Effects of Estrogen Treatment on the Oophorectomized Female Rat Aorta[1] (37699)

HARVEY WOLINSKY[2]

Departments of Medicine and Pathology and the Cardiac-Pulmonary-Renal Research Unit, The Albert Einstein College of Medicine, Bronx, New York 10461

After menopause, morbidity and mortality from atherosclerotic vascular disease rises sharply in women. Although serum lipid changes occur coincident with the decrease in ovarian function (1–3), the possibility that estrogens exert direct vascular effects was postulated decades ago (1). Exogenous estrogen administration results in suppression of oxygen consumption and mucopolysaccharide synthesis by arteries (4, 5) and a decrease in percent of aortic connective tissue (6). We have recently shown that estrogens have a suppressive effect on connective tissue accumulation in the aortas of hypertensive males (7). However, these studies are not directly pertinent to the questions of how the vascular effects of estrogen deprivation in the female are manifest and whether replacement therapy prevents or reverses these effects. Fischer (8) has shown that connective tissue accumulates in the oophorectomized female rat aorta and that estrogen therapy for 3 weeks lowers these levels. However, in that experiment it was not clear to what extent the increased aortic weights of the oophorectomized females were due to their accelerated growth rates. The present study was intended to further clarify the vascular effects of long-term administration of estrogen to oophorectomized mature female rats independent of its effects on their body weights.

Materials and Methods. Mature female Carworth[3] rats (CF-E strain) weighing 230–250 g were used. Bilateral oophorectomy was done within 1 week prior to the onset of the 6-month experimental period. The first experiment consisted of two groups: (Group E) oophorectomized females were given subcutaneous monthly injections of 100 μg of Depo-Estradiol[4] in cottonseed oil, and (Group O) oophorectomized females were given the same amount of cottonseed oil only, on the same dosage schedule. Laboratory rat chow[5] and water were given *ad libitum*. The second experiment consisted of two similar groups, which differed from those of the first experiment only in that food intake of Group O was limited to that ingested by Group E. That it, average food intake in each 48 hr by animals in Group E was calculated, and that amount was given to Group O. Due to the maturity of the animals, day-to-day variation and inter-animal variation of food intake in Group E was quite small, the average being about 14 g per animal. Despite this stability, food intakes were monitored for the entire 6-month period on all animals, each of which was maintained in a metabolic balance cage.

At the conclusion of the experimental period, 11 animals from each group were sacrificed, and heart and uterus weights were calculated. The former was intended to give a rough assessment of hemodynamic status; the latter was used to confirm adequacy of ablative surgery and hormonal therapy. Chemical determinations were made on vessels of 6 animals from each group using intima-media preparations from anatomically-

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[3] Carworth Farms, New City, NY.

[4] Depo-Estradiol cypionate, Upjohn Company, Kalamazoo, MI.

[5] Ralston Purina Company, St. Louis, MO.

TABLE I. Characteristics of Animal Groups.

	Experiment 1		Experiment 2	
	E	O	E	O
Final body weight (g)	237 ± 5[a]	315 ± 6	251 ± 5	257 ± 7
Heart weight (g)	0.81 ± 0.03	0.91 ± 0.02	0.81 ± 0.01	0.84 ± 0.01
Uterus weight (g)	0.53 ± 0.03	0.11 ± 0.01	0.50 ± 0.07	0.10 ± 0.01

[a] Values expressed as mean ± SEM. E, estrogen-treated oophorectomized rats; O, untreated oophorectomized rats.

defined thoracic aortic segments as described elsewhere (9). In each segment, total aortic elastin, collagen, and noncollagenous alkali-soluble proteins (NCASP), a component which we consider to be a good reflection of cell protein, were determined (9). Total DNA content of each of five segments from each of the groups was determined using the modification by Hubbard *et al.* (10) of Ceriotti's method (11). DNA standards were corrected for water content by using inorganic phosphorus as the criterion according to the method of Fiske and Subbarow (12).

Statistical evaluation was carried out using Student's paired t test. Significance was considered to be present at the 5% level or less.

Results. In Table I body and organ weights are shown. In Experiment 1, untreated oophorectomized animals (Group O) were very significantly heavier than estrogen-treated oophorectomized animals (Group E) ($t = 10.01$, $p < 0.001$), which maintained the usual nearly-constant adult body weight of female rats (13). In Experiment 2, weight balancing achieved its goal: equalization of weights of treated and nontreated groups. In Experiment 1, heart weights were significantly greater in untreated animals ($t = 2.77$, $p < 0.02$); however, in Experiment 2, no significant difference was found ($t = 1.59$, $0.2 > p > 0.1$). Uterus weights confirmed the effectiveness of ablation in Groups O and the effects of exogenous estrogen in Groups E of both experiments.

