The Role of Local Estrogen Biosynthesis in Males and Females

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Natural (human) and experimental (mouse) models of estrogen insufficiency have revealed hitherto unexpected roles for estrogens in both males and females. In postmenopausal women, and in men, estrogen no longer has a major role as a circulating hormone, but rather it functions locally as a paracrine or even 'intracrine' factor in tissue sites where it is formed. As a consequence, the tissue-specific nature of aromatase production assumes physiological and pathophysiological significance. The availability of circulating precursors is also important in sites where there is no local supply of C_{19} precursors, particularly in elderly women. The potential clinical significance of these findings in terms of the development of new therapeutic modalities is discussed.

Our understanding of the role of estrogens in both males and females has expanded greatly in recent years. Hitherto unanticipated roles have emerged that question the very definitions of the terms 'estrogen' and 'androgen' as they are currently used. Considerable emphasis has been focused on the regulation of extragonadal estrogen biosynthesis, in particular that which occurs in adipose tissue and bone, and its importance in the wellbeing of the elderly[1]. The regulation of the gene encoding aromatase (*CYP19*) in normal adipose tissue from various body sites, including the breast, has been examined as a function of age[2], and significant changes in the regulation of *CYP19* expression have been documented in mesenchymal cells close to a breast tumor[3–5]. This has led to the conclusion that tumorous epithelium of the breast, and/or macrophages recruited to the tumor site, produce factors such as prostaglandin E_2 (PGE_2), tumor necrosis factor α (TNF-α) and class I cytokines that regulate *CYP19* expression in the surrounding mesenchymal cells of the adipose tissue and of the tumor itself[6–8] (Fig. 1).

Because bone is a favored site for breast cancer metastasis, there has also been interest in aromatase production in bone and in primary cultures of human osteoblastic cells[9,10]. We, and others, have observed that these cells exhibit high aromatase activity, which is regulated primarily by class I cytokines, interleukin 1β (IL-Iβ) and TNF-α. These observations, together with the finding of a markedly altered bone phenotype in men with mutations of either the gene encoding the estrogen receptor α (ERα) or *CYP19*, characterized by undermineralization and failure of epiphyseal fusion[11,12], suggest that local estrogen production in bone cells plays an important role in the maintenance of bone mineralization and the prevention of osteoporosis in men and in women. In an extension of these concepts, we have advanced the hypothesis, also enunciated by Labrie and colleagues[13], that in postmenopausal women, and also in men, extragonadal estrogen biosynthesis in several sites, including adipose tissue, bone, various sites of the brain, vascular endothelial and smooth muscle cells, plays an important but hitherto largely unrecognized physiological and pathophysiological role in a paracrine, and indeed, intracrine, fashion[14]. The long-term health consequences of a decline in estrogen after the menopause include bone loss, urogenital aging, increased cardiovascular disease and perhaps cognitive impairment culminating in dementia. The incidence and pattern of occurrence of all of these disease processes differ significantly between men and women and cannot be explained by gender differences in circulating estrogen levels alone. For example, men have plasma estradiol levels in the postmenopausal range throughout their adult years, but rarely develop osteoporosis until late in life. Hence, our understanding of the peripheral metabolism of precursor steroids in the main estrogen target tissues appears fundamental to ascertaining the mechanisms underlying the development of diseases associated with the decline in circulating estrogen levels after menopause.

Of equal importance is the understanding derived from studies of ERα-knockout (ERKO)[15] and aromatase knockout (ArKO) mice[16,17], of the role of locally produced estrogen in the regulation of male reproduction and, in particular, its role in spermatogenesis. This new understanding of the role of estrogens in the male blurs our definition of male versus female hormones because, at least at the local level, both C_{19} and C_{18} steroids have important roles to play in both sexes.

• The Concept of Local Estrogen Biosynthesis

Although the ovaries are the principal source of systemic estrogen in the premenopausal non-pregnant woman, other sites of estrogen biosynthesis are present throughout the body, and these become the major sources of estrogen after the menopause. These sites include the mesenchymal cells of the adipose tissue and skin (reviewed in Ref. 1) osteoblasts[18] and perhaps chondrocytes[19] in bone, vascular endothelial and aortic smooth muscle cells[20], as well as several sites in the brain, including the medial preoptic/anterior hypothalamus, the medial basal

