Does Postmenopausal Estrogen Administration Increase the Risk of Breast Cancer? Contributions of Animal, Biochemical, and Clinical Investigative Studies to a Resolution of the Controversy (44202)

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Abstract. **Despite nearly six decades of epidemiological studies, meta-analyses, and reviews, there is still considerable controversy in the literature about the question, does postmenopausal estrogen administration increase the risk of breast cancer? In an effort to resolve the controversy, a number of animal, biochemical, and clinical investigative studies in this field have been reviewed. The following summary formulation is proposed:**

1. **Administration of estrogen is inherently capable of promoting the growth of breast cancer, and therefore of increasing the incidence of clinical breast cancer.**
2. **Human response to estrogen is like that of the low-cancer-incidence strains of mice studied by Lacassagne, in that large doses and prolonged administration are required to induce clinical breast cancer.**
3. **The blood levels of estradiol produced by the usual doses of postmenopausal estrogen are relatively low, equivalent to those of the follicular phase of the menstrual cycle. These levels may be near the threshold for producing breast-cancer-promoting effects; therefore, the tumor response will vary greatly in different populations, depending on genetic susceptibility factors:**
   a. **The prevalence of a family history of premenopausal breast cancer in a first-degree relative.**
   b. **The prevalence of abnormal BRCA1, BRCA2, and p53 genes.**
   c. **The prevalence of increased 16 α-hydroxylation of estradiol.**
   d. **The prevalence of smokers who are slow acetylators.**
4. **Consumption of alcohol (5 grams or more daily) along with the postmenopausal estrogen administration results in elevation of blood estradiol levels to values equivalent to those of the periovulatory peak of the menstrual cycle, which may be well above the threshold for producing breast-cancer-promoting effects in all women. The risk for cancer will therefore be uniformly increased in women who use alcohol and take estrogen.**
5. **Increased risk of breast cancer from postmenopausal estrogen administration can be eliminated by taking two synergistic steps:**
   a. **Eliminating alcohol consumption, or at least keeping it well below an average of 5 grams daily (equivalent to 2/3 ounce of whiskey or 3 ounces of wine).**
   b. **Diminishing the capacity to 16 α-hydroxylate estradiol, either through pharmacological agents such as indole-3-carbinol or through increased consumption of cruciferous vegetables.**

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It is concluded that despite the inherent ability of postmenopausal estrogen therapy to increase the risk of breast cancer in theory, the increased risk can be eliminated in practice by minimizing or eliminating consumption of alcohol and ingesting pharmacological or dietary agents that reduce the 16 α-hydroxylation of estradiol. [P.S.E.B.M. 1998, Vol 217]

The notion that administration of estrogen is likely to increase the risk of developing breast cancer feels intuitively right. After all, the breast is a target organ for estrogens, which cause it to grow and undergo epithelial hyperplasia, and such hyperplasia is believed to be the fertile ground in which the somatic mutations that lead to benign and malignant neoplasia can occur. However, an intuitively right feel is not evidence, which can be provided only be empirical studies, experimental or epidemiological. The importance of marshaling the best possible evidence, pro or con, about whether estrogen administration increases the risk of breast cancer cannot be overstated; in view of the reported major beneficial effects of postmenopausal estrogen administration in decreasing osteoporosis (1–3), decreasing coronary disease (4–6), decreasing Alzheimer's disease (7), and decreasing overall mortality (8, 9), it would be wrong to discourage women from taking estrogen merely on the basis of feelings, unsupported by evidence, about possible risks for breast cancer.

In humans, of course, true experimental studies of the link between estrogen administration and the incidence of breast cancer are precluded. The only evidence available comes from epidemiological studies, but animal, biochemical, and clinical investigative studies may help clarify the interpretation of epidemiological studies.

