Mechanisms of Cardioprotection by Estrogens (44201)

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Abstract. **Strong scientific evidence suggests that estrogen therapy offers considerable protection from coronary artery disease. While earlier studies focused mainly on favorable changes in plasma lipid profiles as the mechanism of estrogen action, recent studies have documented other mechanisms of action including direct effects on vascular wall. The other mechanisms include estrogen's role as an antioxidant, its ability to protect from DNA damage, inhibition of cell proliferation, and altering vascular response to vasoactive agents. Considerable emphasis must be placed on research on individual components of the commonly used conjugated estrogens and estrogen structure/function relationship to target specific tissues and minimize undesirable effects while maintaining estrogen's cardioprotective potency.**

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Estrogen replacement therapy is attracting wide interest, both in terms of its benefits and risks. It is estimated that approximately 15%–20% of postmenopausal women are on estrogen replacement therapy (1). Estrogens relieve menopausal symptoms (2) and play a major role in preventing two of the most important health concerns of postmenopausal women, i.e., osteoporosis and coronary artery disease (3, 4). The beneficial effects of estrogen on osteoporosis have been covered by excellent reviews recently (5) and will not be discussed here. It should also be noted that although a number of estrogen preparations are used clinically, most contain either estradiol or conjugated equine estrogens (CEE). Considerable attention will be focused on CEE for four reasons: i) it is the most widely used estrogen preparation in most clinical trials; ii) it represents a mixture of estrogens (as discussed below); iii) the role and biological effect of each component (and their metabolites) is still not clear; and iv) seldom is there an attempt to explain the benefits and risks of estrogen replacement therapy in terms of chemical composition of CEE.

To understand the mechanism of estrogen action, it is essential to understand structural differences among estrogens used in therapy and appreciate the differences in their biological activity and a few aspects of their metabolism. CEE represents a mixture of conjugated estrogens isolated from the urine of pregnant mares and contains at least 9 major components (6, 7). Among these, estrone sulphate is the predominant estrogen (about 45%) with a significant amount of ring B unsaturated equine estrogens (predominantly 17α-dihydroequilin, equilin, equilenin amounting to nearly 51%), and minor amounts of both 17β and 17α estradiol (<4%). Although this estrogen preparation has been in use for the past 50 years, the significance of individual equine estrogen in demonstrated clinical effects is not known. The USP specifications for composition of conjugated estrogen requires that, apart from two of its major components (estrone sulphate and equilin sulphate), the preparation contain 17α-dihydroequilin, equilenin, 17β-dihydroequilin, equilenin, 17β-estradiol, and 8 dehydroesterone (8). Elegant studies by Bhavnani and his collaborators (6) have shown that the formation of ring B unsaturated in mares may involve pathways without squalene and cholesterol as intermediates. The detailed metabolism and pharmacokinetics of major components of conjugated estrogen preparation by Bhavnani, *et al.* (9–11) showed that equilin sulphate is metabolized to 17β-dihydroequiln and 17β-dihydroequilin sulphate. They further noted (11) that a large fraction of radioactivity was associated with metabolites that were more polar polyhydroxy ring B unsaturated estrogens. The identity and biological activities of these polar metabolites are only beginning to be investigated. 17β-

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dihydroequilin sulphate is a far greater uterotrophic agent than equilin (12) and has one of the highest binding affinities for estrogen receptors (13) in the human and rat uterus, but in the rabbit uterus all the equine estrogens were less effective than 17β-estradiol, but better than 17α-estradiol (12). Howard and Keaty (14) examined the clinical potency of CEE components using suppression of urinary gonadotrophic levels in oopherectomized women. They noted that equilin sulphate was the most potent in this respect. However, these studies noted that in terms of uterotrophic potency, 17β-estradiol sulphate and equilin sulphate were the most potent.

One important point is that there is an equilibrium in the body between the inactive sulphoconjugates and the active free phenols (14), and there is evidence that only a proportion of the sulphates are cleaved before their appearance in the system's circulation. Anderson, *et al.* (15) reported that the major, circulating, conjugated oestrogen after oral administration is the parent sulphoconjugate. This suggests that bioavailability of estrogen is influenced by the role of sulphatases (16). Recent studies also suggest that estrogens exist as aryl esters, a factor that could affect estrogen distribution.

Cardioprotective Effect of Estrogen

Cardiovascular disease is the leading cause of death in postmenopausal women, accounting for 53% of all deaths in women over the age of 50 (17). Coronary heart disease (CHD) affects 2 million women and accounts for 500,000 deaths annually in women in the United States (18, 19). The cardioprotective effect of postmenopausal estrogen administration has been demonstrated in a number of studies, with recent publication of a 10-year follow-up of 48,470 postmenopausal women that revealed a relative risk for heart disease of 0.56 (20). This study now joins 21 case control and cohort studies designed to examine the effect of postmenopausal estrogen treatment on fatal and nonfatal cardiovascular disease. Despite the absence of a randomized, placebo-controlled trial on estrogen replacement, the 22 available studies in combination (and by meta-analyses) lead to the conclusion that postmenopausal women have about a 50% reduction in cardiovascular disease with estrogen administration (21, 22).

