

Estrogens in the breast tissue: a systematic review

Lusine Yaghjyan · Graham A. Colditz

Received: 21 June 2010 / Accepted: 17 January 2011
© Springer Science+Business Media B.V. 2011

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

Abbreviations

BMI Body mass index
CV Coefficient of variation
LPAT Laboratory of proteomics and analytical technologies

Electronic supplementary material The online version of this article (doi:10.1007/s10552-011-9729-4) contains supplementary material, which is available to authorized users.

L. Yaghjyan (✉) · G. 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
e-mail: yaghjyanl@wudosis.wustl.edu

G. A. Colditz
e-mail: colditzg@wudosis.wustl.edu

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

MS Mass spectrometry
NCI National Cancer Institute

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 [15–17]. 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 β -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 α -hydroxylation to 16 α -hydroxyestrone (16 α -OHE₁) 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)

Table 1 Summary characteristics of the studies on breast tissue estrogens

Study characteristic	<i>n</i> (%)	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

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].

Table 2 Summary description of the studies on tissue estrogen levels

Author	Country	Study population		Biospecimen source		EM assay	EM studied	Comments	
		Source	Age (years)	Total N	N pre-/post-				
Beattie et al. [56]	USA	BC	NR	44	27/17	Breast tumor	RIA	E ₂	Participants selected from Polish population with 50% lower breast cancer incidence None of the participants were taking hormones; three patients had fibroadenomas Women had no history of metabolic, hepatic, endocrine diseases and did not receive treatment in previous 2 months 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%) Women had no ongoing treatment; all tumors were ER + Women had no HRT for at least 4 weeks before mastectomy and did not use drugs interfering with estrogen disposition 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
Blankenstein et al. [59]	Poland	BC	NR	16	6/10	Breast tumor, breast fat tissue	RIA	E ₁ , E ₂ , E ₁ S, E ₂ S	
Blankenstein et al. [77]	Poland	BC	NR	16	6/10	Breast tumor, fat tissue, normal breast tissue	RIA	E ₁ , E ₂ , E ₁ S, E ₂ S	
Bonney et al. [63]	UK	BC	35–79	20	7/13	Breast tumor	RIA	E ₁ , E ₂	
Chetrite et al. [32]	France	BC	48–75	14	0/14	Breast tumor, surrounding tissue, distal breast tissue	RIA	E ₁ , E ₂ , E ₁ S, E ₂ S	
Ernster et al. [52]	USA	BC, BBD, HC	See comments	164	164/0	Nipple aspirate	RIA	E ₁ , E ₂	
Garvin et al. [57]	Sweden	BC	51–86	10	0/10	Tissue perfusate from microdialysis	IA	E2	
Lonning et al. [61]	Norway	BC	31–81	47	34/13	Breast tumor	RIA	E ₁ , E ₂ , E ₁ S	
Mady et al. [90]	Egypt	BC, BBT	See comment	45	22/23	Breast tumor (malignant or benign)	RIA/IA	E ₁ , E ₂ , E ₁ S, E ₃	

Table 2 continued

Author	Country	Study population		Biospecimen source		EM assay	EM studied	Comments
		Source	Age (years)	Total N	N pre-/post-			
O'Brien et al. [58]	USA	BC	See comments	65	36/29	Adipocytes separated from breast fat tissue	E ₁ , E ₂	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, 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
Pasqualini et al. [27]	France	BC	24–44 48–79	34	11/23	Breast tumor	E ₁ , E ₂ , E ₁ S	Women had no history of endocrine, metabolic, or hepatic diseases and did not use hormones in the last 10 months
Recchione et al. [53]	Italy	BC	50–85	34	0/34	Breast tumor	E ₂	Did not have HRT for at least 6 months, have not been previously treated with chemotherapy or HRT for their disease
Reed, et al. [62]	UK	BC	55–77	8	0/8	Breast tumor, normal tissue	E ₁	Advanced (stage II and IV) BC patients
Thijssen et al. [60]	Poland	BC	NR	16	6/10	Breast tumor, fat tissue	E ₁ , E ₂	Had not received HRT or chemotherapy before operation, participants selected from Polish population with 50% lower BC rates
Van Landenghem et al. [55]	Netherlands	BC, BBL	15–95	113	73/40	Breast tumor, benign lesion, benign tumor	E ₁ , E ₂	
Vermeulen et al. [54]	Belgium	BC	NR	64	0/64	Breast tumor, normal breast tissue	E ₁ , E ₂ , E ₁ 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	E ₂ , E ₁ 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