The Current Status of Epidemiological Studies

From 1941 to 1996, there have been 71 reports of epidemiological studies of the estrogen-breast cancer link, 7 meta-analyses, and uncounted reviews, including one from our laboratory published in 1993 (10). Of the epidemiological studies, 27 (11–37) showed a slight increase in breast cancer risk with estrogen administration, 32 (38–69) showed no difference in risk, and 10 (70–79) showed a slight decrease in risk. Of the meta-analyses, four (80–83) showed a slight increase in risk and three (84–86) showed no difference in risk. Opinions in the reviews have varied across the spectrum. I feel that both the weight of opinion and the weight of evidence indicate that the risk is exceedingly small or nonexistent; for example, Henrich (87) has stated, "... these findings do not support an overall increased risk of breast cancer in women who have ever used postmenopausal estrogens ..." and Goldzieher (88) has stated, "... an increased risk of breast cancer in estrogen users—if any—must be so small that its demonstration challenges the capabilities of the most sophisticated computerized statistical analyses of such data. ..." Nevertheless, there are respected voices that still say there is indeed a risk, though only with prolonged estrogen administration; for example, Hulka and Brinton (89) have stated, "... studies suggest that long-term use (10 years or more) may result in increased relative risks...," and Adami and Persson (90) have stated, "... in most studies there is no overall association between ever using replacement estrogens and breast cancer risk ... [but] there is some consistent evidence of an increased risk after many years of exposure." Thus, a sharp difference of opinion persists despite the fact that new epidemiological studies continue to appear in the literature at a steady pace, in the hope, so far unrealized, that better experimental design will produce the "definitive" study that will resolve the controversy once and for all.

The question of whether estrogen administration favors the development of breast cancer has been the Gordian Knot of endocrinology. Hundreds of workers, over nearly six decades, have attempted to unravel it by epidemiological studies, but without success. In the following sections, animal, biochemical, and clinical investigative studies will be discussed, and they may provide a resolution to the controversy.

Animal Studies on Estrogens and Breast Cancer

Isolation and identification of individual estrogens began in the 1930s, and soon after experimentalists began to study the possibility that administration of estrogen to animals (usually mice) might induce mammary neoplasms. Among the earliest and most comprehensive studies were those of Lacassagne (91–93). Detailed scrutiny of his experiments shows several points that have major relevance to the questions that persist today about the human epidemiological findings concerning estrogens and breast cancer: the ease of producing breast cancers and the time it took to do so depended on the natural susceptibility of the mouse strain that was studied; strains with a high incidence of spontaneous breast cancer regularly and speedily developed breast cancer after estrogen administration; strains with a low incidence of spontaneous breast cancer required very prolonged administration of estrogen, but most animals did eventually develop breast cancer; and strains that never showed spontaneous breast cancer did not develop cancer at all after estrogen administration. These findings indicated that the role of estrogen was that of promoter or cocarcinogen rather than inciter or primary carcinogen, and