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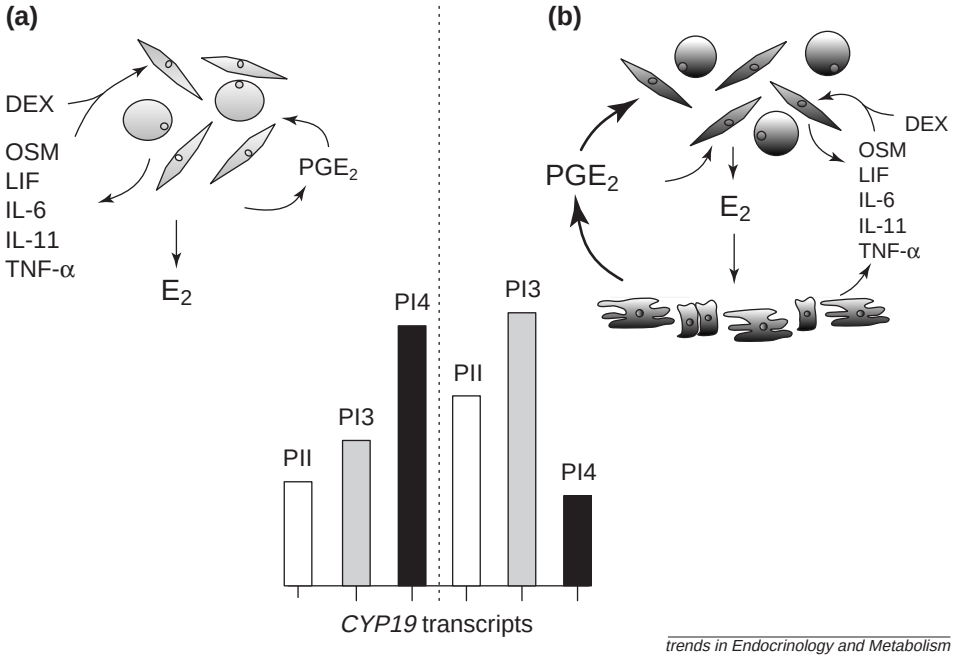

Figure 1. Proposed regulation of expression of the aromatase gene (*CYP19*) in breast adipose tissue from **(a)** cancer-free individuals and **(b)** those with breast cancer. In cancer-free individuals, expression is stimulated primarily by class I cytokines or TNF-α produced locally, in the presence of systemic glucocorticoids. As a consequence, promoter I.4 (PI.4)-specific transcripts of aromatase predominate. In those with breast cancer, PGE_2 produced by the tumorous epithelium, tumor-derived fibroblasts and/or macrophages recruited to the tumor site is the major factor stimulating *CYP19* expression, as evidenced by the predominance of promoter II- (PII) and I.3 (PI.3)-specific transcripts of *CYP19*. The relative expression of each promoter-specific transcript is shown in arbitrary units. Not indicated is the fact that in the presence of a tumor total *CYP19* expression is increased three to fourfold. DEX, dexamethasone; E_2, estradiol; IL, interleukin; LIF, leukaemia inhibitory factor; OSM, oncostatin M; PGE_2, prostaglandin; TNF-α, tumor necrosis factor α.

hypothalamus and the amygdala[21]. These extragonadal sites of estrogen biosynthesis possess several fundamental features that differ from those of the ovaries. Principally, the estrogen synthesized within these compartments, particularly in bone, breast and brain, is probably only biologically active at a local tissue level in a paracrine or 'intracrine' fashion[13]. Thus, the total amount of estrogen synthesized by these extragonadal sites might be small, but the local tissue concentrations achieved are probably quite high, and exert significant biological influence locally. Another important feature is that these sites are dependent on circulating precursor C_{19} steroids for estrogen biosynthesis. Although these extragonadal tissues have the capacity to convert C_{19} steroids to C_{18} steroids, unlike the ovaries they lack the ability to synthesize C_{19} precursors. Hence, estrogen production in adipose tissue, bone and brain is dependent on the availability of circulating C_{19} precursors.

After the menopause, the mesenchymal cells of the adipose tissue become the main source of estrogen[1,22]. Therefore, in the post-reproductive years, the degree of a woman's estrogenization is mainly determined by the extent of her adiposity. This is of clinical importance because corpulent women are relatively protected against osteoporosis[23]. Conversely, obesity is positively correlated with breast cancer risk[24].

In the case of males, it has been estimated that at best the testes can account for 15% of circulating estrogens[25] and hence, in the male, local production of estrogens, both intratesticular and extragonadal, is of physiological significance throughout adult life. For example, the Leydig cells[26] and other cells of the testes, including germ cells in various stages of differentiation[27], produce estradiol, which, as mentioned below, has an important role in spermatogenesis. Estrogen production in bone appears to be as vital for the maintenance of bone mineralization and prevention of osteoporosis in men as it is in women. This is supported by studies of men, either with a mutation of *CYP19* (Refs 12,28) or a mutation of the gene encoding the ER (Ref. 11). These individuals exhibit failure of epiphysial fusion, osteopenia and delayed bone age. Recently, we have observed that male ArKO mice also exhibit alterations in bone histomorphometry characteristic of undermineralization[29].