Although epidemiologic data correlate cholesterol levels with incidence of CHD, the effect of endogenous and exogenous sex steroids on lipids, and subsequent cardiovascular risk is more complex and not well understood. Until recently, much of the cardiovascular effects of sex steroids were felt to be mediated through changes in lipoproteins (23). The cardioprotective effect of estrogen was thought to be substantially mediated by increases in high density lipoproteins (HDL) and the lowering of LDL cholesterol (24). Most studies have clearly established that estrogen therapy decreases total plasma cholesterol (24, 25) and increases (24, 26) or maintains (25) plasma triglyceride levels. However, in the presence of a progestin, estrogen causes a significant decrease in both total cholesterol and triglyceride levels in the plasma (26). The majority of the studies have consistently documented that a significant increase in HDL cholesterol levels in the plasma may be slightly blunted by the addition of a progestin (24–27). HDL_2 cholesterol levels were noted to be significantly elevated either in the estrogen alone or with a progestin (25) with no consistent agreement or changes in HDL_3 levels (28). Estrogen alone or in combination with a progestin significantly reduced LDL cholesterol levels (24–27). One of the interesting aspects of estrogen therapy on plasma lipoprotein is the consistent decrease in Lp(a) levels observed even in the presence of a progestin (26–29). A summary of the effect of estrogens on plasma lipids is shown in Table I.

The significance of overall beneficial effects contributed by blood lipid changes was reevaluated by two studies that analyzed the source of the cardioprotective effect of estrogens and found that only 25%–50% of the estrogen effect could be accounted for by HDL changes (30, 31). Therefore, as much as 50%–75% of the effect of postmenopausal estrogen therapy is not linked to circulating lipid levels. Moreover, measurement of cholesterol levels in lipoprotein fractions does not reflect interaction of lipoproteins with the vessel wall. Animal data also support the lack of correlation between sex steroid induced changes in circulating lipoproteins and cardiovascular pathology. For example, monkeys were fed an atherogenic diet and administered estrogens and progestins in concentrations that resulted in ''unfavorable'' lipoprotein changes (32), less atherogenic change than controls despite a significant reduction in HDL cholesterol. This study indicates a lack of correlation between circulating HDL levels and atherogenesis and provides indirect evidence for a local effect of sex steroids at the vascular wall. The lack of correlation between HDL levels and cardiovascular effects of estrogens should lead to a reevaluation of other mechanisms by which sex steroids may affect cardiovascular disease risk.

It has recently been shown that high doses of estrone, estradiol, and estriol inhibit oxidation of LDL (mediated by copper ions, monocytes, and endothelial cells), whereas testosterone had no effect (33). Thus, estrogens had antioxidant properties in this model system. Other researchers have demonstrated *in vivo* antioxidant effects of estrogen in human subjects using estradiol patch (34), while studies using conjugated equine estrogens yielded conflicting results (35). This could depend upon the metabolic pathways of estrogen metabolism as well. Structural differences in the estrogen molecule have been shown to influence its antioxidant potential (36). In fact, studies from our laboratory have shown that equine estrogens (equilin) are better oxidants (than estradiol) at inhibiting the peroxidation of both fatty acids and cholesterol (37). These observations may be very important *in vivo* since peroxidation of lipoproteins is now believed to play a key role in atherogenesis due to excess uptake of oxidized LDL by macrophages (38). The oxidation of LDL involves not only the formation of malonaldehyde and other

Table I. Postmenopausal Estrogen Therapy and Plasma Lipids

	Unopposed	With progestin	References
Total cholesterol	↓	↓	24–26
Triglycerides	↑→	↑↓	24–26
HDL Cholesterol	↑	↑→↓	24,25,26
HDL_2	↑	↑↓	25,29
HDL_3	↓	↑↓	25,30
LDL Cholesterol	↓	↓	24–27
Lp(a)	↓→	↓	26,28

hydroxy derivatives of fatty acids but also the formation of toxic oxysterols from the cholesterol moiety (39). The mechanism of antioxidant effect of estrogen on LDL oxidation is not clear. Estrogen could play an inhibitory role either in production and/or scavenging of reactive oxygen species, or in protecting the level of endogenous antioxidant such as vitamin E within the LDL molecule. It has recently been shown (40) that estradiol has no effect on superoxide production by human mononuclear cells. However, no studies were done on the effect of estrogen on pathways leading to the formation of specific highly reactive oxygen species such as singlet oxygen, hydrogen peroxide and hydroxyl radicals. Estrogens might be involved in scavenging free radicals or may interfere with oxidative chain reactions, without having a significant effect on superoxide formation. Preliminary studies done in our laboratory suggest that at high concentrations, estradiol-17β might inhibit superoxide formation with no effect on hydroxyl radicals.

