vitamin E may play an important role in the ability of frying oil to withstand thermal oxidative changes. Inclusion of vitamin E to frying oil was found to render polyunsaturated fatty acids more resistant to oxidation (7). During frying, lipids especially polyunsaturated fatty acids (PUFA) undergo oxidation, hydrolysis and polymerization which lead to the production of volatile and non-volatile degradation products (8,9). Polymers and polar compounds in the non-volatile products will remain in the oil (9). Heated frying oils were found to have higher content of free radicals compared to fresh frying oils (10). Deep-fried food contain free radicals as about one third of the dry weight of a deep-fried food is made up of oil absorbed during the process of deep frying (11). The condition is made worse when the oil is used repeatedly for frying foods (12). The degradation products of the deteriorated oils can get into the systemic circulation when the fried foods were ingested (7).

The highly reactive free radicals formed complex such as hydroxyl peroxides, aldehyde and 4-hydroxyalkenals, which can escape from the membrane and cause disturbances at many sites in our body (13). When these free radicals are not destroyed by antioxidants, they cause oxidative stress. They reacted with inorganic and organic chemicals (proteins, lipids and carbohydrates) particularly with the molecules in membrane and nucleic acids, thus producing a range of enzymatically damaging consequences (14). This would increase the risk of hypertension (10,15), atherosclerosis (16) and increased lipoprotein oxidation (17). The excess free radicals generated will also be detrimental to bone metabolism (18,19,20). It may cause excessive bone loss which may lead to osteoporosis (21,22).

Repeatedly heated palm and soy oils were shown to cause detrimental changes on bone histomorphometry in ovariectomized rats (5). Estrogen deficiency state in ovariectomised rats and postmenopausal women has been associated with oxidative stress and excessive bone loss (18,23,24). Intake of repeatedly heated oils may worsen the oxidative stress condition in ovariectomised rats, further exposing the bone to free-radicals. The oxidative condition would activate osteoclastic bone resorptive activity, leading to osteoporosis (18). Palm oil was found to resist degradation better than soy oil and therefore less harmful to bone of ovariectomised rats (5). It is not known if intake of repeatedly heated oils would produce similar effects in normal male rats, which are not under oxidative stress. Therefore, the aim of this study is to determine the effects of repeatedly heated palm and soy oils on the bone structure of normal male rats. Bone histomorphometry was used to measure the structural changes in the bone (25).

## MATERIALS AND METHODS

## EXPERIMENTAL DESIGN

In this study, forty-two male Sprague-Dawley rats aged 3 months old (200-250g) were randomly assigned into seven groups according to their diet. The groups were NC: fed on normal rat chow, FPO: fed on rat chow mixed with fresh palm oil, FSO: fed on rat chow mixed with fresh soy oil, 5HPO: fed on rat chow mixed with five times heated palm oil, 5HSO: fed on rat chow mixed with five times heated soy oil, 10HPO: fed on rat chow mixed with ten times heated palm oil and 10HSO: fed on rat chow mixed with ten times heated soy oil. The rats were kept in cages in groups of two rats per cage at room temperature with 12 hour light and dark cycles. They were allowed to acclimatize for a week before the treatment was started. These rats were fed their respective diet for six months after which they were euthanized with overdosage of ketamine and xylazine and the femora were harvested. This study was approved by the University Research and Animal Ethics Committee (FP/FAR2008/KAMSIAH/9-APR/220-APR-2008-FEB-2011).

## PREPARATION OF DIET

For heated palm and soy oils groups, the oils were heated to 180˚C for frying sweet potato (Ipomoea batatas¬) (Chow Kit, KL, Malaysia). The sweet potatoes were peeled and cut into chunks before they were fried (1.0 kg of sweet potatoes for 2.5 litres of oil ). The frying process was carried out for 10 minutes. Thereafter, the heated oils were allowed to cool down at room temperature for at least 5 hours. Completion of this process would make the the once heated-palm or soy oil (1X). Hence, the same process was repeated according to how many times the oils need to be heated for each group. The heated oils were then mixed with rat chow (Gold Coin Sdn Bhd, Selangor, Malaysia) that had been grinded to powder form. About 150 g of oil was used for every 1 kilogram of rat chow. The mixture was then mixed with sufficient water to aid in the mixing process. Pellets were then formed from the mixture and heated in the oven at 80˚C for 24 hours.