In a similar fashion, it is reasonable to speculate that estrogen production in one or more brain sites has an influence on sexual behavior and, as suggested by recent observational epidemiological studies, might have a role in the maintenance of cognitive function and the prevention of Alzheimer's disease (reviewed in Ref. 30). In this context, it is appropriate to reconsider why osteoporosis is more common in women than in men, and affects women at a younger age in terms of fracture incidence. Similarly, one might question why the incidence of Alzheimer's disease is greater among women than among men.

• Precursor Availability

A key factor in the gender difference in the incidence of these diseases appears to be the availability of precursor C_{19} steroids for aromatization to estrogens in extragonadal sites, a concept also advanced by Labrie and colleagues[31]. In postmenopausal women, the principal source of C_{19} steroid production is the adrenal cortex, which elaborates androstenedione, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). However, the secretion of these steroids and their plasma concentrations decrease markedly with advancing age[32]. Moreover, DHEA must first be converted to androstenedione before aromatization. Another major step is the reduction of the 17-keto group to 17β-hydroxyl, catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD) type I, which is essential for formation of the active estrogen, estradiol. The distribution of this enzyme in the various extragonadal sites of aromatization has not yet been fully established, although it is found in tumorous breast epithelium[33] and in bone[9]. It should be noted

in this context that there is a recent report that 17β-HSD type III, which converts androstenedione to testosterone, is present in visceral fat[34], together with 17β-HSD type II.

In the male circulation, in contrast, the levels of testosterone are at least an order of magnitude greater than those circulating in the plasma of postmenopausal women (10–30 vs 0.5 nmol l^{-1}), whereas the levels of androstenedione are similar (~2.5 nmol l^{-1}). Because the levels of circulating testosterone in the male are similar to the K_m of aromatase (20–30 nmol l^{-1}), it is likely that circulating testosterone can be converted efficiently in extragonadal sites to give rise to local concentrations of estradiol sufficient to transactivate both ERs (α and β) (K_D ~1 nmol l^{-1}). Moreover, although testosterone levels in the plasma of men decrease with advancing years, this decrease is small compared with the decrease in the circulating levels of adrenal C_{19} steroids. Consequently, compared with women, men maintain a high circulating level of the active precursor testosterone throughout life, which is available for conversion to the active estrogen, estradiol, in extragonadal sites. Not only is the level of circulating testosterone in men much greater than that in women, but it is also two orders of magnitude greater than the mean levels of circulating estradiol in postmenopausal women (less than 130 pmol l^{-1}) and in men (≈25–130 pmol l^{-1}). Given that much of the circulating estradiol is bound to sex hormone-binding globulin, it is unlikely to have a major impact on transactivation of the ER, compared with estrogen produced locally as a consequence of conversion of circulating testosterone. Thus, the uninterrupted sufficiency of circulating testosterone in men throughout life supports the local production of estradiol by aromatization of testosterone in estrogen-dependent tissues, and thus affords ongoing protection against the so-called estrogen deficiency diseases. This appears to be important in terms of protecting the bones of men against mineral loss, and might contribute to the maintenance of cognitive function and prevention of Alzheimer's disease in men (Fig. 2).

Currently, there is considerable interest in the use of testosterone as a component of hormone replacement therapy (HRT) for postmenopausal women, but its use is mostly limited to those women who complain of loss of libido. However, postmenopausal testosterone replacement is effective in both the prevention and treatment of osteoporosis[35,36]. Thus, there appears to be a broader role for the use of testosterone in HRT, namely as a circulating precursor for local synthesis of estrogen in target tissues, where the latter acts in an intracrine and paracrine fashion.

