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Palm Vitamin E Prevents Osteoporosis in Orchidectomized Growing Male Rats

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Abstract – Testosterone deficiency increased bone resorption, giving rise to osteoporosis. Testosterone deficiency also increased lipid peroxidation and free radical formation. Free radicals have been shown to be toxic to osteoblasts as well as to activate osteoclasts. In this study, the effects of giving an antioxidant, i.e. vitamin E-rich extract from palm oil on bone mineral density and calcium content was studied. Palm vitamin E prevented the loss in bone mineral density due to orchidectomy, seen in the whole femur, proximal amd midshaft regions, as well as L4 vertebra. Similar observations were seen in bone calcium content of the L5 vertebra. Giving palm olein also prevented the loss in bone mineral density in the femoral midshaft and L4 vertebra; and bone calcium content in the L5 vertebra. In conclusion, vitamin E-rich extract from palm oil was effective in preventing the loss in bone mineral density and calcium content of orchidectomized male rats. This action is probably due to its role as an antioxidant.

Key words – Bone mineral density, orchidectomy, testosterone, palm vitamin E.

Introduction

Complex cellular, physiologic and metabolic factors interplay to maintain bone mineral density. Amongst these the sex hormones estrogen and testosterone are important in maintaining bone density, and deficiency of these hormones, as seen in menopausal women and old men, will predispose to osteoporosis. Increased bone turnover is characteristic of post-menopausal women, while for elderly men bone-specific biochemical markers remained unchanged (Resch *et al.*, 1994; Garnero *et al.*, 1996).

Gunness and Orwoll (1995) found that orchidectomy in mature (4 month old) rats stimulates cancellous bone but diminishes periosteal bone turnover with subsequent decreases in bone volume and mineral density. Earlier studies found that femoral bone mineral density was significantly reduced as early as 1 month (Ima-Nirwana *et al.*, 1998) and 3 months (Rosen *et al.*, 1995) post-orchidectomy in male rats. Therefore, testosterone deficiency appeared to increase bone resorption.

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The process of lipid peroxidation gives rise to free radicals. Orchidectomy was found to result in increased lipid peroxidation in the heart, while administration of testosterone reversed this to a certain extent (Sreelatha *et al.*, 1993). Testosterone treatment was also found to decrease levels of lipid peroxidation products in the liver due to alcohol and paracetamol toxicity (Jaya *et al.*, 1995). Lipid peroxidation was also found to affect bone metabolism. Free radicals were shown to play a role in osteoclast activation (Garrett *et al.*, 1990; Key *et al.*, 1990; Suda, 1991) and also found to be toxic to osteoblasts (Moreau *et al.*, 1998). Ferric, a prooxidant was found to be deposited in osteoblasts and associated with impairment of bone growth (Yukihiro *et al.*, 1995). Vitamin E is an important lipid soluble antioxidant and has been shown to be effective in certain diseases predisposed by free radicals, such as atherosclerosis and cancer (Tan *et al.*, 1991; Qureshi *et al.*, 1991a; Qureshi *et al.*, 1991b; Nesaretnam *et al.*, 1995). Vitamin E was also found to play a role in maintaining bone matrix trophysm (Passeri and Provvedini, 1983) and in stimulating trabecular bone formation (Xu *et al.*, 1995). Impairment of bone calcification induced by

ferric was prevented by vitamin E supplementation (Ebina *et al.*, 1991; Yee and Ima-Nirwana, 1998).

Palm olein, which is the refined, bleached and deodorised palm oil, is widely used as cooking oil in this region. Palm olein is predominantly monounsaturated, containing approximately 40% saturated, 44% monounsaturated and 12% polyunsaturated fatty acids (Marzuki *et al.*, 1991). Palm olein is rich in vitamin E, containing 196 ppm α-tocopherol, 201 ppm α-tocotrienol, 372 ppm γ-tocotrienol and 96 ppm δ-tocotrienol (Elson, 1992). Earlier studies have been done to study the effects of palm olein on blood pressure, serum lipids and lipid peroxidation products (Ima-Nirwana *et al.*, 1995a; Ima-Nirwana *et al.*, 1995b). However, no study has been done to determine the effects of palm oil and palm vitamin E on bone mineral density and metabolism. This study was done to observe the effects of long-term supplementation of palm olein and vitamin E-rich extract from palm oil on bone mineral density and metabolism in normal and orchidectomized growing male rats.

