



Published in final edited form as:

Cancer Lett. 2015 January 28; 356(2 0 0): 231–243. doi:10.1016/j.canlet.2014.04.018.

Estrogen Metabolism and Breast Cancer

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Abstract

There is currently accumulating evidence that endogenous estrogens play a critical role in the development of breast cancer. Estrogens and their metabolites have been studied in both pre- and postmenopausal women with more consistent results shown in the latter population, in part because of large hormonal variations during the menstrual cycle and far fewer studies having been performed in premenopausal women. In this review we describe in detail estrogen metabolism and associated genetic variations, and provide a critical review of the current literature regarding the role of estrogens and their metabolites in breast cancer risk.

Keywords

Breast cancer; Sex hormones; Estrogen; Estrogen metabolites; Postmenopausal women; Premenopausal women

1. Introduction

Breast cancer remains an overwhelming health burden, with an estimated 232,670 new breast cancer cases and 40,000 deaths among women living in the U.S in 2014 [1]. Age is the strongest risk factor for breast cancer. Unlike many cancers that increase beginning at the end of the fifth decade of life, breast cancer begins to rise in the third decade of life, most likely due to the effects of ovarian hormones on breast tissue [2–4]. More than 2/3 of all new cases occur after the age of 55 and women older than 65 have a relative risk greater than 4.0 when compared with those younger than 65.

To date, many additional risk factors for breast cancer have been identified. Some risk factors are non-modifiable, such as age, BRCA1 and BRCA2 gene mutations, family history, reproductive history, and high-dose radiation to the chest. Others are potentially modifiable, such as high endogenous estrogens, hormone therapy, obesity (for postmenopausal breast cancer) and alcohol consumption [2, 3]. There is some controversy regarding whether or not the risk factor of high mammographic density is modifiable [5–9].

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Conflict of Interest

None of the authors has a conflict of interest.

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Since a number of these known risk factors are related to endogenous estrogen levels, the effect of estrogens on breast carcinogenesis has drawn a great deal of attention in the last two decades, with evidence suggesting that estrogens play a causal role in the etiology of breast cancer [10]. In this review, we will discuss the metabolism of estrogens and will present a detailed analysis of published data evaluating the role of circulating and urinary estrogens and their metabolites in human breast cancer.

2. Estrogen Metabolism

All steroid hormones originate from C27 cholesterol (Figure 1). The main source of cholesterol required for the synthesis of steroid hormones (steroidogenesis) is LDL-cholesterol [11]. Cholesterol is metabolized down a number of enzymatic pathways and is converted to the 21-, 19-, and 18-carbon steroid hormones, respectively.

The first step in ovarian steroidogenesis is the movement of cholesterol into the mitochondrion. This step is regulated by the steroidogenic acute regulatory protein (*StAR*) encoded by the *STAR* gene [12]. The next step involves the conversion of cholesterol to preg-nenolone, catalyzed by the mitochondrial side-chain cleavage enzyme complex. Pregnenolone acts as a precursor for all steroid hormones. It is metabolized by different enzymes, and under the action of 17-hydroxylase/17, 20-lyase enzyme, a product of the *CYP17A1* gene is converted to progesterone or androstendione. Androstendione, in turn, is further metabolized to other androgens or estrogens.

Estrogens are among very few aromatic molecules in humans. They are all C18 steroids and consist of one benzene ring, a phenolic hydroxyl group at C3, and a hydroxyl group (17 β -estradiol) or a ketone group (estrone) at C17 (Figure 1). The main estrogens circulating in the human body are estradiol and estrone, as well as 16-hydroxyestradiol (estriol). Although estriol is usually the major estrogen in pregnant women [13], and is the most abundant estrogen in the urine of all women, estradiol is the most biologically active estrogen, primarily secreted by ovarian granulosa cells located next to theca cells and regulated by follicle-stimulating hormone (FSH). Estrone is reversibly converted to estradiol through the action of 17 β -hydroxysteroid dehydrogenase enzyme [14]. Androstenedione, the most important product of the theca cells during the follicular phase of the menstrual cycle, is not biologically active; however, it acts as a precursor for both estrone and testosterone in the ovaries and peripheral tissues. [15]. Testosterone, in turn, is converted to estradiol by the action of aromatase enzyme in the peripheral tissues (Figure 1).

In premenopausal women, estradiol synthesized in the ovaries is the most important estrogen, while in postmenopausal women, estrone synthesized in peripheral tissues is predominant. Aromatase (*CYP19*), encoded by the *CYP19A1* gene, is the rate-limiting enzyme in catalyzing the conversion of androgens to estrogens [16, 17]. Given the importance of this enzyme, blocking aromatase activity is an important pharmacological tool used for the treatment of estrogen-dependent diseases such as breast cancer, endometriosis, and endometrial cancer.

Estradiol and estrone are metabolized by three competitive pathways involving irreversible hydroxylations catalyzed by the NADPH-dependent cytochrome P450 (*CYP*) enzymes

including CYP1A1, CYP1B1, and CYP1A2 (Figure 2). Estrone and estradiol are hydroxylated at positions C2, C4 and C16 and are converted to catechol estrogens (2-hydroxyestrone, 4-hydroxyestrone, 2-hydroxyestradiol, and 4-hydroxyestradiol), and 16 α -hydroxyestrone. Estriol is produced by the hydroxylation of estradiol or 16 α -hydroxyestrone. Catechol estrogens are further metabolized (methylated) to methoxyestrogens (2-methoxyestrone, 4-methoxyestrone, 2-methoxyestradiol and 4-methoxyestradiol) by the catechol-O-methyltransferase (COMT) enzyme (Figures 2 and 3).

In addition to methylation, parent estrogens and catechol estrogens are also conjugated with glucuronic acid and sulfate by hepatic phase II enzymes including UDP-glucuronosyltransferases and sulfotransferases, respectively. Conjugation is considered a detoxification reaction by which hormones either become water soluble and are excreted in the urine or feces, or turn into a more lipophilic moiety with elevated half-lives (Figure 2) [18–20].

2-hydroxylation pathway

Quantitatively, the 2-hydroxylation pathway is the major metabolic pathway compared to the 4- and 16-hydroxylation pathways. The cytochrome P-450 enzymes, including CYP1A1 and CYP1B1, are major phase I enzymes mainly expressed in breast and liver tissues [21]. These enzymes, along with CYP1A2, catalyze the C2 hydroxylation of parent estrogens to their respective catechol estrogens [22]. Two-hydroxylated estrogens possess low binding affinity for the estrogen receptor (ER) [23, 24]. These metabolites demonstrate reduced hormonal potency when compared with estradiol, and both non-estrogenic and anti-estrogenic activities have been attributed to them. There is some evidence from cell culture studies in ER+ human MCF-7 breast cancer cells suggesting that 2-hydroxyestrone and 2-hydroxyestradiol inhibit cell growth and proliferation [25, 26]. In addition, 2-hydroxy metabolites have been associated with normal cell differentiation and apoptosis [27, 28]. Taken together, these findings have led some researchers to classify 2-hydroxyestrone as a “good estrogen” [29]. The lack of tumorigenic activity of 2-hydroxy metabolites has been attributed to a few mechanisms including a high rate of clearance, more rapid rate of *O*-methylation by the COMT enzyme, lower hormonal potency in estrogen target tissues, and methylated products of 2-hydroxyestradiol such as 2-methoxyestradiol, which suppress tumor cell proliferation and angiogenesis [23]. At the same time, it has been shown that 2-hydroxyestrogens can damage DNA and generate free radicals as they go through redox cycling or when COMT is inhibited [30, 31]. It is important to note that high inter-individual variability in 2-hydroxylation has been shown in human liver samples, possibly explaining the high variability of metabolite levels in individuals [32].