- **Regulation of Aromatase Production in Adipose Tissue**

We have suggested previously[1] that aromatase is a marker of the undifferentiated adipose mesenchymal cell phenotype. In support of this, the factors that stimulate its production in adipose tissue of cancer-free individuals are factors that either inhibit or reverse the differentiated phenotype of adipocytes, namely class I cytokines, such as IL-6, oncostatin M and IL-11, or TNF-α (Fig. 1). Moreover, all of these factors act via the mesenchymal promoter I.4 of *CYP19* and require glucocorticoids as co-stimulators (reviewed in Ref. 1). Adipocyte differentiation is driven by transcription factors such as CCAAT/enhancer binding protein (C/EBP)α and -β and also peroxisome proliferator-activator receptor (PPAR) γ (Ref. 37), and although involving the downregulation of aromatase production, the differentiation process also involves the upregulation of markers such as lipoprotein lipase, the insulin receptor and glucose transporter 4 (GLUT4). These actions are antagonized by TNF-α, which is also found in adipocytes[38]. Significantly in this context, mice lacking TNF-α function are protected from obesity-induced insulin resistance[39]. These considerations suggest that factors that stimulate adipocyte differentiation, such as ligands of the PPARγ receptor (for example, troglitazone, BRL 49653 and 15-deoxy-$\Delta^{12,14}$-PGJ-$_2$) would inhibit aromatase production, and this has proved to be the case (Fig. 3)[40]. They also predict that individuals with insulin resistance have higher levels of *CYP19* expression in their adipose tissue, and therefore are at greater risk of developing breast cancer. Although the former has not yet been shown, there is epidemiological evidence to support the latter contention[41]. As shown in Fig. 1, in healthy breast tissue, promoter I.4-specific transcripts predominate, suggestive of a major role of cytokines in the regulation of *CYP19* expression. In the presence of a tumor[3–5], *CYP19* expression increases, mainly because of the induction of ovarian-type promoter II- and I.3-specific transcripts that are regulated by cAMP, probably as a consequence of PGE_2 production by the tumorous epithelium (Fig. 1).

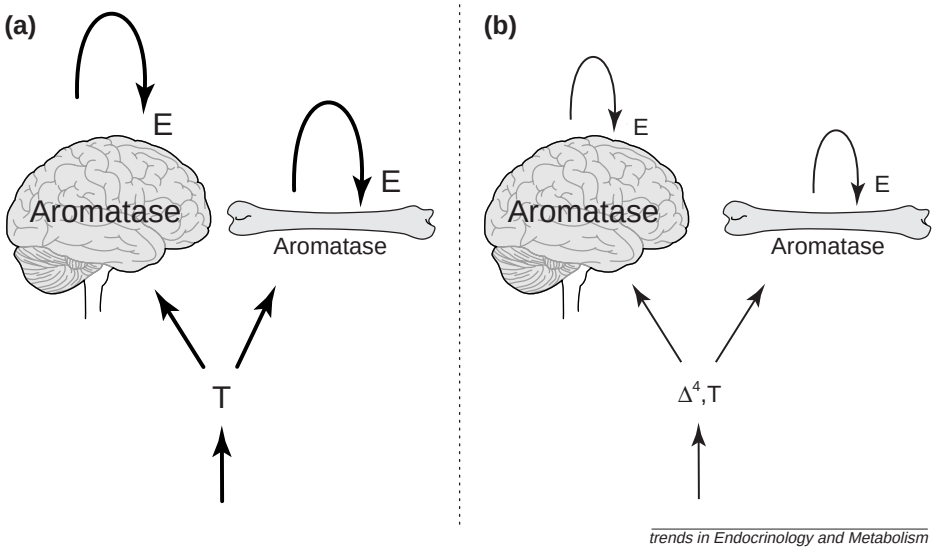

Figure 2. Importance of circulating C_{19} steroids as precursors for extragonadal estrogen biosynthesis in **(a)** men and **(b)** postmenopausal women. Δ^4, androstenedione; E, estrogen; T, testosterone.

