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This paper is dedicated to Professor Włodzimierz Ostrowski
Review

Female hormones act as natural antioxidants — a survey of our research

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The increase in the lipid peroxide level in the serum and liver of female mice after bilateral ovariectomy evidenced antioxidant activities of female hormones. This increase was abolished upon administration of female hormones. Similar increase in the level of lipid peroxide was observed in the serum of women who had undergone bilateral ovariectomy. Injection of 2-hydroxyestradiol suppressed the increase in the lipid peroxide level in the liver of rats receiving whole-body γ-ray irradiation. Considering that the mechanism of coronary atherosclerosis is ascribed at least in part to the increased level of lipid peroxides, estrogen therapy could be applied to women who had undergone bilateral ovariectomy prior to menopause or to normal women after menopause. 2-Hydroxyestradiol might be applied also to men.

In 1977, we reported that the lipid peroxide level in women is lower than that in men of the same age group (30 to 50 years) [1]. Since we thought at that time that an increase in lipid peroxides is causative of atherosclerosis, we have suspected that this lower lipid peroxide level in women might explain the fact found by the Framingham study [2] that the risk factor for atherosclerosis in women is lesser than in men. It was also found that the lipid peroxide level is increased in diabetes with angiopathy [3], atherosclerosis [4], and apoplexy [5]. To verify our hypothesis that the increased level of lipid peroxides in the bloodstream is causative of atherosclerosis, we conducted experiments on the effects of linoleic acid hydroperoxide on the cells of blood vessels, and found that the hydroperoxide injured the endothelial cells of blood vessels *in vivo* [6] and *in vitro* [7]. Linoleic acid hydroperoxide increased also the uptake of low-density lipoprotein (LDL) by smooth muscle cells and macrophages [8]. Recently, we have found generation of hydroxyl radicals from lipid hydroperoxides in LDL upon addition of ferrous iron or epinephrine-iron complexes [9]. Because of high reactivity of hydroxyl radicals, the injury to the endothelial cells of the aorta and other vessels could be ascribed to the action of these hydroxyl radicals. In view of these findings, we predicted that female hormones would be inhibitory to lipid peroxidation *in vivo*, because their structures suggest that they could act as antioxidants through their phenolic hydroxyl group.

In 1986, we found in *in vitro* experiments that female hormone estrogens, such as estrone, estradiol (E_2), and estriol, inhibited the peroxidation of methyl linoleate caused

Abbreviations: LDL, low-density lipoprotein; E_2, estradiol; 2-OHE_1, 2-hydroxyestrone; 2-OHE_2, 2-hydroxyestradiol; 2-OHE_3, 2-hydroxyestriol.

by UV irradiation and also peroxidation of rat liver microsomal lipids caused by the iron-ADP peroxidation system in contrast to the male hormone testosterone [10]. Soon after, Sugioka *et al.* [11] confirmed our results in the report of an inhibitory effect of estrogens on membrane phospholipid peroxidation. Such antioxidant action of various natural and synthetic estrogens was confirmed in 1995 by Lacort *et al.* [12].

Extending our observations to the *in vivo* experiments, we found that administration of E_2 brought about a decrease in serum and liver lipid peroxide levels in female mice, whereas this reduction in male mice was insignificant [13]. However, when 2-hydroxyestradiol (2-OHE_2), a major metabolite of E_2 in the liver, was intraperitoneally injected, the lipid peroxide level in the liver was decreased in both male and female mice [13]. From these results, one may conclude that a remarkable effect of E_2 is due to its metabolite 2-OHE_2.

In 1987 Nakano *et al.* [14] demonstrated that catecholestrogens such as 2-hydroxyestrone (2-OHE_1) and 2-OHE_2 are more potent antioxidants *in vitro* than E_2 and α-tocopherol. Up to now, evidences have accumulated indicating an antioxidant activity of estrogens, catecholestrogens, and related compounds [15–28].