Materials and Methods

Animals – 3-month old male Wistar rats rats weighing between 200-250 g were either sham-operated or orchidectomised. The two groups were further divided into 3 subgroups and given either normal rat chow, normal rat chow and palm vitamin E 30 mg/kg rat weight, or rat chow with added palm olein. Each group consists of ten rats. The rats were kept 5 per cage under 12 hours natural light/dark cycles and tap water *ad libitum.*

Diets – Normal rat chow diet was from Gold Coin, Malaysia. For the palm olein diet, palm olein (Lam Soon, Malaysia) was mixed with rat chow in the ratio of 20% palm olein:80% rat chow, weight:weight. The above diets were given to the respective groups *ad libitum..*

Palm vitamin E – Vitamin E-rich fraction from palm oil was prepared by the Palm Oil Research Institute of Malaysia, and had the following composition per 100 gram: α-tocopherol, 24.4%; α-tocotrienol, 21.6%; γ-tocotrienol, 27.7%; δ-tocotrienol, 11.0%; palm olein, 15.3%. The palm vitamin E was diluted in olive oil (Bertolli, Italy) to obtain the concentration of 30 mg/kg rat weight in 0.1 ml. The mixture was given as oral gavage daily 6 days/week. Olive oil was chosen as the diluent because it contains only 51 ppm α-tocopherol and no tocotrienols.

The above treatments were carried out for 8 months.

Body weights – The rats were weighed at the beginning of the study and monthly until completion of study.

Bone mineral density measurements – Bone mineral density measurements of the left femur and lumbar vertebrae were obtained using the Dual-Energy X-ray Absorptiometer (Norland, USA). Bone mineral density measurements of the left femur (whole, proximal, midshaft and distal parts); and 3rd to 5th lumbar vertebrae, and 4th lumbar vertebra were taken. The proximal part of the femur was marked 1 cm. in length from the hip joint. The distal part was marked 1 cm in length from the knee joint. The midshaft was the area in between the proximal and the distal parts. The 5th lumbar vertebra was identified as the first vertebra from the pelvic bone and the others were counted from there.

The rats were anaesthetised with Ketapex and Xylazil (1:1), and placed prone for the lumbar vertebral measurements. For the femoral readings, the rats was placed supine and the left lower limb was put in external rotation.

Bone calcium content – The animals were sacrificed by cervical dislocation. The left femur and fifth lumbar vertebra were dissected out and cleansed of all soft tissues. The cleaned bones were dried in an oven at 100°C for 24 hours, then ashed in a furnace at 800°C for 12 hours. The ash was weighed and dissolved in 3 ml. nitric acid and then diluted in lanthanum chloride. Calcium chloride was measured with a Atomic Absorption Spectrophotometer (Shimadzu AA-680) at 422.7 nm.

Bone biomarkers – Serum alkaline phosphatase and serum tartrate-resistant acid phosphatase activities were assayed using kits from Sigma, USA (no. 245 and 435 respectively). The serum was stored at 4^{0}C and assayed the day after sacrifice. The optical density was measured using a spectrophotometer (Shimadzu UV-160A) at 405 nm. The coefficient of variation for both assays is 0.99.

Analyses of data – Difference between treatment groups was analysed using the One-way analysis of variance with the Tukey's honestly significant difference as the post-hoc test (Statistical Package for Social Sciences). Means of the orchidectomized rats were compared to its normal control using Students t-test between 2 independent means (Microsoft Excel).

This study has been approved by the Universitys Research and Ethics Committee

Results

Body weights – No difference in body weights of the different groups at every data point were seen. However, body weight was significantly higher at completion of study compared to the beginning for the rats in the sham-operated and sham-operated/ palm vitamin E groups. No significant change in weight was seen in the other groups (Fig. 1).

Bone mineral density – Orchidectomy reduced bone mineral density in all the skeletal regions except the distal femur and L3-L5 vertebrae for the rat chow group. No differences were detected between the orchidectomized and sham-operated animals in the palm vitamin E group. For the palm olein group, orchidectomy reduced bone mineral density only in the whole and proximal femur. No differences in bone mineral density were seen between the three treatment groups for all the skeletal regions of the sham-operated animals. However, for the orchidectomized rats, bone mineral density of the proximal femur was less in the palm olein group compared to the palm vitamin E group (Table 1).