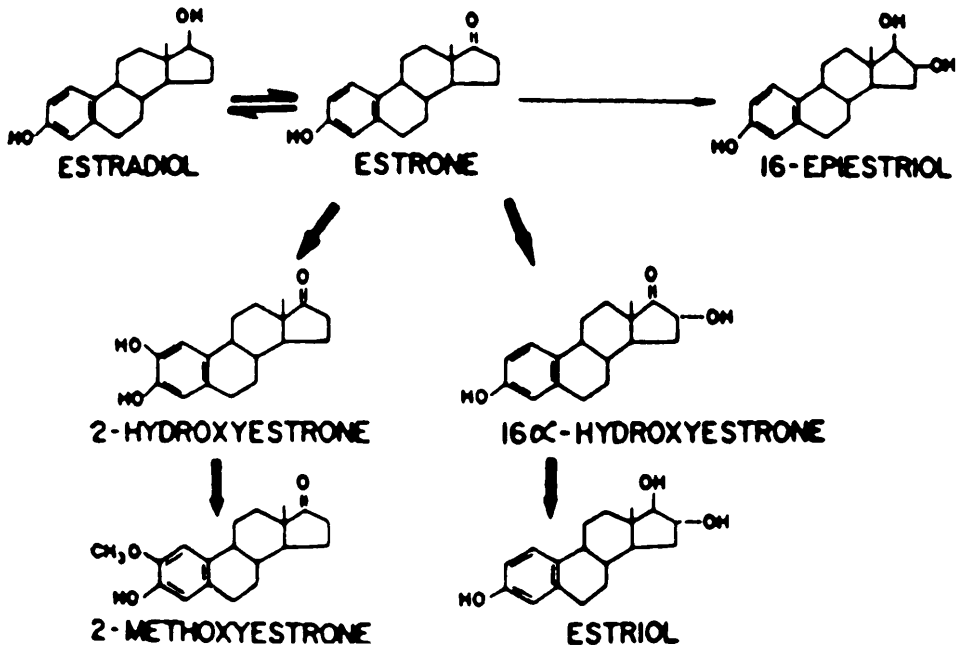

Figure 1. The metabolism of estradiol in humans is primarily oxidative; almost all of it undergoes reversible oxidation to estrone, which can then be oxidized irreversibly by one of two alternative pathways, the 2-hydroxylation pathway, which leads to the non-estrogenic metabolites 2-hydroxyestrone and 2-methoxyestrone, and the 16 α-hydroxylation pathway, which leads to the highly estrogenic metabolites 16 α-hydroxyestrone and estriol (16 α-hydroxyestradiol). Thus the overall estrogenic impact of a given amount of secreted or administered estradiol or estrone depends on the relative magnitudes of the 2-hydroxylation and 16 α-hydroxylation pathways.

that some congenital characteristic(s) of the animals, genetic or other, determined their responsiveness to the tumor-promoting effect. The implications for human epidemiology are readily apparent: since human populations are much less genetically homogeneous than laboratory animal strains, it is to be expected that any breast-cancer-promoting effect of estrogen administration in women would vary greatly from time to time, from place to place, and from group to group. Furthermore, since by laboratory-animal standards the spontaneous breast cancer incidence in humans is quite low, it is to be expected that demonstrating a breast-cancer-promoting effect of estrogen in women, as in low-incidence mice, would require very prolonged administration, and that the effect would be a modest one.

Results similar to those of Lacassagne were also reported by Loeb, *et al.* (94–96), who found that a hereditary factor strongly influenced the development of breast cancer in mice after estrogen administration, that there was an estrogen dose-response relationship for cancer development, and that cancer could develop long after estrogen administration was stopped. These findings, too, correspond closely with the findings in human epidemiological studies.

A few years after the studies of Lacassagne and Loeb, it became apparent that the cancer-promoting effect of estrogen in mice was dependent on the presence of the mouse mammary-tumor virus (MMTV) (97, 98). Thus, the congenital factor that was demonstrated by their findings of profound strain differences in susceptibility to estrogen was apparently the MMTV. At first glance, this does not appear to be relevant to human epidemiology, since MMTV has not been found in humans. However, a recent study by Bradlow, *et al.* (99) suggests that the effect of MMTV on increasing susceptibility to breast cancer is mediated by its effect on increasing the 16 α-hydroxylation of estradiol, which is indeed relevant to human susceptibility, as will be discussed in the next section.

The Role of 16 α-Hydroxylation of Estradiol

The metabolism of estradiol in humans is primarily oxidative (Fig. 1). Almost all of it 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 16 α-hydroxylation pathway, which leads to the highly estrogenic metabolites 16 α-hydroxyestrone and estriol (16 α-hydroxyestradiol) (100). Thus the overall estrogenic impact of a given amount of secreted or administered estradiol or estrone depends on the relative magnitudes of the 2-hydroxylation and 16 α-hydroxylation pathways.

The most consistent abnormality of estrogen secretion or metabolism that has yet been discovered in the long search for hormonal abnormalities in breast cancer (101) is increased 16 α-hydroxylation of estradiol. In 1966, our laboratory reported that men with breast cancer showed markedly increased 16 α-hydroxylation of estradiol (102). We subsequently reported increased 16 α-hydroxylation in women with breast cancer as well (103). Bradlow and Fishman and their co-workers have greatly expanded the studies of 16 α-hydroxylation in breast cancer, with the following findings:

1. Increased 16 α-hydroxylation was confirmed in women with breast cancer (104).
2. Increased 16 α-hydroxylation was found in women at familial high risk for breast cancer (105).
3. Increased 16 α-hydroxylation was found in mouse strains with a high incidence of breast cancer; the de-

gree of increase paralleled the degree of increased cancer incidence in different strains (98).