## BONE HISTOMORPHOMETRY

The distal portion of the right femur samples were kept in 70 % alcohol. They were dehydrated and embedded in methyl methacrylate (Osteo-Bed Bone Embedding Kit; Polysciences, USA). The block was sectioned at 8 μm thickness with a microtome (Leica RM2155, Wetzlar, Germany) and stained with the Von Kossa stain. Structural parameters were measured form the samples using Nikon Eclipse 80i microscope (Nikon Instrument Inc., US) with an image analyzer software Pro-Plus v. 5.0 (Media Cybernatics, Silver Spring, MD, USA). The measurements were performed at the metaphyseal region, located about 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

The food intake was measured daily while the body weights were measured weekly. The structural parameters measured were Trabecular volume (BV/TV, %), Trabecular thickness (TbTh, μm), Trabecular number (TbN, #/mm$^2$) and Trabecular separation (TbSp, μm). The parameters were measured according to the American Society of Bone Mineral Research Histomorphometry Nomenclature Committee 1987 ( 26 ).

## STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. Data were analysed using SPSS for Windows software (version 17.0). The normally distributed data was analyzed using ANOVA followed by Tukey’s hsd while not normally distributed data was analysed using Kruskal-Wallis and Mann-Whitney test.

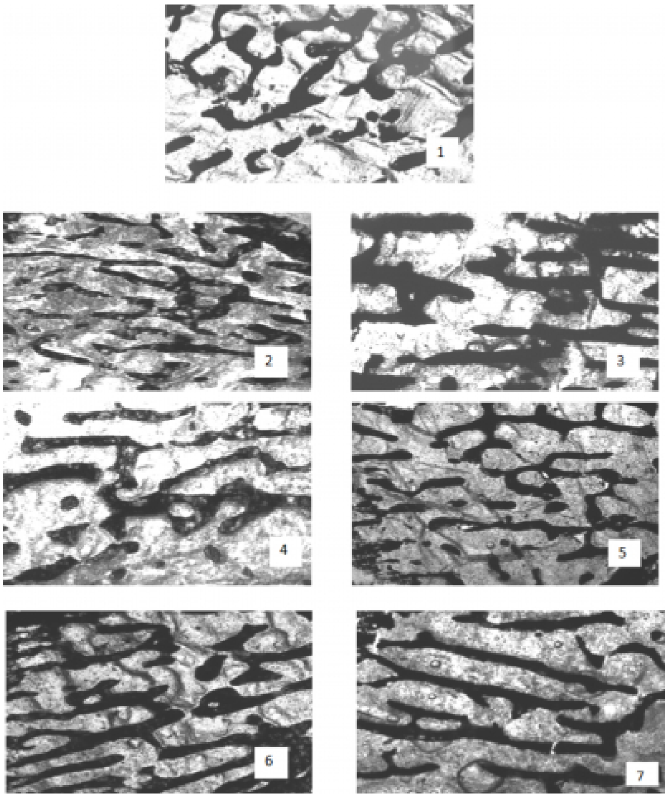
Changes were considered significant for p values less than 0.05.

## RESULTS

There was no significant difference in diet intake or body weight between the groups ($p<0.05$) (data not shown). The trabeculae of femoral bone that were stained black with Von Kossa for each group were shown in Figure 1. Grossly, there were no significant differences in the trabeculae density between the groups. Results of the structural histomorphometric analysis revealed that there were no significant difference in the Trabecular volume (BV/TV), Trabecular thickness (TbTh) , trabecular separation (TbSp) and Trabecular number (TbN) parameters between the groups ( Figure 2, 3, 4 and 5).