Methoxyestrogens, including 2-methoxyestradiol, have been shown to inhibit carcinogenesis by suppressing cell proliferation and estrogen oxidation due to effects on microtubule stabilization [33–35]. Lottering *et al.* [36] investigated the effects of 17- β estradiol and its metabolites on cell cycle in MCF-7 cells and reported that 2-methoxyestradiol acts as a cytostatin and inhibits mitosis.

4-hydroxylation pathway

CYP3A4/3A5 has been shown to be the primary enzyme in the 4 hydroxylation of estradiol in human liver microsomes [32]. 4-hydroxylated catechol estrogens possess carcinogenic potential due to their ability to cause DNA damage by forming depurinating adducts, which in turn, generate mutations with subsequent oxidative damage and initiation of breast cancer [37]. In microsomal preparations of human mammary fibroadenoma and adenocarcinoma, formation of 4-hydroxyestradiol was four times higher than 2-hydroxyestradiol formation, indicating that the ratio of 4-/2-hydroxyestradiol may be used as a biomarker for detection of malignant breast tumors [38]. In addition, it has been shown that the ratios of quinone-estrogen DNA adducts to their parent or conjugated catechol estrogens were significantly higher in women with breast cancer or at high risk of breast cancer compared with control women [39]. On the other hand, it has been suggested that the 4-methoxyestrogens prevent oxidative metabolism of estradiol [35] and oxidative DNA damage [40]. These findings are in agreement with a more recent study in which inhibition of COMT enzyme activity was associated with higher levels of depurinating 4-hydroxyestrone (estradiol)-1-N³Adenine and 4-hydroxyestrone (estradiol)-1-N⁷Guanine adducts in MCF-10F cells [41].

16-hydroxylation pathway

16 α -hydroxyestrone is the most important metabolite of the 16-hydroxylation pathway. 16 α -hydroxyestrone is a potential tumor initiator, which promotes unscheduled DNA synthesis and anchorage independent growth in mouse mammary epithelial cells [42–44]. Animal studies have shown that urinary concentrations of 16 α -hydroxyestrone are associated with increased proliferation of mammary cells [43, 44], Ras oncogene expression [45], and mammary tumor incidence [46]. Osborne *et al.* investigated the extent of estradiol 16 α -hydroxylation in relation to the risk of developing breast cancer in human breast tissue. They reported that 16 α -hydroxyestrone levels were eight-fold higher in cancerous mammary terminal duct lobular units than nearby mammary fat tissue, suggesting that 16 α -hydroxyestrone production may play an important role in breast cancer induction [47].

There are currently substantial data suggesting a link between concentrations of individual metabolites or the ratio of specific metabolites and breast cancer risk in humans; this will be discussed in further details later in this review.

3. Role of Genetic Variation in Estrogen Metabolism

It has been postulated that genetic polymorphisms in genes encoding enzymes involved in estrogen metabolism pathways and the genes encoding the ERs are associated with breast cancer risk. Polymorphic variations in genes encoding COMT, CYP1A1, CYP1B1, estrogen receptor alpha (ER α), estrogen receptor beta (ER β), CYP17A1, and CYP19A1 have received extensive attention within the last decade.

COMT is a phase II enzyme that inactivates catechol estrogens by conjugating them into nongenotoxic methoxyestrogens [48]. COMT also prevents biotransformation of catechol estrogens to quinone-DNA adducts and development of reactive oxygen species (ROS) capable of damaging cellular macromolecules such as DNA, lipids, and proteins [49–51]. COMT, located on chromosome 22q11 [52], is polymorphic; a single G to A transition at

codon 158 of *COMT* (single nucleotide polymorphism (SNP) rs4680) results in a 3- to 4-fold decrease in enzymatic activity (GG vs. AA genotype). Individuals with heterozygous genotype (A/G) show intermediate levels of *COMT* activity [53–54].

Given the role of *COMT* in the conversion of catechol estrogens to methoxyestrogens, genetic variations in this enzyme may influence the risk of breast cancer as a result of significant changes in catechol estrogen and methoxyestrogen levels [55]. It has been hypothesized that women possessing the low activity *COMT* genotype (AA or Met/Met) might be at greater risk of breast cancer due to higher concentrations of catechol estrogen intermediates [56–58]. Unexpectedly, results from the most recent meta-analysis [59] of 56 studies including 34,358 breast cancer cases and 45,429 controls show no evidence of significant associations between the *COMT* Val158Met polymorphism and breast cancer risk in any genetic model (comparing recessive and dominant models with each other). Although these findings did not change in subgroup analyses by ethnicity, source of controls, or menopausal status, they must be interpreted with caution because of the large heterogeneity between studies and lack of data for adjustment of other covariates such as age, body mass index (BMI), lifestyle, and environmental factors.

In addition to the hydroxylation of parent estrogens to catechol estrogens, CYP along with peroxidase enzymes catalyze the oxidation of catechol estrogens to estrogen semiquinones and quinones, which are carcinogenic metabolites of estrogens (Figure 3) [60]. It has been hypothesized that polymorphic variations in *CYP1A1* and *CYP1B1* genes are linked with increased risk of breast cancer. In a case-control study, Taioli *et al.* [61] demonstrated that African-American breast cancer cases with the *Msp*I homozygous variant polymorphism in *CYP1A1* had an odds ratio (OR) of 8.4 (95% confidence interval: 1.7–41.7) compared with controls. This association was not observed in Caucasian women. In contrast, Miyoshi [62] reported an inverse association between the *Msp*I (6235(T/C)) and breast cancer risk in Japanese women (OR= 0.60; 95% CI, 0.41–0.88). Taken together, these findings are mixed and more research is needed to clarify this discrepancy.

SNPs in *CYP1B1* genes have also been investigated in relation to breast cancer risk [63–66]. In a population-based case-control study conducted by Reding *et al.* [67] of 891 breast cancer cases and 878 controls, women homozygous with the T allele in *CYP1B1**2 (Ser¹¹⁹; rs1056827) were compared with women homozygous with the G allele. Those homozygous for the *CYP1B1**2 Ser allele (T/T) had a 1.69 times higher risk of breast cancer (95% CI, 1.17–2.46); however, results from this study were not in agreement with an earlier meta-analysis [68] that showed no overall associations of breast cancer risk with *CYP1B1* polymorphisms. This inconsistency may be due to selection bias or the result of chance in Redwing's study, or limitations of meta-analysis, such as variability in populations and publication bias.

In a meta-analysis of more than 10,000 mostly Caucasian breast cancer cases, multiple potentially functional SNPs in ER α including rs2234693, rs9340799, rs1801132, rs3798577, and rs2228480 have been studied. The only SNP that showed borderline significant association with reduced risk of breast cancer was rs2234693 (CC genotype vs. TT; OR=0.92; 95 % CI, 0.86–0.99; *P* =0.08 for heterogeneity test) [69]. Polymorphisms in the

ER β gene and breast cancer risk have also been studied in a recent systematic review [70] that reported that rs2987983, and rs4986938 SNPs are significantly associated with overall breast cancer risk

Most recently, Chattopadhyay *et al.* [71] reported significant associations between SNPs in ER α (rs2234693), ER β (rs2987983), CYP17A1 (rs743572) and CYP19A1 (rs700519) and breast cancer risk in a case control study performed in North India. The study included 360 cases with corresponding controls matched on age, sex, ethnicity, and geographical location. For the ER α genetic polymorphism, the CC genotype was the reference genotype for all comparisons. The CT and CT+TT genotypes were positively associated with postmenopausal status ($P=0.018$ and $P=0.017$; respectively) and histological grade I and II cases ($P=0.022$ and $P=0.008$; respectively), and negatively associated with advanced clinical stage (III+IV) ($P=0.008$ and $P=0.021$; respectively). However, in each case the significance was lost after conducting Bonferroni corrections for multiple comparisons. For the other SNPs, a number of associations remained statistically significant after performing Bonferroni corrections and adjusting for age. For ER β , the TC+CC genotypes were inversely correlated with premenopausal status when compared with the TT genotype (OR=0.31; 95 % CI, 0.15–0.62; P value=0.001). For CYP17A1, the TC+CC genotypes were positively associated with ER– status compared with TT genotype (OR=2.77; 95 % CI, 1.52–5.04; P value =0.001). Finally, a SNP in CYP19A1 or the aromatase gene (rs700519) was linked with an increased risk among postmenopausal women (CT+TT vs. CC; OR=2.72; 95 % CI, 1.47–5.10; P value=0.001).

