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## Estrogens in the breast tissue: a systematic review

**Lusine Yaghjian** and

Division of Public Health Sciences, Department of Surgery, Washington University in St. Louis School of Medicine, Campus Box 8100, 660 S. Euclid Avenue, St. Louis, MO 63110, USA

**Graham A. Colditz**

Division of Public Health Sciences, Department of Surgery, Washington University in St. Louis School of Medicine, Campus Box 8100, 660 S. Euclid Avenue, St. Louis, MO 63110, USA

Institute for Public Health, Washington University in St. Louis, St. Louis, MO, USA

Lusine Yaghjian: yaghjianl@wudosis.wustl.edu; Graham A. Colditz: colditzg@wudosis.wustl.edu

### Abstract

The role of estrogens in breast carcinogenesis has been investigated at the level of whole body (plasma) and cell (molecular, receptors, etc.). Growing attention focused on the breast tissue being an intracrine organ, with potentially important local estrogen production in the breast. However, very little is known about the local breast tissue estrogen levels. Understanding the role of the tissue estrogens in breast carcinogenesis might open new avenues in breast cancer prevention. This systematic review summarizes published studies that measured local estrogen levels in the breast and offers suggestions for strategies to fill gaps in our existing scientific knowledge.

### Keywords

Estrogen metabolites; Tissue estrogen metabolism; Breast carcinogenesis

### Introduction

The role of estrogens in hormonal carcinogenesis has been investigated over the last few decades in cell culture, animal models, and humans. Numerous studies have linked the use of exogenous hormones to the risk of breast, ovarian, and, possibly, prostate cancer [1, 2]. Both direct and indirect mechanisms support estrogen's contribution to the initiation and promotion of breast cancer. Estradiol and, to lesser degree, other estrogens, increase proliferation of breast epithelium and stroma and, consequently, increase the chances of mutation in rapidly proliferating epithelium [3–11]. Those effects accumulate with increasing cumulative exposure to estrogens [10, 12]. Importance of accumulated exposure to hormones is supported by the increase in breast cancer risk with early menarche, late first full-term pregnancy, and late menopause [12–14].

Some metabolites formed as the result of 4- and 16-hydroxylation of estrogens have genotoxic properties. In contrast, the products of 2-hydroxylation inhibit tumorigenesis [15–

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Correspondence to: Lusine Yaghjian, yaghjianl@wudosis.wustl.edu.

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17]. In addition, estrogen stimulates prolactin secretion and production of local growth promoters (growth factors), implicated in cancer promotion [12, 18–20]. The hormonal microenvironment surrounding the breast tissue may play an important role in the breast carcinogenesis.

Studies relating endogenous hormones to breast health-related outcomes measured hormone levels in urine and/or serum. However, tissue levels of endogenous hormones might be a more relevant measure of exposure of the breast tissue to their hormonal influences [21, 22]. The blood estrogen levels might not reflect the actual levels of estrogens and their metabolites in peripheral tissues, including the breast, due to the local metabolism of estrogens. Active estrogens in the breast are formed as the result of the local estrogen synthesis (aromatization) and the uptake of estrogen sulfates from circulation and their subsequent desulfation [22–25]. Studies in postmenopausal women show that levels of estradiol in uterine endometrium and breast cancer tissues can be 10- to 50-fold higher than those levels in blood [22, 23, 26, 27]. Moreover, the ratios of the major estrogen metabolites also differ between the circulation and the breast tissue [28]. Breast tissue acting as an intracrine organ with local estrogen production highlights the potential importance of this intracrine function [24, 29]. However, only a few studies evaluated breast tissue estrogen levels and their involvement in breast carcinogenesis.

## Estrogen metabolism

Estrogen synthesis takes place primarily in the ovary (especially membrana granulosa and luteinized granulosa cells) in premenopausal women and primarily in peripheral tissues in postmenopausal women [12, 22, 30]. The aromatization of androgens into estrogens is the most important source of estrogens in the breast tissue [22]. Some active estrogens are also formed from circulating estrone sulfate or  $17\beta$ -estradiol sulfate as the result of de-conjugation by sulfatase [1, 22, 31, 32]. Local release of biologically active estrogens from conjugates and their further metabolism prolong the effect of estrogen on peripheral tissues [22]. In addition, concentration of conjugating enzymes in some peripheral tissues is low, which may result in the accumulation of active (unconjugated) metabolites and an increased effect of estrogens on the target tissue [22]. In postmenopausal women, estrogen production is constant in contrast to cyclic production in premenopausal women. Consequently, the exposure of breast tissue to estrogen in postmenopausal women is continuous [22]. This continuous exposure to high local levels of the estrogen could in part explain more consistent findings on the association between the hormones and breast health outcomes in postmenopausal women [33–38].

