Exploring Breast Cancer Estrogen Disposition: The Basis for Endocrine Manipulation

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Abstract

Although normal breast tissue and breast cancer estrogens are known to be elevated compared with plasma estrogen levels, the mechanism behind this phenomenon has been an issue of debate for 2 decades. If local estrogen aromatization were to be confirmed as the main estrogen source in breast cancer tissue, tissue-specific inhibition of estrogen production, avoiding systemic side effects, would become a potentially attractive option for breast cancer treatment and prevention. Based on recent results from our groups exploring tissue estrogens, together with estrogen-synthesizing and estrogen-regulated gene expression levels, we propose a new model to explain elevated breast tissue estrogen levels. Although local estrogen production may be important, the local contribution is overruled by rapid plasma-to-tissue equilibration, including active uptake of circulating estrogens or enhanced tissue binding. As for breast cancer tissue levels, elevated levels of estradiol may be explained to a large extent by estrogen receptor binding and local conversion of estrone into estradiol. This model indicates that effective suppression of benign and malignant tissue estrogens as a treatment for ER+ breast cancer requires systemic suppression and will not be markedly affected by local enzyme targeting.

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Introduction

Estrogens play a pivotal role in breast cancer development and sustained tumor growth. Observational studies conducted before the era of estrogen replacement therapy revealed that early loss of ovarian function reduced breast cancer risk by 60% to 70% (1, 2). More recently, a number of groups reported that high postmenopausal plasma estradiol (E2) levels are associated with a subsequent risk of breast cancer (3). In addition, a number of studies have indicated that early loss of ovarian function reduces subsequent breast cancer incidence by 50% (6) among BRCA1 carriers; however, about 80% of the breast cancers that arise in this group are estrogen receptor–negative (ER−) basal-like (7, 8). This finding supports a role for estrogen during the early stages of cancer development. The notion that estrogens sustain the growth of ER+ breast cancer cells is confirmed by the dramatic effects achieved through antihormonal therapy for ER+ breast cancer (9, 10). The stimulation of ER+ breast cancer cell proliferation in vitro by estrogens, particularly E2, strongly suggests that these clinical growth effects result from direct effects of estrogens on ER+ breast cancer and not by intermediary pathways.

Although multiple estrogen metabolites expressing varying estrogen-agonistic activity have been identified, in general 4 estrogen hormones are considered of interest: estradiol (E2), estrone (E1), estrone sulfate (E1S), and the estrone conjugate estrone sulfate (E1S). Although E3 plays a major role in pregnancy, it is of little importance in nonpregnant women and will not be further considered here. Estrone sulfate is derived by sulfate conjugation of E1 and may be deconjugated by sulfatases, leaving conjugated and unconjugated estrogens at equilibrium (11, 12). Estradiol is the active hormone that binds the ER (13) with a high affinity (kDa < 1 nmol/L). Although E1 and E1S are biologically inactive, many tissues express enzymes (sulfatase and reductases) that convert E1S and E1 into active E2 (14, 15).

In premenopausal women, the main estrogen source for breast tissue is circulating E2, which is secreted by the ovaries. At menopause, ovarian production of estrogen ceases. In postmenopausal women, circulating E1, which is produced in different tissues (e.g., skin, soft tissue, muscle, and liver) by aromatization of androstenedione taken up from the circulation, is the main unconjugated plasma estrogen. However, due to conflicting evidence, the
Breast Cancer Estrogen Levels

Translational Relevance

The contribution of local estrogen production to normal breast and breast cancer tissue estrogen levels remains controversial. If local production is the main factor regulating normal breast and breast cancer tissue estrogen levels, tissue-specific inhibition of estrogen production may become an attractive option for breast cancer treatment as well as preventive measures. Measurements obtained by highly sensitive assays together with studies correlating estrogen-regulating enzymes and estrogen-regulated gene expression profiles with tissue estrogen levels, are providing new evidence on the subject. Although local estrogen production may be important, the data suggest a state of rapid plasma-to-tissue equilibrium, including active uptake of circulating estrogens or enhanced tissue binding. As for breast cancer tissue levels, intratumor estradiol correlates with the estrogen receptor level and local conversion of estrone into estradiol. This model indicates that effective suppression of benign and malignant tissue estrogens as a treatment for ER+ breast cancer requires systemic suppression.