Table 2 continued

Author	Country	Study population		Biospecimen source	EM assay	EM studied	Comments
		Source	Age (years)	Total N	N pre-/post-		
Dabrosin et al. [79]	Sweden	HW	22–34	12	12/0	Tissue perfusate from microdialysis, RIA/IA (E ₁ , E ₂ , E ₁ S, E ₂)	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 absolute concentrations in the tissue. Estrone was not detectable in microdialysis sample.
Dabrosin et al. [20]	Sweden	HW	22–30 60–65	16	11/5	Tissue perfusate from microdialysis, breast biopsy IA E2	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 breast cancer, EM estrogen metabolites, NR not reported, Pre premenopausal, Post postmenopausal, E₁ estrone, E₂ estradiol, E₁S estrone sulfate, E₂S estradiol sulfate, 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

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

Thijssen 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 $\leq 5\%$. 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.

Acknowledgments Dr. Graham Colditz is supported by ACS Clinical Research Professorship.

Conflict of interests The authors declare that they have no conflict of interests.

References

1. Eliassen AH, Hankinson SE (2008) Endogenous hormone levels and risk of breast, endometrial and ovarian cancers: prospective studies. *Adv Exp Med Biol* 630:148–165
2. Giese RW (2003) Measurement of endogenous estrogens: analytical challenges and recent advances. *J Chromatogr A* 1000(1–2):401–412
3. Pattarozzi A, Gatti M, Barbieri F et al (2008) 17 β -estradiol promotes breast cancer cell proliferation-inducing stromal cell-derived factor-1-mediated epidermal growth factor receptor transactivation: reversal by gefitinib pretreatment. *Mol Pharmacol* 73(1):191–202
4. LaMarca HL, Rosen JM (2007) Estrogen regulation of mammary gland development and breast cancer: amphiregulin takes center stage. *Breast Cancer Res* 9(4):304
5. Russo J, Fernandez SV, Russo PA et al (2006) 17-Beta-estradiol induces transformation, tumorigenesis in human breast epithelial cells. *FASEB J* 20(10):1622–1634
6. Seeger H, Deuringer FU, Wallwiener D, Mueck AO (2004) Breast cancer risk during HRT: influence of estradiol metabolites on breast cancer and endothelial cell proliferation. *Maturitas* 49(3):235–240
7. Johansson CM, Anderson TJ, Bergstrom R, Lindgren A, Persson IR (1998) Epithelial proliferation in the normal human breast in relation to endogenous hormones and oral contraceptive use. *Breast* 7:162–167
8. Sutherland RL, Prall OW, Watts CK, Musgrove EA (1998) Estrogen/progestin regulation of cell cycle progression. *J Mammary Gland Biol Neoplasia* 3(1):63–72
9. Foidart JM, Colin C, Denoo X et al (1998) Estradiol, progesterone regulate the proliferation of human breast epithelial cells. *Fertil Steril* 69(5):963–969
10. Pike MC, Spicer DV, Dahmouch L, Press MF (1993) Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev* 15(1):17–35
11. Henderson BE, Feigelson HS (2000) Hormonal carcinogenesis. *Carcinogenesis* 21(3):427–433
12. Clemons M, Goss P (2001) Estrogen and the risk of breast cancer. *N Engl J Med* 344(4):276–285
13. Rosner B, Colditz GA (1996) Nurses' health study: log-incidence mathematical model of breast cancer incidence. *J Natl Cancer Inst* 88(6):359–364
14. Paffenbarger RS Jr, Kampert JB, Chang HG (1980) Characteristics that predict risk of breast cancer before, after the menopause. *Am J Epidemiol* 112(2):258–268
15. Zhu BT, Conney AH (1998) Functional role of estrogen metabolism in target cells: review, perspectives. *Carcinogenesis* 19(1):1–27
16. Westerlind KC, Gibson KJ, Evans GL, Turner RT (2000) The catechol estrogen, 4-hydroxyestrone, has tissue-specific estrogen actions. *J Endocrinol* 167(2):281–287