Recently attention has been focused on the effect of estrogen metabolites on lipid peroxidation. In the liver and some extrahepatic tissues (breast, uterus, kidney, etc.) estradiol undergoes reversible oxidation to estrone, which can then be oxidized irreversibly by one of two alternative pathways, the 2-hydroxylation pathway, which leads to the non-estrogenic metabolites 2-hydroxyestrone and 2-methoxyestrone, and the 16α-hydroxylation pathway, which leads to the highly estrogenic metabolites 16α-hydroxyestrone and estriol (16α-hydroxyestradiol). Thus, the overall estrogenic and metabolic impact of administered estradiol depends on the relative magnitudes of the 2-hydroxylation and 16α-hydroxylation pathways (41). The C2-hydroxylation of estradiol-17β is primarily carried out by the dioxin-inducible CYP1A2 group of cytochrome P450 (42). There is precedence for compounds that stimulate C2-hydroxylation (Dioxin or polycyclic aromatic hydrocarbon) to cause decreased estrogenic activity (43–47). *In vivo* studies have shown that women who smoke cigarettes generally have lower urinary estrogen levels than nonsmoking women (45). Not as much is known about the specificity of the P450-mediated C16α-hydroxylation except that the activity is inducible by phenobarbitol but not polycyclic hydrocarbon (46). These characteristics suggest that the P450 enzyme mediating the C16α-hydroxylation might be a member of the CYP2 or CYP3 families (42).

Our laboratory examined (48) the effect of estrogen metabolites 4-hydroxy estrone and 17α-dihydroequilin (metabolites of estradiol-17β and equilin, respectively) and compared their antioxidant effect on plasma and lipoprotein lipid peroxidation with parent estrogens. Lipid peroxidation was evaluated by products of both fatty acid (thiobarbutric acid reactive substances (TBARS)) and cholesterol (oxysterols) oxidation from lipoproteins or whole plasma. Whereas all estrogens significantly reduced lipid peroxidation, 4-hydroxyesterone was far more potent than either 16α-hydroxyestrone, equilin or 17α-dihydroequilin in inhibiting TBARS formation in lipoproteins induced by Cu^{++} (Table II). Similar effects were also noted on TBARS formation in THP-I macrophages in culture. However, 17α-dihydroequilin (along with equilin) strongly inhibited oxysterol formation whereas 4-hydroxyesterone was ineffective (Table III). These studies suggest that different estrogens might act preferentially on distinct lipid substrates in exhibiting antioxidant effects. Therefore, *in vivo* effect of estrogens as antioxidants might depend upon the predominance of pathways (16α-hydroxylation versus 2α- and 4α-hydroxylation) of metabolism.

The antioxidant ability of estrogens was compared to Vitamin E and β-carotene (49) in terms of lipid peroxidation. In our study (49) the antioxidant effect of E_2 was compared with fat-soluble antioxidants [alpha-tocopherol and beta-carotene] in terms of both fatty acid (thiobarbutric acid) and cholesterol oxidation. Addition of α-tocopherol (54 μM) inhibited oxidation (as measured by tiobarbutric acid assay) of LDL by 92.6% and HDL by 76.5% but inhibited HDL oxidation by only 55.4%. BC had no antioxidant effect. By the diene conjugation method (Figure 1), lag times for α-tocopherol and β-carotene were reduced, but E_2 exhibited a linear curve (no change in lag time). In terms of

Table II. Effect of Specific Estrogen Metabolites on VLDL and LDL Peroxidation[a]

Group	TBARS Formation (nMoles)	
	VLDL	LDL
Control	31.75 ± 1.39	49.77 ± 0.95
Equilin	14.21 ± 0.95	15.98 ± 0.23[b]
17 α-dihydroequilin	11.31 ± 0.60[b]	14.50 ± 0.61[b]
17 β-dihydroequilin	13.23 ± 1.21[b]	17.56 ± 0.53[b]
17 β-dihydroequilenin	14.68 ± 2.88[b]	18.12 ± 0.77[b]
4-hydroxy estrone	5.04 ± 0.20[b,c]	5.44 ± 0.98[b,c]
16-hydroxy estrone	25.22 ± 0.95[b]	26.77 ± 0.39[b]

Note. Mean ± SD.
[a] VLDL (130 μg protein) and LDL (125 μg protein) were incubated with cupric sulfate 50 μl (50 mM) and estrogens (7.5×10^{-5}M) for a period of 1 hr at 37°C. The formation of TBARS was measured by the method of Satoh (15). Each data point is a mean of 3–4 determinations.
[b] Significantly different from control ($p < 0.01$).
[c] Significantly different from other estrogens ($p < 0.01$).
Taken from Ref. 48 with permission.

Table III. Effect of Estrogen Metabolites on Formation of Plasma Oxysterols[a]

Group	Oxysterols (μg/mL)
Control	16.75 ± 2.12
Equilin	1.36 ± 0.44*†
17α-Dihydroequilin	1.02 ± 0.92*†
4-Hydroxyestrone	13.51 ± 3.70

Note. Mean ± SD.
* Significantly different from control ($p < 0.1$).
† Significantly different from 4-hydroxyestrone ($p < 0.1$).
[a] Taken from Ref. 48 with permission.

cholesterol oxidation, E_2 was far more effective than either of the antioxidants in inhibiting oxysterol formation. This study shows that E_2 is as effective an antioxidant as α-tocopherol in terms of fatty acid peroxidation but far more effective than α-tocopherol or β-carotene in terms of cholesterol peroxidation.