Relative contribution from circulating versus locally synthesized estrogens to intratumor E2 levels in postmenopausal women has been controversial for 2 decades. Although intratumor aromatase expression has been detected at mRNA (16) and protein (17) levels, and aromatase activity has been detected in tumor specimens in vitro (18), the quantitative importance of local estrogen production within tumors and adjacent benign tissues versus circulatory uptake has not been defined.

The contribution of locally synthesized versus systemically delivered E2 to intratumor hormone levels may have important therapeutic implications. First, if local aromatization is the main source of intratumor E2, intratumor aromatase expression should predict sensitivity to aromatase inhibitors (19). Second, although the aromatase protein is similar in all body compartments, gene transcription is regulated by tissue-specific promoters (20) that are sensitive to stimulation by different ligands (see below). This raises the possibility of using pharmacologic organ- or tissue-specific estrogen deprivation as an improved means of targeted therapy. For example, breast-specific estrogen deprivation could avoid unwanted side effects in bone and genito-urethral tissue while suppressing breast cancer estrogen levels. Third, a strong quantitative contribution from alternative pathways, such as E1S deconjugation, would in contrast indicate a role for other classes of drugs, such as sulfatase or dehydrogenase inhibitors, in breast cancer therapy.

The aim of this review is to examine the evidence evaluating the contributions from different sources to normal breast tissue and intratumor E2. During this process, we focus on 4 topics. First, we discuss data from recent sensitive and specific analyses of plasma, benign breast, and breast tumor estrogen levels. Second, we compare these findings with previous data from in vivo tracer infusion studies. Third, we correlate the expression of hormone synthesizing enzymes in normal breast and breast cancer tissue with hormone levels, with the aim of identifying the key synthetic pathways regulating estrogen production in breast cancer. Finally, we consider potential correlations between plasma E2 values and intratumor expression of estrogen-regulated genes. An integration of these lines of evidence provides a new perspective on the importance of different compartments for the estrogenic stimulation of ER+ breast cancer.

Plasma Estrogen Levels, Production, and Metabolic Pathways

Although ovarian estrogen synthesis ceases at menopause, E2, E1, and E1S are all detected in the plasma of postmenopausal women.

Estrogens arise through aromatization (Fig. 1) of androgens only (21). The gene coding for the aromatase enzyme (CYP19) contains at least 10 promoters, each of which gives rise to mRNAs with unique untranslated first exons but to identical proteins because the translational start site is located in exon II (20). Of note, these promoters are differentially activated in different tissue compartments, and there are differences between benign and malignant tissues derived from the breast; thus, promoter 1.4 seems to act as the major regulator of aromatase expression in benign breast tissue, in contrast to breast cancers, in which promoters 1.3, 1.7, and PH seem to be the most active. Each tissue has its own active promoters [e.g., 1.3, 1.4, and PI in adipose tissue; 1.1, 1.2, and 2a in the placenta; and 1.4 and 1.6 in bone (see ref. 20 for details and references to original work)]. Although aromatase has been detected in most normal tissue compartments (see references in ref. 21), the quantitative contribution from individual organs and tissue types to circulating estrogens has not been formally addressed.

Once thought in error to be a major source of circulating estrogens in postmenopausal women, the adrenal cortex is the main source of circulating androgens, although conflicting evidence suggests some minor ovarian contribution to circulating testosterone (22–24). Plasma androstenedione (A) and testosterone (T) levels average 5 to 7 nmol/L and 0.5 to 1 nmol/L, respectively, in postmenopausal women (25). Overall, about 1% to 4% of circulating A (and <1% of circulating T) undergoes aromatization (see references to original works in refs. 21 and 26); thus, E1 and E2 synthesis represents the major and minor pathways, respectively, of estrogen production in postmenopausal women. Although to a substantial degree E2 may be synthesized by reduction of E1 (27), the relative contribution from each of these 2 pathways (aromatization of T to E2 versus reduction of E1 to E2) to plasma and tissue E2 has not been formally assessed (Fig. 1).

Except during the first few days of the menstrual cycle, E2 constitutes the major unconjugated plasma estrogen in premenopausal women. In postmenopausal women,

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plasma E1 levels average about 70 to 90 pmol/L, with E2 levels of about 15 to 25 pmol/L, although there is substantial variation among individuals (25, 28). In contrast, after menopause, levels within individuals vary only modestly over a number of years (29). In addition, circulating E1S concentrations may average from 5 to 600 pmol/L (30). Estrone sulfate is synthesized by sulfate conjugation of E1 (31); no direct secretion of estrone sulfate is known to occur.