17. Telang NT, Suto A, Wong GY, Osborne MP, Bradlow HL (1992) Induction by estrogen metabolite 16 α -hydroxysterone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. *J Natl Cancer Inst* 84(8):634–638
18. McCormack VA, Dowsett M, Folkard E et al (2009) Sex steroids, growth factors and mammographic density: a cross-sectional study of UK postmenopausal Caucasian and Afro-Caribbean women. *Breast Cancer Res* 11(3):R38
19. Levina VV, Nolen B, Su Y et al (2009) Biological significance of prolactin in gynecologic cancers. *Cancer Res* 69(12):5226–5233
20. Dabrosin C (2005) Positive correlation between estradiol and vascular endothelial growth factor but not fibroblast growth factor-2 in normal human breast tissue in vivo. *Clin Cancer Res* 11(22):8036–8041
21. Tamimi RM, Byrne C, Colditz GA, Hankinson SE (2007) Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 99(15):1178–1187
22. Zhu BT (1998) Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* 19:1–27
23. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC (2002) Production and actions of estrogens. *N Engl J Med* 346(5):340–352
24. Sasano H, Miki Y, Nagasaki S, Suzuki T (2009) In situ estrogen production, its regulation in human breast carcinoma: from endocrinology to intracrinology. *Pathol Int* 59(11):777–789
25. Sasano H, Nagasaki S, Miki Y, Suzuki T (2009) New developments in intracrinology of human breast cancer: estrogen sulfatase and sulfotransferase. *Ann N Y Acad Sci* 1155:76–79
26. Recchione CVE, Manzari A, Cavalleri A, Martinetti A, Secreto G (1995) Testosterone, dihydrotestosterone and oestradiol levels in postmenopausal breast cancer tissues. *Steroid Biochem Molec Biol* 52(6):541–546
27. Pasqualini JR, Chetrite G, Blacker C et al (1996) Concentrations of estrone, estradiol, estrone sulfate, evaluation of sulfatase, aromatase activities in pre-and postmenopausal breast cancer patients. *J Clin Endocrinol Metab* 81(4):1460–1464
28. Geisler J (2003) Breast cancer tissue estrogens and their manipulation with aromatase inhibitors and inactivators. *J Steroid Biochem Mol Biol* 86:245–253
29. Labrie F, Luu-The V, Labrie C et al (2003) Endocrine, intracrine sources of androgens in women: inhibition of breast cancer, other roles of androgens, their precursor dehydroepiandrosterone. *Endocr Rev* 24(2):152–182
30. Guillemette C, Belanger A, Lepine J (2004) Metabolic inactivation of estrogens in breast tissue by UDP-glucuronosyltransferase enzymes: an overview. *Breast Cancer Res* 6(6):246–254
31. Shatalova EG, Walther SE, Favorova OO, Rebbeck TR, Blanchard RL (2005) Genetic polymorphisms in human SULT1A1 and UGT1A1 genes associate with breast tumor characteristics: a case-series study. *Breast Cancer Res* 7(6):R909–R921
32. Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR (2000) Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. *J Steroid Biochem Mol Biol* 72(1–2):23–27
33. Riza E, dos Santos Silva I, De Stavola B et al (2001) Urinary estrogen metabolites, mammographic parenchymal patterns in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 10(6):627–634
34. Greendale GA, Palla SL, Ursin G et al (2005) The association of endogenous sex steroids and sex steroid binding proteins with mammographic density: results from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study. *Am J Epidemiol* 162(9):826–834
35. Tamimi RM, Hankinson SE, Colditz GA, Byrne C (2005) Endogenous sex hormone levels and mammographic density among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 14(11 Pt 1):2641–2647