We further examined (50) the ability of estrogens to protect against DNA damage induced by either hydrogen peroxide or arachidonic acid alone or in combination with Cu^{2+}. DNA-strand breaks were determined by conversion of double-stranded, supercoiled ΘX-174 RFI DNA to double-stranded, open, circular DNA and linear, single-stranded DNA (Figure 2). Estradiol-17β significantly decreased the formation of single- and double-strand breaks in DNA induced by H_2O_2 alone or with Cu^{2+}. Equilin (an equine estrogen) was more effective than estradiol-17β at the doses tested. Arachidonic acid in the presence of Cu^{2+} caused the formation of high levels of linear DNA that was protected by estrogen with equilen being more effective. These studies suggest that estrogens through this protective effect on DNA damage might contribute to cardioprotection.

Another area of considerable interest in evaluating estrogen effect is whether lipoprotein/estrogen interaction plays a significant role in the distribution of E_2 within plasma. Studies of the cardioprotective effect of estrogen have revealed that estrogen significantly decreases LDL levels and increases HDL levels in plasma (31). Further studies of HDL subfractions have shown that estrogen causes major increases in HDL_2 or HDL_{2a} fraction and apoAI levels (51). While there are considerable advances made in the effects of estrogen on lipoproteins (52), knowledge of its distribution and transport in plasma compartment and factors influencing its cellular uptake are limited. Estrogen is believed to exist in plasma predominantly bound to steroid hormone binding globulin (SHBG) or albumin and as free estrogen (37, 53). The free estrogen is believed to enter the cells passively bound to an intracellular estrogen receptor (54). Recently elegant studies by Leszczynski and Schafer (55, 56) showed that a variety of steroid hormones can bind to plasma lipoproteins using equilibrium dialysis techniques. Furthermore, they noted that steroid hormones were converted into less polar compounds (presumably fatty acid esters), especially in high density lipoproteins (HDL). Indeed, fatty acid esters of estrogen have been identified in human blood (57). These studies suggested that lipoprotein bound steroid hormones would provide an alternative means of transporting estrogen into the cells. However, quantitative aspects of distribution of estrogen within various lipoproteins and non-lipoprotein compartments and their entry into the cells are not known. Our recent studies (58) showed that addition of labeled estradiol to plasma followed by ultracentrifugation suggested that E_2 was predominantly bound to HDL (50%) and lipoprotein-free fraction (presumably albumin and SHBG) with very little in VLDL and LDL fraction (<2%). Increasing hydrophobicity of estradiol (conversion to acetates) increased its binding to LDL.

Recently important new developments are occurring on the role of estrogens in modulating blood flow and re-

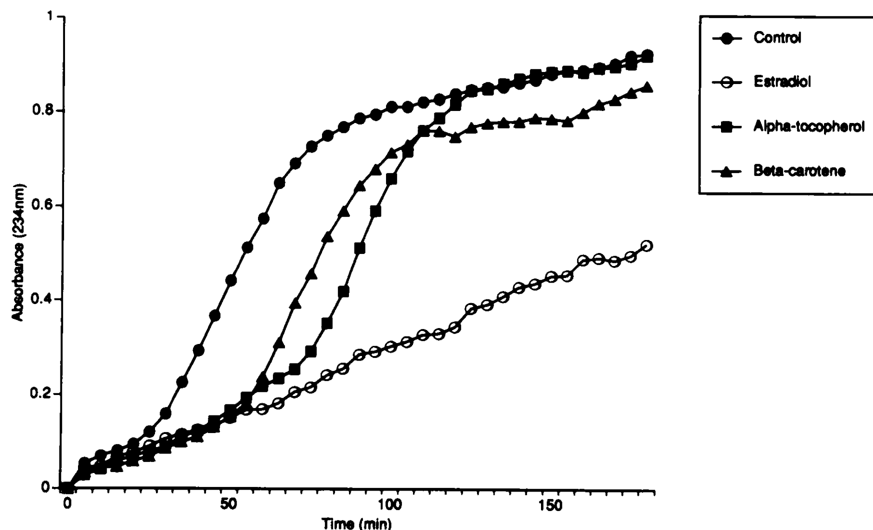

Figure 1. Time course of the formation of conjugated dienes during AAPH-induced oxidation. LDL (60 μg) in PBS were oxidized with 11 mM/L AAPH. All additions of estrogens and vitamins were 28 μM/L. Diene increase was measured by continuously monitoring the increase of the 234 nM absorbance. The initial 234 nM absorbance of the LDL solution was set to 0.1 and then the increase was recorded over 3 hr. No blank cuvete was used in the experiment. Taken from Ref. 49 with permission.