EFFECT OF OVARIECTOMY ON LIPID PEROXIDE LEVELS OF FEMALE MICE

Since a question arose as to whether or not endogenous estrogens are indeed effective in preventing an increase in lipid peroxide levels *in vivo*, we examined changes in the lipid peroxide level in mice after ovariectomy [29]. Female mice of the ddY strain (12–13 weeks old) were subjected to bilateral ovariectomy by the back approach under anesthesia with ether, and control mice only to laparotomy as a sham-operation. Animals of both groups were sacrificed at 1, 2, and 3 months after operation for the measurement of the lipid peroxide level in the serum by the method of Yagi [30] and in the liver according to Ohkawa *et al.* [31]. As shown in Table 1, the serum lipid peroxide level expressed per ml of serum in the ovariectomized animals tended to increase at 1 month after the operation and was significantly higher than that in the sham-operated control mice after 2 and 3 months. When serum lipid peroxide level was expressed in terms of peroxides per mg of serum lipids, the level in the ovariectomized mice was significantly higher than that in the sham-operated control mice at only 3 months after the operation.

Table 1. Effect of bilateral ovariectomy on serum lipid peroxide level and lipid content in female mice [29].

Mean ± S.E. is given. Number of mice in each group was 10. Significant difference from the corresponding values of the sham-operation group: $^*P < 0.05$.

	Time after operation (month)		
	1	2	3
Lipid peroxide level (nmol/ml serum)			
Sham-operation	3.6 ± 0.4	3.7 ± 0.3	3.3 ± 0.3
Ovariectomy	4.3 ± 0.5	5.1 ± 0.6*	4.3 ± 0.3*
Lipid peroxide level (nmol/mg serum lipids)			
Sham-operation	1.0 ± 0.1	1.2 ± 0.1	0.9 ± 0.1
Ovariectomy	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.1*
Lipid content (mg/dL serum)			
Sham-operation	356 ± 19	317 ± 20	407 ± 38
Ovariectomy	368 ± 24	416 ± 38*	359 ± 32

Table 2 presents changes in liver lipid peroxide levels in the ovariectomized and control mice. The level expressed as nmol/100 mg of liver was significantly increased in the ovariectomized mice at 1 month after operation as compared with that in the sham-operated control mice. The increase in the level continued, at least, up to 3 months after the operation. At 2 and 3 months, the levels expressed per mg of liver lipids increased.

increased by bilateral ovariectomy. To understand the mechanism underlying the increase in the lipid peroxide level caused by ovariectomy, we examined the enzyme activities involved in decomposition of lipid peroxides and found that the activities of glutathione peroxidase, glutathione *S*-transferase, glutathione reductase, and glucose 6-phosphate dehydrogenase in the liver of ovariectomized mice were not changed.

Table 2. Effect of bilateral ovariectomy on liver lipid peroxide level and lipid content in female mice [29].

Mean ± S.E. is given. Number of mice in each group was 10. Significant difference from the corresponding values of the sham-operation group: $^{*}P < 0.05$, $^{**}P < 0.005$.

	Time after operation (month)		
	1	2	3
Lipid peroxide level (nmol/100 mg liver)			
Sham-operation	23.4 ± 1.4	28.7 ± 2.2	26.2 ±1.4
Ovariectomy	29.1 ± 1.6*	37.8 ± 2.6*	41.7 ± 4.6**
Lipid peroxide level (nmol/mg liver lipids)			
Sham-operation	16.4 ± 2.1	16.2 ± 1.2	13.6 ± 1.1
Ovariectomy	16.4 ± 1.0	22.3 ± 1.9*	20.0 ± 2.0*
Lipid content (mg/g liver)			
Sham-operation	15.5 ± 1.2	17.6 ± 0.7	20.0 ± 1.2
Ovariectomy	18.0 ± 0.8	17.4 ± 1.0	20.6 ± 0.6

EFFECT OF ADMINISTRATION OF ESTROGEN AND CATECHOLESTROGEN ON SERUM AND LIVER LIPID PEROXIDE LEVELS OF OVARIECTOMIZED FEMALE MICE

When E_2 or 2-OHE_2 was injected subcutaneously at the dose of 0.2 or 2.0 mg/kg body weight 3 times per week for 4 weeks, the lipid peroxide level in the serum of ovariectomized female mice at 2 days after the last injection was significantly decreased as compared with that of the control mice given only the vehicle. A significant decrease was also observed in the lipid peroxide level in the liver of the ovariectomized female mice, upon administration of E_2 or 2-OHE_2 (Table 3).