Total DNA contents of the aortic wall did not differ between treated and untreated animals in each experiment. The respective values, expressed as total micrograms of DNA per aorta (mean ± SEM), were: Experiment 1—Group E, 18.0 ± 1.0; Group O, 18.0 ± 2.0. Experiment 2—Group E, 14.4 ± 1.2; Group O, 12.4 ± 1.1. We have no explanation for the differences between experiments, which barely reached significance ($t = 2.52$, $p < 0.05$). The lack of effects of estrogen or ablation on total cells, as reflected by DNA, probably reflects the degree of maturity of the animals at the outset of the experiment (13).

In Fig. 1 aortic dry weights of the groups in Experiments 1 and 2 are shown. Whether weights were balanced or not, values for the untreated groups were significantly greater than those of groups given estrogen ($t = 3.23$, $p < 0.01$ and $t = 4.87$, $p < 0.001$ for Experiments 1 and 2, respectively).

Figure 2 shows the absolute amounts of wall components in each of the experiments.

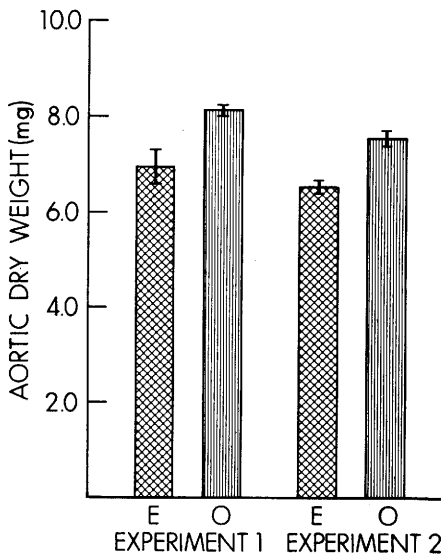

FIG. 1. Total dry aortic weights in each of the groups (mean ± SEM). In both experiments, aortas from untreated oophorectomized animals (Group O) were significantly heavier than those of oophorectomized animals given estrogen (Group E).

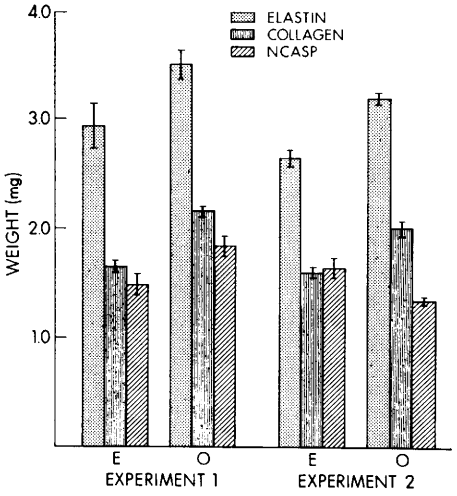

FIG. 2. Weights of aortic wall components in the groups studied (mean ± SEM). In both experiments, absolute weights of aortic elastin and collagen were significantly greater in untreated oophorectomized animals (Group O) than in estrogen-treated oophorectomized animals (Group E). The third component, NCASP, was increased in Group O of Experiment 1, but was decreased in Group O of Experiment 2.

In either case, whether weight was balanced or not, total elastin and collagen were both significantly increased in vessels from animals who were oophorectomized and not given replacement therapy. The results were remarkable in that similar increments of aortic elastin and collagen were found in untreated oophorectomized animals of both experiments. That is, about 0.5 mg of extra elastin and 0.5 mg of extra collagen accumulated in the aorta of an animal not given estrogen, over a 6-month period, regardless of its body weight. The findings are less consistent for NCASP, which apparently reflects cellular proteins. In Experiment 1, the untreated group had significantly more of this aortic component ($t = 2.60$, $p < 0.05$); in Experiment 2, significantly less of this component was present in the untreated group ($t = 3.00$, $p < 0.02$). The reasons for this difference are not evident, but the effects on percentages of components in the vessel wall are unmistakable.

In Table II the percentages of aortic components based on dry weights are shown. In both experiments, despite the equal increments of elastin and collagen in the untreated groups, only the collagen percents were were significantly higher in those groups ($t = 3.22$, $p < 0.01$ and $t = 3.27$, $p < 0.01$, respectively). The lack of changes in percentages of elastin in both experiments and of percentages of NCASP in Experiment 1 further reinforces the importance of expressing results in relative *and* absolute terms if all net changes in multicomponent tissues are to be detected. The above-mentioned difference in the absolute amount of the aortic NCASP component in Experiment 2 was manifest as a significant reduction in percentage of that component, as well ($t = 5.82$, $p < 0.001$).

Discussion. Demonstrated beneficial effects of replacement estrogen therapy in women with reduced ovarian function now include retardation or arrest of postmenopausal osteoporosis (14) and achievement of "lower-risk" profiles of the various plasma lipoproteins (15, 16) and serum cholesterol (17). Clinical studies suggest that premature surgically-induced menopause is associated with an accelerated appearance of coronary artery disease (3). The above-mentioned changes in circulating lipids, direct vascular effects of the hormone, or both could be responsible. The known actions of estrogen on vascular metabolism have been alluded to earlier. In

TABLE II. Percentages of Aortic Wall Components.