36. Bremnes Y, Ursin G, Bjurstam N et al (2007) Endogenous sex hormones, prolactin and mammographic density in postmenopausal Norwegian women. *Int J Cancer* 121(11):2506–2511
37. Johansson H, Gandini S, Bonanni B et al (2008) Relationships between circulating hormone levels, mammographic percent density, breast cancer risk factors in postmenopausal women. *Breast Cancer Res Treat* 108(1):57–67
38. Thomas H, Reeves G, Key T (1997) Endogenous estrogen and postmenopausal breast cancer: a quantitative review. *Cancer Causes Control* 8(6):922–928
39. Sheila Dawling DLH, Nady R, Fritz FP (2004) In vitro model of mammary estrogen metabolism: structural and kinetic differences between catechol estrogens 2- and 4-hydroxyestradiol. *Chem Res Toxicol* 17:1258–1264
40. Leane Lehmann JW (2008) Gene expression of 17 β -estradiol-metabolizing isozymes: comparison of normal human mammary gland to normal human liver and to cultured human breast adenocarcinoma cells. *Adv Exp Med Biol Hormonal Carcinogenesis* V 617:617–624
41. Rosselli MDR (2006) Estrogen metabolism and reproduction—is there a relationship? *J für Fertilität und Reproduktion* 16:19–23
42. Liehr JG (2001) Genotoxicity of the steroidal oestrogens estrone and oestradiol: possible mechanism of uterine and mammary cancer development. *Human Reproduction Update* 2001 7(3):273–281
43. Westerlind KCGK, Evans GL, Turner RT (2000) The catechol estrogen, 4-hydroxyestrone, has tissue-specific estrogen actions. *J Endocrinol* 167:281–287
44. Telang NTSA, Wong GY, Osborne MP, Bradlow HL (1992) Induction by estrogen metabolite 16 α -hydroxysterone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. *J Natl Cancer Inst* 84:634–638
45. Osborne MPBH, Wong GYC, Telang NT (1993) Upregulation of estradiol C16 α -hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. *J Natl Cancer Inst* 85:1917–1920
46. Santen RJ, Santner SJ, Pauley RJ et al (1997) Estrogen production via the aromatase enzyme in breast carcinoma: which cell type is responsible? *J Steroid Biochem Mol Biol* 61(3–6):267–271
47. Shekhar M, Pauley R, Heppner G (2003) Host microenvironment in breast cancer development: Extracellular matrix-stromal cell contribution to neoplastic phenotype of epithelial cells in the breast. *Breast Cancer Res* 5(3):130–135
48. Reed MJ, Purohit A (1997) Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis. *Endocr Rev* 18(5):701–715
49. Roy D, Cai Q, Felty Q, Narayan S (2007) Estrogen-induced generation of reactive oxygen, nitrogen species, gene damage, estrogen-dependent cancers. *J Toxicol Environ Health B Crit Rev* 10(4):235–257
50. Mueck AO, Seeger H (2007) Breast cancer: are oestrogen metabolites carcinogenic? *Maturitas* 57(1):42–46
51. Li T, Sun L, Miller N et al (2005) The association of measured breast tissue characteristics with mammographic density, other risk factors for breast cancer. *Cancer Epidemiol Biomarkers Prev* 14(2):343–349
52. Ernster VL, Wrensch MR, Petrakis NL et al (1987) Benign, malignant breast disease: initial study results of serum, breast fluid analyses of endogenous estrogens. *J Natl Cancer Inst* 79(5):949–960
53. Recchione C, Venturelli E, Manzari A et al (1995) Testosterone, dihydrotestosterone, oestradiol levels in postmenopausal breast cancer tissues. *J Steroid Biochem Mol Biol* 52(6):541–546
54. Vermeulen A, Deslypere JP, Paridaens R et al (1986) Aromatase, 17 β -hydroxysteroid dehydrogenase, intratissular sex hormone