Thus, it is clear that both serum and liver lipid peroxide levels in female mice were

Therefore, we assumed that the increase in the lipid peroxide level due to ovariectomy resulted from a diminished effect of estrogen as an antioxidant. In fact, administration of E_2 or 2-OHE_2 suppressed the increase in lipid peroxide levels caused by ovariectomy. This result substantiates the antioxidant activity of estrogens and catecholestrogens *in vivo*.

EFFECT OF OVARIECTOMY ON SERUM LIPID PEROXIDE LEVELS IN WOMEN

The above-mentioned animal experiments revealed that endogenous estrogen and catecholestrogen prevent an increase in lipid peroxide levels in animals. Consequently, we

Table 3. Effects of E_2 and 2-OHE_2 on lipid peroxide levels in bilaterally ovariectomized female mice [29].

Mean ± S.E. is given. Number of mice in each group was 10. Significant difference from the values of the control group after ovariectomy: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

Group	Dose	Lipid peroxide levels	
	(mg/kg)	Serum (nmol/ml serum)	Liver (nmol/100 mg liver)
Ovariectomy	–	5.9 ± 0.6	39.4 ± 3.9
Ovariectomy + E_2	0.2	3.7 ± 0.4*	28.4 ± 2.5*
	2.0	3.4 ± 0.2***	27.7 ± 1.8*
Ovariectomy + 2-OHE_2	0.2	4.1 ± 0.4*	29.4 ± 1.3*
	2.0	3.8 ± 0.2**	26.0 ± 2.3*

have carried out a clinical investigation in humans [32]. First, we compared serum lipid peroxide levels of normal pre- and postmenopausal women. The level in 18 premenopausal women (age, 35.9 ± 6.2 years old) was 2.66 ± 0.59 nmol/ml serum. In the case of 10 postmenopausal women (age 57.6 ± 6.1 years old), it was 3.37 ± 0.27 nmol/ml serum, indicating a significant increase ($P < 0.05$) in serum lipid peroxides after natural menopause.

In order to examine changes in serum lipid peroxide levels after ovariectomy, 7 premenopausal women who had undergone bilateral ovariectomy were examined. The average age of 7 subjects was 45 years. In 5 out of 7 patients, serum lipid peroxide levels were measured at least twice after the operation (Fig. 1). The lipid peroxide level in the serum was increased in all patients. In the most typical case, the level before the operation was relatively low (1.31 nmol/ml), but became elevated with time and reached 5.91 nmol/ml 60 days after the operation. This patient suffered from unidentified clinical symptoms such as a serious hot flush, palpitation, and nervousness after the operation. Table 4 presents statistical analysis of the changes in serum lipid peroxide level. Before the operation the levels in women who have undergone bilateral ovariectomy were normal, but were significantly increased 15, 30, and 60 days after the operation, when expressed either per ml of serum or per mg of total serum lipids. On the other hand, the lipid peroxide level in the serum of 9 premenopausal women (average age 44.8 years) who have undergone hysterectomy, with unilateral ovariectomy, did not change significantly.

