Natural Product Sciences
1(1) : 17 ~ 24 (1995)

Combined Effects of Sex Hormones and Dietary Oils on Lipid Peroxidation

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Abstract—The effects of orchildectomy with/without testosterone replacement in male rats, and ovarectomy with estrogen replacement in female rats on lipid peroxidation were studied in male and female rats fed with diets fortified with 20% w/w soybean oil or palm oil for 4 months. Serum, liver and heart homogenates were assayed for malonaldehyde and conjugated diene levels. Orchidectomy was found to reduce levels of lipid peroxidation products in the serum, liver and heart. Testosterone replacement did not increase the lipid peroxidation products to levels in the non-orchildectomised rats, while estrogen did not influence lipid peroxidation significantly. Palm oil decreased, but soybean oil increased lipid peroxidation in the liver and heart of both the castrated and sex hormone-replaced male and female rats.

Keywords—lipid peroxidation · testosterone · estrogen · soybean oil · palm oil

Peroxidation of polyunsaturated fatty acids (PUFA) are implicated in various pathological processes, such as atherosclerosis, ageing and cancer. Oxidative modification of low-density lipoproteins (LDL) predispose then to be uptaken by macrophages to form the foam cells of atheroma (Fogelman *et al.*, 1980; Graziano and Heneken, 1993). The sex hormones estrogen and testosterone were found to influence lipid peroxidation. Testosterone treatment and/or ovarectomy in ferric nitrilotriacetate (Fe-NTA)-treated female rats increased lipid peroxidation in the renal cortical proximal tubules. In contrast, estriol or estradiol treatment and/or orchidectomy in Fe-NTA treated male rats decreased lipid peroxidation in the renal cortical proximal tubules (Toyokuni *et al.* 1990). The oxidation of human LDL was inhibited by estrogens, while testosterone did not have any effect at all (Maziere *et al.*, 1991).

Human and animal studies have found that different dietary oils have different effects on lipid peroxidation. Fish oil supplements increased levels of lipid peroxidation products in plasma (Brown and Wahle, 1990) and urine (Piche *et al.*, 1988). Other researchers have found that the more polyunsaturated soybean oil produced significantly higher plasma TBARS than the more monounsaturated olive oil (Scaccini *et al.*, 1992), while the more monounsaturated palm oil was found to produce lower levels of serum malonaldehyde compared to the more saturated butterfat (Pereira *et al.*, 1991).

In this study the combined effects of testosterone and estrogen with dietary soybean oil or palm oil on lipid peroxidation in rats were studied.

Experimental

Animals and castration - Male and female

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Rattus norwegicus rats, weighing between 145-165 g (age aproximately 2 months) were used. Orchidectomy was done via the scrotum and ovarectomy was done through laparotomy. A group of non-orchidectomised male rats were used as controls.

Hormone preparations - Testosterone propionate (Halewood Chemicals, Middlesex, England) and beta-estradiol (Sigma, St. Louis, USA) were dissolved in corn oil (Mazola, CPC/AJI, Kuala Lumpur, Malaysia). 1 mg testosterone in 0.1 ml oil was injected subcutaneously to the orchildectomised male rats every morning (Erdstein *et al.*, 1989). The ovarectomised female rats were given 25 μg estrogen in 0.1 ml oil subcutaneously every morning (Legan *et al.*, 1975). The groups not given hormonal replacement were given subcutaneous 0.1 ml corn oil daily.

Diets - The rats were fed rat chow (Gold Coin, Port Klang, Malaysia, Table 1) or rat chow +20% weight/weight (w/w) soybean oil (Yee Lee Corporation, Ipoh, Malaysia) or palm oil (palm olein, Lam Soon, Petaling Jaya, Malaysia) *ad libitum*. The diets and hormonal injections were started concurrently 1 week post castration.

The approximate fatty acid composition of the oils are given in Table 2.

Table 1. Compositon of rat chow

Contents	Composition % (w/w)
Crude protein (min.)	20.0
Crude fibre (max.)	5.0
Crude fat (max.)	2.5
Moisture (max.)	13.0
Ash (max.)	7.0
Calcium	0.7-1.4
Total phosphorous	0.6-1.2
Nitrogen- ree extract (approx.)	51.0

(by courtesy of Gold Coin, Malaysia Ltd., Port Klang, Selangor, Malaysia)

Table 2. Fatty acid composition of the oils used in this study.