Studies assessing different SNPs in the CYP19A1 and CYP17A1 genes in relation to breast cancer have yielded inconclusive results. Talbott *et al.* [72] have demonstrated that polymorphic variations in the CYP 19 gene in rs1008805 (A/G) SNP with at least one G allele, but not rs730154 (C/T) SNP, is linked with higher risk of premenopausal breast cancer (OR = 1.72; 95% CI, 1.20–2.49). There was no association between postmenopausal breast cancer and rs1008805 SNP. Yet Reding and other investigators [73] from the Women's Contraceptive And Reproductive Experiences (CARE) study observed no substantial association with breast cancer risk for neither SNPs in CYP19A1, nor for CYP17A1, ER α , COMT, CYP1A1, or CYP1B1 genes in more than 1,600 White and Black cases. Similar results have been noted from the Shanghai Breast Cancer Study for 19 SNPs in CYP19A1 gene [74]. Polymorphic variations in the CYP17A1 gene in relation breast cancer have been also considerably evaluated, but findings are mixed and no firm conclusion can be drawn at this time [75–78].

Further studies with more homogenous populations and larger sample sizes are required to substantiate the role of ERs and estrogen-metabolizing gene polymorphisms in breast cancer risk pathogenesis.

4. Estrogens and Breast Cancer Risk

There is increasing evidence from epidemiological, animal, and *in vitro* studies that endogenous estrogens are involved in breast carcinogenesis [79]. Evidence suggesting a hormonal role in breast cancer development began with an early observation that bilateral

oophorectomy significantly reduces breast-cancer risk, and that risk reduction is greater if the ovaries are removed earlier in life [80]. In addition, some of the well-established risk factors for breast cancer, including early onset of menarche (<12 years), late menopause (>55 years), nulliparity or having child late in life, are related to lifetime exposure of breast tissue to sex hormones. Given that approximately 2/3 of breast tumors are ER positive (ER+) [81] and responsive to circulating estrogens, and that almost all ER negative (ER-) cases are resistant to endocrine therapy, it is important to elucidate the specific mechanisms by which estrogens are related to elevated breast cancer risk. We will discuss the association of estrogens with breast cancer risk in the following sections separately based on menopausal status. Assessing the role of androgens and progesterone in breast cancer is beyond the scope of this review and will not be discussed here.

4.A. Postmenopausal Women

4.A.1. Circulating primary hormones—In postmenopausal women, increased circulating concentrations of estradiol, estrone, estrone-sulfate, and androstendione have been associated with higher breast cancer risk, whereas higher levels of sex hormone binding globulin (SHBG) have been associated with lower risk [82–89]. Key *et al.* [90], in a pooled analysis of 9 prospective studies of 663 women who developed breast cancer and were not on any exogenous sex hormones, showed that risk of breast cancer significantly increases with higher levels of total estradiol, free estradiol, estrone, estrone-sulfate, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and testosterone. The relative risk (RR) and 95% CI for the highest quintile versus the lowest quintile of estradiol levels was 2.0 and 1.47–2.71, respectively (Table 1). Since this analysis was published, a few more prospective and case-control studies have been reported that have found similar results [91–95]. It should be noted that the majority of populations studied were general populations with average breast cancer risk who were not taking any exogenous sex hormones. However, when breast cancer risk category (low versus high based on Gail or Rosner and Colditz risk model scores) [96, 97] was taken into consideration, no difference in the association between sex hormones and breast cancer was observed in low versus high breast cancer risk subjects [94, 98]. These findings should be evaluated in further studies.

Surprisingly, the results from the Women's Health Initiative (WHI) are in disagreement with those from observational studies. Manson and colleagues [99] have recently published updated data from the WHI with 13 years of cumulative follow-up. Briefly, WHI randomized 27,347 postmenopausal women 50–79 years to take either conjugated equine estrogens alone (CEE) for 7.2 years or CEE plus medroxyprogesterone acetate (MPA) for 5.6 years. Compared with the placebo group, the CEE group showed a 21% reduced risk of invasive breast cancer (95% CI, 0.65–0.97) while the CEE+MPA group showed a 28% increased risk (95% CI, 1.11–1.48) (Table 1). As suggested by Chlebowski and others [100, 101] the discrepancy between observational studies and the WHI may be due to methodological issues. For example, in non-research setting, women using hormones usually have more screening mammograms than non-hormone users, and consequently breast cancer is detected earlier; however, in the WHI study, all participants were required to receive screening mammograms at baseline and annually. In addition, there were a relatively small

number of breast cancer cases in the estrogen-alone group (n=168 for the intervention group and n=216 for the control group).

There are a number of limitations of observational studies, including the collection of a single blood sample. It has been suggested that one blood sample with a long period of follow up time, which is characteristic of epidemiological studies, may not be a good predictor of breast cancer risk. However, Zhang *et al.* in a nested case-control analysis within the Nurses' Health Study, showed that one single measurement of blood reproductive hormones is sufficient to predict ER+/PR+ breast cancer in postmenopausal women 16–20 years following blood draw. In addition, when two blood measurements, collected 10 years apart, were compared, the intra-class correlation coefficients were found to be 0.69 (95 % CI, 0.61–0.75) for estradiol, and 0.74 (95 % CI, 0.67–0.80) for SHBG, indicating that hormone levels are well correlated over long period of time [102].

Due to scarce available data, it is unclear if the relationship between circulating sex hormones and breast cancer differs according to receptor status. In a recent short review by Key, results from four studies were compared, and it was reported that estradiol was directly linked with ER+ breast cancer in postmenopausal women. However, since the number of ER – breast cancer cases was very small, no firm conclusion could be established [103].

A number of known breast cancer risk factors have been proposed to influence risk via effects on estrogens. Obesity, defined as BMI > 30 kg/m², raises the risk of postmenopausal breast cancer, and this has been attributed to the higher circulating levels of estrogens synthesized in the adipose tissue of obese women. At the same time, an inverse association between obesity and SHBG blood levels has been reported, which in turn, contributes to higher concentrations of free estradiol (bioavailable fraction) in the circulation.

The magnitude of the associations of estrogens with a number of breast cancer risk factors including obesity, reproductive, demographic, and life style factors has been investigated by the Endogenous Hormones and Breast Cancer Collaborative Group in several studies. In a pooled analysis of eight prospective studies in postmenopausal women, adjusting data for free estradiol concentrations attenuated breast cancer risk by 17% for each 5 kg/m² increase in BMI, resulting in a loss of statistical significance for the association between BMI and breast cancer risk [104]. In another cross-sectional analysis of 13 prospective studies by the same group, estrogen and androgen levels were positively associated with obesity, smoking (15+ cigarettes daily) and alcohol consumption (20+g alcohol daily), and inversely linked with age. By contrast, SHBG concentrations were greater in older women and lower in obese women and those consuming alcohol [105].

Mammographic density, a known risk factor for breast cancer development, is a measure of the amount of fibroglandular tissue that appears on a mammogram [106]. It has been hypothesized that sex steroid effects on breast cancer are mediated through mammographic density [107, 108]; however, available data do not consistently support the hormonal basis for mammographic density mostly because of the confounding influence of BMI [109–111].