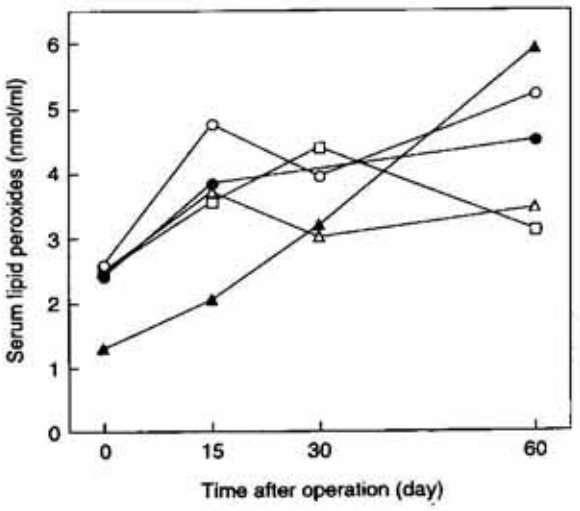

Figure 1. Changes in serum lipid peroxide levels of women after bilateral ovariectomy [32].

○, Patient A, 51 years, myoma; ●, patient B, 44 years, endometriosis; △, patient C, 55 years, myoma; ▲, patient D, 43 years, myoma; □, patient E, 46 years, myoma.

These results are in good agreement with those obtained in animal experiments [29], and substantiated our conclusion that female hormones have an important biological function as endogenous antioxidants.

Table 4. Changes in serum lipid peroxide levels in women following bilateral ovariectomy [32].

Mean ± S.D. is given. Significant difference from the values before operation was calculated by the paired *t*-test: $^{*}P = 0.06$, $^{**}P < 0.05$, $^{***}P < 0.01$.

Number of subjects	Time of sampling	Serum lipid peroxide level	
		(nmol/ml)	(nmol/ mg lipids)
5	Before operation	2.25 ± 0.53	0.38 ± 0.12
	15 days after operation	3.58 ± 0.96***	0.74 ± 0.19***
6	Before operation	2.54 ± 0.76	0.46 ± 0.18
	30 days after operation	3.64 ± 0.50**	0.66 ± 0.13**
5	Before operation	2.25 ± 0.53	0.38 ± 0.12
	60 days after operation	4.44 ± 1.16**	0.70 ± 0.20*

PROTECTIVE EFFECT OF ESTROGENS AND CATECHOLESTROGENS ON RADIATION INJURY

Since it is well known that lipid peroxides are generated by radiation and cause damage to organs and tissues, we have expected that catecholestrogens would have a protective effect on radiation injury. Three phases of radiation syndrome are considered to occur in rats after an 8-Gy single dose of whole-body γ-ray irradiation; 1) an initial phase up to 4 days after the irradiation, in which prostration, fasting, and diarrhea occur; 2) a remission phase from 5 to 7 days, in which the animals regain normal feeding and mobility; and 3) a final phase from 8 days, in which hemorrhage occurs, which leads to death. A marked increase in the lipid peroxide level in the liver was observed in the initial phase [33]. In male mice of BALB/c strain 4 days after 8-Gy irradiation, we found that the lipid peroxide level in the liver was almost doubled the control level [34] (Table 5). When the animals were subcutaneously injected with 2-OHE_2 3 h before and 3 h after the irradiation at the dose of 2 mg/kg body weight, the increase in the lipid peroxide level caused by irradiation was significantly suppressed.

Under the above experimental conditions, we followed the survival of irradiated animals for 30 days (Fig. 2). In control mice, the first death occurred on the 10th day after the irradiation, and the percent survival of mice after 30 days was only 5%. When 2-OHE_2 was injected, the onset of the first death was delayed to the 15th day after the irradiation, and the survival rate after 30 days remark-

Table 5. Effect of 2-OHE_2 on liver lipid peroxide level in mice after ^{60}Co γ-ray irradiation [34].

Mice received whole-body irradiation with an 8-Gy single dose of ^{60}Co γ-ray. 2-OHE_2 was subcutaneously injected at the dose of 2 mg/kg body weight 3 h before and 3 h after the irradiation. Lipid peroxide level was measured 4 days after the irradiation. Mean ± S.E. is given. Number of mice in each group was 9. Significant difference from the corresponding values for group 1: $^{*}P < 0.01$.