Fatty Acid	Soybean oil	Palm oil
12:0	–	0.2
14:0	0.1	1.0
16:0	11.0	39.8
18:0	4.0	4.4
18:1	23.4	42.5
18:2	53.2	11.2
18:3	7.8	0.4
20:0	–	0.4
Saturates	15.1	45.4
Monounsaturates	23.4	42.5
Polyunsaturates	61.0	11.6

(adapted from Chong and Ng 1991)

There were eight rats per group and they were placed in groups of four per cage under natural light/dark cycles. Tap water was given *ad libitum*. All rats survived the duration of experiment.

The treatment was carried out for 4 months. The rats were sacrificed by exsanguination and samples of serum, liver and heart were taken for analyses of malonaldehyde and conjugated diene concentrations.

Sample preparation - Blood was taken from the common carotid artery, clotted and centrifuged (Chilspin MSE FISONS) at 3,000 rpm for 25 minutes. The serum was divided into aliquots and stored at –70°C (Jouan VX 350) until assayed.

Liver and heart homogenates were prepared according to Stocks *et al.* (1974). All work was done in ice to minimise peroxidation *in vitro*. Aliquots of the samples were stored at –70°C until used.

Biochemical analyses - Measurement of malonaldehyde was modified according to Ledwozyw *et al.* (1986) and Yagi (1976). 0.1 ml distilled water was added to 0.4 ml of the serum or homogenate samples and mixed (Stuart autovortex). 2.5 ml trichloroacetic asid

(TCA) (1.22M TCA in 0.6M HCl) was added to the mixture and mixed and left to stand at room temperature for 15 minutes. 1.5 ml thiobarbituric acid (TBA) (0.67% TBA in 0.05M NaOH) was added to the mixture, mixed and incubated in boiling water for 30 minutes.

After cooling to room temperature, 4 ml n-buthanol was added to the mixture and mixed. The top layer consisting of n-buthanol was taken and the fluorescence measured using a spectrofluorometre (Shimadzu RF-5000) at excitation and emmission wavelengths 515 nm and 553 nm, respectively. MDA concentration was measured according to the formula modified from Yagi (1976).

Measurement of conjugated dienes was described by Buege and Aust (1978).

Determination of total protein - The above measurements were expressed as per g protein. Total protein content of the samples were determined using the computerised auto-analyser, Hitachi 717, based on the Biuret method.

Analyses of data - The results obtained were analysed via analysis of variance and Student's t test. $p<0.05$ was considered significant.

This study was approved by the Research and Ethical Committee, Medical Faculty, Universiti Kebangsaan Malaysia, and confirmed by the University's Central Research Committee.

Table 3. Effects of dietary fat and orchidectomy with/witout testosterone replacement of malonaldehyde concentrations (umol/g protein).

Rat Preparation	Diet		
	Chow	Soybean oil	Palm oil
Serum			
Non-orchidectomised	0.79±0.08	0.77±0.12	0.82±0.12
Orchidectomised	0.49±0.08*	0.50±0.08*	0.50±0.06*
Orchidectomised + Testosterone	0.50±0.07*	0.49±0.07*	0.53±0.09*
Liver			
Non-orchidectomised	1.40±0.30[a]	2.10±0.60	1.90±0.30
Orchidectomised	0.53±0.04*[a,b]	0.98±0.18*[b]	0.68±0.10*[a]
Orchidectomised + Testosterone	0.72±0.12*[@]	0.65±0.10*[@]	0.66±0.17*
Heart			
Non-orchidectomised	3.6±1.1[a]	5.5±0.9	4.3±0.8[a]
Orchidectomised	2.0±0.5*	2.2±0.5*	2.4±0.6*
Orchidectomised + Testosterone	1.8±0.5*	1.5±0.3*	1.8±0.4*

*=significant from non-orchidectomised rats
@=significant from orchidectomised rats
a=significant from soybean group
b=significant from palm oil group
Significant level is at $p<0.05$.
Values are in mean±s.d. (n=6-8).

Results

Male rats - Removal of the testis decreased the malonaldehyde concentrations in the serum, liver and heart. Replacement doses of testosterone did not bring the levels back to the non-orchidectomised group. Malonaldehyde concentrations were lower in the palm oil group compared to the soybean group in liver homogenates of the orchidectomised rats, and in heart homogenates of the non-orchidectimised rats (Table 1).

