Aromatization of androgens in women: current concepts and findings

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Objective: To review the role of circulating C_{19} steroids as precursors of estrogens in postmenopausal women.

Design: Review of current published literature.

Result(s): In postmenopausal women as in men, estradiol no longer functions as a circulating hormone, because it ceases to be formed by the ovaries at the time of menopause. Estradiol continues to be formed in a number of extragonadal sites, however, including breast, bone, vascular smooth muscle, and various sites in the brain. At these sites of formation, local estradiol levels can be quite high, but the production rate is insufficient to affect the body in a global fashion; thus, estrogen action at these extragonadal sites of synthesis is primarily at a local level and serves a paracrine or even intracrine role.

Because of this, in postmenopausal women as in men, circulating estrogen levels do not drive growth and development of target tissues. Instead, they reflect the metabolism of estradiol at these extragonadal sites. Estrogen that is not metabolized at these sites reenters the circulation, and, consequently, circulating levels of estradiol reflect its synthesis and action in extragonadal sites. Thus, they are reactive instead of proactive. An important difference between estrogen production at these extragonadal sites and estrogen that is synthesized in the ovary is that the former is absolutely dependent on a supply of circulating C_{19} androgenic substrate.

Conclusion(s): Circulating levels of testosterone begin to decline in the mid-reproductive years, and the levels of adrenal androgenic steroids, namely adrostenedione and DHEA, decrease throughout postmenopausal life. Therefore, the circulating levels of these adrogenic steroids may serve an important role in the maintenance of local estrogen synthesis, for example, in the bone and brain where estrogen has a profound influence on the maintenance of mineralization on the one hand, and possible cognitive function on the other. (Fertil Steril® 2002;77(Suppl 4):S6–10. ©2002 by American Society for Reproductive Medicine.)

Key Words: Androgens, circulating pro-hormone, extragonadal conversion, estrogens, paracrine actions, intracrine actions

Testosterone can be regarded as a circulating pro-hormone, which is converted in target on the one hand to 5α-dihydrotesterone (DHT), and on the other hand to estradiol. The former is the principal ligand for androgen receptors, and the latter is the principal endogenous ligand for the estrogen receptors.

Testosterone circulates at concentrations which are an order of magnitude greater than those of estradiol in the blood of postmenopausal women (Table 1). An obvious implication of this realization is that androgens have an important role to play in female physiology. It is now recognized that much of the physiology of androgens is explicable in terms of the concept that testosterone functions as a circulating pro-hormone, which is converted in target tissues, on the one hand to 5α-dihydrotestosterone (DHT) and on the other hand to

estradiol (Fig. 1). The former is the principal ligand for androgen receptors, and the latter is the principal ligand for both estrogen receptor α and β isoforms. Therefore in any consideration of the roles of testosterone in either women or men, it is necessary to discuss both of these pathways of testosterone metabolism.

The situation is complicated by the fact that, in postmenopausal women, only about 25% of circulating testosterone is derived by direct secretion from the ovaries (Fig. 2). The rest is formed largely from circulating precursors derived either from the adrenal cortex or from the ovaries. These are principally androstenedione, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS). Whereas DHEAS is secreted entirely from the adrenals, androstenedione and DHEA are formed in both adrenals and ovaries. Dehydroepiandrosterone

Plasma steroid levels in men and in postmenopausal women.

	Women (nmol/L)	Men (nmol/L)
T	0.6	12
Δ^4	2.5	4
E_1	0.10	0.13
E_2	0.04	0.10
DHEA	15	10
DHEAS	2500	2000

Note: T = testosterone; E_1 = estrone; E_2 = estradiol; DHEA = dehydro-epiandrosterone; DHEAS = dehydroepiandrosterone sulfate.

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and DHEAS are present in the circulation in concentrations which are orders of magnitude greater that those of the active sex steroids (Table 1). Consequently, they form a large reservoir of precursor, which is available for conversion to testosterone and thus to estrogens in numerous peripheral tissue sites; it is also relatively insensitive, in the short term, to changes in secretion rates.