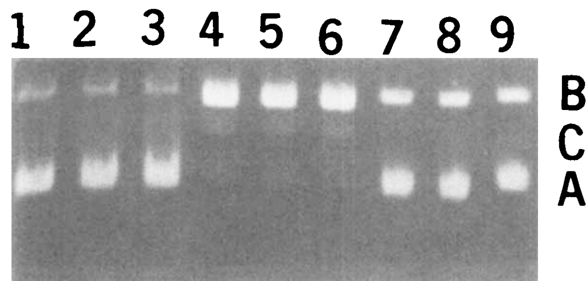

Figure 2. Agarose gel (stained with ethidium bromide) of ΘX-174 RFI plasmid DNA incubated with H_2O_2 in the presence of or absence of estrogen at 37°C for 30 min. Lanes 1–3: control DNA; lanes 4–6: DNA + H_2O_2 (15 mM); lanes 7–9: DNA + H_2O_2 (15 μM) + E_2 (18 μM). A, B, and C represent supercoiled, open circular and linear forms of DNA, respectively. Taken from Ref. 50 with permission.

sponses of the coronary artery to vasoconstriction agents. Previous studies have already documented that estradiol-17β can induce relaxation of a contracted coronary artery, reverse acetylcholine-induced vasoconstriction (59, 60), and improve exercise-induced myocardial ischemia in women with coronary artery disease (61, 62). In an important study Collins, et al. (63) showed that estradiol-17β modulates acetylcholine-induced coronary artery responses only in women, but not in men. This phenomenon might operate through enhancement of endothelium dependent nitric oxide synthesis (64, 65). Many details of vascular response in terms of growth and vasomotor effects to estrogen can be found in an excellent review by Farhat, et al. (66).

No discussion on estrogen therapy is complete without examining the serious concern that exogenous estrogen might increase the incidence of breast cancer (67). Whereas estrogens promote breast cancer in experimental animal models, tamoxifen (a competitive inhibitor of estrogen binding) significantly reduces the carcinogensis (68). These data, together with studies relating plasma estrogen levels to risk of breast cancer, suggest that estrogen levels may influence the risk of disease among postmenopausal women (69).

However, the results in humans are not consistent. In one study the risk of breast cancer with menopausal estrogen replacement increased slowly with duration of therapy, but only after the first five years of therapy (70). Two other studies showed no significant increase in breast cancer risk with estrogen replacement (71, 72). Other studies showed that short-duration (less than 15 years) use of estrogen did not increase breast cancer risk, whereas long-term use might pose a 25% increase in risk (73). Interesting studies by Colditz, et al. (74) found that the small increase in risk that they noted in women who took estrogen was related to the consumption of alcohol. The results of the PEPI Study published recently (75) did note an increase in endometrial hyperplasia in women taking estrogens.

A relationship between the pathways of estrogen metabolism and its potential role in pathology was the recent study of Osborne, et al. (76) who reported an association between elevated estradiol-17β 16α-hydroxylase activity in human breast tissue and increased risk of breast cancer. The C16α-hydroxylation leads to formation of the electrophilic metabolite 16α-hydroxyestrone, which has been shown to cause DNA damage (77). On the other hand the formation of catechol estrogens (2-OH E1 and 4-OHE$_1$) and their effects have also received considerable attention.

These compounds are non-uterotrophic, bind to estrogen receptors (78), and are able to form DNA adducts (79). These compounds may reduce proliferation activity of cells and function as antiestrogen (80). Under the influence of metals these compounds cause severe DNA damage (81). Therefore, an understanding of differences in metabolism of E_2 is critical to evaluate estrogen induced carcinogenesis.

The reader is referred to excellent reviews on the estrogen therapy in relation to cancer risk by Zumoff (41) and Speroff (82).

Future Research

One area that needs immediate attention is to determine which components of the complex CEE mixture are responsible for the demonstrated clinical benefits (and risks) in humans. If one can identify a single component responsible for most of the effects, is it necessary to consume a complex mixture for therapy? In view of the fact that the metabolism and biological effects of the major components in CEE are not known, it is time to sort the mixture out and identify a single compound to be used in therapy. Secondly, there are challenges to developing an ideal estrogen (i.e., one that produces beneficial effects in the cardiovascular system and bone with little effect on breast and reproductive tissues). It is possible that selectivity and specificity can be achieved via the estrogen receptor interaction. A recent review by Kaufmann and Bryant (83) alludes to this topic with important suggestions on potential antiestrogenic compounds that are in development. Finally, I feel strongly that further knowledge on the distribution of estrogens in plasma may help identify factors to deliver estrogen selectively to target tissues.

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1. Cauley JA, Cummings SR, Blade DM, et al. Prevalence and determinants of estrogen replacement therapy in elderly women. Am J Obstet Gynecol **163:**1438–1444, 1990.
2. Hammond CB, Maxson WS. Current status of estrogen therapy for menopause. Fertil Steril **37:**5–25, 1982.
3. Hutchinson TA, Polansky SM, Feinstein AD. Post menopausal estrogens protect against fracture of hip and distal radius. Lancet **2:**705, 1979.
4. Ross RK, Paganini-Hill A, Gerkins V, et al. A case controlled study of menopausal estrogen therapy and breast cancer. Lancet **1:**858–860, 1980.
5. Lindsay R. The menopause and osteoporosis. Obstet Gynecol **87**(Supplement):16S–19S, 1996.