Estrogen metabolism in the peripheral tissues and that in the liver are different [22, 39, 40]. In peripheral tissues, including the breast, 4-hydroxylation of estrogens is the dominant pathway of estrogen metabolism [22]. Some of the intermediate metabolites in this pathway interact with the estrogen receptor at a reduced dissociation rate compared to estradiol resulting in a longer effect [22, 41]. Semiquinones and quinones formed as the result of 4-hydroxylation of estrogens have DNA-damaging properties realized through non-receptor-mediated mechanisms [22, 42]. The metabolites formed through 2-hydroxylation of estrogens, on the other hand, have much weaker hormonal potency than estradiol; they are rapidly metabolized and suspected to be strong inhibitors of tumor cell proliferation and angiogenesis [22]. Small amounts of estrogens are metabolized through  $16\alpha$ -hydroxylation to  $16\alpha$ -hydroxyestrone ( $16\alpha$ -OHE<sub>1</sub>) which induces genotoxic DNA damage [22, 43–45]. Ratios of estrogen metabolites with different biological properties in tissues can differ from their ratios in the blood [28].

Studies of aromatase activity in the breast showed its presence predominantly in tumoral stroma and adipocytes, while in normal breast tissue, aromatase expression is almost exclusive to fibroblasts of the adipose tissue [24, 28]. Aromatase activity is induced by factors secreted as a result of complex cell–matrix interactions. [24]. Santen et al. have shown that stromal cells in breast carcinomas are the major source of estrogens in the tumor [46]. Later, it was suggested that the local estrogen production by stromal fibroblasts in early stages of breast carcinogenesis drives epithelial expansion (paracrine mechanism, estrogen receptor dependent), which is later replaced or additionally supplied by production of estrogen by epithelial cells themselves (autocrine) [47]. In addition to proliferative effects, estradiol regulates cytokine production and induces recruitment of macrophages and lymphocytes into tumor mass [48, 49]. On the other hand, cytokines produced by macrophages and lymphocytes further stimulate the estradiol production [49]. Thus, breast tumor initiation and progression appear to result from disruption of the normal epithelial–stromal interactions [24].

Several factors could affect the breast tissue levels of estrogens by modifying the local metabolism and changing the relative amounts of metabolites with different biological properties [12, 22, 50]. In previous studies, breast health risk factors reflective of hormonal changes (for example age, menopausal status, parity) were associated with the breast tissue features such as total nuclear area, nuclear area of both epithelial and non-epithelial cells, proportion of collagen, and area of glandular structures indicative of the cell proliferation which might result from the local hormonal changes [51]. Increase in the proportion of breast adipose tissue could influence the estrogen levels by changing the local aromatization rates [52].

Very little is known about the levels of local estrogens in the breast tissue, especially in healthy women, and their association with breast health risk factors. Understanding the role of the tissue estrogens in breast carcinogenesis might open new avenues in breast cancer prevention. The purpose of this review is to summarize published studies that measured local estrogen levels in the breast.

## Literature search

Published studies were identified using the PubMed Central (U.S. National Institutes of Health [NIH]), BioMed Central, Embase, Biosis, and Scopus literature search (through Washington University in St. Louis). We limit this review to studies published between January 1980 and January 2010 that were accessible in full-text format, were published in English, and have measured at least one estrogen metabolite in breast using any type of biospecimen. Articles were searched using the terms “tissue estrogen”, “tissue estrogen measurement”, “tissue estrogen level”. We also searched for studies on specific metabolite, for example, for estradiol, the terms “tissue estradiol”, “tissue estradiol measurement”, “tissue estradiol level” were used. Bibliography of the articles found through electronic searches helped to identify additional relevant references that were then hand-searched. Studies that looked at the effect of aromatase inhibitors on estrogen levels without reporting the estrogen concentrations and studies that focused on analytical method development were excluded from this review.