The present discussion focuses on the role of androgens as precursors of estrogens in postmenopausal women. Whereas in premenopausal women, the ovaries are the principal source of estrogen, which functions as a circulating hormone to act on distal target tissues, in postmenopausal women, and in m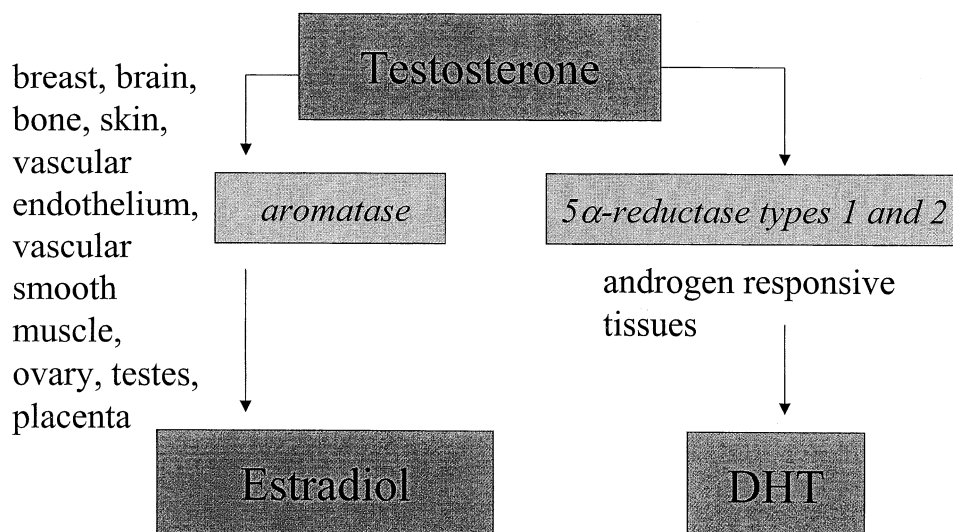en, when the ovaries cease to produce estrogens, this is no longer the case. Under these circumstances, estradiol is no longer solely an endocrine factor. Instead, it is produced in a number of extragonadal sites and acts locally at these sites as a paracrine or even intracrine factor (1, 2). These sites include the mesenchymal cells of adipose tissue, osteoblasts and chondrocytes of bone, the vascular endothelium and aortic smooth muscle cells, and numerous sites in the brain. Thus, circulating levels of estrogens in postmenopausal women and in men are not the drivers of estrogen action, they are reactive rather than proactive. This is because circulating estrogen in this situation originates in extragonadal sites where it acts locally; if it escapes local metabolism, it subsequently enters the circulation. Therefore, circulating levels reflect, instead of direct, estrogen action in postmenopausal women and men.

NONSEXUALLY DIMORPHIC ROLES OF ANDROGENS AND ESTROGENS

Studies employing models of estrogen insufficiency have revealed new and unexpected roles for estradiol in both females and males (2). These models include mutations in humans of the aromatase gene, of which ten cases are documented, three of whom are men (3, 4), and one case of a man with a mutation in the estrogen receptor (ER) α (5). They also include mice with targeted disruptions of ERα and ERβ; the double ERα- and β-knockout mouse (6–9) as well

Testosterone is a precursor for DHT and estradiol in extragonal sites.

breast, brain, bone, skin, vascular endothelium, vascular smooth muscle, ovary, testes, placenta