Serum conjugated dienes increased with orchidectomy, and remained elevated even with testosterone replacement. Liver conjugated dienes after orchidectomy remained unchanged in the group fed rat chow, increased in the group fed soybean oil, and decreased in the group fed palm oil. Testosterone replacement decreased the conjugated dienes in the soybean group to levels lower than the non-orchidectomised rats. Orchidectomy reduced the conjugated diene concentrations in the heart homogenates of the rats fed chow, while testosterone replacement reduced it in the soybean and palm oil groups. Conjugated diene levels were consistently lower in the palm oil-fed rats compared to the chow and soybean-fed rats in the liver and heart of all the three different rat preparations (Table 2).

Female rats - Estrogen replacement increased liver malonaldehyde in the chow-fed group only. Liver malonaldehyde concentra-

Table 4. Effects of dietary fat and orchidectomy with/without testosterone replacement on conjugated diene concentrations (OD/g protein).

Rat Preparation	Diet		
	Chow	Soybean oil	Palm oil
Serum			
Non-orchidectomised	11.0±1.8	10.8±1.9	9.0±2.6
Orchidectomised	15.6±1.6*	14.0±2.8*	13.4±1.9*
Orchidectomised + Testosterone	13.6±2.1*	14.1±3.6*	13.6±3.1*
Liver			
Non-orchidectomised	69.0±8.3	66.9±10.9	68.2±4.1
Orchidectomised	58.0±13.5[a]	82.0±16.7*	48.8±8.4*[a]
Orchidectomised + Testosterone	60.0±6.4*[b]	53.7±4.7*@[b]	42.5±11.6*
Heart			
Non-orchidectomised	133.8±36.8[b]	107.1±9.5	75.9±11.8[b]
Orchidectomised	91.3±12.1[a]	120.7±22.3	77.0±17.2[a]
Orchidectomised + Testosterone	74.8±14.9*[a,b]	55.8±13.0*@	54.1±11.1*@

*=significant from non-orchidectomised rats
@=significant from orchidectomised rats
a=significant from soybean group
b=significant from palm oil group
Significant level is at $p<0.05$.
Values are in mean±s.d. (n=6-8).

tions were lower in the palm oil group compared to the soybean group for the ovarectomised rats, and lower than both the chow and soybean-fed groups for the estrogen-replaced rats (Table 3).

Estrogen replacement reduced liver and heart conjugated dienes only in the palm oil group. The rats fed palm oil had lower liver conjugated dienes than both the chow and soybean-fed rats, and lower heart conjugated dienes than the soybean-fed rats. (Table 4).

Discussion

We found that orchidectomy reduced lipid peroxidation in serum, liver and heart. The changes were more obvious for malonaldehyde rather than conjugated dienes, but the trend is similar. These results are in agreement with earlier reports. Toyokuni and co-workers (1993) found that orchidectomy reduced lipid peroxidation in renal tubules of Fe-NTA treated mice, however giving testosterone to similarly treated female mice increased lipid peroxidation. In our study, testosterone replacement did not increase the lipid peroxidation back to levels in non-orchidectomised male rats. This could mean that hormones other than testosterone such as dehydroepiandrosterone and androstenedione produced by the testis may play an important role in the lipid peroxidation process. Also, testosterone may have different effects in male and female animals. The different peroxidative stress imposed and different organs studied may also account for the differences in observations. Rosenblum *et al.* (1985) observed that testis exposed to ethanol-induced injury had decreased PUFA and increased malonaldehyde and conjugated diene concentrations, associated with decrease in plasma testosterone levels. But the effects seen here could be complicated by the alcohol given and not merely due to reduced testosterone levels.

Adding 20% w/w palm oil did not increase

Table 5. Effects of dietary fat and ovarectomy with estrogen replacement on malonaldehyde concentrations (umol/g protein).

Rat Preparation	Diet		
	Chow	Soybean oil	Palm oil
Serum			
Ovarectomised	0.47±0.07	0.50±0.09	0.48±0.08
Ovarectomised + Estrogen	0.54±0.09	0.47±0.07	0.44±0.07
Liver			
Ovarectomised	0.74±0.11[a]	1.12±0.32	0.75±0.23[a]
Ovarectomised + Estrogen	1.16±0.29[@b]	1.13±0.10[b]	0.82±0.26
Heart			
Ovarectomised	2.2±0.6	2.2±0.4	2.3±0.6
Ovarectomised + Estrogen	1.9±0.6	2.1±0.6	2.0±0.5

@=significant from ovarectomised rats
a=significant from soybean group
b=significant from palm oil group
Significant level is at $p<0.05$.
Values are in mean±s.d. (n=6-8).