Group	^{60}Co	2-OHE_2	Lipid peroxide level	
			(nmol/100 mg liver)	(nmol/mg lipids)
1	–	–	51.9 ± 5.4	21.7 ± 2.1
2	+	–	101.6 ± 8.5*	41.1 ± 2.2*
3	+	+	43.0 ± 6.9	21.6 ± 2.5

ably increased up to 70%. In the case of mice injected with 2-OHE_1 or 2-hydroxyestriol (2-OHE_3), the survival rate was always higher than that of the control animals. The injection of *dl*-α-tocopheryl acetate also resulted in a significant protection, but only during 21 days. In conclusion, among the catecholestrogens tested, 2-OHE_2 was the most effective in protecting mice from death. Recently we have also reported that 2-OHE_2 has a beneficial effect to cure leucopenia induced by irradiation [35].

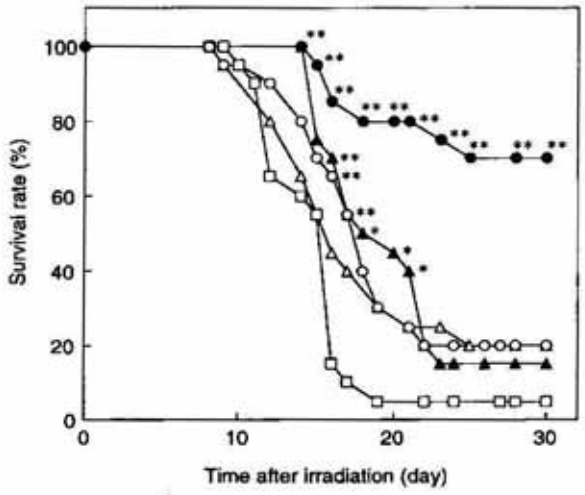

Figure 2. Effect of catecholestrogens on survival rate of mice after ^{60}Co γ-ray irradiation [34].

Mice received whole-body irradiation with an 8-Gy single dose of ^{60}Co γ-ray. Each catecholestrogen was injected 3 h before and 3 h after the irradiation. ○, 2-OHE_1; ●, 2-OHE_2; △, 2-OHE_3; ▲, *dl*-α-tocopheryl acetate; □, control. Number of mice in each group was 20. Significant difference from the corresponding control value as analyzed by the chi-square test: $^*P < 0.05$; $^{**}P < 0.01$.

IMPLICATIONS OF ESTROGENS AND CATECHOLESTROGENS IN VARIOUS DISEASES

It is well recognized that premenopausal women have a markedly lower incidence of atherosclerotic heart diseases than men of corresponding age [2]. It is also known that menopause and bilateral ovariectomy prior to menopause result in an increasd risk of myocardial infarction and coronary atherosclerosis in women [36, 37]. Estrogen replacement therapy is beneficial for reduction of risk of coronary heart disease [38]. The mechanism of the beneficial effect in coronary heart diseases has been considered to be mediated through changes in lipids and lipoproteins. However, in the light of our earlier hypothesis that the increase in the level of lipid peroxides in the blood initiates and propagates atherogenesis [39, 40], we believe that the estrogen replacement therapy is at least partly mediated through its antioxidant activity. In fact, recent studies have clarified the estrogen-mediated inhibition of LDL peroxidation [15–18, 20, 21, 27, 28]. The catecholestrogen 2-OHE_2 is more potent than E_2 in inhibiting lipid peroxidation of LDL [41, 42]. We further observed that 2-OHE_2 was effective in preventing cultured endothelial cells from being injured by lipid hydroperoxides [42]. All these data suggest that estrogen or catecholestrogen, serving as an antioxidant, could be beneficial in protection of cardiovascular disease. It might be noted that catecholestrogen could be applied to men, since its hormonal effect is nil or, if any, less than 1/1000 of that of estrogen [43].