Testosterone

aromatase

5α-reductase types 1 and 2

androgen responsive tissues

Estradiol

DHT

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FIGURE 2

Androgen production into the blood of postmenopausal women.
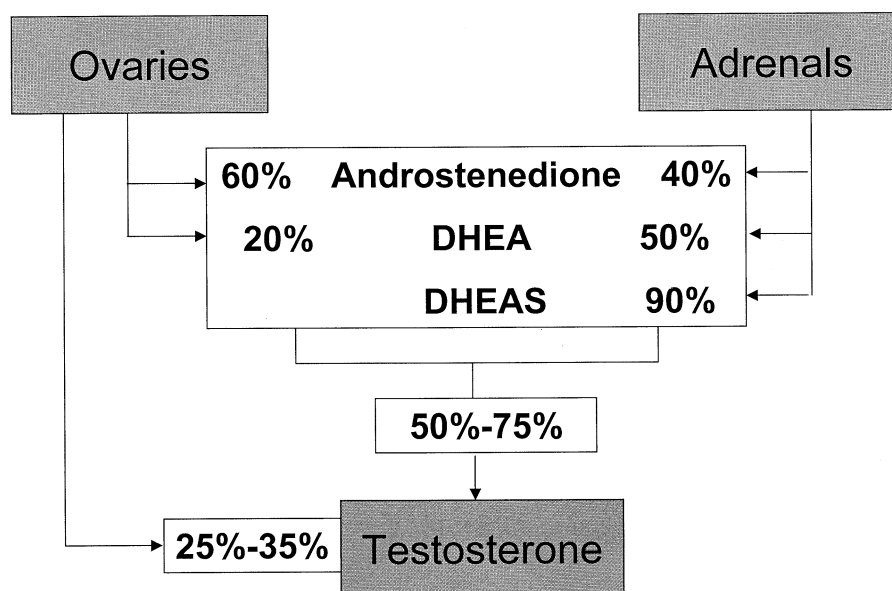
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as the aromatase knockout (ArKO) mouse (10–12). Recently described consequences of estrogen deprivation challenge the traditional beliefs of gender-specificity of sex steroid actions. For example, the lipid and carbohydrate phenotype of estrogen insufficiency is not sexually dimorphic and appears to apply to both males and females (13, 14), as does the bone phenotype of undermineralization and failure of epiphyseal closure (15). Even more dramatically, the roles of estradiol in male germ cell development and efferent duct fluid transport would indicate that, in this local context, estradiol might be more appropriately defined as an androgen (16–18).

As indicated previously there is a growing appreciation that both androgens and estrogens have general metabolic roles that are not directly involved in reproductive processes and apply, to a greater or lesser extent, to both sexes. This is perhaps more readily understood when placed in the context of the emerging knowledge of the evolution of steroidogenic genes on the one hand and those encoding steroid hormone receptors on the other.

Largely from the work of Callard and her colleagues, it is now recognized that the biosynthesis of estrogens occurs throughout the entire vertebrate phylum, including mammals, birds, reptiles, amphibians, teleosts, and elasmobranch fish as well as agnatha (hagfish and lampreys), and in protochordates such as amphioxus (19, 20). To the author's knowledge, estrogen biosynthesis has not been reported in non-chordate animal phyla. Consistent with this, phylogenetic analysis of steroid receptors in lower vertebrates indicates that the first steroid receptor was an estrogen receptor, followed by a progesterone receptor (21). No equivalents of the "classical" steroid receptors have been found in any species outside the vertebrates, although an orthologue of the estrogen-related receptor (ERR) is present in *Drosophila* namely, the ecdysone receptor.

Genome mapping and phylogenetic analysis indicate that the full complement of mammalian steroid receptors evolved from these ancient receptors by two, large-scale genomic expansions—one before the advent of jawed vertebrates and one after (21). Specific regulation of physiological processes by androgens and corticoids are relatively recent innovations that emerged after these duplications. Thus, we might speculate that the role of C_{19} steroids was, in the first instance, merely to serve as a precursor for the estrogenic steroids, and that specific physiological roles for C_{19} steroids only emerged later. On this basis, it is reasonable to expect that estrogens should play important physiological roles in males just as they do in females. It is also consistent with the knowledge that, at least in placental mammals, the female phenotype is the default phenotype, and that the difference between "maleness" and "femaleness" is not an absolute one, but instead is governed by a subtle balance of the ratios of estrogenic vs. androgenic actions.

THE CONCEPT OF LOCAL ESTROGEN BIOSYNTHESIS

Extragonadal sites of estrogen biosynthesis possess several fundamental features that differ from those of the ovaries. First, the estrogen synthesized within these compartments acts predominantly at the local tissue level in a paracrine or "intracrine" fashion (22, 23). Thus, the total amount of estrogen synthesized by these extragonadal sites may be small, but the local tissue concentrations achieved are probably high and exert biological influence locally. As a consequence, extragonadal estrogen biosynthesis plays an important, yet largely unrecognized, physiological and pathophysiological role.