Estrogens are metabolized and eliminated by a 2-step process (Fig. 2). The first part involves hydroxylations executed by multiple cytochrome P450 enzymes (CYP) (32, 33). The estrogen steroid nucleus may be exposed to hydroxylations at multiple positions (32). The main metabolic pathways involve 2-hydroxylations and (to a lesser degree) 4-hydroxylations, generating catechol estrogens, and hydroxylations at the 16α position, generating 16α-hydroxysterone followed by reduction into E3 (34, 35). Although these enzymes have both E1 and E2 as substrates, some of them express a selective substrate preference for either E1 or E2 (32, 36, 37). Of note, such hydroxylations take place in different body compartments, including breast cancer and liver tissue, partly executed by different members of the CYP family. Thus, whereas estrogen-metabolizing CYPs such as CYP1A2 and CYP3A5 play a major role in metabolizing estrogens in the liver (2- and 16α-hydroxylation in particular), in the breast CYP1A1 and CYP1B1 are the main executors of 2- and 4-hydroxylations, respectively (38, 39). In a second step, these metabolites are subjected to conjugation (Fig. 2). Although a minor amount is sulfate-conjugated and excreted in the bile, followed by substantial intestinal deconjugation and recycling (40), the bulk is subsequently glucuronidated and excreted through the urine (41). Similarly, circulating E1S may be deconjugated by sulfatase and subsequently metabolized as unconjugated E1 (34).

So far, we have gained only a limited understanding of which factors determine plasma and tissue estrogen concentrations in postmenopausal women. Several growth factors and cytokines have been shown to regulate aromatase expression in vitro (42), but little is known with respect to aromatase activity regulation in vivo. No feedback mechanisms regulating plasma estrogen levels have been identified. Although in vivo aromatization of androstenedione correlates with body weight (see references in ref. 21), the correlation between plasma estrogen levels and in vivo aromatase activity is moderate (43), probably due to interindividual variation in the estrogen elimination rate. Germline mutations in the aromatase gene are rare but have severe consequences. Although a single germline gain of function aromatase mutation has been described (44), aromatase deficiency due to inactive mutations that reveal a clinical phenotype resembling inactivating mutations that
affect the ER receptor gene (45) has been described in a few families. Multiple aromatase polymorphisms have been detected, but few of these affect the coding region (46). Of interest, one of these intronic variants was reported to be associated with improved prognosis in patients treated with letrozole (47), and recently 2 variants, located upstream of the promoter area, were found to be associated with enhanced aromatase activity (48).

The different CYPs are subject to multiple polymorphic variants (33). However, although plasma E$_1$ and E$_2$ clearance rates vary substantially (31, 49), so far no distinct CYP polymorphism has been correlated with plasma estrogen levels (33).

Analyzing Plasma and Tissue Estrogen Levels

Before discussing data on estrogen concentrations in detail, we must consider certain methodologic limitations related to plasma and tissue estrogen measurements in postmenopausal women. Measuring estrogen in plasma and particularly in tissue is not trivial, and nonoptimized methodologies have contributed significantly to the variable data in the literature. Circulating estrogen levels in postmenopausal women are among the lowest of all steroids assessed in clinical research or practice. Thus, plasma estrogen assessment requires radioimmunoassays (RIA) specifically derived for that purpose.

Over the years, several studies have evaluated plasma and tissue estrogen levels (see below). Due to the relatively low specific activity of $^3$H-labeled estrogens (50–150 mCi/mmoll), assays based on $^3$H-isotopes in general do not provide the sensitivity required for such purposes. Our groups have made considerable efforts to develop $^{125}$I-based RIAs (specific activity about 2,000 Ci/mmol) for estrogen measurement in the low postmenopausal concentration range (28, 50–53). With such methods, we have achieved sensitivity limits in the range of 0.67 pmol/L, 1.14 pmol/L, and 0.53 pmol/L for plasma E$_2$, E$_1$, and E$_1$S,
respectively (52). Although these methods for plasma (52) and tissue (51) estrogen measurement are highly sensitive, precise, and devoid of cross-reactivity, they are labor-intensive because they require multiple prepurification steps. The need for such sensitive assays, particularly for assessing estrogen levels in patients undergoing treatment with aromatase inhibitors, is illustrated by recent findings (53) revealing that the majority of patients who receive treatment with the third-generation aromatase inhibitors anastrozole or letrozole achieve plasma estrogen levels below these sensitivity limits. Recently, commercial assays such as the DSL-8700 E1 RIA applying 125I-labeled E1, and the Spectria estradiol-sensitive RIA and DSL-4800 ultrasensitive RIA using 125I-labeled E2 have become available. Such kits in general do not involve any chromatographic prepurification procedures, highlighting the need for high specificity of the antibody applied. The theoretical sensitivity limit for DSL-8700 is 4.44 pmol/L, and theoretical sensitivity limits for E2 kits are in the range of 5 to 8.14 pmol/L. Although such assays may determine plasma estrogen levels reliably in the majority of postmenopausal women, some individuals reveal plasma E2 concentrations even below 5 pmol/L.