6. Bhavnani BR. The saga of ring B unsaturated equine estrogens. Endocr Rev **9**:396–416, 1988.
7. Pillai GK, McErlane KM. Quantitative determination of conjugated estrogens in formulation by capillary GLC. J Pharm Sci **70**:1072–1075, 1981.
8. Adams WP, Hasegawa J, Johnson RN, and Havins RD. Conjugated estrogens bioinequivalence: Comparison of four products in postmenopausal women. J Pharm Sci **68**:986–991, 1977.
9. Bhavnani BR, Wollever CA, Benoit H, Wong T. Pharmacokinetics of equilin and equilin sulphate in normal postmenopausal women and men. J Clin Endocrinol Metab **56**:1048–1056, 1983.
10. Bhavnani BR, Cerutti A. Pharmacokinetics of dihydroequilin sulphate and 17β-dihydroequilin in normal postmenopausal women. J Clin Endocrinol Metab **78**:197–204, 1994.
11. Bhavnani BR, Cerutti A, Wallace D. Metabolism of [H^3] 17β-dihydroequilin and [H^3] 17β-dihydroequilin sulphate in normal postmenopausal women. Steroids **59**:389–394, 1994.
12. Stern MD. Pharmacology of conjugated estrogen. Maturitas **4**:333–339, 1982.
13. Bhavnani BR, Woolever CA. Interaction of ring B unsaturated estrogens with estrogen receptors of human endometrium and rat uterus. Steroids **56**:201–209, 1991.
14. Howard KP, Keaty EC. Evaluation of equilin 3-monosulphate and other estrogens. Ann Intern Med **128**:224–234, 1978.
15. Anderson AB, Sklovsky E, Sayers L, Steel PA, Turnbull AC. Comparison of estrogen concentration in postmenopausal women taking oestrone sulphate and estradiol. Biochem J **1**:140–142, 1978.
16. Stevens RL, Flutarty AL, Killgrove AR, Kihara H. Aryl sulphates of human tissue: Studies on a form of aryl sulphate B found predominantly in the brain. Biochim Biophys Acta **481**:549–550, 1977.
17. Bush TL. The epidemiology of cardiovascular disease in postmenopausal women. Ann NY Acad Sci **592**:263–271, 1990.
18. Lobo RA. Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. J Clin Endocrinol Metab **73**:925, 1991.
19. Lobo RA. Cardiovascular implications of estrogen replacement therapy. Obstet Gynecol **75**:18S, 1990.
20. Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the Nurses Health Study. N Engl J Med **325**:756–762, 1991.
21. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. JAMA **256**:1861, 1991.
22. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: A quantitative assessment of the epidemiologic evidence. Prev Med **20**:47, 1991.
23. Seed M. Sex hormones, lipoproteins, and cardiovascular risk. Atherosclerosis **90**:1–7, 1991.
24. The Writing Group for the PEPI Trial. Effect of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA **273**:199–208, 1995.
25. Paganini-Hill A, Dworsky R, Krauss RM. Hormone replacement therapy, hormone levels, and lipoprotein cholesterol concentration in elderly women. Obstet Gynecol **174**:897–902, 1996.
26. Farish E, Spowart K, Barnes JF, Fletcher CD, Hart PM, Dagen MM. Effects of postmenopausal hormone replacement therapy on lipoproteins including lipoprotein(a) and LDL subfractions. Atherosclerosis **126**:77–84, 1996.
27. Lahdenpara S, Puolakka J, Pyorala T, Luotola H, Taskinen MR. Effects of postmenopausal estrogen/progestin replacement therapy on LDL particles: Comparison of transdermal and oral treatment regimens. Atherosclerosis **122**:153–162, 1996.
28. Miller VT, Muesing RA, LaRosa JC, Phillips EA, Stillman RJ. Effects of conjugated estrogen with and without three different progestogens on lipoproteins, high density lipoprotein subfractions, and apolipoprotein AI. Obstet Gynecol **77**:235–240, 1991.
29. Soma MR, Oswago I, Paoletti R, Fumagalli R, Morriset JD, Moschia M, Crosignani P. The lowering of lipoprotein(a) induced by estrogen plus progesterone replacement therapy in postmenopausal women. Arch Intern Med **153**:1402–1408, 1993.