## Results

We identified 19 eligible studies. In Table 1, we summarize the key characteristics of the studies. The majority of the published studies originated in Europe (73.7%). In most of the studies (84.2%), the biospecimens were obtained from women diagnosed with breast cancer; only three studies attempted to investigate the estrogen levels in healthy cancer-free women.

Most studies were very small: the mean number of participants was 41 (median 34, range 8–164) with the total number of 462 pre- and 323 postmenopausal women across all 19 studies. Study populations differed with respect to women's menopausal status. Most studies (12 or 63.2%) included both pre- and postmenopausal women, 5 studies had only postmenopausal (including perimenopausal) participants, and 2 studies focused only on premenopausal women.

Types of biological samples used to measure the local estrogen levels in the breast included breast tumor tissue [11], normal glandular tissue distant from the tumor site [4], breast fat tissue [3] and adipocytes separated from breast fat tissue [1], breast nipple aspirate [2], perfusate obtained from the breast microdialysis [3], benign breast lesions [1], and benign breast tumor [2]. All studies have reported some details of the specimen collection and processing. In all the studies, either radioimmunoassay or immunoassay techniques were used to measure the estrogens. Most studies (57.9%) measured only one or two estrogen metabolites, while eight investigated up to 4 analytes.

Most studies excluded women who were using hormonal preparations before or at the time of the biospecimen retrieval, but a few studies did not consider use of hormones as an exclusion criterion. More than half of the papers have reported their findings only as mean levels of the estrogen metabolites (57.9%). Half of the studies did not report measures of assay precision and variability (intra- and interassay coefficient of variation).

In Table 2, we present for each study the characteristics of study population, biospecimen source, investigated estrogen metabolites, and assays used for estrogen measurement (see Table 2).

## Key findings from the previous studies

Diversity of the studies with respect to analytical methods of estrogen measurement, study populations, presentation of the results, and types of biological specimens makes comparison across the studies very difficult. Those issues are discussed in details later.

Below, we summarize general findings related to the levels of estrogen metabolites in the breast as presented in Supplementary Table 1.

### Tumor tissue versus blood levels

The levels of estrone ( $E_1$ ) and estradiol ( $E_2$ ) measured in breast tumor tissue from postmenopausal women were markedly higher than their levels in plasma, and the difference was more prominent for estradiol [27, 52–55]. Compared to blood levels, breast tumor tissue concentration of estrone sulfate ( $E_1S$ ) was higher in postmenopausal women but was lower in premenopausal women [27, 54]. In other studies, no correlation was observed between plasma and tissue levels of estradiol and estrone in either pre- or postmenopausal women [52, 55, 56]. A significant positive correlation between estradiol levels in plasma and normal breast tissue was found in postmenopausal women [57]. Across six studies, results remain inconsistent.

### Estrogen levels in pre- versus postmenopausal women

The tissue estradiol concentrations were found to be similar in normal breast tissue from both pre- and postmenopausal women, but the levels were higher in breast tumor tissue from premenopausal women compared to postmenopausal women [55]. However, across six studies that explicitly reported estradiol concentrations in tumor tissue by menopausal status, Mann–Whitney–Wilcoxon test for difference in estradiol levels between pre- and postmenopausal women did not reach statistical significance ( $p > 0.05$ ). Higher levels of

both estrone and estradiol in breast adipocytes/fatty tissue were reported in premenopausal women compared with postmenopausal women; the difference was more pronounced for estradiol [58, 59]. The tissue concentration of estrone sulfate was significantly higher in postmenopausal women compared with premenopausal women and in postmenopausal women.  $E_1S$  was dominating the other tissue estrogens [27]. Across four studies, the pattern differed by the tissue type (normal, fatty, malignant) and by the estrogen metabolite.

### **Estrogen levels in different types of breast tissue**

Tissue estrone concentrations were reported to be higher in fatty or normal tissue compared with the breast tumor tissue [60, 61]. Two studies, however, reported that  $E_1$  concentrations in the normal breast tissue were in most cases lower than those in the tumor tissue [60, 62]. One of the earliest studies found no difference between estrone concentration in the malignant and non-malignant tissues in premenopausal women [55].