Many investigators consider methods such as gas chromatography/tandem mass spectrometry (GC/MS-MS) and liquid chromatography/tandem mass spectrometry (LC/MS-MS) as gold standards with respect to estrogen level measurements. However, some MS methods lack sensitivity for determining plasma E2 levels in many postmenopausal women (54), particularly during treatment with estrogen-suppressing compounds. Nonetheless, using GC/MS-MS, Santen and colleagues (55) reported average plasma levels of E2 in postmenopausal women similar to those recorded by our RIA, and others have lower values than most of the sensitive RIAs (56). Although such methods, including assays for plasma E1S and tissue estrogen levels, are in rapid development (57–59), it is clear that the choice and validation of assays based on MS are as important as for those based on RIA.

Method sensitivity problems are even greater when measuring tissue estrogen concentrations. As mentioned above, breast tissue contains enzymes involved in estrogen metabolism. Although major catechol estrogens, such as 2-hydroxyestrone, are rapidly cleared from the circulation (60), the fact that these metabolites may account for about 50% of total estrogen metabolites in the urine (61) indicates a high production rate. In general, we lack knowledge regarding tissue concentrations of estrogen metabolites, but they are a potential source of interference in RIA that must be eliminated during sample preparation for assay.

Benign Breast Tissue Estrogen Concentrations and Synthesis

Although our focus is on the source of estrogens for breast cancers, a brief account of estrogen disposition in benign breast tissue is merited because (i) this is potentially a source of estrogens for the malignant tissue per se, (ii) it is an important comparator for the arguments made with malignant tissue, and (iii) it may be important in considering prevention strategies for breast cancer.

It has been known for 3 decades that E2 levels in breast cancer tissue exceed those in plasma by a factor of about 10; however, the mechanism behind this phenomenon remains obscure. In a previous study (30) we measured tissue estrogen levels using a 125I-based RIA (51) after prepurification through several steps, including high-performance liquid chromatography. Although we detected lower tissue levels of the different estrogen fractions compared with previous studies that applied RIAs without prepurification (62–64), we detected a benign tissue/plasma concentration ratio of E1, the main estrogen product in postmenopausal women, of 6 to 7 (30). This is consistent with the benign tissue/plasma 14C E1 ratio of 5 to 6 found by Larionov and colleagues (65) when they applied presurgical steady-state infusions of 14C-labeled E1 to breast cancer patients.

The benign tissue/plasma gradient »1 (as will be seen for tumor tissue as well) has provoked much discussion about what the potential mechanism might be. Several explanations have been proposed, including active uptake from the circulation, local estrogen synthesis, and enhanced tissue binding. From their studies infusing 3H-androstenedione and 14C E1 in concert, by determining the 3H/14C isotope ratio in the tissue compared with plasma E1 fractions, Larionov and colleagues (65) estimated that local tissue production (based on an elevated 3H/14C ratio) may account for a median of 15% of local E1 concentrations. In light of the potential clinical implications of these findings, we have explored the robustness of a pharmacokinetic model (66, 67). Given the specialized nature of the calculations, we provide a detailed analysis in the Supplementary Material and summarize the key findings here.

Overall, pharmacokinetic modeling is most consistent with a model suggesting rapid plasma/benign tissue exchange of the estrogen compounds, in which case the actual local contribution may not be determined (66). Although an elevated tissue/plasma gradient may be due either to enhanced tissue binding or, alternatively, a mechanism of active uptake of circulating estrogens in the tissue, regrettably there is no pharmacokinetic model by which these 2 processes may be evaluated separately in vivo (67).