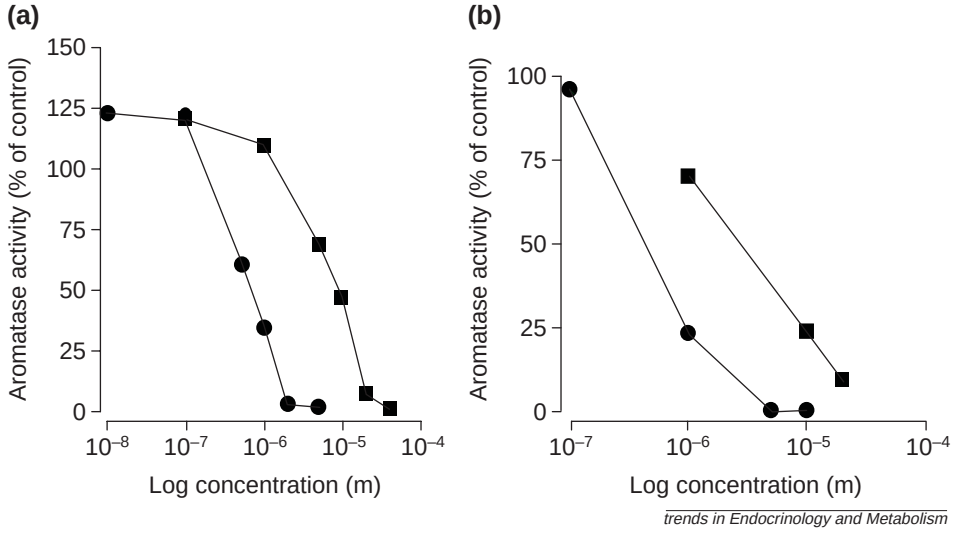

Figure 3. Inhibition of aromatase activity of human breast adipose stromal cells by the peroxisome proliferator-activator receptor (PPAR)γ ligands troglitazone, BRL 49653 (closed squares), and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (closed circles). **(a)** In the presence of tumor necrosis factor α (TNF-α) and dexamethasone; **(b)** in the presence of oncostatin M and dexamethasone[40].

- **Testicular Estrogen and Spermatogenesis**

Following our recent observations that male ArKO mice develop a progressive infertility such that by the age of one year most are infertile, we have examined the phenotype of these animals[17]. We observed that the seminiferous tubules are grossly dysmorphic, with an absence of mature spermatids and developmental arrest at the level of early round spermatid differentiation. In some cases, multinucleate and apoptotic cells can be seen sloughing into the lumen. There is also marked Leydig cell hyperplasia, presumably as a consequence of the raised luteinizing hormone (LH) levels. In some cases, a normal tubule is seen side by side with a dysmorphic one. This phenotype is first noticed at around 20–23 weeks of age, and differs from that of the ERKO mice, which are infertile throughout life and have distended seminiferous tubules, apparently as a consequence of pressure build-up, owing to failure of fluid transport across the epithelium of the head of the epididymis[42]. This does not appear to happen in the ArKO mice. These observations point to important roles for intratesticular estradiol production in male reproduction and, in particular, spermatogenesis. This estradiol could be derived from the Leydig cells[26] or from the germ cells because aromatase production[27] and activity[43], as well as the presence of ERβ, have been reported in male germ cells in various stages of development[44,45].

- **Clinical Considerations**

An important issue pertaining to the role of estrogen in the development of breast cancer in postmenopausal women is the relationship between HRT and breast cancer risk. A collaborative analysis of a large body of the available epidemiological data found that systemic administration of estrogen with/without progestin to postmenopausal women is associated with an overall 1.35-fold increase in breast cancer risk[46]. The increase in risk was reported to be greater in women of very low body mass index (BMI) but diminished with increasing BMI and was negated by obesity. This reinforces the hypothesis that high local concentrations of estradiol[47] as a consequence of synthesis within the breast stimulate cancer development, and that the increase in circulating estrogen as a consequence of HRT has little influence on intratumoral levels, except in women with minimal adiposity. The action of locally produced estrogen appears to be largely paracrine in nature, and mediated via the classic estrogen receptor(s) or else via DNA adduct formation by quinone intermediates[48].

In conclusion, we believe that the results of recent studies reveal the importance of local estrogen production in the physiology and pathophysiology of elderly women and men, in particular its importance in the development of breast cancer, and in the maintenance of bone mineralization, as well as its role in male reproduction. Local estrogen production might also play a role in the prevention of cardiovascular disease and in the maintenance of cognitive function. These studies not only throw light on the role of locally produced estrogens in health and disease processes, but might also lead to new and hitherto unexpected modalities of therapy. This is already apparent from the observation that tumor-derived PGE_2 is a major factor stimulating local aromatase synthesis in the breast fat of cancer patients[18] (Fig. 1), which leads to the consideration that prostaglandin synthesis inhibitors such as aspirin and ibuprofen might be beneficial in breast cancer prevention or treatment[8,49]. Similarly, the observation that PPARγ ligands, such as the thiazolidinediones, inhibit aromatase synthesis suggests that these compounds, some of which are available in the USA for the treatment of insulin-resistant diabetes, might also have a role in breast cancer prevention. Finally, the concept that estrogen biosynthesis in postmenopausal women is a local phenomenon, and that estrogens act in a paracrine and intracrine fashion, together with the tissue-specific nature of the regulation of aromatase synthesis, implies that it should be possible to develop novel tissue-specific inhibitors of aromatase synthesis, analogous to the development of selective estrogen receptor modulators (SERMs). Such selective aromatase modulators (SAMS) would be desirable as second-line adjuvant therapy following breakthrough from tamoxifen treatment, since currently used aromatase inhibitors block total body estrogen biosynthesis, with the risk of bone demineralization and other sequelae. However, they could also be developed for first-line adjuvant therapy, and offer an alternative to current preventative modalities.

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