Concentration of estradiol was consistently reported to be higher in the breast tumor tissue or tissue with benign breast disease compared with either normal glandular or fatty tissue [32, 52, 59, 60, 63]; sometimes, the difference was more prominent in postmenopausal women [59]. Interestingly, a more recent study demonstrated that the difference between the normal and malignant tissue estradiol levels was dependent on estrogen receptor (ER) status in both pre- and postmenopausal women [61]. An increase in estradiol levels was seen in estrogen receptor-positive breast cancers, contrasting lower estradiol levels in ER-negative breast cancers [55, 61]. Another study found a positive correlation between estradiol concentration and estrogen receptor expression in ER-positive breast cancers, but there was no correlation seen for estrone or estrone sulfate [54]. Higher concentrations of estrone sulfate were found in the breast tumor tissue compared to the normal tissue [32, 54].

Across the studies, the malignant tissue (except that from ER negative tumors) had higher levels of estrogen metabolites compared with normal breast tissue.

### **Correlation between tissue estrogen metabolites**

Thjussen et al. have reported a significant correlation ( $p < 0.01$ ) between estradiol and estrone in the adipose tissue in both pre- and postmenopausal women ( $r = 0.76$  and  $0.73$ , respectively) [60]. In the tumor tissue, however, the significance of correlation was limited to premenopausal women ( $r = 0.56$ ,  $p < 0.05$ ) [60]. Greater levels of estrone compared to estradiol concentrations were found in adipocytes; the difference was more pronounced in postmenopausal women [58]. Concentration of estradiol was higher in the tumor tissue compared to estrone, but in the normal tissue, concentrations of those metabolites were similar [63]. The findings across three studies were inconsistent.

### **Correlation between tissue estrogen metabolites and breast cancer risk factors**

Only two studies examined the correlation of tissue estrogen levels with breast cancer risk factors. In postmenopausal women, both estrone and estradiol were positively and significantly correlated with body mass index (BMI,  $r = 0.48$  and  $0.52$ , respectively) [58]. These findings were similar to the correlation between blood estrogen levels and BMI found in a large cohort [35, 64]. In premenopausal women, both analytes were positively and significantly correlated with time since the last menses ( $r = 0.55$  and  $0.62$ , respectively) and time since last full-term pregnancy and were inversely correlated with use of oral contraceptives and the duration of breastfeeding [52, 58]. In addition, parity and breastfeeding were inversely correlated with estradiol levels [52]. A positive correlation of smoking with estrone levels and a positive correlation of alcohol consumption with estradiol levels in current drinkers were also reported in premenopausal women [52]. The findings from two studies suggest that different breast cancer risk factors can affect breast tissue

estrogen levels in pre- and postmenopausal women, but this issue needs further investigation.

## Discussion

The role of tissue estrogen levels in the breast carcinogenesis and the levels of metabolites in healthy breast tissue remain poorly understood. We have identified 19 published studies that measured a limited number of estrogens in different biological specimens. The use of different assays, standardization approaches, and measurement units makes difficult comparison of the absolute levels of estrogens across the studies. Accessibility to a healthy tissue impedes investigation into the estrogen levels in normal breast of healthy women. As the result, vast majority of the studies were conducted using tissue from breast cancer patients. Exploration of tissue estrogens becomes challenging in cases of small samples, estrogen multiplicity, and low concentrations in biological samples [2]. Below, we discuss methodological issues that make difficult comparison of the results across the studies and could explain inconsistent findings.

As mentioned earlier, most of the studies used tissue specimens from women diagnosed with breast cancer. Some of those studies used the breast tissue distant from the tumor for the comparison. The distant tissue was considered as “normal breast tissue”. In women diagnosed with breast cancer, the changes in hormonal microenvironment of the breast might not necessarily be restricted to the immediate affected area and could be already present in the distant tissue in both ipsilateral and contralateral breast, perhaps to the lesser degree. Only three studies measured estrogens in healthy women (without history of breast cancer); the total number of participants across the studies was 75 (70 pre- and 5 postmenopausal women). In those studies, the samples from premenopausal women were obtained in the luteal phase of their menstrual cycle. It is unknown whether fluctuations of estrogens during the menstrual cycle [1, 52] result in similar cyclic fluctuations in breast tissue estrogen levels. Previous studies showed that a single blood sample is sufficient to estimate woman’s long-term breast cancer risk [65]. However, in the studies of breast health-related outcomes, a single blood sample might not reflect the long-term hormone levels [65, 66]. Although one blood sample might be enough for measurements of estrone sulfate in premenopausal women and would reflect the metabolite’s average levels over a 3-year period, for estrone and estradiol the correlation over time is moderate (interclass correlation range 0.22–0.38) and use of only one sample might result in a measurement error [66]. A single blood or urine sample is reflective of only phase-specific (follicular or luteal) levels of estrogen [67]. Similarly, if the cyclic changes in the breast tissue exist, measurement of tissue estrogens only in the luteal phase might not provide an accurate measure of the woman’s exposure to local estrogens and might underestimate the average estrogen levels and the exposure throughout the menstrual cycle and, consequently, underestimate the total exposure to estrogens in epidemiologic studies.