It has long been of concern that circulating estrogen may not be an appropriate surrogate for breast tissue levels. Some studies have shown that estrogen concentrations in normal or

breast tumor tissue are greater than in the circulation [112–113]. It has been suggested that inhibition of local estrogen aromatization in tumor tissue may be an appropriate breast cancer prevention strategy. This notion has recently been rejected by Lønning *et al.* [114] who proposed a model in which plasma-to-tissue equilibration explains the high estrogen levels in breast tissue. According to this model, malignant breast tumors are constantly exposed to circulating estrogens through ER binding or active uptake of estrogens. Therefore, they propose that systemic suppression of estrogen production may be superior to targeting local aromatase enzyme in hormone responsive breast cancer. Given the strong positive association observed between blood sex hormones and breast cancer in postmenopausal women, circulating estrogens seem to be an appropriate marker of tissue exposure, as suggested by Hankinson and Eliassen [115].

4.A.2. Urinary and circulating estrogen metabolites—The role of estrogen metabolites in breast cancer has been the subject of discussion for the last three decades; however, compared to circulating primary estrogens, few studies have investigated the association between breast cancer and individual estrogen metabolites, their pathways or ratios. Among catechol estrogens, 16 α -hydroxyestrone and 4-hydroxy metabolites are relatively more estrogenic and have genotoxic potential while 2-hydroxy metabolites are considered to have little estrogenic activity or antiestrogenic properties [29].

Findings from small observational studies [25, 116, 117] have led to the hypothesis that a lower ratio of urinary 2-hydroxyestrone to 16 α -hydroxyestrone (2/16-hydroxyestrone) is a breast cancer risk factor. The most recent combined analysis [118] reviewed the results of 5 trials comprised of 385 invasive breast cancer cases and 723 controls. All metabolites were analyzed utilizing an enzyme linked immunosorbent assay (ELISA), and odds ratios adjusted for known breast cancer risk factors were calculated. Results comparing the women in the lowest tertiles with those in the highest tertiles did not reveal any significant association for either the 2/16-hydroxyestrone ratio (OR=1.02; 95% CI, 0.71–1.48) (Table 2) or any individual metabolites (OR for 2-hydroxyestrone =0.93; 95% CI, 0.67–1.30, and OR for 16 α -hydroxyestrone =1.01; 95% CI, 0.73–1.41). Additionally, when data were stratified by ER status, no significant difference in the relative risk was found. The pattern of results was similar in a systematic review by Obi *et al.* [119] in which 6 prospective and 3 retrospective studies including 1189 breast cancer cases and 1888 matched controls were reviewed. Women in the top category of either urinary or circulating 2/16-hydroxyestrone did not differ in risk of breast cancer compared to those in the bottom category (Table 2). Risk associations were not changed when ER subtype was also taken into account.

Recently, Fuhrman *et al.* [120] examined the associations of circulating levels of 15 estrogens and estrogen metabolites individually and grouped by pathway, as well as metabolic pathway ratios, with breast cancer risk in a prospective case-control study nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Participants were 277 invasive breast cancer cases and 423 matched controls, all postmenopausal women not using exogenous hormones at the time of the blood draw. In contrast to previous studies, an assay with high sensitivity and specificity, liquid chromatography–tandem mass spectrometry (LC-MS/MS), was utilized to measure circulating levels of hormones and their metabolites. The study demonstrated positive associations between breast cancer risk and

unconjugated estradiol levels (HR = 2.07; 95% CI, 1.22–3.51; $P_{\text{trend}}=0.01$) and the ratio of 4-hydroxylation pathway catechol estrogens to 4-hydroxylation pathway methylated catechol estrogens (HR = 1.76; 95% CI, 1.06–2.93; $P_{\text{trend}}=0.02$) comparing the highest to the lowest quintile. Interestingly, the ratio of 2-hydroxylation pathway to parent estrogens was found to be inversely associated with breast cancer risk (HR $_{Q5 \text{ vs. } Q1} = 0.54$; 95% CI, 0.32–0.90; $P_{\text{trend}}=0.003$) (Table 2).

Since the publication of the Fuhrman *et al.* results, another two studies have been conducted by the same lab and core investigators with inconsistent findings. Dallal *et al.* [121] reproduced similar results in a prospective case-cohort study within the Breast and Bone Follow-up to the Fracture Intervention Trial (B~FIT), of 407 cases and 496 controls. In this study, high circulating levels of estradiol were associated with elevated breast cancer risk (HR_{top vs. bottom quintile}=1.86; 95% CI, 1.19–2.90; $P_{\text{trend}}=0.04$). Additionally, increased ratios of the 2-hydroxylation pathway to parent estrogens, and 2:16-hydroxylation pathways were associated with lower risk (HR=0.69; 95% CI, 0.46–1.05; $P_{\text{trend}}=0.01$; and, HR=0.60; 95% CI, 0.40–0.90; $P_{\text{trend}}=0.002$, respectively) (Table 2). Surprisingly, the ratio of 4-hydroxylation pathway to parent estrogens was reported to be inversely related to breast cancer risk (HR_{top vs. bottom quintile} =0.61; 95% CI, 0.40–0.93; $P_{\text{trend}}=0.004$), which is not in agreement with the findings from previous studies. In contrast to Fuhrman and Dallal studies, no statistically significant results were observed in another nested case-control study by the same lab using blood samples from the Columbia Missouri Serum Bank [122]. The reasons for the discrepancies among these results are not quite clear, however, the investigators believe they might be due to differences in COMT polymorphisms or simply due to chance alone.

The association between circulating endogenous estrogens and breast cancer risk in postmenopausal women has been conclusively established, and compelling evidence exists to support a causal relationship. Epidemiological studies have consistently shown a 2–3 fold increase in breast cancer risk in women with elevated blood estradiol levels. On the other hand, findings from estrogen metabolite investigations are mixed. This inconsistency in estrogen metabolite results may be due to methodological differences in participant characteristics, study design and follow-up length (for some studies insufficient), number of cases (some studies under-powered), and high inter-individual variation in serum and urinary concentrations of estrogen metabolites, or limitations associated with estrogen metabolite measurement. Of particular note are differences in assay methodologies. Until recently, the leading methodology for measurement of estrogen metabolites was ELISA, a method that has limited specificity and sensitivity. This is of particular importance for analysis of samples from postmenopausal women, whose levels are extremely low. Recently, some groups have used liquid chromatography–tandem mass spectrometry (LC–MS/MS), which has much higher sensitivity and specificity.

4. B. Premenopausal Women

4. B.1. Circulating primary hormones—Much less research has been performed on the effects of endogenous estrogens on breast cancer risk in premenopausal women than in postmenopausal women. Thus, the role of estrogens in breast carcinogenesis in this

population is not thoroughly understood and remains relatively unclear. This is likely due to the much smaller number of breast cancer cases in premenopausal women. Another potential reason may be the large inter- and intra-individual variations in sex hormone concentration during the menstrual cycle. To the best of our knowledge, only nine prospective studies [123–132] have evaluated the associations between serum estrogens and breast cancer risk in premenopausal women.

Hankinson and Eliassen reviewed the results of seven of these studies in 2010 [133]. Briefly, three cohort studies [123, 124, 127] failed to show significant associations between breast cancer risk and estradiol, estrone, or estrone-sulfate, possibly due to the fact they were small studies with less than 50 cases, or had not adjusted for the timing of the menstrual cycle at the blood draw. Although the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort [128], Guernsey cohort [126], and Rosenberg studies [125] had larger sample sizes with 285, 62 and 79 cases respectively and accounted for the phase of menstrual cycle, no statistically significant results were found for associations between breast cancer risk and estrone, estradiol, or SHBG.