In addition, Behl *et al.* [23] found that E_2 can protect hippocampal cells from the oxidative stress-induced cell death caused by neurotoxins, amyloid β-protein, hydrogen peroxide, and glutamate and that the neuronal protection afforded by E_2 was estrogen receptor independent. More recently, Green *et al.* [44] also reported on the neuroprotective effects of estrogens, though they did not mention a role of estrogens as an antioxidant. Since oxidative injury has been implicated in various neurodegenerative diseases, such as Parkinson's disease [45] and Alzheimer's disease [46], the antioxidant activity of estrogens or catecholestrogens may become useful also for the prevention and treatment of these neurodegenerative diseases.

REFERENCES

1. Suematsu, T., Kamada, T., Abe, H., Kikuchi, S. & Yagi, K. (1977) Serum lipoperoxide level in patients suffering from liver diseases. *Clin. Chim. Acta* **79**, 267–270.

2. Castelli, W.P. (1984) Epidemiology of coronary heart disease: The Framingham study. *Am. J. Med.* **76**, 4–12.

3. Sato, Y., Hotta, N., Sakamoto, N., Matsuoka, S., Ohishi, N. & Yagi, K. (1979) Lipid peroxide level in plasma of diabetic patients. *Biochem. Med.* **21**, 104–107.

4. Plachta, H., Bartnikowska, E. & Obara, A. (1992) Lipid peroxides in blood from patients with atherosclerosis of coronary and peripheral arteries. *Clin. Chim. Acta* **211**, 101–112.

5. Kawamoto, M., Kagami, M. & Terashi, A. (1986) Serum lipid peroxide level in apoplexia. *J. Clin. Biochem. Nutr.* **1**, 1–3.

6. Yagi, K., Ohkawa, H., Ohishi, N., Yamashita, M. & Nakashima, T. (1981) Lesion of aortic intima caused by intravenous administration of linoleic acid hydroperoxide. *J. Appl. Biochem.* **3**, 58–65.

7. Sasaguri, Y., Nakashima, T., Morimatsu, M. & Yagi, K. (1984) Injury to cultured endothelial cells from human umbilical vein by linoleic acid hydroperoxide. *J. Appl. Biochem.* **6**, 144–150.

8. Yagi, K., Inagaki, T., Sasaguri, Y., Nakano, R. & Nakashima, T. (1987) Formation of lipid-laden cells from cultured aortic smooth muscle cells and macrophages by linoleic acid hydroperoxide and low density lipoprotein. *J. Clin. Biochem. Nutr.* **3**, 87–94.

9. Yagi, K., Komura, S., Ishida, N., Nagata, N., Kohno, M. & Ohishi, N. (1993) Generation of hydroxyl radicals from lipid hydroperoxides contained in oxidatively modified low-density lipoprotein. *Biochem. Biophys. Res. Commun.* **190**, 386–390.

10. Yagi, K. & Komura, S. (1986) Inhibitory effect of female hormones on lipid peroxidation. *Biochem. Int.* **13**, 1051–1055.

11. Sugioka, K., Shimosegawa, Y. & Nakano, M. (1987) Estrogens as natural antioxidants of membrane phospholipid peroxidation. *FEBS Lett.* **210**, 37–39.

12. Lacort, M., Leal, A.M., Liza, M., Martín, C., Martínez, R. & Ruiz-Larrea, M.B. (1995) Protective effect of estrogens and catecholestrogens against peroxidative membrane damage *in vitro*. *Lipids* **30**, 141–146.

13. Yoshino, K., Komura, S., Watanabe, I., Nakagawa, Y. & Yagi, K. (1987) Effect of estrogens on serum and liver lipid peroxide levels in mice. *J. Clin. Biochem. Nutr.* **3**, 233–240.

14. Nakano, M., Sugioka, K., Naito, I., Takekoshi, S. & Niki, E. (1987) Nobel and potent biological antioxidants on membrane phospholipid peroxidation: 2-Hydroxy estrone and 2-hydroxy estradiol. *Biochem. Biophys. Res. Commun.* **142**, 919–924.