Levels of circulating estrogens are correlated with BMI in both pre- and postmenopausal women [12, 68–74]. The local aromatization of estrogens takes place in adipose and other peripheral tissues [24]. In obese individuals, increased BMI could induce the cortisol synthesis and thus indirectly affect local estrogen production [52]. For both reasons, the adjustment of estrogen levels for BMI becomes important while comparing the absolute levels of local estrogens across the studies.

The levels of hormones in blood are considerably higher in premenopausal than in postmenopausal women [75, 76], and tissue levels of estrogens in postmenopausal women could greatly exceed their levels in the circulation [22, 23, 26, 27]. Important differences in physiology and estrogen synthesis in pre- and postmenopausal women make important



reporting the results separately for each group. Despite a mixed study population, not all of the studies reported the results by menopausal status [63].

Distribution of endogenous hormones is skewed [68, 75]. Most of the studies on tissue estrogens did not elaborate on the shape of the distribution of tissue estrogens and reported their findings as mean levels. If deviation from normality exists (similar to the circulating estrogens), comparison of the median concentrations becomes more relevant than comparison of the means. However, out of 19 studies, only 4 have reported findings as median levels; one study has reported geometric means.

Characteristics of women in the studies are important for both validity and generalizability of the findings. As mentioned earlier, most of the studies did recognize the importance of excluding women who used hormonal preparations prior to sample retrieval due to their potential altering effect on endogenous hormonal levels. For a similar reason, a few studies have also excluded women with metabolic, hepatic, or endocrine problems [27, 32]. In four of the studies, the participants came from a population with low breast cancer incidence [59, 60, 77] and from an established diet intervention study [78] where women, as reported by the authors, were more physically active and did not reflect the general population. As the result, findings of those studies have a restricted generalizability.

Selection of an appropriate biospecimen is crucial in studies of local breast estrogen levels. Depending on the tissue preparation method, tissue homogenate can either reflect both extracellular and cell bound estrogen levels or represent only intracellular concentrations in contrast to blood levels reflective of extracellular estrogen only [55, 79]. Measurement of estrogens in nipple aspirate fluid might not accurately reflect the exposure of the breast epithelium to estrogens. In addition, woman's capacity to produce nipple aspirate fluid has been previously associated with increased risk of breast cancer. Thus, cancer-free women in such studies might represent a high-risk population with respect to breast cancer [78]. The content of the perfusate obtained from microdialysis of the breast tissue can be affected by tissue pressure, temperature, and molecular size. In a defined study, these parameters are kept constant. However, the perfusate collection conditions may vary across different studies and may affect final perfusate concentrations of metabolites.

Tissue estrogen levels measured in picograms per weight unit (pg/mg) cannot be compared with blood levels measured in picograms per volume unit (pg/mL). Similarly, estrogen levels measured in adipose tissue cannot be compared with those levels in circulation because the concentrations are standardized for the oil content of the tissue (reported per grams of oil). In addition, a higher aromatase activity in the adipose tissue compared with glandular breast tissue [60] could result in higher estrogen levels and overestimate the exposure of the breast epithelium and connective tissue to the local estrogens. Breast tissue retrieved during routine reduction mastectomy or breast surgeries with removal of healthy breast tissue in cancer-free women (breast lift procedures, breast implant exchanges where a "capsulectomy" is performed, correction of constricted or tuberous breast deformity, etc.) might be the best source of biospecimen for studying the local estrogens in healthy women. Separation of the area rich in glandular structures and stroma by a trained pathologist would prevent contamination with the adipose elements of the final sample used for the estrogen assay.