On the other hand, early follicular blood samples from breast cancer cases ($n=197$) in the Nurses Health Study II (NHSII) demonstrated a significantly elevated breast cancer risk in women with higher total and free estradiol ($RR_{Q4 \text{ vs. } Q1} = 2.1$; 95% CI, 1.1–4.1; and, $RR_{Q4 \text{ vs. } Q1} = 2.4$; 95% CI, 1.3–4.5; respectively). Importantly, the magnitude of the effect estimate was more pronounced among the ER+/PR+ cases compared with all breast cancer cases ($RR_{Q4 \text{ vs. } Q1} = 2.7$; 95% CI, 1.2–6.0 for follicular total estradiol) consistent with the classical role of ERs in stimulating higher cell proliferation and mutagenesis [134]. On the other hand, no evidence of a relationship between breast cancer risk and estrone, estrone-sulfate, or SHBG was seen [130].

In a 2011 meta-analysis of those seven nested case–control studies with a total of 693 cases and 1609 controls, only a weak relationship between circulating estradiol and breast cancer risk was reported (OR for a doubling of estradiol=1.10;95% CI, 0.96–1.27) (Table 3) [135].

Following this meta-analysis, results of three studies and a new systematic review have been published. Dorgan *et al.* [129] reported no association between total or bioavailable estradiol or SHBG and breast cancer risk, in a prospective case-control study of 98 breast cancer cases nested in the Columbia, MO, Serum Bank cohort matched with 168 controls on factors such as the day of blood draw and menstrual cycle phase. Similarly, data from 104 cases of the Italian Hormones and Diet in the Etiology of Breast Tumors (ORDET) cohort did not suggest any associations with risk when the highest tertiles of estradiol and SHBG levels were compared with the lowest tertiles [131]. The Nurses' Health Study II has recently published updated data regarding the relationship of plasma sex hormones to breast cancer risk [132]. Since the last data were published in 2006, more than 400 additional cases were identified, bringing the total number of cases to 634. Estrogens were measured by radioimmunoassay or LC–MS/MS, and SHBG was measured by chemiluminescence immunoassay. Overall, after adjusting for known breast cancer risk factors, no significant associations were reported between breast cancer risk and early follicular or mid-luteal total estradiol, free estradiol, estrone, or SHBG. There was weak evidence indicating that mid-

luteal estradiol was positively related with ER+/PR+ breast cancer (OR_{Q5 vs. Q1} = 1.7; 95% CI, 1.0–2.9; $P_{\text{trend}} = 0.02$).

Interestingly, results from the most recent pooled analysis of data by the Endogenous Hormones and Breast Cancer Collaborative Group [136] were not consistent with the findings of the previous studies. This inconsistency may be due to the exclusion of data from four of the previously discussed studies [123, 125, 127, 131]. Participants included in the analyses were premenopausal women below 50 years of age who were not using any exogenous sex hormones at the time of blood collection. Cases and controls were matched for age, menstrual cycle day, and blood draw date, and an odds ratio associated with a doubling in hormone levels was calculated separately for each hormone. Data from 600 women with incident breast cancer and 1375 controls were used to calculate the odds ratio for breast cancer in relation to serum estradiol. The odds ratio for circulating estrone was estimated based on data from 477 cases and 933 controls. Findings showed that a doubling in concentrations of estradiol and estrone was associated with 19% and 27% elevated risk of breast cancer, respectively (OR for estradiol = 1.19; 95% CI, 1.06–1.35; $P_{\text{trend}} = 0.004$; OR for estrone = 1.27; 95% CI, 1.05–1.54; $P_{\text{trend}} = 0.01$) (Table 3). SHBG was not related to breast cancer risk. These risk estimates were not changed significantly after adjustment for hormonal breast cancer risk factors. When data were evaluated based on the tumor ER subtype, no significant differences in results were observed between ER+ or ER– cases, although odds ratios were generally larger among the ER+ subjects.

Overall, observational data regarding the associations of circulation estrogen concentrations with breast cancer risk in premenopausal are not as strong as postmenopausal women. Therefore, no conclusions can be drawn until larger well-designed studies with enhanced analytical methods are conducted.

4.B.2. Urinary and circulating estrogen metabolites—Four prospective studies have investigated the relationships between breast cancer incidence and concentrations of individual estrogen metabolites, their ratios or metabolic pathways in premenopausal women. The ratio of 2/16-hydroxyestrone has been studied in either urine or blood samples with inconsistent results. Muti *et al.* [137] reported that relative to the lowest quintile, cases in the highest quintile of luteal phase urinary 2/16-hydroxyestrone had a 45% reduction in breast cancer risk ($n = 67$ cases; adjusted RR = 0.55; 95% CI, 0.23–1.32). Additionally, both 2-hydroxyestrone and 16 α -hydroxyestrone were positively but non-significantly linked with risk. Consistent with these results was a study conducted by Meilahn *et al.* [117] in which 60 breast cancer cases were matched to 184 controls on age, baseline visit date, and menstrual cycle phase. Results showed that women in the top tertile of the 2/16-hydroxyestrone ratio had a lower breast cancer risk compared to those in the bottom tertile (OR = 0.75; 95% CI, 0.35–1.62; P value = 0.46).

The New York University Women's Health Study [138] is the only trial that has reported circulating levels of the 2/16-hydroxyestrone ratio. In this study, 377 cases were matched with 377 controls on day and phase of menstrual cycle. Results revealed no significant associations between breast cancer risk and 2-hydroxyestrone or 16 α -hydroxyestrone, or their ratio (OR for 2-hydroxyestrone: 16 α -hydroxyestrone = 1.13; 95% CI, 0.68–1.87; $P_{\text{trend}} = 0.5$).

Along the same lines, in the subgroup analysis based on ER status when comparing the highest quartile with the lowest quartile among ER+ cases, the 2/16-hydroxyestrone ratio was associated with more than doubling of breast cancer risk (OR=2.15; 95% CI, 0.88–5.27; $P_{\text{trend}}=0.09$).

Contrary to the previous studies that had analyzed 2-hydroxyestrone, 16 α -hydroxyestrone, and their ratio with the ELISA method, the NHSII quantitated a comprehensive list of 15 urinary estrogens and estrogen metabolites utilizing the gold standard method, LC-MS/MS [139]. Mid-luteal urine samples were collected by 247 cases and 485 matched controls. Findings indicated that 2- and 4-hydroxylation pathway estrogen metabolites, but not 16-hydroxylation pathway, were inversely associated with risk, but results did not reach statistical significance. Likewise, the 2/16-hydroxyestrone ratio was not linked to risk (RR_{Q4 vs. Q1}=0.90; 95% CI, 0.57–1.41; $P_{\text{trend}} = 0.86$). The only estrogen metabolite positively associated with risk was 17-epiestriol, which is a metabolite in the 16-hydroxylation pathway (RR_{Q4 vs. Q1}= 1.74; 95% CI, 1.08–2.81; $P_{\text{trend}} = 0.01$). Interestingly, when comparing the highest quartile with the lowest quartile, elevated urinary concentrations of estrone and estradiol were associated with 48% and 49% reductions in risk, respectively (RR for estrone = 0.52; 95% CI, 0.30–0.88; RR for estradiol = 0.51; 95% CI, 0.30–0.86). Additionally, elevated ratio of 16-pathway estrogen metabolites to parent estrogen metabolites was associated with higher risk (RR_{Q4 vs. Q1}= 1.61; 95% CI, 0.99–2.62; $P_{\text{trend}} = 0.04$), and parent estrogen metabolites to non-parent estrogen metabolites ratio was negatively linked to risk (RR_{Q4 vs. Q1}= 0.58; 95% CI, 0.35–0.96; $P_{\text{trend}} = 0.03$). It is difficult to interpret these findings since the directions of the associations for the primary estrogens in the urine are not in agreement with that observed in circulating primary estrogens. Also, due to a lack of research done in this area, sufficient data do not exist for comparison. One possible explanation for these results is that higher levels of primary estrogens are excreted into urine prior to finding any chance to convert into genotoxic metabolites. Also, one may speculate that urine is not as relevant as blood to breast tissue exposure.

