J Musculoskel Neuron Interact 2003; 3(4):370-373

Perspective Article

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The role of estrogen metabolism in aging

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Keywords: 2-hydroxyestrone, 16α-hydroxyestrone, Cancer, Osteoporosis

Estrogens are implicated in numerous diseases and conditions that affect our aging population. Originally conceived to be an effect of the two main estrogens – estradiol and estrone, it has become increasingly clear that the metabolites of these two estrogens may be responsible for much of the effect and that how an individual metabolizes his or her estrogen may be predictive of disease risk, prognosis, and efficacy of therapy.

Estradiol metabolism occurs primarily by oxidation in both men and women. The first stage is the transformation of estradiol to estrone by oxidation at the C17 position by 17ß-hydroxysteroid-dehydrogenase, a process that is reversible. The balance largely favors the formation of estrone which is characterized by the fact that estradiol is rapidly converted to estrone but the reverse reduction of estrone to estradiol occurs considerably more slowly. Further metabolism of estrone occurs irreversibly through two mutually exclusive pathways – 2-hydroxylation and 16α-hydroxylation[1]. 4-Hydroxylation also occurs and, although a "minor" player in terms of quantity, its role as a genotoxic agent has been well described[2] and justifies research attention as interventions are tested to modulate the two dominant pathways. Other sites on estrone and estradiol are also hydroxylated and numerous oxidative metabolites are generated[1]. In addition, estrogens are metabolized by glucoronidation, sulfonation, and O-methylation[1]. Their relative contribution to the metabolite pool is less than 2, 16α- or even the 4-hydroxyestrogens and little research attention has been devoted to their biological effects. This is not to infer however that they have no effect. Members of the cytochrome P450 family are the major enzymes catalyzing the oxidative metabolism of the estrogens to the hydroxylated metabolites[3]. Although most of the oxidative metabolism takes place in the liver, some estrogen-metabolizing isoforms of the cytochrome P450s are selectively expressed in extrahepatic tissue (e.g., brain, breast, prostate). The implications of this are enormous as the metabolites, whether endogenous or exogenously given, will show tissue specificity[3].

It is clear from decades of animal and *in vitro* research that the estrogen metabolites are not simply inert breakdown products, but they have significant biological activity. 2-hydroxyestrogens have been shown to have essentially no estrogen action and may act as anti-estrogens[4,5]. Methylation of 2-hydroxyestradiol leads to the generation of a powerful anti-angiogenic, apotosis-stimulating metabolite[6] that is currently in clinical trials as a chemotherapeutic/preventive agent. 2-hydroxyestrone has been considered the "good estrogen"[7] relative to cancer development although the data are not entirely consistent[8]. Oxidative hydroxylation on the D ring at position 16 leads to 16α-hydroxyestrone (16α-OHE1). This estrogen metabolite exhibits estrogenicity comparable to that of estradiol, has a low binding affinity for sex hormone binding globulin[9], and can covalently bind to the estrogen receptor causing a long lasting hyper-estrogenic stimulus including proliferation and upregulation of oncogenes[10]. Although not discussed in detail because of the paucity of human data, the second set of catechol estrogens, the 4-hydroxyestrogens also have estrogenic activity[1,11]. They have been implicated in cancer development because they undergo redox cycling to generate reactive oxygen species. These in turn may damage DNA and other cell constituents, induce cell transformation, and initiate tumorigenesis[12].

Because of the different biological activities of the metabolites and the competing nature of the 2-hydroxylation and 16α-hydroxylation pathways for the limited estrone pool, the ratio of 2/16α-OHE1 has been proposed to be a marker of disease risk. Further, it has been proposed that it is possible to modulate estrogen metabolism through lifestyle or therapeutic interventions. If this proves to be accurate, measuring estrogen metabolites may provide a useful biological marker of disease risk as well as of the efficacy of a particular intervention to alter that risk. The next section will briefly overview diseases/conditions for which estrogen metabolism has been implicated. This will be followed by a brief description of factors that have been implicated to

The author has no conflict of interest.

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Accepted 1 August 2003

influence estrogen metabolism.

Estrogen metabolism has been most strongly implicated in estrogen-related cancers. Specifically, a lower 2/16α-OHE1 ratio has been associated with an increased risk of breast[13,14], endometrial[15], and cervical cancer[16]. Breast cancer patients have been reported to have elevated 16α-OHE1 and lower 2-OHE1 in serum and urine relative to controls[13,14]. The concentration of 16α-OHE1 has also been found to be higher in normal breast tissue as well as in cancerous tissue from breast cancer patients compared to normal breast tissue obtained from reduction mammoplasty[17]. An increase in cervical neoplasia and severity of disease has been associated with a reduction in 2/16α-OHE1[16]. In men, we recently reported that, like breast cancer, a lower 2/16α-OHE1 ratio was associated with increased risk for prostate cancer and increased severity of disease[18]. Benign prostatic hypertrophy has also been associated with lower 2-hydroxyestrogens[18]. Other diseases associated with estrogen metabolism include head and neck cancers (decreased 2/16α-OHE1)[19], systemic lupus erythrematosus (increased 16α-OHE1)[20,21], recurrent respiratory papillomatosis (increased 16α-OHE1)[22], and thyroid cancer (increased 2/16α-OHE1)[23]. Less information is available on the role of estrogen metabolism and osteoporosis although it is now evident that the metabolites exert specific and differential effects in cortical and cancellous bone[11,24,25]. Specifically 16α-OHE1 acts very similar to estradiol, 2-OHE1 has no estrogenic effect whatsoever, and 4-OHE1 is somewhat intermediate. Two reports[26,27] suggest that higher concentrations of the 2- hydroxyestrogens and a greater 2/16α-OHE1 ratio are associated with increased risk for osteoporosis. Given the relationship between bone mineral density (BMD) and breast cancer[28,29] (increased BMD = increased breast cancer risk), it is plausible that an increase in 2/16α-OHE1 will be good for the breast but bad for the bone.