### Figure 1

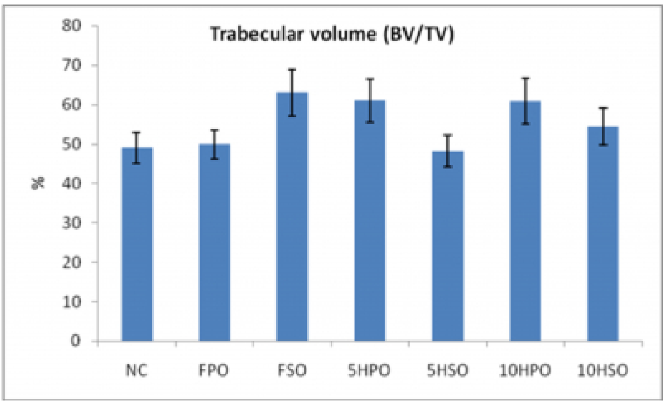
Figure 1: Photomicrographs of the bone trabeculae stained black with Von Kossa (X 5).




1: Normal Control (NC), 2: Fresh palm oil (FPO), 3: Fresh soy oil (FSO), 4: Five times heated palm oil (5HPO), 5: Five times heated soy oil (5HSO), 6: Ten times heated palm oil (10HPO), 7: Ten times heated soy oil (10HSO).

### Figure 2

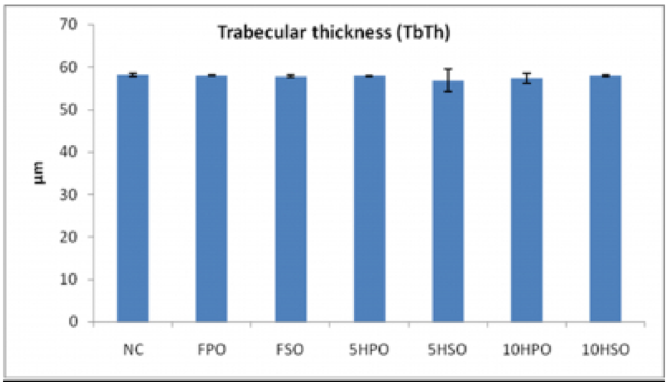
Figure 2: Effects of dietary heated oils on bone volume per tissue volume ( BV/TV ) in normal male rats




NC: normal control, FPO: fresh palm oil, FSO: fresh soy oil, 5HPO: five times heated palm oil, 5HSO: five times heated soy oil, 10HPO: ten times heated palm oil, 10HSO: ten times heated soy oil. Value is expressed as mean ± SEM (n=6).

### Figure 3

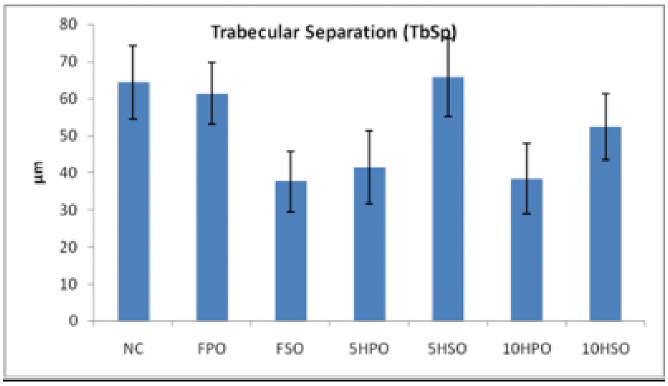
Figure 3: Effects of dietary heated oils on trabecular thickness ( TbTh ) in normal male rats




NC: normal control, FPO: fresh palm oil, FSO: fresh soy oil, 5HPO: five times heated palm oil, 5HSO: five times heated soy oil, 10HPO: ten times heated palm oil, 10HSO: ten times heated soy oil. Value is expressed as mean ± SEM (n=6)..

**Figure 4**

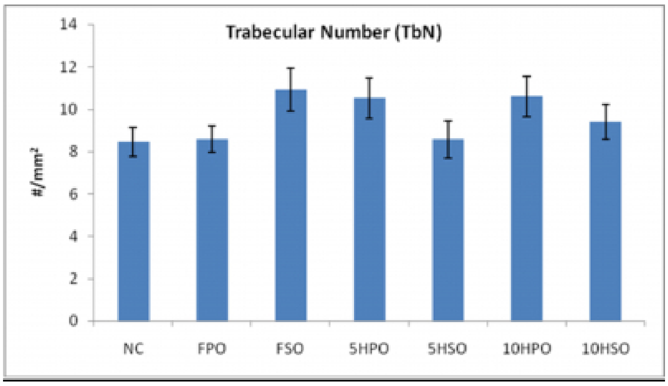
Figure 4: Effects of dietary heated oils on trabecular separation ( TbSp ) in normal male rats




NC: normal control, FPO: fresh palm oil, FSO: fresh soy oil, 5HPO: five times heated palm oil, 5HSO: five times heated soy oil, 10HPO: ten times heated palm oil, 10HSO: ten times heated soy oil. Value is expressed as mean ± SEM (n=6).