30. Ottosson U, Johansson B, Von Schoultz B. Subfractions of high-density lipoprotein cholesterol during estrogen replacement therapy: A comparison between progestogens and natural progesterone. Am J Obstet Gynecol **151**:746–750, 1985.
31. Bush TL, Barrett-Connor E, Cowan LD, Criquie MH, Wallace RB, Suchinddran CM, Tyroler HA, Rifkind BM. Cardiovascular mortality and non-contraceptive estrogen use in women: Results from the Lipid Research Clinics Program Follow-Up Study. Circulation **75**:1002–1009, 1987.
32. Adams MR, Clarkson TB, Shively CA, Parks JA, Kaplan JR. Oral contraceptives, lipoproteins, and atherosclerosis. Am J Obstet Gynecol **163**:1388–1393, 1990.
33. Maziere C, Auclair M, Ronveaux MC, Salmon S, Santus R, Maziere JC. Estrogen inhibits copper and cell-mediated modification of low density lipoprotein. Atherosclerosis **89**:175–182, 1991.
34. Sack MN, Rader DJ, Cannon RO. Oestrogen and inhibition of oxidation of low density lipoproteins in postmenopausal women. Lancet **343**:269–270, 1994.
35. Guetta V, Lush RM, Figg WD, Waclawiw MA, Cannon RO. Effect of antiestrogen tamoxifen on low density lipoprotein concentration and oxidation in postmenopausal women. J Amer Cell Cardiol **76**:1072–1073, 1995.
36. Mukai K, Daifuku K, Yokoyama S, Nakano M. Stopped flow investigation of antioxidant effects of estrogen. Biochim Biophys Acta **1035**:348–352, 1990.
37. Subbiah MTR, Kessel B, Agrawal M, Rajan R, Abplanalp W, Rymaszewski Z. Antioxidant potential of specific estrogens on lipid peroxidation. J Clin Endocrinol Metab **77**:1095–1097, 1993.
38. Heinecke JW. Free radical modification of low density lipoproteins: Mechanism and biological consequences. Free Radic Biol Med **3**:65–73, 1991.
39. Arshad MAQ, Bhadra S, Cohen RM, Subbiah MTR. Plasma lipoprotein peroxidation potential: A method to determine individual susceptibility to peroxidation. Clin Chem **37**:1756–1758, 1991.
40. Rifici VA, Khachadurian AK. The inhibition of low-density lipoprotein oxidation by 17-β estradiol. Metabolism **41**:1110–1114, 1992.
41. Zumoff B. Biological and endocrinological insights into the possible breast cancer risk from menopausal estrogen replacement therapy. Steroids **58**:196, 1993.
42. Nelson DR, Kawataki F, Waxman DH, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Garsulus IC, Gotoh O, Okuda K, Nebert DW. The P-450 superfamily: Update on new sequence, gene mapping, accession numbers, early trivial names, and nomenclature. DNA Cell Biol **12**:1, 1993.
43. Ashtroff B, Rowlands C, Dickerson R, Safe S. 2378 tetrachlorodibenzo-p-dioxin inhibition of estradiol-17β induced increases in rat uterine epidermal growth factor receptor binding activity and gene expression. Mol Cell Endocrinol **72**:247, 1990.
44. Spink D-C, Lincoln DW, Dickermann HW, Gierthy GF. 2378 tetrachlorodibenzo-p-dioxin causes an extensive alteration of 17β-estradiol metabolism in MCF-7 breast tumor cells. Proc Natl Acad Sci USA **87**:6917, 1990.
45. MacMahan B, Trichopoulos D, Cole P. Cigarette smoking and urinary estrogens. N Engl J Med **307**:1062, 1982.
46. Schneider J, Sasia S, Kappas A. Metabolism of estradiol in liver cell culture: Differential references of C-2 and C-16 oxidations to drugs and other chemicals that induce selective species of cytochrome P-450. J Clin Invest **72**:1420, 1983.
47. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the autoestrogen effect of cigarette smoking. N Engl J Med **315**:1305, 1986.
48. Tang M, Abplanalp W, Ayres S, Subbiah MTR. Superior and distinct antioxidant properties of selected estrogen metabolites on lipid peroxidation. Metabolism **45**:411–414, 1996.

