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.

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 (E$_2$) levels are associated with a subsequent risk of breast cancer development (see ref. 3 for references to original work). Whether this relates only to growth stimulation of already transformed cells or includes promotion of carcinogenesis (4, 5) is not fully understood. Of note, oophorectomy 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-positive (ER$^+$) cells is confirmed by the dramatic effects achieved through antihormonal therapy for ER$^+$ tumors in both adjuvant (9) and metastatic (10) settings. The stimulation of ER$^+$ breast cancer cell proliferation in vitro by estrogens, particularly E$_2$, 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: E$_2$, estrone (E$_1$), estriol (E$_3$), and the estrone conjugate estrone sulfate (E$_1$S). Although E$_3$ 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 E$_1$ 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 E$_1$ and E$_1$S are biologically inactive, many tissues express enzymes (sulfatase and reductases) that convert E$_1$S and E$_1$ into active E$_2$ (14, 15).

In premenopausal women, the main estrogen source for breast tissue is circulating E$_2$, which is secreted by the ovaries. At menopause, ovarian production of estrogen ceases. In postmenopausal women, circulating E$_1$, 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
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 prevention. Tissue estrogen 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 tissue estrogen levels. 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 PI 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,
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-hydroxyestrone 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 E1 and E2 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/mmol), 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 0.55 pmol/L for plasma E2, E1, and E1S,
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 E$_1$ RIA applying $^{125}$I-labeled E$_1$, and the Spectria estradiol-sensitive RIA and DSL-4800 ultra-sensitive RIA using $^{125}$I-labeled E$_2$ 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 E$_2$ 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 E$_2$ concentrations even below 5 pmol/L.

Many investigators consider methods such as gas chromatography/tandem mass spectroscopy (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 E$_2$ 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 E$_2$ 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 E$_1$/S 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 catecholestrogens, 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 E$_2$ 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 $^{125}$I-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 E$_1$, the main estrogen product in postmenopausal women, of 6 to 7 (30). This is consistent with the benign tissue/plasma $^{14}$C E$_1$ ratio of 5 to 6 found by Larionov and colleagues (65) when they applied presurgical steady-state infusions of $^{14}$C-labeled E$_1$ 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 $^3$H-androstenedione and $^{14}$C E$_1$ in concert, by determining the $^3$H/$^{14}$C isotope ratio in the tissue compared with plasma E$_1$ fractions, Larionov and colleagues (65) estimated that local tissue production (based on an elevated $^3$H/$^{14}$C ratio) may account for a median of 15% of local E$_1$ 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 E$_1$ and E$_2$ 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 (E$_1$ averaging 5–6, E$_2$ averaging 1.5–2). These findings are consistent with both active uptake and enhanced tissue binding. In premenopausal women, the great majority of plasma E$_2$ is ovarian in origin. Considering the difference in tissue E$_2$ 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>ER/PGR</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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</table>

Postmenopausal women

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>E2 (95% CI)</th>
<th>E1 (95% CI)</th>
<th>E1S (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>30</td>
<td>29.2 (19.3–44.1)</td>
<td>121.5 e (63.6–620.9)</td>
<td>99.5 e (53.8–184.0)</td>
</tr>
<tr>
<td>ER+</td>
<td>21</td>
<td>31.1 (19.5–44.9)</td>
<td>267.1 b,e (159.9–646.4)</td>
<td>56.1 (29.3–107.4)</td>
</tr>
<tr>
<td>ER−</td>
<td>9</td>
<td>25.1 (8.1–69.6)</td>
<td>19.3 (6.1–61.5)</td>
<td>48.8 (17.0–140.5)</td>
</tr>
</tbody>
</table>

Premenopausal women

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>E2 (95% CI)</th>
<th>E1 (95% CI)</th>
<th>E1S (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Total</td>
<td>13</td>
<td>453.0 (314.3–656.7)</td>
<td>615.9 e (230.0–1646.3)</td>
<td>398.7 (243.2–563.4)</td>
</tr>
<tr>
<td>ER+</td>
<td>7</td>
<td>398.7 (243.2–653.4)</td>
<td>1621.8 a,d (863.2–3047.9)</td>
<td>245.4 c (117.0–514.5)</td>
</tr>
<tr>
<td>ER−</td>
<td>6</td>
<td>552.9 (253.2–1092.4)</td>
<td>198.7 (32.7–1207.5)</td>
<td>148.5 e (46.7–471.6)</td>
</tr>
</tbody>
</table>

Statistically significant difference compared with ER+ subgroup of patients:

*P < 0.01.

Statistically significant difference compared with benign tissue:

*P < 0.10, *P < 0.05.