This model is supported by recent results from our own studies measuring plasma and benign breast tissue estrogens in pre- and postmenopausal women (Tables 1 and 2). Average benign tissue levels of E1 and E2 in premenopausal women exceed those in postmenopausal women by a factor of about 3 and 15, respectively. On the other hand, the tissue/plasma concentration gradient is remarkably similar in both groups (E1 averaging 5–6, E2 averaging 1.5–2). These findings are consistent with both active uptake and enhanced tissue binding. In premenopausal women, the great majority of plasma E2 is ovarian in origin. Considering the difference in tissue E2 concentrations between pre- and postmenopausal women and the similarity with respect to the tissue/plasma concentration gradient, these...
Table 1. Tissue estrogen levels (fmol/g wet weight) given as geometric mean levels with 95% confidence interval in benign and malignant breast samples from the same breast in correlation with menopausal status and ER status.

<table>
<thead>
<tr>
<th></th>
<th>E2 (benign)</th>
<th>E2 (tumor)</th>
<th>E1 (benign)</th>
<th>E1 (tumor)</th>
<th>E1S (benign)</th>
<th>E1S (tumor)</th>
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<tbody>
<tr>
<td><strong>Postmenopausal women</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Total group</td>
<td>n = 30</td>
<td>29.2 (19.3–232.0)</td>
<td>477.2 (366.7–620.9)</td>
<td>143.7 (93.3–221.3)</td>
<td>53.8 (32.0–90.4)</td>
<td>99.5 (53.8–184.0)</td>
</tr>
<tr>
<td>ER+ subgroup</td>
<td>n = 21</td>
<td>31.1 (19.5–46.7)</td>
<td>475.6 (338.2–616.4)</td>
<td>167.6 (100.1–280.9)</td>
<td>56.1 (29.3–107.4)</td>
<td>98.6 (44.8–217.1)</td>
</tr>
<tr>
<td>ER- subgroup</td>
<td>n = 9</td>
<td>19.3 (6.1–69.6)</td>
<td>407.6 (265.3–676.8)</td>
<td>100.3 (39.9–252.3)</td>
<td>48.8 (17.0–140.5)</td>
<td>99.5 (31.8–252.2)</td>
</tr>
<tr>
<td><strong>Premenopausal women</strong></td>
<td></td>
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<tr>
<td>Total group</td>
<td>n = 13</td>
<td>39.1 (19.5–69.6)</td>
<td>475.6 (338.2–616.4)</td>
<td>167.6 (100.1–280.9)</td>
<td>56.1 (29.3–107.4)</td>
<td>98.6 (44.8–217.1)</td>
</tr>
<tr>
<td>ER+ subgroup</td>
<td>n = 7</td>
<td>39.1 (19.5–69.6)</td>
<td>475.6 (338.2–616.4)</td>
<td>167.6 (100.1–280.9)</td>
<td>56.1 (29.3–107.4)</td>
<td>98.6 (44.8–217.1)</td>
</tr>
<tr>
<td>ER- subgroup</td>
<td>n = 6</td>
<td>25.8 (12.2–92.0)</td>
<td>475.6 (338.2–616.4)</td>
<td>167.6 (100.1–280.9)</td>
<td>56.1 (29.3–107.4)</td>
<td>98.6 (44.8–217.1)</td>
</tr>
</tbody>
</table>

Statistically significant difference compared with ER subgroup of patients: *p < 0.01, *p < 0.001 (Mann-Whitney U test). Statistically significant difference compared with benign tissue: **p < 0.05, ***p < 0.001 (Wilcoxon signed rank test).

Another intriguing finding relates to the tissue/plasma concentration ratios for the different estrogen fractions. A comparison of E2, E1, and E1S reveals that E1 is the least polar (i.e., the most fat-soluble) compound, and E1S is the most polar (i.e., the most water-soluble). The tissue/plasma concentration ratios for E1, E2, and E1S are about 5–6, 1.5–2, and 0.1, respectively. Lipophilicity and small-molecule size both predict for rapid membrane transfer (67). Because of its sulfate conjugation, E1S behaves as a weak acid, a group of compounds known in general to reveal a small volume of distribution as compared with basic drugs (67). In contrast, E1 and E2 both behave as neutral compounds at physiologic pH. Taken together, these findings are consistent with (but do not definitely prove) the notion that tissue/plasma estrogen concentration ratios may be explained by the physiochemical properties of the respective molecule.