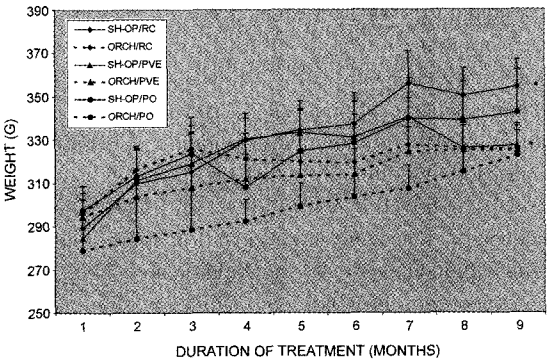

Fig. 1. Body weights of orchidectomised male rats given palm vitamin E or palm oil.

Table 1. Bone Mineral Density (mg/cm^2) of Male Orchidectomized Rats given Palm Vitamin E or Palm Olein

Groups	Rat Chow		Palm Vitamin E		PALM Olein	
Skeletal Region	SH-OP	ORCH	SH-OP	orch	sh-op	orch
Whole Femur	0.175 ± 0.003	0.163 ± 0.004*	0.167 ± 0.003	0.168 ± 0.004	0.171 ± 0.006	0.158 ± 0.003*
Proximal Femur	0.195 ± 0.005	0.174 ± 0.005*	0.182 ± 0.005	0.185 ± 0.005^a	0.185 ± 0.006	0.164 ± 0.004*^{a}
Midshaft Femur	0.164 ± 0.004	0.151 ± 0.004*	0.158 ± 0.004	0.1556 ± 0.003	0.165 ± 0.006	0.150 ± 0.005
Distal Femur	0.167 ± 0.003	0.162 ± 0.005	0.155 ± 0.005	0.1624 ± 0.005	0.163 ± 0.009	0.162 ± 0.003
L3-L5 Vertebrae	0.159 ± 0.003	0.150 ± 0.004	0.162 ± 0.004	0.158 ± 0.004	0.157 ± 0.004	0.148 ± 0.003
L4 Vertebra	0.153 ± 0.004	0.138 ± 0.005*	0.144 ± 0.003	0.150 ± 0.007	0.150 ± 0.005	0.144 ± 0.004

*indicates significant difference from the sham-operated group given the same treatment ($p<0.05$).
same alphabetical superscript indicates significant difference between treatment groups ($p<0.05$).
SH-OP = sham-operated, ORCH = orchidectomized.

Table 2. Bone Calcium Content (mg) of Male Orchidectomized Rats given Palm Vitamin E or Palm Olein.

Groups	Rat Chow		Palm Vitamin E		Palm Olein	
Bone	SH-OP	ORCH	SH-OP	ORCH	SH-OP	ORCH
Left Femur	137.6 ± 3.9	131.8 ± 3.1	135.4 ± 5.1	132.3 ± 4.9	137.7 ± 8.4	144.9 ± 8.0
L5 Vertebra	44.1 ± 1.7	39.2 ± 1.2*	42.6 ± 1.3	39.2 ± 1.2	42.4 ± 2.3	49.0 ± 4.5

*indicates significant difference from the sham-operated group given the same treatment ($p<0.05$).
SH-OP = sham-operated, ORCH = orchidectomized.

Table 3. Bone Biomarkers of Male Orchidectomized Rats given Palm Vitamin E or Palm Olein.

Geoups	Rat Chow		Palm Vitamin E		Palm Olein	
Enzyme	SH-OP	ORCH	SH-OP	ORCH	SH-OP	ORCH
ALP (IU/L)	86.3 ± 10^a	80.3 ± 3.8^c	109.9 ± 5.7^b	84.8 ± 6.4*^{d}	165.3 ± 11.3^{ab}	184.2 ± 7.0^{cd}
TRAP (IU/L)	5.3 ± 0.9^a	3.8 ± 0.5	2.5 ± 0.6^a	2.9 ± 0.3	4.2 ± 0.3	4.3 ± 0.8

*indicates significant difference from the sham-operated group given the same treatment ($p<0.05$).
same alphabetical superscript indicates significant difference between treatment groups ($p<0.05$).
SH-OP = sham-operated, ORCH = orchidectomized.
ALP = alkaline phosphatase, TRAP = tartrate resistant acid phosphatase.

Bone calcium content – Orchidectomy reduced bone calcium in the fifth lumbar vertebra of the rats given rat chow. No other significant findings were obtained (Table 2).