- concentrations in cancerous, normal glandular breast tissue in postmenopausal women. *Eur J Cancer Clin Oncol* 22(4):515–525
55. van Landeghem AA, Poortman J, Nabuurs M, Thijssen JH (1985) Endogenous concentration, subcellular distribution of estrogens in normal, malignant human breast tissue. *Cancer Res* 45(6):2900–2906
 56. Beattie CW, Briele HA, Hart G, Das Gupta TK (1983) Estradiol and progesterone in human breast cancer cytosols and their relationship to receptor incidence. *Cancer Invest* 1(3):199–206
 57. Garvin S, Dabrosin C (2008) In vivo measurement of tumor estradiol and vascular endothelial growth factor in breast cancer patients. *BMC Cancer* 8:73
 58. O'Brien SN, Anandjiwala J, Price TM (1997) Differences in the estrogen content of breast adipose tissue in women by menopausal status, hormone use. *Obstet Gynecol* 90(2):244–248
 59. Blankenstein MA, Maitimu-Smeele I, Donker GH et al (1992) On the significance of in situ production of oestrogens in human breast cancer tissue. *J Steroid Biochem Mol Biol* 41(3–8):891–896
 60. Thijssen JH, Blankenstein MA, Donker GH, Daroszewski J (1991) Endogenous steroid hormones and local aromatase activity in the breast. *J Steroid Biochem Mol Biol* 39(5B):799–804
 61. Lonning PE, Helle H, Duong NK et al (2009) Tissue estradiol is selectively elevated in receptor positive breast cancers while tumour estrone is reduced independent of receptor status. *J Steroid Biochem Mol Biol* 117(1–3):31–41
 62. Reed MJ, Aherne GW, Ghilchik MW, Patel S, Chakraborty J (1991) Concentrations of oestrone and 4-hydroxyandrostenedione in malignant and normal breast tissues. *Int J Cancer* 49(4):562–565
 63. Bonney RC, Reed MJ, Davidson K, Beranek PA, James VH (1983) The relationship between 17 beta-hydroxysteroid dehydrogenase activity, oestrogen concentrations in human breast tumours, in normal breast tissue. *Clin Endocrinol (Oxf)* 19(6):727–739
 64. Hankinson SE, Willett WC, Manson JE et al (1995) Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. *J Natl Cancer Inst* 87(17):1297–1302
 65. Hankinson SE, Eliassen AH (2007) Endogenous estrogen, testosterone, progesterone levels in relation to breast cancer risk. *J Steroid Biochem Mol Biol* 106(1–5):24–30
 66. Missmer SA, Spiegelman D, Bertone-Johnson ER et al (2006) Reproducibility of plasma steroid hormones, prolactin, insulin-like growth factor levels among premenopausal women over a 2-to 3-year period. *Cancer Epidemiol Biomarkers Prev* 15(5):972–978
 67. Michaud DS, Manson JE, Spiegelman D et al (1999) Reproducibility of plasma, urinary sex hormone levels in premenopausal women over a one-year period. *Cancer Epidemiol Biomarkers Prev* 8(12):1059–1064
 68. Thomas HV, Key TJ, Allen DS et al (1997) A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on the island of Guernsey. *Br J Cancer* 75(7):1075–1079
 69. Boyapati SM, Shu XO, Gao YT et al (2004) Correlation of blood sex steroid hormones with body size, body fat distribution, other known risk factors for breast cancer in post-menopausal Chinese women. *Cancer Causes Control* 15(3):305–311
 70. Potischman N, Swanson CA, Siiteri P, Hoover RN (1996) Reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. *J Natl Cancer Inst* 88(11):756–758
 71. Kopelman PG, Pilkington TR, White N, Jeffcoate SL (1980) Abnormal sex steroid secretion, binding in massively obese women. *Clin Endocrinol (Oxf)* 12(4):363–369
 72. Zumoff B (1982) Relationship of obesity to blood estrogens. *Cancer Res* 42(8 Suppl):3289s–3294s