Recently, two systematic reviews and combined analyses have examined the relationships between circulating or urinary 2-hydroxyestrone, 16 α -hydroxyestrone, and their ratio with breast cancer risk. Obi *et al.* [119], in a study of 682 premenopausal cases and 1027 matched controls, concluded that the urinary 2/16-hydroxyestrone ratio, but not circulating levels, is non-significantly associated with lower risk of breast cancer (range of ORs=0.5–0.75; 95% CI, 0.25–1.01 and 0.35–1.62; respectively) (Table 3). Similarly, Dallal *et al.* [118], in a combined analysis of 726 women ($n=183$ cases) demonstrated that elevated urinary 2/16-hydroxyestrone is suggestive of lower breast cancer risk (OR_{top tertile vs. low tertile}= 0.74; 95% CI, 0.45–1.23) (Table 3). Additionally, data from the same analysis showed that higher urinary 2/16-hydroxyestrone is indicative of decreased risk of breast cancer for ER– cases (OR_{top tertile vs. low tertile}= 0.33; 95% CI, 0.13–0.84). This latter finding is based on small number of 31 cases; therefore, it may be due to chance and needs more research to be confirmed. A summary of data for both circulating and urinary estrogens and their metabolites is presented in Table 3.

5. Conclusions

There is currently convincing evidence that endogenous estrogens are associated with breast cancer in postmenopausal women; however, this relationship has not been firmly established in premenopausal women, possibly due to the large variations in hormone levels during the menstrual cycle, the small number of studies that have been performed, and the small number of cases. Of recent interest are genetic polymorphisms in the enzymes involved in estrogen metabolism that may modify breast cancer risk in relation to sex hormones.

The role of estrogen metabolites in the etiology of breast cancer has been studied, but available data are mixed and no firm conclusion can be drawn in either pre- or postmenopausal women. Developments in mass spectrometry have greatly enhanced the sensitivity and specificity in the quantification of estrogens and estrogen metabolites in both blood and urine.

Further studies with particular attention to factors such as hormone receptor subtype, controlling for hormones variations during menstrual cycle in premenopausal women, are needed to improve our understanding of the importance of estrogen to breast cancer risk. Taken together, there are not sufficient data to confirm the role of estrogen metabolites as predictors of breast cancer, but it can be concluded at this point that any intervention which leads to reduced circulating levels of primary estrogens have the potential to lower the risk of breast cancer in postmenopausal women.

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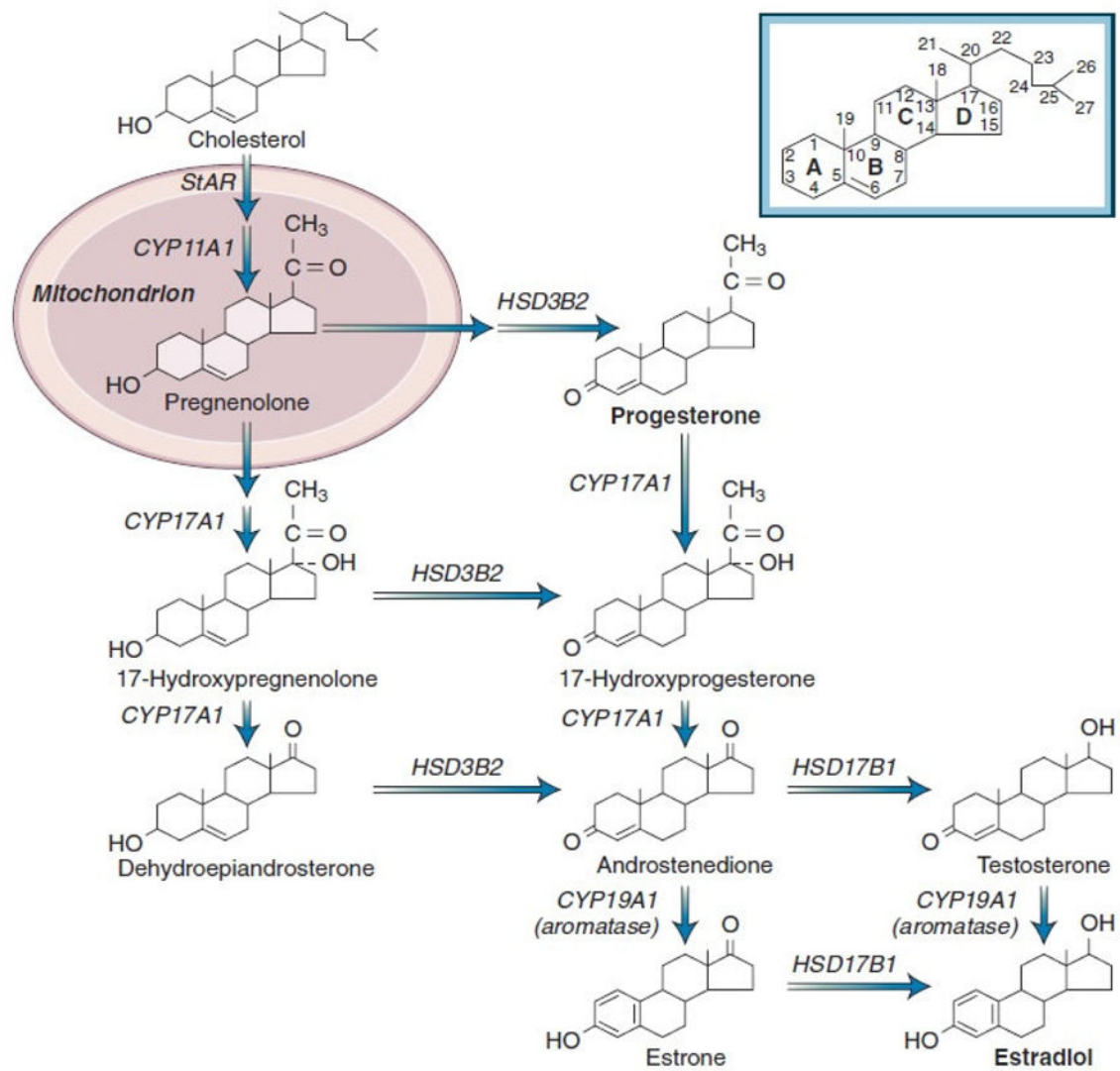


Figure 1. Pathways of steroid hormone synthesis in humans

Abbreviations: StAR, steroidogenic acute regulatory protein; CYP11A1, side-chain cleavage of P450; CYP17A1, 17-hydroxylase/17,20-lyase; HSD3B2, 3 β -hydroxysteroid dehydrogenase- 5,4 isomerase type 2; ; CYP19A1, aromatase; HSD17B1, 17 β -hydroxysteroid dehydrogenase type 1 [13].