4. Elevated 16 α-hydroxylation in mice was inherited as an autosomal dominant (98).
5. The presence of MMTV was associated with elevated 16 α-hydroxylation; introduction of virus into animals without it raised hydroxylation, and deletion of virus from animals containing it lowered hydroxylation (101).

The interesting finding that the increased 16 α-hydroxylation in mice is related to the presence of MMTV and can be manipulated up or down by controlling the amount of virus present in the mice brings us back full circle to Lacassagne's studies (91–93), which have been interpreted, as we mentioned earlier, as showing that the cancer-promoting effect of estrogen administration is dependent on the presence of MMTV (97, 98). The findings of Bradlow, *et al.* (105) suggest that one can restate the situation as follows: the cancer-promoting effect of estrogen administration in mice is dependent on high activity of the 16 α-hydroxylation pathway of estrogen metabolism. If the same is true in humans too (recall that elevated 16 α-hydroxylation activity has been shown in women with breast cancer [104] and women with familial susceptibility to breast cancer [105]), one would expect great variability in the cancer-promoting effect of estrogens in different populations, depending on the prevalence of elevated 16 α-hydroxylation in these populations. Lemon (106) reported that 16 α-hydroxylation capacity in humans had an approximately trimodal distribution, and speculated that some of the variation in susceptibility to spontaneous breast cancer might be due partly to this genetic variation in 16 α-hydroxylation, a line of thought similar to the speculation in this review.

Very recent studies originating at an apparently unrelated starting point appear to provide further substantiation of a possible important role of increased 16 α-hydroxylation in breast cancer: Zhang, *et al.* (107) and Cauley, *et al.* (108) reported that there was a significant correlation between increased bone mass and increased risk of breast cancer in postmenopausal women, and Kim, *et al.* (109) subsequently reported that there was a significant correlation between increased bone mass and increased 16 α-hydroxylation of estradiol. These findings suggest that increased 16 α-hydroxylation may be the causal link between increased bone mass and increased risk of breast cancer.

Elevated 16 α-hydroxylation capacity, which is easily measurable (110), might prove to be a useful marker of increased susceptibility to breast cancer in individuals or populations. It might also be a highly useful point of attack for preventive therapy, since Bradlow, *et al.* showed that pharmacologically decreasing 16 α-hydroxylation in mice with a high incidence of spontaneous breast cancer greatly decreased the incidence of cancer (111). If this result is applicable to human breast cancer, its importance cannot be overstated. It should be mentioned that 16 α-hydroxylation activity can also be lowered by ingestion of cruciferous vegetables (112).

The Role of Genetic Factors

It has been reported that women with a first-degree relative (mother or sister) who had premenopausal breast cancer are at increased risk for spontaneous breast cancer (112). It is not clear whether this applies to increased risk for estrogen-promoted breast cancer as well.

More recently, abnormalities in certain genes, BRCA1 (114), BRCA2 (115), and p53 (116), have also been reported to increase susceptibility to spontaneous breast cancer. It is likewise not clear whether this applies to increased risk for estrogen-promoted breast cancer.

It is not known which of these genetic factors for susceptibility to breast cancer may be linked with genetic variations in 16 α-hydroxylation capacity.

The Role of Smoking

After considerable controversy, a consensus had developed that the effect of smoking on the risk of breast cancer was very small or nonexistent (117). This conclusion has been rendered untenable by a recent study (118), which showed that the population is divided about equally into slow acetylators and fast acetylators, with the former showing increased risk of breast cancer with smoking and the latter showing no increased risk and possibly slight protection (as would be predicted from the fact that smoking decreases 16 α-hydroxylation of estradiol [119]). In studies of breast-cancer risk with estrogen administration, it now becomes essential to analyze the population studied for the number of smokers who are slow acetylators.