These results have potential implications for the future clinical development of estrogen-modulating compounds. Because of its high plasma levels, the estrogen conjugate E1S has received much attention as a potential source of intratumoral estrogens (68). Thus, the findings from previous studies of high levels of plasma (25) and tissue E1S (63) as compared with unconjugated E1 and E2 suggest that circulating E1S, taken up by the tissue with subsequent deconjugation and reduction, is an important source of intratumoral E2 (68). Although our own studies (69, 70) have confirmed high plasma levels of E1S, our findings of low tissue E1S and a low tissue/plasma concentration ratio argue against the hypothesis that this conjugate may be a major contributor to tissue E2. Second, although we cannot directly assess benign breast tissue estrogen synthesis in postmenopausal women, rapid equilibration between plasma and tissue compartments, which is supported by the highly significant correlation between plasma and tissue estrogen concentrations for E1 and E2 (Table 2), indicates that plasma estrogen levels predict benign breast tissue levels well in both pre- and postmenopausal women. Of even more importance, whatever the magnitude of local estrogen production within benign breast tissue, rapid equilibration of breast tissue with the plasma compartment indicates the role of local synthesis to be of minor importance. Whether there is a substantial local synthesis (or not), this will have little impact on local estrogen levels in benign tissues as long as local concentrations are at equilibrium with plasma estrogen levels.

Breast Cancer Estrogen Concentrations and Synthesis

In contrast to the finding that intratumoral concentrations of E2 in ER+ tumors resembled concentrations in benign tissue (30), we found substantially elevated levels of E2 in ER+ tumors as compared with normal tissue from the same breast in postmenopausal women (Tables 1 and 2; Fig. 3). Surprisingly, however, we detected tumor levels...
Elevated estrogen (E1) tumor than in benign tissue. Of interest, they recorded an intratumor E1 in only 3 of 24 tumors. The variation in local synthesis was found to account for more than 50% of the median value of 15% in benign tissue. On the other hand, cells, and the other antibody detected aromatase staining (72) revealed major protein staining related to stromal confounding results were obtained with 2 antibodies: One staining for aromatase protein expression remained a con-modulation may have significant implications. Immuno-tissue compartment (see details outlined in Supplementary Material). Similar results were obtained in a smaller study by Reed and colleagues (71). Although this difference in diffusion rates between compartments (see Supplementary Material), a lower concentration of 14C-E1 in breast cancer compared with normal breast tissue (75), and intratumor aromatization of 3H-labeled androstenedione. Although enzymatic aromatase activity may be detected in most fresh breast cancer samples (19), these findings, Although individual variation was substantial, the results revealed a median contribution from local aromatization accounts for about 30% of intratumor E1 levels, compared with a median value of 15% in benign tissue. On the other hand, local synthesis was found to account for more than 50% of intratumor E1 in only 3 of 24 tumors. The variation between tumors, however, was substantial. This may be due not only to variable local synthesis but also to variation in diffusion rates between compartments (see Supplementary Material). Similar results were obtained in a smaller study by Reed and colleagues (71). Although this difference between breast cancer and normal breast tissue could indicate increased intratumor estrogen synthesis, alternatively it may be due to a slower uptake of E1 from the circulation into the tumor compared with a normal breast tissue compartment (see details outlined in Supplementary Material). The potential impact of intratumor steroid synthesis or modulation may have significant implications. Immuno-staining for aromatase protein expression remained a controversial subject for 2 decades. For several years, conflicting results were obtained with 2 antibodies: One study by Reed et al. (30) and normal breast tissue. No correlations between benign breast tissue and plasma, indicating intratumor concentrations of E1 in benign tissue from the same breasts to either intratumoral E1 or E2 levels has been recorded. When ER+ tumors were analyzed separately, statistically higher intratumoral E2 but not E1 levels were recorded in the group of ARO677+ tumors as compared with ARO677– tumors (74). In the same material (75), we detected low levels of aromatase mRNA in breast cancer and normal breast tissue. No correlations between benign and malignant tissue aromatase mRNA expression and either E2 (Spearman rs = 0.02 and 0.05, respectively) or E1 (Spearman rs = −0.11 for both) levels were recorded. Although enzymatic aromatase activity may be detected in most fresh breast cancer samples (19), these findings, in similarity to the data presented above, do not address the quantitative role of tumor aromatization in vivo.