	Experiment 1		Experiment 2	
	E	O	E	O
Elastin	42.15 ± 1.03[a]	43.00 ± 1.24	40.38 ± 1.45	42.24 ± 0.54
Collagen	23.79 ± 0.53	25.97 ± 0.41	24.39 ± 0.42	26.65 ± 0.55
NCASP	20.29 ± 1.06	22.50 ± 1.06	24.95 ± 1.03	17.93 ± 0.63

[a] Values expressed as mean ± SEM. E, estrogen-treated oophorectomized rats; O, untreated oophorectomized rats.

this study, we have shown that an unmistakeable increase in vascular connective tissue occurs in the oophorectomized rat. No clear-cut statement is, of course, yet possible concerning what, if any, deleterious effects these fibrous changes may incur and what their relationship is to concomitant serum lipid alterations. However, it has been shown that vascular damage, represented largely by increased connective tissue, greatly enhances the susceptibility of the vessel wall to lipid deposition (18). The mechanism by which this alteration occurs is not certain, but it may be related to changes in wall permeability (19), to direct binding of lipid to the connective tissue (20), or to as yet unrecognized effects.

Summary. The postmenopausal state appears to be associated with an increased incidence of atherosclerotic vascular disease in women. Connective tissue and cellular changes in the female rat thoracic aorta were studied in oophorectomized mature rats with and without estrogen replacement. In one study, no attempt was made to control the increased growth of the oophorectomized female. However, because of the possible contribution of different growth rates to results, in another study, weights of the treated and nontreated groups were balanced by pair-feeding. Both studies showed the same qualitative results. Total DNA contents of anatomically-defined thoracic aortic segments were not affected by oophorectomy or by estrogen replacement. Significant and similar increments in total aortic elastin and collagen were seen in untreated oophorectomized groups compared to estrogen-treated ones; a significant increase in percentage of collagen was found. Accelerated accumulation of connective tissue in the rat aortic wall is associated with reduction of endogenous estrogen levels and is unrelated to changes in body weight; estrogen replacement prevents these changes.

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1. Pick, R., Clarke, G. B., and Katz, L. N., *in* "Progress in Biochemical Pharmacology; Recent Advances in Atherosclerosis" (C. J. Miras, A. M. Howard, and R. Paoletti, eds.), Vol. 4, p. 354. S. Karger, New York (1968).

2. Rivin, A. V., and Dimitroff, S. P., Circulation **9**, 533 (1954).

3. Oliver, M. F., and Boyd, G. S., Lancet **ii**, 690 (1959).

4. Malinow, M. R., Moguilevsky, J. A., and Gershenson, L., Circulation Res. **14**, 364 (1964).

5. Priest, R. E., Koplitz, R. M., and Benditt, E. P., J. Exp. Med. **112**, 225 (1960).

6. Cembrano, J., Lillo, M., Val, J., and Mardones, J., Circulation Res. **8**, 527 (1960).

7. Wolinsky, H., Circulation Res. **30**, 341 (1972).

8. Fischer, G. M., Endocrinology **91**, 1227 (1972).

9. Wolinsky, H., Circulation Res. **28**, 622 (1971).

10. Hubbard, R. W., Matthew, W. T., and Dubowick, D. A., Anal. Biochem. **38**, 190 (1970).

11. Ceriotti, G., J. Biol. Chem. **198**, 297 (1952).

12. Fiske, C. H., and Subbarow, Y., J. Biol. Chem. **66**, 375 (1925).

13. Looker, T., and Berry, C. L., J. Anat. **113**, 17 (1972).

14. Riggs, B. L., Jowsey, J., Goldsmith, R. S., Kelly, P. J., Hoffman, D. L., and Arnaud, C. D., J. Clin. Invest. **51**, 1659 (1972).

15. Oliver, M. F., and Boyd, G. S., Circulation **13**, 82 (1956).

16. Marmorston, J., Magidson, O., Lewis, J. J., Mehl, J., Moore, F. J., and Bernstein, J., New Eng. J. Med. **258**, 583 (1958).

17. Ho, K-J., and Taylor, C. B., Proc. Soc. Exp. Biol. Med. **143**, 810 (1973).

18. Cox, G. E., Truehart, R. T., Kaplan, J., and Taylor, C. B., Arch. Pathol. **76**, 166 (1963).

19. Sinex, F. M., *in* "Treatise on Collagen" (R. Gould and G. N. Ramachandran, eds.), Vol. 2, p. 409. Academic Press, New York (1968).

20. Kramsch, D. M., Franzblau, C., and Hollander, W., J. Clin. Invest. **50**, 1666 (1971).

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