The Role of Alcohol Consumption

Because the literature had arrived at a consensus that alcohol consumption is a risk factor for spontaneous breast cancer (120), the large Nurses' Health Study (32), which sought to evaluate the role of estrogen administration as a risk factor for breast cancer, was designed to stratify for alcohol consumption as a possible associated risk. Analysis of the data yielded a startling finding: only women who consumed alcohol manifested an increased risk of breast cancer with estrogen administration; those who did not consume alcohol showed no increase in risk. The authors noted this finding in their discussion but did not place great emphasis on it despite its potentially profound implications; they merely commented that "... the apparent difference in risk due to estrogens according to level of alcohol intake is unexpected and intriguing. Further study is needed ..." One might think that other epidemiologists would hasten to redesign their studies and reanalyze their data in the light of this important observation, but only one has done so, the almost equally large Iowa Women's Health Study, reported by Gapstur, *et al.* (34). The data from that study are in agreement with those of Colditz, *et al.* in the Nurses' Health

Study (83): only women who consumed 5 grams of alcohol or more per day manifested an increased risk of breast cancer with estrogen administration; those who consumed less alcohol than that or none at all showed no increase in risk (Table I).

It should be emphasized that no one has published data that disagree with the findings of these two studies.

Gapstur, *et al.* were also hesitant about placing great emphasis on their finding about the role of alcohol because they were concerned that there was no "biologically plausible" mechanism by which alcohol could exert the observed effect, but recently a biologically plausible mechanism has been reported: Ginsburg, *et al.* (121) have found that when a postmenopausal woman receiving estrogens consumes alcohol, her blood level of estradiol rises acutely, by about 300% (Fig. 2).

Our laboratory proposes the following hypothesis to account for the apparently critical role of alcohol in susceptibility to the breast-cancer-promoting effect of estrogen, based on the findings of Colditz, *et al.* (32), Gapstur, *et al.* (34), and Ginsburg, *et al.* (121): The elevation of blood estradiol by administration of postmenopausal estrogen replacement therapy alone is only modest, i.e., from castrate levels to levels characteristic of the follicular phase of the menstrual cycle (121); these levels may be near a threshold value for breast-cancer-promoting effects, so that some women will manifest increased risk and some will not, depending on their varying genetic susceptibility factors, including family history of breast cancer; abnormal BRCA1, BRCA2, and p53 genes; and elevated capacity to 16 α-hydroxylation estradiol. When alcohol is consumed along with estrogen, the blood estradiol is raised to levels characteristic of the periovulatory peak in the menstrual cycle, which may be above the threshold of cancer-promoting effects for all women, regardless of genetic background, resulting in a clearly demonstrable increase in risk in whole populations.

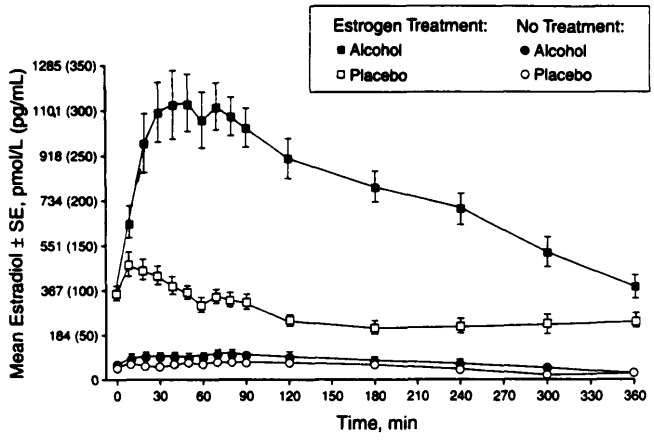

Figure 2. Data from Ref. 121. Postmenopausal women who are not using estrogen replacement show castrate levels of serum estradiol, and alcohol consumption does not increase these levels. This corresponds to the absence of any effect of alcohol on increasing breast-cancer risk in women who have never used estrogens (see Fig. 1). Postmenopausal women who are on estrogens have basal serum estradiol levels corresponding to those of the follicular phase of the menstrual cycle; consumption of alcohol results in a rise of serum estradiol to values corresponding to those of the periovulatory peak in the menstrual cycle; the effect lasts for more than 6 hr. (Reprinted with permission from Journal of the American Medical Association)

On the basis of available data, it seems reasonable to conclude that whatever increase in breast cancer incidence