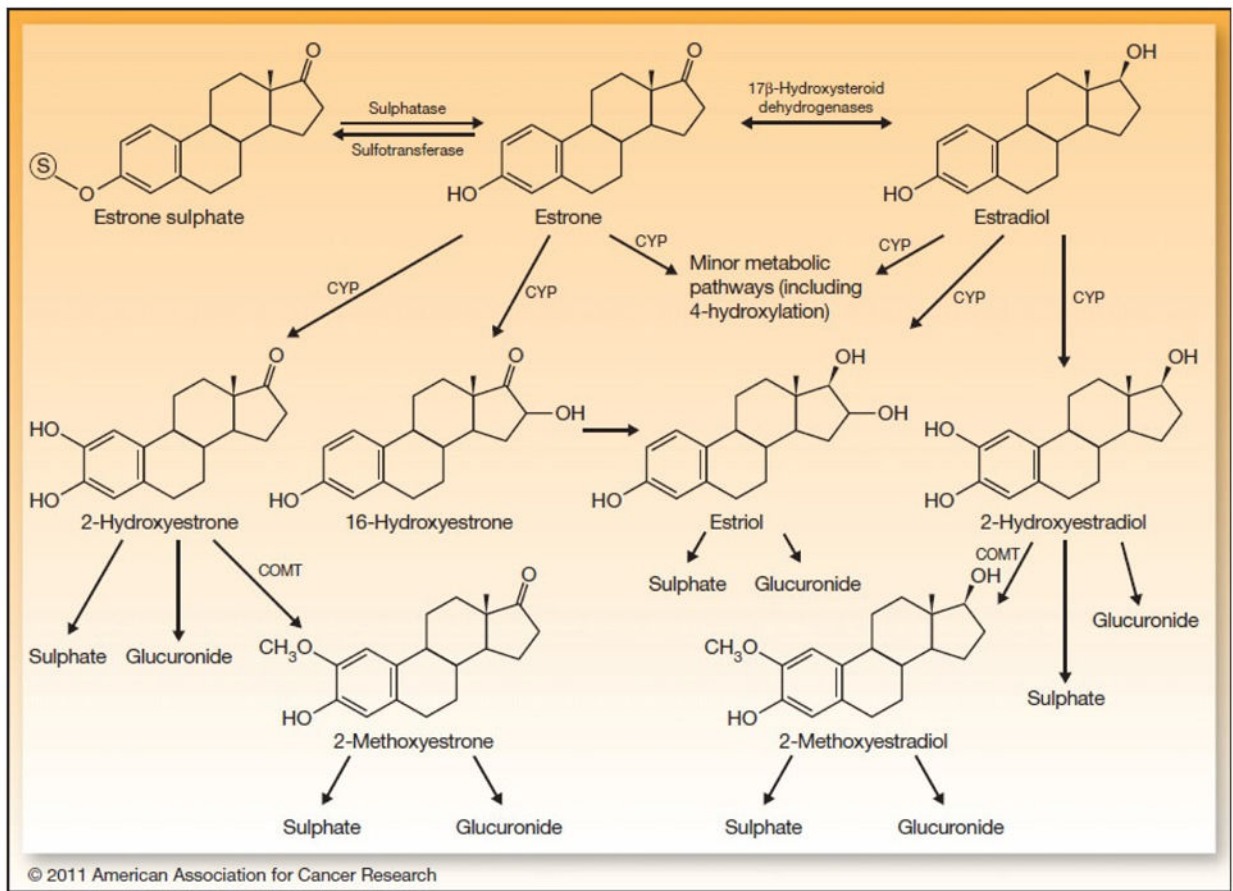


Figure 2. Endogenous estrogen metabolism in human

The parent estrogens estrone and estradiol are reversibly inter-converted, catalyzed by the 17 β -hydroxysteroid dehydrogenase enzyme. They are also converted to catechol estrogens including 2-hydroxestrogens, 4-hydroxestrogens, 16-hydroxestrone, or estriol through the action of CYP enzymes. Catechol estrogens, in turn, are metabolized to 2-methoxyestrogens and 4-methoxyestrogens. Estrone, catechol estrogens, and methoxyestrogens can be conjugated to glucuronic acid and sulfate.

Abbreviations: COMT, catechol-O-methyltransferase; CYP, cytochrome P-450 enzyme [114].

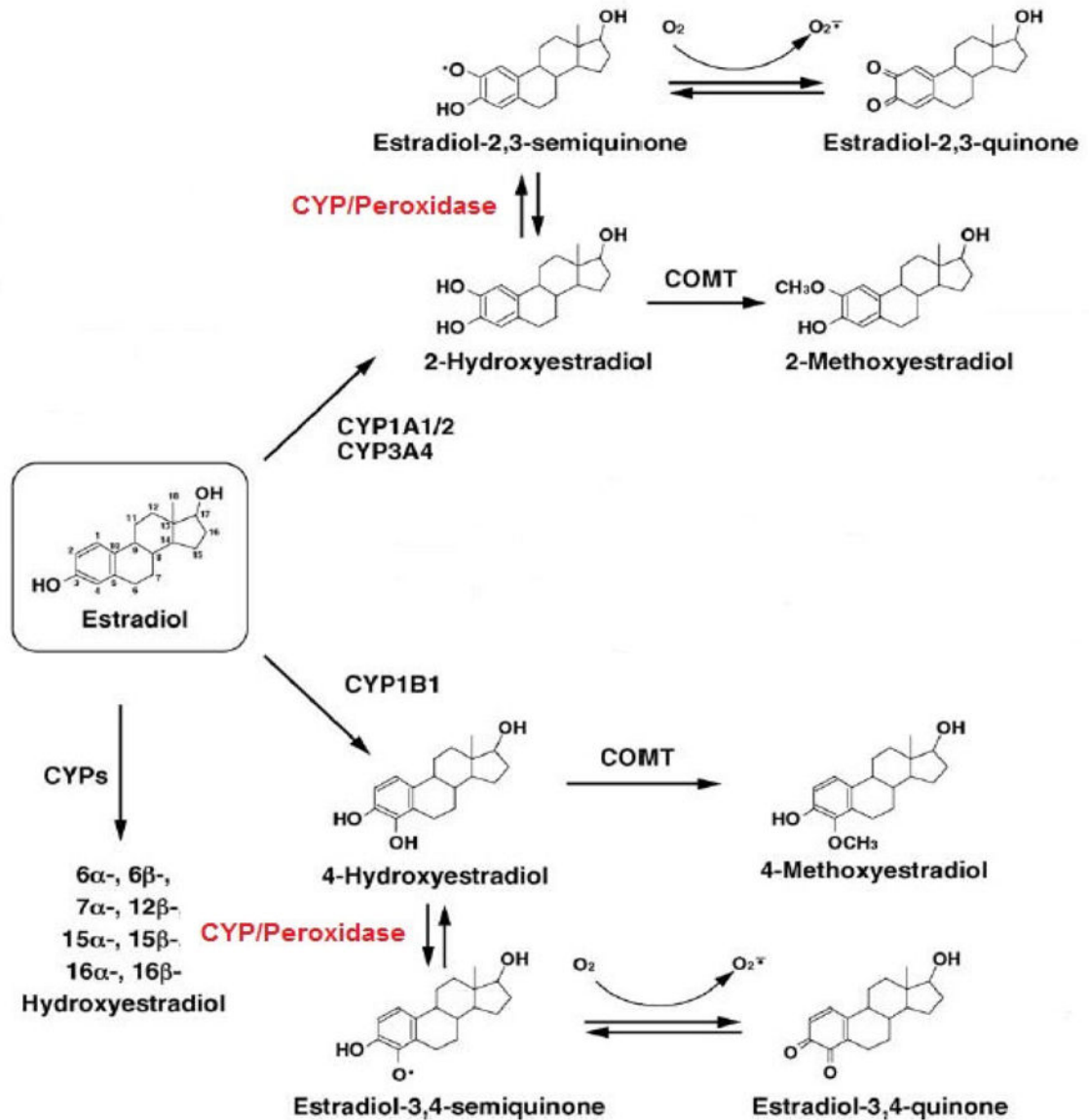


Figure 3. Estradiol metabolism and DNA adduct formation

Estradiol catechol estrogens, including 2-hydroxyestradiol and 4-hydroxyestradiol, can go through reductive-oxidative cycling and produce mutagenic free radicals. These reactions are catalyzed by CYP and peroxidase enzymes. Estrogen semiquinones and quinones are reactive and carcinogenic intermediate metabolites of redox cycling pathways and can cause DNA damage.

Abbreviations: CYP, cytochrome P-450 enzyme; COMT, catechol-O-methyltransferase [17].

Table 1

Risk estimates for estrogens and breast cancer risk in postmenopausal women in studies of over 300 cases only.