The power of local estrogen biosynthesis is illustrated by the cases of men in whom aromatase expression in adipose tissue is greatly increased, whereas aromatase expression present in the testes is unaffected. This results in florid gynecomastia and short stature due to premature epiphyseal fusion (24, 25). This condition is a consequence of chromosomal rearrangements that result in the insertion of a constitutive promoter upstream of the start of translation of the aromatase gene (25). Another example relates to postmenopausal breast cancer (26). It has been determined that the concentration of estradiol present in breast tumors of postmenopausal women is at least 20-fold greater than that present in the plasma. With aromatase inhibitor therapy, there is a precipitous drop in the intratumoral concentrations of estradiol and estrone together with a corresponding loss of intratumoral aromatase activity, indicating that this activity within the tumor and the surrounding breast adipose tissue is responsible for these high tissue concentrations (27).

In bone, aromatase is expressed primarily in osteoblasts and chondrocytes (28), and aromatase activity in cultured osteoblasts is comparable to that present in adipose stromal cells (29). Thus, it appears that in bone also, local aromatase expression is the major source of estrogen responsible for the maintenance of mineralization, although this is extremely difficult to prove due to sampling problems. For both breast tumors and for bone, therefore, it is likely that circulating estrogen levels have little impact on the relatively high endogenous tissue estrogen levels. Instead, the circulating levels merely reflect the sum of local formation in its various sites. This is a fundamental concept for the interpretation of relationships between circulating estrogen levels in postmenopausal women and estrogen insufficiency in specific tissues.

The second important point is that estrogen production in these extragonadal sites is dependent on an external source of C_{19} androgenic precursors because, as discussed earlier, these extragonadal tissues are incapable of converting cholesterol to the C_{19} steroids (22, 23). As a consequence, circulating levels of testosterone and androstenedione as well as DHEA and DHEAS become extremely important in

terms of providing adequate substrate for estrogen biosynthesis in these sites (Fig. 1).

The fact that circulating testosterone levels in the postmenopausal woman are an order of magnitude greater than circulating estradiol levels (Table 1) suggests that circulating androgens might be more important for maintaining local estrogen levels in extragonadal sites than are circulating estrogens. Moreover, in men, circulating testosterone levels are an order of magnitude greater than those in postmenopausal women. In postmenopausal women, the ovaries secrete about 25% of the circulating testosterone directly. The remainder is formed peripherally from androstenedione, DHEA, and DHEAS (Fig 2). The secretion of these steroids and their plasma concentrations, however, decrease markedly with advancing age (1). Moreover, DHEA must first be converted to androstenedione prior to aromatization. Another major step is the reduction of the 17-keto group to the 17β-hydroxyl catalyzed by one or more members of the 17β-HSD family, which is essential for formation of the active estrogen or estradiol. The distribution of these enzymes in various extragonadal sites of aromatization has not yet been fully established, although reductive and/or oxidative members are expressed in many tissues.

In this context, one may consider why osteoporosis is more common in women than in men and affects women at a younger age. This review has suggested that 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 may also contribute to the maintenance of cognitive function and prevention of Alzheimer's disease (2).

CONCLUSIONS

It is now apparent that circulating androgenic steroids have an important role to play in the physiology and pathophysiology of women. This role, however, is not primarily to serve as a circulating endocrine factor as such, but to function instead as a precursor of downstream active metabolites, namely, 5α-dihydrotestosterone and estradiol, which are formed in extragonadal sites. The peripheral conversion of circulating androgens to estrogens becomes of major significance in the postmenopausal woman, when the ovaries cease to synthesize estrogens, and estradiol no longer has a primary role as a circulating hormone.

The ability of C_{19} steroids such as DHEAS, DHEA, and androstenedione to be metabolized to active sex steroids adds a further complexity because, in some extragonadal sites, these may be converted to testosterone, whereas in others, they may be metabolized through to estrogens. In such cases, the testosterone formed may never enter the

circulation, only its metabolic products (22, 23). Thus, determination of circulating levels of testosterone may not be reflective of its fate or function in a particular tissue. On the other hand, realization of the precursor role of circulating C_{19} androgenic steroids leads to the prediction that lower than average levels of these steroids could lead to inadequate synthesis of estradiol in peripheral tissues such as bone and brain. Changes that have been traditionally considered ensuing sequelae of estrogen insufficiency, such as loss of bone mineralization and possibly changes in brain-derived functions, may paradoxically turn out to be a consequence of insufficiency of circulating androgenic steroids.

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