Table I. Interaction Between Alcohol Consumption and Noncontraceptive Estrogen Use* on Risk of Breast Cancer in Postmenopausal Women, Iowa Women's Health Study, 1986–1989

Alcohol intake (g/day)	Noncontraceptive estrogen use†									
	Never					Ever				
	No. of cases	Total person-years	RR‡	95% CI§	*p* for trend	No. of cases	Total person-years	RR†	95% CI	*p* for trend
0	162	47,308	1.0			80	27,564	0.88	0.67–1.15	
<1.5	32	7,895	1.25	0.86–1.83		15	5,056	0.93	0.55–1.58	
1.5–4.9	46	12,238	1.16	0.84–1.62		29	8,181	1.13	0.76–1.68	
5.0–14.9	19	8,908	0.71	0.44–1.15		35	6,130	1.88	1.30–2.72	
≥15.0	18	5,605	1.07	0.65–1.74		23	4,189	1.83	1.18–2.85	
					0.46					0.0001

Note. n = 493 cases. Data from Ref. 34. The relative risk (RR) of breast cancer in women who do not consume alcohol and have never used estrogens is set at 1.0. Increasing amounts of alcohol consumption have no significant effect on RR in these never-users of estrogens. Women who have used estrogen but consume little or no alcohol show no significant increase in breast-cancer risk—RRs do not differ significantly from 1.0; in contrast, women who have used estrogen and consume 5 grams of alcohol per day or more (5 grams corresponds to ⅔ ounce of whiskey or 3 ounces of wine) show a highly significant increase in risk (RR of slightly over 1.8).

* −2 log likelihood test for interaction ($\chi^2 = 16.48$, df = 4, $p < 0.005$).

† Number of cases adds to less than 493 because of missing data.

‡ Relative risk (RR) of breast cancer, adjusted for age, body mass index, age at menarche, age at first livebirth, and family history of breast cancer using Cox proportional hazards regression.

§ CI, confidence interval.

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is produced by administration of normal doses of postmenopausal estrogen replacement may require mediation by alcohol consumption, *i.e.* that without alcohol consumption, such estrogen administration poses *no* risk.

Summary/Formulation

1. Administration of estrogen is inherently capable of promoting the growth of breast cancer, and therefore of increasing the incidence of clinical breast cancer.
2. Human response to estrogen is like that of the low-cancer-incidence strains of mice studied by Lacassagne, in that large doses and prolonged administration are required to induce clinical breast cancer.
3. The blood levels of estradiol produced by the usual doses of postmenopausal estrogen are relatively low, equivalent to those of the follicular phase of the menstrual cycle. These levels may be near the threshold for producing breast-cancer-promoting effects; therefore, the tumor response will vary greatly in different populations, depending on genetic susceptibility factors:
   a. The prevalence of a family history of premenopausal breast cancer in a first-degree relative.
   b. The prevalence of abnormal BRCA1, BRCA2, and p53 genes.
   c. The prevalence of increased 16 α-hydroxylation of estradiol.
   d. The prevalence of smokers who are slow acetylators.
4. Consumption of alcohol (5 grams or more daily) along with the postmenopausal estrogen administration results in elevation of blood estradiol levels to values equivalent to those of the periovulatory peak of the menstrual cycle, which may be well above the threshold for producing breast-cancer-promoting effects in all women. The risk for cancer will therefore be uniformly increased in women who use alcohol and take estrogen.
5. Increased risk of breast cancer from postmenopausal estrogen administration can be eliminated by taking two synergistic steps:
   a. Eliminating alcohol consumption, or at least keeping it well below an average of 5 grams daily (equivalent to 2/3 ounce of whiskey or 3 ounces of wine).
   b. Diminishing the capacity to 16 α-hydroxylate estradiol, either through pharmacological agents such as indole-3-carbinol or through increased consumption of cruciferous vegetables.

Conclusion

Despite the inherent ability of postmenopausal estrogen therapy to increase the risk of breast cancer in theory, the increased risk can be eliminated in practice by minimizing or eliminating consumption of alcohol and ingesting pharmacological or dietary agents that reduce the 16 α-hydroxylation of estradiol.

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