Until recently, the existing assays used to measure estrogens had capacity to measure only a very limited number of metabolites. Disadvantages of the existing methods used to measure endogenous estrogens [2, 32, 53, 55, 62, 80, 81] include poor sensitivity and specificity, insufficient accuracy and/or reproducibility, large quantities of the specimen required for the assay, and degradation of selected estrogens (catechol estrogens) during the intermediate

analytical steps of the assay. In addition, some of the methods are extremely labor-intensive [81, 82].

Majority of the previous studies on tissue estrogens used radioimmunoassays to measure the estrogens. Sometimes, striking differences in the levels of hormones measured with this technique could be accounted for by several methodological issues. Technological advances over the 30-year period could also contribute to the differences across the studies. While some studies conducted in 1980s applied a pre-purification step prior to radioimmunoassay analyses [55], much of the studies conducted at that time performed direct radioimmunoassays. Most commercially available kits for estradiol measurement are unreliable for measurement of low estradiol concentrations (insufficient sensitivity) [83, 84]. In earlier studies, cross-reactivity of estradiol antibodies with estrone was another issue that could affect the results and varied by estrone concentration [83]. Furthermore, non-specific binding (part of bound radioactivity that is not explained by antigen–antibody interaction) can result in falsely low or high concentrations in the samples if non-specific binding differs from that in the assay calibrators [83].

Considerable intra- and interlaboratory variation in plasma estradiol concentrations measured by RIA was previously reported [85]. The assay had a tendency to overestimate low concentrations and to have worse assay precision (intra- and interassay coefficient of variation [CV] at concentrations approaching assay's limit of detection [84, 85]. Assay precision varied significantly across the studies of tissue estrogen levels reporting CVs ranging between 2.8 and 20%. Only four studies reported CVs < 5%. Majority of the reported CVs were within 5–10% range (moderate precision), but few studies reported CVs between 15 and 20%. Precision was the best for estrone, followed by estrone sulfate and estradiol. Most of the studies investigated a very limited number of estrogens. The overall effect of endogenous hormones on the breast could result from a combined effect of metabolites formed as the result of different estrogen metabolism pathways that have similar biological properties rather than a single metabolite. Thus, a summary measure of the exposure might be more relevant for the epidemiologic studies of the carcinogenic effects of estrogens on the breast tissue.

Recently, the Laboratory of Proteomics and Analytical Technologies (LPAT) at National Cancer Institute (NCI) has developed a high-performance liquid chromatography–mass spectrometry (MS) method for measurement of endogenous estrogens in biological specimens (urine, serum, tissue sections, and peritoneal fluid) [81, 82, 86–89]. The method allows to measure simultaneously 15 estrogen metabolites in a relatively small amount of tissue and brings a new approach to measuring exposure to tissue estrogens in epidemiologic studies as well as potential to expand the studies using urine and blood samples to include more estrogen metabolites. The assay's limit of the detection for quantification of estrogens in serum is 8 pg/ mL. The method has shown a good reproducibility in the study on frozen sections from different tissues and with further validation could become widely used in research. Understanding the distribution and effects of tissue estrogens on the risk of breast cancer could open new avenues for breast cancer prevention and development of individualized approaches to the breast cancer prevention.

## Conclusions

Despite an increasing recognition of the intracrine role of the breast tissue, very little is known about the levels of estrogen metabolites in the breast, especially in healthy women, and their effects on breast carcinogenesis. Measurement of local estrogen metabolites has been challenging. However, recent advances in laboratory science pave a way for more



comprehensive investigation into breast estrogen levels and further evaluation of their effects in epidemiologic studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

<b>BMI</b>	Body mass index
<b>CV</b>	Coefficient of variation
<b>LPAT</b>	Laboratory of proteomics and analytical technologies
<b>MS</b>	Mass spectrometry
<b>NCI</b>	National Cancer Institute