First author, year	Hormone	Cases/controls	Design/Biospecimen	RR (95% CI) for the top vs. bottom hormone category level	P _{trend}
Key, 2002 ^{*,†} [90]	Total estradiol	656/1709		2.00 (1.47–2.71)	<0.001
	Free estradiol	478/980		2.58 (1.76–3.78)	<0.001
	Estrone	469/1188		2.19 (1.48–3.22)	<0.001
	Estrone-sulfate	310/651		2.00 (1.26–3.16)	<0.001
	SHBG	373/1160	Pooled analysis/blood	0.66 (0.43–1.00)	0.041
	Androstenedione	375/1000		2.15 (1.44–3.21)	<0.001
	DHEA	231/423		2.04 (1.21–3.45)	0.018
	DHEAS	578/1501		1.75 (1.26–2.43)	0.002
	Testosterone	585/1574		2.22 (1.59–3.10)	<0.001
	Estradiol			2.1 (1.5–3.2)	<0.001
Missmer, 2004 ^{††} [92]	Free estradiol			1.9 (1.2–2.9)	<0.001
	Estrone			1.7 (1.1–2.6)	<0.001
	Estrone-sulfate			2.4 (1.6–3.8)	<0.001
	SHBG	322/643	Nested case-control study within the Nurses' Health Study/blood	0.8 (0.6–1.3)	0.14
	Androstenedione			1.5 (1.0–2.3)	0.04
	DHEA			1.4 (0.9–2.2)	0.02
	DHEAS			1.7 (1.1–2.7)	0.003
	Testosterone			1.6 (1.0–2.4)	<0.001
				HR (95% CI)	P value
	Manson, 2013 ^{†††} [99]	Treatment intervention	# of BC events in treatment/placebo	Design	
CEE (Premarin)		168/216	RCT	0.79 (0.65–0.97)	0.02
	CEE+MPA (Prempro)	434/323		1.28 (1.11–1.48)	<0.001

Abbreviations: SHBG, sex hormone binding globulin; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; RR, relative risk; HR, hazard ratio; CI, confidence interval; CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; RCT, randomized controlled trial; BC, breast cancer.

* From individual studies cited in references [82–89], [124], [127], and [140–142]. Reported relative risks are based on quintile comparisons.

† Cases and controls are matched on different factors such as age, date of blood collection, etc.

†† Cases and controls are matched on age and date and time of blood collection. Reported relative risks are based on quartile comparisons.

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††† With a median cumulative follow-up of 13 years. Median intervention was 7.2 year for CEE trial and 5.6 years for CEE+MPA trial.

Note: Results from Manjer [91], Kaaks [93], Eliassen [94], Baglietto [95], and Beattie [98] studies are not shown in this table. Number of cases in these studies includes 173, 285, 197, 197, and 135, respectively.

Table 2

Risk estimates for estrogen metabolite pathways or ratios and breast cancer risk in postmenopausal women in studies of over 300 cases only.

First author, year	Estrogen metabolite pathways or ratios*	Cases/controls	Design/Biospecimen	Risk estimates [†] (95% CI) for the top vs. bottom hormone category level	P _{trend}
Dallal, 2013 ^a [118]	2-OHE ₁ /16α-OHE ₁ Ratio	385/723	Combined analysis/urine	1.02 (0.71–1.48)	0.9
Obi, 2011 ^{b,c} [119]	2-OHE ₁ /16α-OHE ₁ Ratio	1189/1888	Systematic review/urine& blood	0.75 (0.35–1.62) to 1.31 (0.53–3.18)	N/A
Dallal, 2014 ^d [121]	2-OHE ₁ /16α-OHE ₁ Ratio	407/496	Case-cohort study within the B-FIT/blood	0.88 (0.59–1.32)	0.8
	2-pathway/16-pathway			0.6 (0.40–0.90)	0.002
	2-pathway/parent estrogens			0.69 (0.46–1.05)	0.01

Abbreviations: N/A, not available; 2-OHE₁, 2-hydroxyestrone; 16α-OHE₁, 16-alpha-hydroxyestrone; CI, confidence interval; PLCO, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; B-FIT, the Breast and Bone Follow-up to the Fracture Intervention Trial.

* Parent estrogens include estrone and estradiol.

2-hydroxylation pathway: 2-hydroxyestrone, 2-methoxyestrone, 2-hydroxyestradiol, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether.

16-hydroxylation pathway: 16α-hydroxyestrone, estriol, 17-epiestriol, 16-ketoestradiol, and 16-epiestriol.

[†] Risk estimates for [118] and [119] references are odds ratios, but risk estimates for [121] reference are hazard ratios.

^a From individual studies cited in references [117], [137], and [143–145]. Reported odds ratio is based on tertile comparison.

^b Range of odds ratios with respective CIs. Reported odds ratios are based on tertile and quintile comparisons, respectively.

^c From individual studies cited in references [117], [137], and [144–148].

^d Reported hazard ratios are based on quintile comparisons.

Note: All studies adjusted or matched for different covariates affecting breast cancer risk. Also, results from Kabat [116], Fuhrman [120], and Falk [122] studies are not shown in this table. Number of cases in these studies includes 42, 277, and 215, respectively.

Table 3

Risk estimates for estrogens and their metabolites and breast cancer risk in premenopausal women: results from meta-analysis, systematic review, or individual studies of over 300 cases only.

First author, year, reference	Hormone	Cases/controls	Design/Biospecimen	Odds ratio (95% CI) for the top vs. bottom hormone category level	P_{trend}
Fortner, 2013 ^a [132]	Estradiol				
	Follicular	462/909		1.0 (0.7 to 1.5)	0.76
	Luteal	479/959		0.9 (0.6 to 1.4)	0.65
	Free estradiol				
Key, 2013 ^{b,c} [136]	Follicular	447/886	Nested case-control/blood	0.8 (0.5 to 1.2)	0.48
	Luteal	469/944		1.0 (0.7 to 1.5)	0.99
	Estrone				
	Follicular	469/920		1.0 (0.7 to 1.4)	0.62
Walker, 2011 ^d [135]	Luteal	500/1005		0.9 (0.7 to 1.3)	0.89
	SHBG	624/1246		1.2 (0.8 to 1.6)	0.23
	Total estradiol	600/1375		1-19 (1-06-1-35)	0-0042
	Free estradiol	587/1341		1-17 (1-03-1-33)	0-014
	Estrone	477/933	Pooled analysis/blood	1-27 (1-05-1-54)	0-014
	SHBG	767/1699		1-07 (0-94-1-23)	0-29
	Androstenedione	569/1177		1-30 (1-10-1-55)	0-0026
	Estradiol	693/1609	Meta-analysis/blood	1-10 (0-96-1-27)	N/A
	2-OHE1/16 α -OHE1 Ratio	183/543	Combined analysis/urine	0.74 (0.45-1.23)	0.25
	2-OHE1/16 α -OHE1 Ratio	682/1027	Systematic review/urine	0.5 (0.25-1.01) to 0.75 (0.35-1.62)	0.05, N/A
Arsian, 2009 ^e [138]	2-OHE1/16 α -OHE1 Ratio	377/377	Nested case-control/blood	1.13 (0.68-1.87)	0.51

Abbreviations: N/A, not available; SHBG, sex hormone binding globulin; 2-OHE1, 2-hydroxyestrone; 16 α -OHE1, 16- α -hydroxyestrone; OR, odds ratio; CI, confidence interval.

^a Reported odds ratio is based on quintile comparisons.

^b ORs for a doubling in concentrations of hormones.

^c From individual studies cited in references [124], [126], and [128-132].

^d From individual studies cited in references [123-128], and [130].

^e From individual studies cited in references [117], [137], and [143]. Reported odds ratio is based on tertile comparison.

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^fRange of ORs with respective CIs. Reported odds ratios are based on tertile comparisons.

^gFrom individual studies cited in references [117], [137], and [146].

^hReported odds ratios is based on quartile comparisons.

Note: All studies adjusted or matched for different covariates affecting breast cancer risk, but not necessarily for phase of menstrual cycle. Studies with less than 300 cases are part of meta-analysis or systematic review studies reported above.