A striking observation is the elevated tumor/benign-tissue E2 ratio in ER+ breast cancer tissue. In contrast, tumor tissue levels of E1 are reduced as compared with benign tissue concentrations (Fig. 3). The balance between E2 and E1 is regulated through multiple dehydrogenase isoforms. Some of these isoforms (e.g., HSD17B1, B5, B7, and B12) act reductively in catalyzing activation of E1 into E2 (76–79), whereas others (e.g., HSD17B2, B10, and B14) favor oxidation, converting E2 into E1 in different tissues, including benign breast tissue (80–82). Of interest, both B2 and B7 were found to be elevated in breast cancer as compared with normal breast tissue (75), and intratumor of E1 to be consistently lower as compared with tissue concentrations of E1 in benign tissue from the same breasts independently of tumor ER status. Consistent with this observation, in their tracer infusion studies, Larionov and colleagues (65) found a lower concentration of 14C-E1 in breast cancer tissue. Larionov and colleagues (65) found a lower concentration of 14C-E1 in breast cancer tissue compared with ER status ER postmenopausal women, premenopausal women Postmenopausal women Premenopausal women

<table>
<thead>
<tr>
<th>ER status</th>
<th>Postmenopausal women</th>
<th>Premenopausal women</th>
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<tbody>
<tr>
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<td>ER&lt;sub&gt;a&lt;/sub&gt;</td>
<td>ER&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>NT-E1/PL-E2</td>
<td>2.0 (1.3–3.2)</td>
<td>1.3 (0.5–3.2)</td>
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<td>NTE1/PL-E1</td>
<td>6.3 (4.4–9.0)</td>
<td>4.4 (2.8–6.9)</td>
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<tr>
<td>NTE1/PL-E1S</td>
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<td>0.1 (0.0–0.4)</td>
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<tr>
<td>TU-E1/PL-E1</td>
<td>16.4&lt;sup&gt;c&lt;/sup&gt; (10.8–24.9)</td>
<td>1.2 (0.3–5.3)</td>
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<tr>
<td>TU-E1/PL-E1S</td>
<td>2.1 (1.5–3.0)</td>
<td>1.4 (0.5–4.2)</td>
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<tr>
<td>TU-E1S/PL-E1S</td>
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<tr>
<td>TU-E1S/TU-E1</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt; (0.9–2.6)</td>
<td>0.2 (0.0–1.1)</td>
</tr>
</tbody>
</table>

Abbreviations: E1, estrone; E2, estradiol; E1S, estrone sulfate; ER, estrogen receptor; NT, normal (benign) tissue of the same breast; PL, plasma; TU, tumor tissue.

Statistically significant difference compared with ER<sup>-</sup> subgroup of patients:

<sup>a</sup>P < 0.05.

<sup>b</sup>P < 0.01.

<sup>c</sup>P < 0.001 (Mann–Whitney test).

Data from Lønning et al. (30).
levels of E₂ were found to be negatively correlated with HSD17B2 expression but positively correlated with B7 expression. These findings support an additional mechanism of intratumoral E₂ synthesis, i.e., reduction of E₁ into E₂, which would explain elevated levels of tumor E₂ as compared with benign tissue E₂ levels.

Finally, we observed a strong correlation between intratumoral E₂ concentration and ER expression levels, suggesting an affinity effect of the ER (75). Before immunostaining became standard practice for ER assessment, the general cut-off limit for ER positivity in ligand-binding assays was 10 fmol/mg protein (83). Assuming that 1 fmol/mg protein may correspond to about 100 fmol/g wet weight of tissue or 100 pmol/L, the ER concentration of tumors considered positive by this cutoff would far exceed the concentration of E₂ in ER⁺ tumors. The estradiol binding affinity for the ER is less than 1 nmol/L (13); thus, at the concentrations measured, on average we may envision the bulk of intratumor E₂ to be receptor bound.

Taken together, the findings with respect to intratumor E₁ and E₂ versus plasma levels differ significantly from what we observed with respect to benign tissue hormonal concentrations. Although plasma E₁ levels in postmenopausal women correlated with intratumor E₁ and E₂ levels, there were no significant correlations in pre- or postmenopausal women for plasma E₂ and intratumor estrogen levels, or for plasma E₁ in premenopausal women. This suggests that the concentration equilibrium seen for benign tissue may not apply to intratumor levels. Although we cannot estimate the quantitative contribution from intratumoral aromatization, it is notable that the lack of correlation between aromatase expression at the mRNA and protein levels (immunostaining) contrasts with the correlations between intratumor E₂ and dehydrogenase enzymes as well as ER level expression. On the contrary, the observation that tumor E₂ concentration correlated positively to HSD17B7 but negatively to HSD17B2 expression strongly supports a role for intratumor conversion of E₁ into E₂ as a source of intratumor E₂.