 73. De Pergola G, Giorgino F, Cospite MR et al (1993) Relation between sex hormones, serum lipoprotein, lipoprotein(a) concentrations in premenopausal obese women. *Arterioscler Thromb* 13(5):675–679
 74. Grenman S, Ronnema T, Irjala K, Kaihola HL, Gronroos M (1986) Sex steroid, gonadotropin, cortisol, prolactin levels in healthy, massively obese women: correlation with abdominal fat cell size, effect of weight reduction. *J Clin Endocrinol Metab* 63(6):1257–1261
 75. Van Beek AP, de Ruijter-Heijstek FC, Jansen H, Erkelens DW, de Bruin TW (2004) Sex steroids, plasma lipoprotein levels in healthy women: The importance of androgens in the estrogen-deficient state. *Metabolism* 53(2):187–192
 76. Caron P, Audet-Walsh E, Lepine J, Belanger A, Guillemette C (2009) Profiling endogenous serum estrogen and estrogen-glucuronides by liquid chromatography-tandem mass spectrometry. *Anal Chem* 81(24):10143–10148
 77. Blankenstein MA, van de Ven J, Maitimu-Smeele I et al (1999) Intratumoral levels of estrogens in breast cancer. *J Steroid Biochem Mol Biol* 69(1–6):293–297
 78. Chatterton RT Jr, Geiger AS, Khan SA et al (2004) Variation in estradiol, estradiol precursors, estrogen-related products in nipple aspirate fluid from normal premenopausal women. *Cancer Epidemiol Biomarkers Prev* 13(6):928–935
 79. Dabrosin C (2005) Increased extracellular local levels of estradiol in normal breast in vivo during the luteal phase of the menstrual cycle. *J Endocrinol* 187(1):103–108
 80. Yue W, Wang JP, Hamilton CJ, Demers LM, Santen RJ (1998) In situ aromatization enhances breast tumor estradiol levels and cellular proliferation. *Cancer Res* 58(5):927–932
 81. Xu X, Veenstra TD, Fox SD et al (2005) Measuring fifteen endogenous estrogens simultaneously in human urine by high-performance liquid chromatography-mass spectrometry. *Anal Chem* 77(20):6646–6654
 82. Falk RT, Xu X, Keefer L, Veenstra TD, Ziegler RG (2008) A liquid chromatography-mass spectrometry method for the simultaneous measurement of 15 urinary estrogens, estrogen metabolites: assay reproducibility, interindividual variability. *Cancer Epidemiol Biomarkers Prev* 17(12):3411–3418
 83. Carlstrom K (1996) Low endogenous estrogen levels—analytical problems and tissue sensitivity. *Acta Obstet Gynecol Scand Suppl* 163:11–15
 84. Nelson RE, Grebe SK, DJ OK, Singh RJ (2004) Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol, estrone in human plasma. *Clin Chem* 50(2):373–384
 85. Bay K, Andersson AM, Skakkebaek NE (2004) Estradiol levels in prepubertal boys, girls—analytical challenges. *Int J Androl* 27(5):266–273
 86. Blonder J, Johann DJ, Veenstra TD et al (2008) Quantitation of steroid hormones in thin fresh frozen tissue sections. *Anal Chem* 80(22):8845–8852
 87. Xu X, Roman JM, Issaq HJ et al (2007) Quantitative measurement of endogenous estrogens and estrogen metabolites in human serum by liquid chromatography-tandem mass spectrometry. *Anal Chem* 79(20):7813–7821
 88. Xu X, Keefer LK, Ziegler RG, Veenstra TD (2007) A liquid chromatography-mass spectrometry method for the quantitative analysis of urinary endogenous estrogen metabolites. *Nat Protoc* 2(6):1350–1355
 89. Xu X, Othman Eel D, Issaq HJ et al (2008) Multiplexed quantitation of endogenous estrogens, estrogen metabolites in human peritoneal fluid. *Electrophoresis* 29(12):2706–2713
 90. Mady EA, Ramadan EE, Ossman AA (2000) Sex steroid hormones in serum and tissue of benign and malignant breast tumor patients. *Dis Markers* 16(3–4):151–157