49. Ayres S, Tang M, Subbiah MTR. Estradiol-17β as an antioxidant: Some distinct features when compared to fat soluble vitamins. J Lab Clin Med **128**:367–375, 1996.
50. Tang M, Subbiah MTR. Estrogens protect against hydrogen peroxide and arachidonic acid induced DNA damage. Biochim Biophys Acta **1299**:155–159, 1996.
51. Applebaum-Bowden D, McLean P, Steinmetz A, Fontana D, Matthys C, Warnick GR, Albers JJ, Hazzard WR. Lipoprotein, apoprotein, and lipolytic enzyme changes following estrogen administration. J Lipid Res **30**:1895, 1989.
52. Leuven JG. Sex steroids and lipoprotein metabolism. Pharmacol Ther **64**:99–126, 1994.
53. Anderson D. Sex hormone binding globulin. Clin Endocrinol **3**:69–96, 1974.
54. Rao GS. Mode of entry of steroid and thyroid hormones into cells. Mol Cell Endocrinol **21**:97–108, 1981.
55. Leszczynski DE, Schafer RM. Characterization of steroid hormone association with human plasma lipoproteins. Steroids **54**:37–53, 1989.
56. Leszczynski DE, Schafer RM. Metabolic conversion of six steroid hormones by human plasma high density lipoprotein. Biochim Biophys Acta **1083**:18–28, 1991.
57. Janucko L, Hochberg RB. Estradiol fatty acid esters occur naturally in human blood. Science **222**:1334–1336, 1983.
58. Tang M, Abplanalp W, Subbiah MTR. Association of estrogens with human plasma lipoproteins: Studies with estradiol-17β and its hydrophobic derivative. J Lab Clin Med **129**:447–452, 1997.
59. Jiang C, Sarrel PM, Lindsay D, Poole-Wilson PA, Collins P. Acute effects of 17β-estradiol on rabbit coronary artery contractile response to endothelin-a. Am J Physiol 263H-271-H275, 1992.
60. Williams JK, Adams MR, Herrington DM, Clarkson TB. Short-term administration of estrogens and vascular response of atherosclerotic coronary arteries. J Am Coll Cardiol **20**:452–457, 1992.
61. Rees SE, Gluth ST, Blumenthal RS, Resar JR, Zacar HA, Gerstenblith G, Brinker JA. Ethynyl estradiol acutely attenuates abnormal coronary vasomotor response to acetylcholine in postmenopausal women. Circulation **89**:52–60, 1994.
62. Rosano GMC, Sarrel RM, Poole-Wilson PA, Collins D. Beneficial effect of oestrogen on exercise induced myocardial ischemia in women with coronary artery disease. Lancet **362**:133–136, 1993.
63. Collins P, Giuseppe MC, Rosano MC, Sarrel PM, Ulrich L, Adamopoulous S, Beale CM, McNeill JG, Poole-Wilson PA. 17β-Estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. Circulation **92**:24–30, 1995.
64. Van Buren G, Yang D, Clark KE. Estrogen induced uterine vasodilitation is antagonized by L-nitro organism methylester, an inhibitor of nitric-oxide synthesis. Am J Obstet Gynecol **16**:828–833, 1992.
65. Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Mancada S. Induction of calcium dependent nitric oxide synthesis by sex hormones. Proc Natl Acad Sci USA **91**:5212–5216, 1994.
66. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effect of estrogen. FASEB J **10**:615–624, 1996.
67. Hoover K. Menopausal estrogens and breast cancer. N Engl J Med **295**:401–405, 1976.
68. Li JJ and Li SA. Estrogen carcinogenesis in hamster tissues: A critical review. Endocrine Rev **11**:524–531, 1990.
69. Key TJA, Pike MC. The role of oestradiol and progestins in epidemiology and prevalence of breast cancer. Eur J Cancer Clin Oncol **24**:29–43, 1989.
70. Steinberg KK, Thacker SB, Smith SJ, Stroup DF, Zak MM, Flanders WD, Berkelman RL. A meta-analysis of the effect of estrogen replacement therapy on the risk of breast cancer. JAMA **265**:1985–1990, 1991.
71. Dupont WD, Page DL. Therapy and breast cancer. Arch Intern Med **151**:67–72, 1991.
72. Armstrong BK. Oestrogen therapy after the menopause—boon or bane? Med J Aust **148**:213–219, 1988.
73. Grady D, Ernster V. Does post menopausal hormone therapy cause breast cancer? Am J Epidemiol **134**:1390–1400, 1991.
74. Colditz GA, Stampfer MJ, Willett WC, Hennekens CH, Rosner B, Speizer F. Prospective study of estrogen replacement therapy and the risk of breast cancer in post menopausal women. JAMA **264**:2648–2653, 1990.
75. The Writing Group for the PEPI Trial. Effect of hormone replacement therapy on endometrial histology in postmenopausal women. JAMA **275**:370–375, 1996.
76. Osborne MD, Bradlow HC, Wong GYC, Telang NT. Upregulation of estradiol P-16α hydroxylation in human breast tissue: A potential biomarker of breast cancer risk. J Natl Cancer Inst **85**:1917, 1993.
77. Telang NJ, Sato A, Wang GY. Induction of estrogen metabolite 16α-hydroxy estrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. J Natl Cancer Inst **84**:636, 1992.
78. Vanderwalle P, Peyrat JP, Bonneterre J, Lefebvre J. Catechol binding sites in breast cancer. J Steroid Biochem Mol Biol **23**:603, 1985.
79. Liehr JG. Genotoxic effects of estrogen. Mutat Res **238**:269, 1990.
80. Vanderwalle P, Lefebvre J. Opposite effects of estrogen and catechol estrogens on hormone sensitive breast cancer cell line growth and differentiation. Mol Cell Endocrinol **61**:239, 1989.
81. Li Y, Trush MA, Yager JD. DNA damage caused by reactive oxygen species originating from a copper dependent oxidation of 2-hydroxy catechol of estradiol. Carcinogenesis **15**:1421–1427, 1994.
82. Speroff L. Postmenopausal hormone therapy and breast cancer. Obstet Gynecol **87**:448–545, 1996.
83. Kaufmann RF, Bryant HV. Selective estrogen receptor modulators. Drugs **8**:531–539, 1995.