15. Huber, L.A., Scheffler, E., Poll, T., Ziegler, R. & Dresel, H.A. (1990) 17 β-estradiol inhibits LDL oxidation and cholesteryl ester formation in cultured macrophages. *Free Radical Res. Commun.* **8**, 167–173.

16. Mazière, C., Auclair, M., Ronveaux, M.-F., Salmon, S., Santus, R. & Mazière, J.-C. (1991) Estrogens inhibit copper and cell-mediated modification of low density lipoprotein. *Atherosclerosis* **89**, 175–182.

17. Rifici, V.A. & Khachadurian, A.K. (1992) The inhibition of low-density lipoprotein oxidation by 17 β-estradiol. *Metabolism* **41**, 1110–1114.

18. Subbiah, M.T.R., Kessel, B., Agrawal, M., Rajan, R., Abplanalp, W. & Rymaszewski, Z. (1993) Antioxidant potential of specific estrogens on lipid peroxidation. *J. Clin. Endocrinol. Metab.* **77**, 1095–1097.

19. Wachnik, A., Biro, G., Biro, L., Korom, M., Gergely, A. & Antal, M. (1993) Effect of sex hormones on copper, zinc, iron nutritional status and hepatic lipid peroxidation in rats. *Nahrung* **37**, 28–34.

20. Keaney, J.F., Jr., Shwaery, G.T., Xu, A., Nicolosi, R.J., Loscalzo, J., Foxall, T.L. & Vita, J.A. (1994) 17 β-estradiol preserves endothelial vasodilator function and limits low-density lipoprotein oxidation in hypercholesterolemic swine. *Circulation* **89**, 2251– 2259.

21. Sack, M.N., Rader, D.J. & Cannon, R.O. III (1994) Oestrogen and inhibition of oxidation of low-density lipoprotein in postmenopausal woman. *Lancet* **343**, 269–270.

22. Ruiz-Larrea, M.B., Leal, A.M., Liza, M., Lacort, M. & de Groot, H. (1994) Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. *Steroids* **59**, 383–388.

23. Behl, C., Widmann, M., Trapp, T. & Holsboer, F. (1995) 17 β-estradiol protects neurons from oxidative stress-induced cell death *in vitro*. *Biochem. Biophys. Res. Commun.* **216**, 473–482.

24. Takahashi, K., Watanabe, K. & Yoshizawa, I. (1995) On the inhibitory effect of C17-sulfo-conjugated catechol estrogens upon lipid peroxidation of rat liver microsomes. *Biol. Pharm. Bull.* **18**, 1120–1125.

25. Tranquilli, A.L., Mazzanti, L., Cugini, A.M., Cester, N., Garzetti, G.G. & Romaini, C. (1995) Transdermal estradiol and medroxyprogesterone acetate in hormone replacement therapy are both antioxidants. *Gynecol. Endocrinol.* **9**, 137–141.

26. Tang, M., Abplanalp, W., Ayres, S. & Subbiah, M.T.R. (1996) Superior and distinct antioxidant effects of selected estrogen metabolites on lipid peroxidation. *Metabolism* **45**, 411–414.

27. Miller, C.P., Jirkovsky, I., Hayhurst, D.A. & Adelman, S.J. (1996) In vitro antioxidant effects of estrogens with a hindered 3-OH function on the copper-induced oxidation of low density lipoprotein. *Steroids* **61**, 305–308.

28. Wilcox, J.G. Hwang, J., Hodis, H.N., Sevanian, A., Stanczyk, F.Z. & Lobo, R.A. (1997) Cardioprotective effects of individual conjugated equine estrogens through their possible modulation of insulin resistance and oxidation of low-density lipoprotein. *Fertil. Steril.* **67**, 57–62.