Although there are numerous limitations to the human studies conducted to date including small sample sizes, multiple confounders, and the difficulty in assaying the metabolites, it appears that the metabolism of estrogen in men and women may be an important factor in disease risk. Concordantly, if we can alter estrogen metabolism, we may be able to alter that risk. The bulk of the research has focused on increasing 2-hydroxylation as a means to decrease cancer risk. Very little attention has been focused on the impact of these changes on bone and/or other tissues or systems that might benefit for a more "estrogenic" environment. It will be important to consider these as future research continues on the estrogen metabolites.

The most extensively studied factors have been dietary. Brassica vegetables (e.g., cabbage, broccoli, cauliflower, Brussels sprouts) and their derivatives (indole-3-carbinole, dindolymethane, and indolylcarbazole) ingestion either in food or supplement form, result in significant increases in 2-hydroxylation and an increase in the ratio of 2/16α-OHE1[22,30]. Similarly, flaxseed (lignans)[31] and soy (isoflavanoids)[32] intake are associated with increased 2-hydroxylation whereas fiber (e.g., wheat bran) has been shown to have no effect[31]. Interestingly, a recent study reported decreased bone strength with flaxseed consumption in young rats[33]. Estrogen metabolites were not measured but the increase in the non-estrogenic metabolite with flaxseed could potentially lead to reduced bone mass/strength. A high fat diet has been shown to be associated with a decreased 2/16α-OHE1 ratio[34] and a high body mass index (a measure of obesity) is also associated with lower 2/16α-OHE1[14,35]. More recently, the ratio of saturated fat/soluble fiber has been found to be inversely associated with the 2/16α-OHE1 ratio[36]. Across most studies, 16α-OHE1 appears to be relatively constitutive and does not appear to be readily altered.

Smoking's anti-estrogenic effect has been suggested to be due to the associated increase in 2-hydroxylation[37]. Alcohol has not been shown to have an effect[37]. Various medications – anticonvulsants, antidepressants, thyroid medication, have been shown to alter estrogen metabolism[38,39]. Recently we observed that physical activity is positively associated with the 2/16α-OHE1 ratio, i.e., more active premenopausal women had higher 2/16α-OHE1 ratios (data unpublished). The higher 2/16α-OHE1 ratio may help to explain the reduction in breast cancer risk that is observed with increased physical activity[40]. Stress has been associated with an increased cancer risk. An increase in stress is associated with elevated catecholamines which compete with catechol estrogens for methylation[41]. An increase in catecholamines would be associated with a decrease in 2-methoxyestradiol, a reduction in methylation of the 4-hydroxyestrogens, and a potential increase in cancer risk.

Although suggestive and etiologically plausible, the data in humans are still mixed, inconsistent, and occasionally controversial. In addition to definitively determining if and how estrogen metabolites affect disease risk, it is important to understand what role genetics have to play in estrogen metabolism. No difference in the 2/16α-OHE1 ratio has been observed in women with or without a family history of breast cancer[42]. In a small pilot study in our lab, families of women (mother, daughter, grandmother, siblings) were evaluated and found to have almost identical 2/16α-OHE1 ratios within the family although vastly different concentrations due to menopausal/pubertal status[43]. It is important to understand and identify who will respond to factors that modulate estrogen metabolism and who will not. Does the ability to shift metabolism alter the efficacy of treatments (e.g., hormone replacement therapy)? Does it help us to identify who would be best served by treatment? Most of the research, by necessity, has utilized serum and urine measurements. Yet many tissues have cytochrome P450 enzymes to metabolize estrogens and it is unknown what effect different interventions may have at a specific tissue site and/or how enzyme activity is related to disease risk. Metabolism of hormones in target cells may be a general mechanism for markedly increasing the original hormones activity and cellular specificity. Estrogen metabolism in men has received almost no attention. The effect of the metabolites on

Alzheimers, cognition, and the aging brain has received little attention. The effect of estrogen metabolism on the skeleton – peak bone mass attainment, speed of bone loss at menopause, and response to treatment, remains unknown. Similarly, the role that estrogen metabolites play in coronary heart disease risk is relatively unexplored although the catechol metabolites have been shown to have pro- and anti-oxidant properties[44] and to affect LDL oxidation[45].

In summary, it is well appreciated that estrogens have profound influences on numerous tissues and in the development of numerous diseases. It has become increasingly accepted that we must not only consider the parent estrogens, estradiol and estrone, when we evaluate disease risk but also the estrogen metabolites. Estrogen metabolites exert tissue-specific effects in many target cells and appear to be amenable to modulation through a variety of interventions. Their role in aging-related diseases is not well understood but the bulk of the evidence suggests that they may have important roles and should be evaluated to identify who is at risk, who would benefit from treatment and which treatment, and how serious a particular case might be.

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