Bone biomarkers – Serum alkaline phosphatase activity, was higher in the sham-operated rats compared to the orchidectomised ones of the palm vitamin E group. The alkaline phosphatase activity of the sham-operated and orchidectomized rats in the palm olein group was higher than those of the rat chow and palm vitamin E groups (Table 3). Serum tartrate-resistant acid phosphatase was lower for the sham-operated rats of the palm vitamin E group compared to the that of the rat chow group. No other differences were observed (Table 3).

Discussion

After 8 months post-orchidectomy, the bone mineral density was reduced in most of the skeletal regions (except the distal femur and L3-L5) of the rats fed rat chow. This was consistent with earlier studies (Gunness and Orwoll, 1995, Rosen *et al.*, 1995, Ima-Nirwana *et al.*, 1998). Supplementation with palm vitamin E was able to prevent the loss in density due to testosterone deficiency in all the skeletal regions affected. The mechanism whereby palm vitamin E was beneficial in maintaining bone mineral density was probably due to its antioxidant effect. Orchidectomy, resulting in testosterone deficiency, increased lipid peroxidation (Sreelatha *et al.*, 1993) which would also increase bone resorption (Garrett *et al.*, 1990; Key *et al.*, 1990; Suda, 1991) leading to the loss in bone density seen in the orchidectomized rats. Palm vitamin E, being an antioxidant, was able to prevent these changes. Adding palm olein to the diet was able to partially protect the bone from loss post-orchidectomy, i.e. specifically in the midshaft and distal femur, and the lumbar vertebrae L3-L5 and L4 only. This may be because palm olein contain some amount of vitamin E (Elson, 1992) which could have helped to prevent the loss of bone density due to lack of testosterone. No difference between sham-operated rats of the different treatment groups was seen probably because the vitamin E content of rat chow was sufficient for optimal bone density. Further addition of palm vitamin E did not increase the bone density any more.

For the rat chow group, both the femoral and fifth lumbar vertebra bone calcium content of the orchidectomized rats were lower than the sham-operated rats, however, statistical significance was reached only for the lumbar vertebra. This could be because the lumbar vertebra was made up of 100% cancellous bone, making it more susceptible to loss of calcium compared to the femoral bone which has a large proportion of compact bone concentrated in the midshaft region. Other researchers found decreased tibial calcium content in rats 3 and 4 months post-orchidectomy (Schoutens *et al.*, 1984; Vanderscheuren *et al.*, 1993). Upon adding palm vitamin E or palm olein, bone calcium content of the orchidectomized group equalised with that of the sham-operated group. These observations were in accordance with that seen with the bone mineral density measurements.

The changes seen in bone mineral density and one calcium content cannot be attributed to body weight changes since there was no difference in body weights between all the treatment groups at all data points. Furthermore, no weight gain was recorded in all three orchidectomized groups, while the loss in bone density and calcium content was observed mainly in the groups given rat chow only.

Alkaline phosphatase is the enzyme released by osteoblasts upon laying down new bone (Delmas, 1992). The sham-operated and orchidectomized rats given palm olein had markedly raised alkaline phosphatase activity compared to the the rat chow and palm vitamin E groups. However, since alkaline phosphatase is also secreted by hepatocytes, and the method used here measured total serum alkaline phosphatase, this apparent rise could be due to the liver isoenzyme. Long term treatment with a high fat diet could have induced fatty changes in the liver, leading to raised liver enzymes. Other studies did not find any significant changes in serum total alkaline phosphatase activity 3 and 4 months post-orchidectomy (Vanderscheuren *et al.*, 1993, Rosen *et al.*, 1995). Acid phosphatase is released by osteoclasts while resorbing bone. The tartrate-resistant variant is specific for the bone isoenzyme (Delmas, 1992). In this study, the tartrate-resistant acid phosphatase activity was lower in the sham-operated group given palm vitamin E compared to the ones on rat chow. No other significant differences were seen. Increased bone turnover, reflected by increased osteoblasts and osteoclasts surfaces and numbers, were seen as early as 4 weeks post-orchidectomy[3]. However, this is a long term study (8 months), therefore the enzymes might have reached a steady state and might account

for the lack of significant changes seen. As was mentioned earlier, the raised alkaline phosphatase activity of the palm olein fed group could be due to liver pathology.

In conclusion, vitamin E from palm olein was effective in preventing the loss in bone mineral density and bone calcium in testosterone-deficient male rats. The mechanism of action is probably via its antioxidant properties.

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