Data from Lønning et al. (30).
of $E_1$ to be consistently lower as compared with tissue concentrations of $E_1$ 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 $^{14}C-E_1$ in tumor than in benign tissue. Of interest, they recorded an elevated $E_1/^{14}C-E_1$ ratio in breast cancer compared with benign breast tissue and plasma, indicating intratumor aromatization of $^3H$-labeled androstenedione. Although enzymatic aromatase activity may be detected in most fresh breast cancer samples (19), these findings, in contrast to the data presented above, do not address the quantitative role of tumor aromatization in vivo (73). 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 $E_1$ 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. Immunostaining for aromatase protein expression remained a controversial subject for 2 decades. For several years, conflicting results were obtained with 2 antibodies: One (72) revealed major protein staining related to stromal cells, and the other antibody detected aromatase staining in tumor cells (17). Based on these conflicting results, an international consortium was formed with the goal of developing and validating an antibody that could provide uniform results. This has now been achieved with the ARO677 antibody, which has been validated by different laboratories (73).

No significant correlation of ARO677 immunostaining to either intratumoral $E_1$ or $E_2$ levels has been recorded. When ER$^+$ tumors were analyzed separately, statistically higher intratumoral $E_2$ but not $E_1$ 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 $E_2$ (Spearman $r_s$ = -0.02 and 0.05, respectively) or $E_1$ (Spearman $r_s$ = -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 $E_2$ ratio in ER$^+$ breast cancer tissue. In contrast, tumor tissue levels of $E_1$ are reduced as compared with benign tissue concentrations (Fig. 3). The balance between $E_2$ and $E_1$ is regulated through multiple dehydrogenase isoforms. Some of these isoforms (e.g., HSD17B1, B5, B7, and B12) act reductively in catalyzing activation of $E_1$ into $E_2$ (76–79), whereas others (e.g., HSD17B2, B10, and B14) favor oxidation, converting $E_1$ into $E_2$ in different tissues, including benign breast tissue (80–82). Of interest, both $B_2$ and $B^7$ were found to be elevated in breast cancer as compared with normal breast tissue (78), and intratumor

**Table 2. Ratios of diverse plasma and tissue estrogen fractions in subgroups of patients (geometric mean with 95% confidence interval)**

<table>
<thead>
<tr>
<th></th>
<th>Postmenopausal women</th>
<th>Premenopausal women</th>
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<tbody>
<tr>
<td></td>
<td>$E_1$ $n=21$</td>
<td>$E_1$ $n=9$</td>
</tr>
<tr>
<td>NT-E/$E_1$/PL-E$_2$</td>
<td>2.0 (1.3–3.2)</td>
<td>1.3 (0.5–3.2)</td>
</tr>
<tr>
<td>NTE$_1$/PL-E$_1$</td>
<td>5.9 (5.0–7.0)</td>
<td>6.3 (4.4–9.0)</td>
</tr>
<tr>
<td>NTE$_1$/PL-E$_1$/S</td>
<td>0.1 (0.0–0.2)</td>
<td>0.1 (0.0–0.4)</td>
</tr>
<tr>
<td>TU-E$_1$/PL-E$_2$</td>
<td>16.4$^c$ (10.8–24.9)</td>
<td>1.2 (0.3–5.3)</td>
</tr>
<tr>
<td>TU-E$_1$/PL-E$_1$</td>
<td>2.1 (1.5–3.0)</td>
<td>1.4 (0.5–4.2)</td>
</tr>
<tr>
<td>TU-E$_1$/PL-E$_1$/S</td>
<td>0.2 (0.1–0.4)</td>
<td>0.2 (0.0–0.8)</td>
</tr>
<tr>
<td>TU-E$_2$/TU-E$_1$</td>
<td>1.5$^a$ (0.9–2.6)</td>
<td>0.2 (0.0–1.1)</td>
</tr>
</tbody>
</table>

Abbreviations: $E_1$, estrone; $E_2$, estradiol; $E_1$S, estrone sulfate; ER, estrogen receptor; NT, normal (benign) tissue of the same breast; PL, plasma; TU, tumor tissue.

Statistically significant difference compared with ER$^+$ subgroup of patients:

$aP < 0.05$.

$bP < 0.01$.

$cP < 0.001$ (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 intratumoral 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
in vitro experiments (88). When 104 breast cancers were studied (87), a strong positive correlation between plasma E2 levels and intratumor ER expression on the one hand and intratumor expression levels of several classical E2-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 (rs) of 0.51 indicated that plasma E2 levels on average predicted intratumor E2 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 E2 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 E2 and E1 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 E2 levels may be due mainly to a concerted mechanism related to uptake of circulatory (and potentially benign tissue) E1 with intratumor reduction of E1 into E2 and, to a greater degree, an affinity effect of the ER on E2. On the contrary, indirect evidence suggests that intratumor aromatization plays a minor role, and there is no evidence advocating a role of E1S as a potential source of intratumoral E2.

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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