No correlations between intratumor levels of either E₁, E₂, or E₁S and plasma E₁S levels or intratumor estrone sulfatase levels were observed (30, 75). Furthermore, the average expression level of estrone sulfatase in tumor tissue was about one third the level recorded in benign breast tissue. Taken together, these findings do not suggest a major contribution from circulating E₁S to intratumor E₂ levels.

Plasma Estrogen Concentrations and Intratumor Expression of Estrogen-Related Genes

The development of multigene expression assays (84) has allowed investigators to study tumor gene expression profiling in relation to estrogen suppression with aromatase inhibitors (85), as well as to explore potential correlations between circulating estrogen levels and expression of estrogen-regulated genes within tumor tissue. Although germline polymorphisms in estrogen-synthesizing and metabolizing genes (33) should affect all body compartments, as stated above, this may also have tissue- or plasma-specific effects due to organ-specific expression. Although the CYP19 SNPs rs10046 and TCT/C6 were both found to be significantly associated with plasma E₂ levels, they explained only a small percentage of interindividual E₂ variation (86).

In a recent study, some members of our group (87) assessed intratumor expression profiles of genes under estrogen regulation in postmenopausal women. Genes upregulated or downregulated by estrogen stimulation of ER⁺ breast cancer cells were previously indentified from

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**Figure 3.** Ratio between intratumor and benign breast tissue estrogen levels related to ER expression and menopausal status. A, postmenopausal women. B, premenopausal women. *, P < 0.05, **, P < 0.01, and ***, P < 0.001. Reproduced from Lønning et al. (30) with permission.
in vitro experiments (88). When 104 breast cancers were studied (87), a strong positive correlation between plasma E\textsubscript{2} levels and intratumor ER expression on the one hand and intratumor expression levels of several classical E\textsubscript{2}- regulated genes on the other hand were found. When genes such as trefoil factor 1 (TFF1), GREB1, PDZK1, and PgR were combined into an index, a correlation coefficient of (r)\textsubscript{p} of 0.51 indicated that plasma E\textsubscript{2} levels on average predicted intratumor E\textsubscript{2} levels by 27%. In this study, neither breast cancer nor benign tissue estrogen concentrations were available. The data, however, provide strong supporting evidence for the hypothesis that plasma/ benign-tissue estrogens exert a strong biological influence on breast cancer tissue.

Integrative Model

The topic of the contribution of intratumor synthesis versus circulating estrogens to intratumor E\textsubscript{2} levels and ER stimulation in breast cancer has been controversial for decades. We believe that the recent evidence as reviewed here adds a significant contribution to our understanding of the topic. Although we are not formally able to assess (and probably will not be able to in the near future) the quantitative contribution from each individual organ to plasma estrogen levels in postmenopausal women, we believe that a relatively straightforward model can explain our recent observations and clarify previous contradictory evidence with respect to plasma as well as benign and malignant breast tissue estrogen concentrations. As for benign tissue estrogen concentrations, due to rapid equilibration between the tissue and plasma compartments, plasma estrogens become good surrogate markers for benign tissue levels. Although a tissue/ plasma gradient for E\textsubscript{2} and E\textsubscript{1} exists, the fact that this gradient is similar in pre- and postmenopausal women supports the hypothesis that this gradient may be due either to active tissue uptake or simply to enhanced tissue steroid binding or depot (fat) storage. High intratumor E\textsubscript{2} levels may be due mainly to a concerted mechanism related to uptake of circulatory (and potentially benign tissue) E\textsubscript{1} with intratumor reduction of E\textsubscript{1} into E\textsubscript{2} and, to a greater degree, an affinity effect of the ER on E\textsubscript{2}. On the contrary, indirect evidence suggests that intratumor aromatization plays a minor role, and there is no evidence advocating a role of E\textsubscript{2} as a potential source of intratumoral E\textsubscript{2}.

Our data suggest that local enzyme inhibition of the aromatase enzyme or sulfatase is less important than inhibition of total-body estrogen synthesis. Although inhibition of local tumor dehydrogenases could potentially have a role in breast cancer therapy, the evidence argues against targeting local aromatization or sulfate deconjugation as a therapeutic strategy in breast cancer.

Disclosure of Potential Conflicts of Interest

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