lipid peroxidation in all three rat preparations studied. This observation is seen in the liver and heart homogenates, while no inter-diet group differences were seen in the serum. On the other hand, addition of 20% w/w soybean oil increased lipid peroxidation in the non-orchidectomised and orchidectomised rats. The increased peroxidation with soybean-enriched diets was not unexpected since soybean oil has a much higher percentage of PUFA compared to palm oil, which is richer in monounsaturates (Chong and Ng 1991). PUFA is prone to peroxidation while monounsaturates are more resistant. Huang and Fwu (1992) observed higher TBARS in animals fed diets high in soybean oil, while Reaven *et al.* (1991) observed lower conjugated dienes in LDL from humans fed diets rich in the monounsaturated fatty acid, oleate as compared to the polyunsaturated fatty acid, linoleate. Another reason could be that palm oil is rich in the antioxidant tocotrienol (Chong and Ng 1991), which could have effectively reduced lipid peroxidation in the tissues of our expreimental animals. The results of this study are in agreement with our earlier study (Ima-Nirwana, *in press*). Soybean oil, though rich in the antioxidant tocopherol, is devoid of tocotrienol (Elson 1992) and did not succeed in reducing the levels of lipid peroxidation products in our study. Testosterone replacement in the soybean-fed groups attenuated the increase in tissue lipid peroxidation due to orchidectomy, again demonstrating a beneficial effect of testosterone on lipid peroxidation process.

We did not use non-ovarectomised female rats in this study because their estrogen levels fluctuate according to the menstrual cycle of each rat. We found that the effects of estrogen replacement in ovarectomised female rats on lipid peroxidation was not so conclusive. Estrogen replacement increased malonaldehyde, but reduced conjugated dienes in the liver. Reports in the literature also differed.

Table 6. Effects of dietary fat and ovarectomy with estrogen replacement on conjugated diene concentrations (OD/g protein).

Rat Preparation	Diet		
	Chow	Soybean oil	Palm oil
Serum			
Ovarectomised	13.4±2.3	15.5±2.4	13.1±2.9
Ovarectomised + Estrogen	11.1±2.5	11.8±3.1	10.4±2.6
Liver			
Ovarectomised	89.3±8.6	77.9±10.9	74.3±21.4
Ovarectomised + Estrogen	73.5±12.3$^{@b}$	83.7±14.8^{b}	53.3±7.1$^{@}$
Heart			
Ovarectomised	94.7±27.0	78.8±24.3	99.6±22.9
Ovarectomised + Estrogen	84.9±10.3^{b}	78.0±14.7	67.5±2.8$^{@}$

@=significant from ovarectomised rats
a=significant from soybean group
b=significant from palm oil group
Significant level is at $p<0.05$.
Values are in mean±s.d. (n=6-8).

Estrogen was found to increase the susceptibility of red blood cells to peroxidation as measured by the amount of TBARS generated (Le Petit-Thevenin *et al.* 1991). On the other hand, Maziere *et al.* (1991) observed that estrogen inhibited monocyte mediated oxidation of LDL. Toyokuni *et al.* (1990) reported that estrogen reduced the formation of fluorescent products and TBARS in renal tubules of Fe-NTA exposed male mice. The beneficial effect of estrogen on atherosclerosis is via several mechanisms, i.e. by a direct effect on arterial wall (Wagner *et al.* 1992, Haarbo *et al.* 1992), by prevention of uptake and degradation of LDL by the arterial wall (Adams *et al.* 1991) and by reduction of lipid peroxidation process (Toyokuni *et al.* 1990, Maziere *et al.* 1991). Therefore, our results may just portray part of the overall effects of estrogen.

As in the male rats, addition of palm oil to the diet of the female rats reduced liver and heart lipid peroxidation, whereas addition of soybean oil maintained lipid peroxidation at similar concentrations as in the group fed only rat chow. This again demonstrates that palm oil significantly improves lipid peroxidation compared to soybean oil.

In conclusion, orchidectomy has a favourable effect on peroxidation of PUFA. Testosterone did not increase lipid peroxidation while estrogen did not influence lipid peroxidation significantly. Palm oil significantly improved lipid peroxidation, while the reverse is true for soybean oil. Testosterone attenuated the effects of soybean oil on lipid peroxidation in the male rats.

Acknowledgements

The authors thank Baharom Suboh for his technical assistance. This study was supported by research grants IRPA 3-07-03-025 from the Ministry of Science, Technology and Environment, and RD 66/91 from Universiti Kebangsaan Malaysia.

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(Accepted 19 October 1995)