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**Table 1**

Summary characteristics of the studies on breast tissue estrogens

Study characteristic	n (%)	Sample size range	Total sample size across all studies
Year			
1980–1989	5 (26.3)	20–164	405
1990–1999	7 (36.8)	8–65	189
2000–2001/2010	7 (36.8)	10–47	191
Study population			
Breast cancer patients	16 (84.2)	8–164	698
Premenopausal	11 (57.9)	3–164	386
Postmenopausal	15 (78.9)	8–64	312
Healthy women	3 (15.8)	12–47	75
Premenopausal	3 (15.8)	11–47	70
Postmenopausal	1 (5.3)	5	5
Breast biospecimen source			
Tumor tissue	12 (63.2)	16–113	451
Adipose (fat) tissue	3 (15.8)	16	48
Adipocytes separated from the tissue	1 (5.3)	65	65
Normal tissue	4 (21.1)	8–64	102
Nipple aspirate	2 (10.5)	47–164	211
Microdialysis perfusate	3 (15.8)	10–16	38
Benign breast lesions	1 (5.3)	113	113
Benign breast tumor	2 (10.5)	113	125
Estrogen assay *			
Radioimmunoassay (RIA)	17 (89.5)	8–164	695
Immunoassay (IA)	4 (21.1)	10–45	83
Estrogen metabolites measured in the study			
1–2	11 (57.9)	8–164	537
3–4	8 (42.1)	12–64	248

\* Two studies used both methods



**Table 2**

Summary description of the studies on tissue estrogen levels

Author	Country	Study population		Total N	N pre-/post-	Biospecimen source	EM assay	EM studied	Comments
		Source	Age (years)						
Beattie et al. [56]	USA	BC	NR	44	27/17	Breast tumor	RIA	E <sub>2</sub>	
Blankenstein et al. [59]	Poland	BC	NR	16	6/10	Breast tumor, breast fat tissue	RIA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S, E <sub>2</sub> S	
Blankenstein et al. [77]	Poland	BC	NR	16	6/10	Breast tumor, fat tissue, normal breast tissue	RIA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S, E <sub>2</sub> S	Participants selected from Polish population with 50% lower breast cancer incidence
Bonney et al. [63]	UK	BC	35-79	20	7/13	Breast tumor	RIA	E <sub>1</sub> , E <sub>2</sub>	None of the participants were taking hormones; three patients had fibroadenomas
Cheritte et al. [32]	France	BC	48-75	14	0/14	Breast tumor, surrounding tissue, distal breast tissue	RIA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S, E <sub>2</sub> S	Women had no history of metabolic, hepatic, endocrine diseases and did not receive treatment in previous 2 months
Ernst et al. [52]	USA	BC, BBD, HC	See comments	164	164/0	Nipple aspirate	RIA	E <sub>1</sub> , E <sub>2</sub>	Mean age ranged 30.6 to 47.0 depending on race and disease status. Predominantly white cohort (>73%), 13% African-American. Woman's capacity to produce nipple aspirate fluid has been previously associated with increased risk of breast cancer. Excluded pregnant, lactating women, women using OCs within 3 months of the sample collection, current thyroid hormone use, and with ethnicity "other". Woman's capacity to produce nipple aspirate fluid has been previously associated with increased risk of BC. High refusal rate (41.2-64.7%)
Garvin et al. [57]	Sweden	BC	51-86	10	0/10	Tissue perfusate from microdialysis	IA	E <sub>2</sub>	Women had no ongoing treatment; all tumors were ER +
Lonning et al. [61]	Norway	BC	31-81	47	34/13	Breast tumor	RIA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S	Women had no HRT for at least 4 weeks before mastectomy and did not use drugs interfering with estrogen disposition
Mady et al. [90]	Egypt	BC, BBT	See comment	45	22/23	Breast tumor (malignant or benign)	RIA/IA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S, E <sub>3</sub>	Mean age ranged between 26 and 57 depending on menopausal status and menstrual cycle phase. 12 women in follicular stage and 10 women in luteal stage; 12 women with benign and 33 women with malignant tumors. Women did not receive hormonal treatment in the 6 months prior to enrollment and did not have bilateral ovariectomy or a history of cancer or liver disease
O'Brien et al. [58]	USA	BC	See comments	65	36/29	Adipocytes separated from breast fat tissue	RIA	E <sub>1</sub> , E <sub>2</sub>	Mean age ranged between 26 and 64 depending on menopausal status and hormone use. 7 premenopausal women were taking low-dose OCs. 10 postmenopausal women were on HRT,