29. Nakagawa, Y., Yoshino, K., Komura, S., Ishihara, M. & Yagi, K. (1989) Effect of ovariectomy on serum and liver lipid peroxide levels of female mice. *J. Clin. Biochem. Nutr.* **6**, 87–94.

30. Yagi, K. (1976) A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.* **15**, 212–216.

31. Ohkawa, H., Ohishi, N. & Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351–358.

32. Asada, Y., Komura, S., Ohishi, N., Kasugai, M., Suganuma, N., Mizutani, S., Tomoda, Y. & Yagi, K. (1990) Effect of ovariectomy on serum lipid peroxide levels in women. *J. Clin. Biochem. Nutr.* **8**, 247–252.

33. Kergonou, J.-F., Bernard, P., Braquet, M. & Rocquet, G. (1981) Effect of whole-body gamma irradiation on lipid peroxidation in rat tissues. *Biochimie* **63**, 555–559.

34. Tomatsu, A., Komura, S., Ohishi, N., Nakanishi, M. & Yagi, K. (1993) Effect of catecholestrogens on mortality and liver lipid peroxide level of mice irradiated with ^{60}Co γ-ray. *J. Clin. Biochem. Nutr.* **15**, 43–49.

35. Tomatsu, A., Nakanishi, M., Sakanaka, M., Komura, S., Ohishi, N. & Yagi, K. (1995) Protective effect of catecholestrogen on injury caused by ^{60}Co-irradiation on mice. *J. Clin. Biochem. Nutr.* **18**, 49–54.

36. Weinreb, H.L., German, E. & Rosenberg, B. (1957) A study of myocardial infarction in women. *Ann. Int. Med.* **46**, 285–300.

37. Robinson, R.W., Higano, N. & Cohen, W.D. (1959) Increased incidence of coronary heart disease in women castrated prior to the menopause. *Arch. Int. Med.* **104**, 908–913.

38. Stampfer, M.J., Colditz, G.A., Willett, W.C., Manson, J.E., Rosner, B., Speizer, F.E. & Henekens, C.H. (1991) Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. *N. Eng. J. Med.* **325**, 756–762.

39. Yagi, K. (1984) Increased serum lipid peroxides initiate atherogenesis. *BioEssays* **1**, 58–60.

40. Yagi, K. (1986) A biochemical approach to atherogenesis. *Trends Biochem. Sci.* **11**, 18–19.

41. Taniguchi, S., Yanase, T., Kobayashi, K., Takayanagi, R., Haji, M., Umeda, F. & Nawata, H. (1994) Catechol estrogens are more potent antioxidants than estrogens for the Cu^{2+}-catalyzed oxidation of low or high density lipoprotein: Antioxidative effects of steroids on lipoproteins. *Endocr. J.* **41**, 605–611.

42. Komura, S., Nagata, N., Ohishi, N. & Yagi, K. (1995) Protective effect of catecholestrogen on lipid hydroperoxide-induced injury to cultured bovine aortic endothelial cells and on

lipid peroxidation of low-density lipoprotein. *J. Clin. Biochem. Nutr.* **19**, 71–77.

43. Gordon, S., Cantrall, E. W., Cekleniak, W. P., Albers, H.J., Mauer, S., Stolar, S.M. & Bernstein, S. (1964) Steroid and lipid metabolism. The hypocholesteremic effect of estrogen metabolites. *Steroids* **4**, 267–271.

44. Green, P.S., Bishop, J. & Simpkins, J.W. (1997) 17α-estradiol exerts neuroprotective effects on SK-N-SH cells. *J. Neurosci.* **17**, 511–515.

45. Dexter, D.T., Carter, C.J., Wells, F.R., Javoy-Agid, F., Agid, Y., Lee, A., Jenner, P. & Marsden, C.D. (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.* **52**, 381–389.

46. Butterfield, D.A., Hensley, K., Harris, M., Mattson, M. & Carney, J. (1994) β-Amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence specific fashion: Implications to Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **200**, 710–715.