**Figure 5**

Figure 5: Effects of dietary heated oils on trabecular number (TbN) in normal male rats




NC: normal control, FPO: fresh palm oil, FSO: fresh soy oil, 5HPO: five times heated palm oil, 5HSO: five times heated soy oil, 10HPO: ten times heated palm oil, 10HSO: ten times heated soy oil. Value is expressed as mean ± SEM (n=6).

## DISCUSSIONS

In this study we have focused on the effects of free radicals exposure from unhealthy dietary practice on the bone structure of healthy rats. Oxidative stress can occur in estrogen deficiency state as in ovariectomised rats and postmenopausal women (23,24). Free radicals have been shown to activate osteoclast bone resorptive activity in vivo and in vitro, which can lead to bone loss and osteoporosis (18,27,28). They are also toxic to osteoblasts, the cells responsible for bone formation (19). Bone is prone to osteoporosis when the oxidative stress is made worse with intake of degradation products containing free radicals. This was demonstrated in previous studies whereby ovariectomised rats fed with heated oils exhibited characteristics of osteoporosis (5,29).

We expected that in non-stressed rat model, the repeatedly heated palm and soy oils would not compromise the bone structure as the vitamin E content would protect these oils against oxidative degradation. Therefore, we have conducted a study to investigate the effects of these oils on the bone of normal male rat. Male rat was chosen as there was less hormonal variation compared to female rat. Rat is a good model as the bone remodeling cycle is only six to seven days and any bone structural changes can be observed within shorter duration (30). The dietary intake and body weights were not different among the groups, indicating that the frying oils have minimal effects on the rats' appetite. Based on the histomorphometric parameters measured, we did not find any deterioration of the bone structure when the rats were fed with repeatedly heated palm or soy oil.

There are several reasons why the bone structure was not compromised. Palm oil is rich in carotenoids (pro Vitamin A), tocotrienols and tocopherols (Vitamin E) which are potent antioxidants while soy oil contains a mixture of four tocopherol isomers. The antioxidant vitamin E in these frying oils may have destroyed the free-radical generated during heating, thus reducing free-radicals exposure by the rat bone.

Our male rat model was not under stress and its internal anti-oxidant defense was still intact. This internal defense is made up of antioxidant enzymes such as superoxide dismutase, catalase and glutathione (31). They can convert free radicals from the heated frying oils to less reactive forms and protect the body tissues against oxidative damage. These enzymes are also found in bone and therefore can directly protect bone against oxidative stress from within. Whatever vitamin E which remains in the frying oils may also be beneficial to the anti-oxidant enzymes in the bone. Sandra et al. (32) have shown that vitamin E were able to boost the anti-oxidant enzymes activities within the rat bone. Therefore, even when there was an increased free radicals intake from repeatedly heated oils, the internal antioxidant enzymes within the body especially in the bone were able to handle them. This maybe another reason why the bone structure was maintained our rat model.

Normal male rats have androgens like testosterone, dihydrotestosterone (DHT) and non-aromatizable androgens which were able to suppress the osteoclast formation (33) and increase the osteoblast proliferation to maintain bone density (34,35). The protective role of androgens in bone regulation was supported by other studies (36,37,38,39). Estrogen was also shown to play an important role in maintaining the bone density in males (40). Therefore, the intact hormonal system would also help in maintaining the bone structure of our male rat model.

In conclusion, the bone structure of normal male rats was not affected by fresh nor repeatedly heated palm and soy oils. This was probably due to vitamin E content in the frying oils and the intact internal anti-oxidative defense and hormonal system. Though it is not recommended, it is still safe for healthy individual to use repeatedly heated palm or soy oils in term of bone health.

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