Author	Country	Study population		Biospecimen source	EM assay	EM studied	Comments		
		Source	Age (years)					Total N	N pre-/post-
Pasqualini et al. [27]	France	BC	24–44 48–79	34	11/23	Breast tumor	RIA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S	4 premenopausal women had hysterectomy, and 3 postmenopausal women were taking Tamoxifen or megestrol acetate. 5 pre- and 9 postmenopausal women had previous bilateral oophorectomies
Recchione et al. [53]	Italy	BC	50–85	34	0/34	Breast tumor	RIA	E <sub>2</sub>	Women had no history of endocrine, metabolic, or hepatic diseases and did not use hormones in the last 10 months
Reed, et al. [62]	UK	BC	55–77	8	0/8	Breast tumor, normal tissue	RIA	E <sub>1</sub>	Did not have HRT for at least 6 months, have not been previously treated with chemotherapy or HRT for their disease
Thijssen et al. [60]	Poland	BC	NR	16	6/10	Breast tumor, fat tissue	RIA	E <sub>1</sub> , E <sub>2</sub>	Advanced (stage II and IV) BC patients
Van Landingham et al. [55]	Netherlands	BC, BBL	15–95	113	73/40	Breast tumor, benign lesion, benign tumor	RIA	E <sub>1</sub> , E <sub>2</sub>	Had not received HRT or chemotherapy before operation, participants selected from Polish population with 50% lower BC rates
Vermeulen et al. [54]	Belgium	BC	NR	64	0/64	Breast tumor, normal breast tissue	RIA	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S	Women did not receive HRT for at least 12 months and had no metastases
Chatterton et al. [78]	USA	HW	NR	47	47/0	Nipple aspirate	RIA	E <sub>2</sub> , E <sub>1</sub> S	In the total cohort, the mean age is 34.8 years (range 20–40). Women selected from a predominantly Caucasian cohort (>83%). Women had regular menstrual periods between 25–35 days (samples collected in luteal phase), had not taken OCs for at least 6 months, were not taking any medication interfering with ovarian function, were not pregnant/did not plan pregnancy, and had not lactated for at least 6 months. Subjects were part of the study of hormonal responses to a high-fiber and low-fat diet, were more physically active, self-selected, and may not reflect the general population. Woman's capacity to produce nipple aspirate fluid has been previously associated with increased risk of breast cancer
Dabrosin et al. [79]	Sweden	HW	22–34	12	12/0	Tissue perfusate from microdialysis,	RIA/IA (E <sub>2</sub> )	E <sub>1</sub> , E <sub>2</sub> , E <sub>1</sub> S,	Women were free of medication containing hormones for at least 3 months and had regular menstrual periods (27–34 days, samples collected in luteal phase), no previous breast disease or surgery. Diffusion of substances during microdialysis can be affected by tissue temperature, tissue pressure and molecular size microdialysis sample might not be reflective of

Author	Country	Study population		Age (years)	Total N	Biospecimen source		EM assay	EM studied	Comments
		Source	HW			N pre-/post-	IA			
Dabrosin et al. [20]	Sweden	HW	22–30	60–65	16	11/5	Tissue perfusate from microdialysis, breast biopsy	IA	E2	absolute concentrations in the tissue. Estrone was not detectable in microdialysis sample. Women were free of medication containing hormones for at least 3 months; premenopausal women had regular menstrual cycles (27–34 days, samples collected in luteal phase from 7 women and in follicular phase from 4 women). Diffusion of substances during microdialysis can be affected by tissue temperature, tissue pressure and molecular size microdialysis sample might not be reflective of absolute concentrations in the tissue

*Abbreviations:* RIA radioimmunoassay, IA immunoassay, OCs oral contraceptives, HRT hormone replacement therapy, BC<sup>+</sup>breast cancer, EM estrogen metabolites, NR not reported, Pre premenopausal, Post postmenopausal, E1 estrone, E2 estradiol, E3 estrone sulfate, E2S estradiol sulfate, E3 estriol, BC<sup>-</sup>breast cancer patients, BBT patients with benign breast tumor, HW/healthy women (cancer-free), BBL patients with benign breast lesions, HC healthy controls came from departments in allergology, dermatology, ear, nose and throat; eye; gastrointestinal; general internal medicine; gynecology (routine visits); orthopedics, plastic and reconstructive surgery (other than breast); screening and acute care, surgery; urology; and vascular