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Review

Epidemiologic studies of estrogen metabolism and breast cancer

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ABSTRACT

Early epidemiologic studies of estrogen metabolism measured only 2-hydroxyestrone and 16α-hydroxyestrone and relied on direct enzyme immunoassays without purification steps. Eight breast cancer studies have used these assays with prospectively collected blood or urine samples. Results were inconsistent, and generally not statistically significant; but the assays had limited specificity, especially at the low concentrations characteristic of postmenopausal women. To facilitate continued testing in population-based studies of the multiple laboratory-based hypotheses about the roles of estrogen metabolites, a novel liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay was developed to measure concurrently all 15 estrogens and estrogen metabolites in human serum and urine, as unconjugated and total (glucuronidated + sulfated + unconjugated) concentrations. The assay has high sensitivity (lower limit of quantitation ~1–2 pmol/L), reproducibility (coefficients of variation generally ⩽5%), and accuracy. Three prospective studies utilizing this comprehensive assay have demonstrated that enhanced 2-hydroxylation of parent estrogens (estrone + estradiol) is associated with reduced risk of postmenopausal breast cancer. In the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) cohort, the serum ratio of 2-hydroxylation pathway metabolites to parent estrogens was associated with a 28% reduction in breast cancer risk across extreme deciles (p-trend = .05), after adjusting for unconjugated estradiol and breast cancer risk factors. Incorporating this ratio into a risk prediction model already including unconjugated estradiol improved absolute risk estimates substantially (by ⩾14%) in 36% of the women, an encouraging result that needs replication. Additional epidemiologic studies of the role of estrogen metabolism in the etiology of hormone-related diseases and continued improvement of estrogen metabolism assays are justified.

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1. Endogenous estrogen and breast cancer risk

It is widely recognized that endogenous estrogen is associated with increased risk of postmenopausal breast cancer. Persuasive evidence comes from a pooled analysis of individual participant data from nine prospective studies [1]. Included were 663 breast cancer cases and 1765 matched controls, all postmenopausal and not taking exogenous hormones at cohort entry. Breast cancer risk was statistically significantly increased, by 30–50%, with a doubling of circulating estradiol, bioavailable estradiol [estradiol not bound to sex hormone-binding globulin (SHBG)], free estradiol (estradiol not bound to SHBG or albumin), estrone, or estrone sulfate. Across extreme quintiles of circulating estradiol, relative risk (RR) doubled [RR = 2.00; 95% confidence interval (CI) = 1.47–2.71; p-trend < .001]. This strong positive association had been suspected, but not demonstrated in epidemiologic studies until the 1990s, because of the limited sensitivity and accuracy of estradiol assays at the low concentrations characteristic of postmenopausal women.

More recently, in a pooled analysis including seven prospective studies, 767 premenopausal breast cancer cases and 1699 matched controls, none of whom were taking exogenous hormones at cohort entry, endogenous estrogen was associated with increased risk of premenopausal disease, though not as strongly as with postmenopausal disease [2]. Breast cancer risk was statistically significantly increased, by 20–30%, with a doubling of circulating estradiol, free estradiol, or estrone, Across extreme quintiles of circulating estradiol, relative risk increased by 40% (RR = 1.41; 95% CI = 1.02–1.95; p-trend = .004. The ongoing pooled analysis of postmenopausal breast cancer now includes more than 5000 cases, practically all the data available worldwide. Nonetheless, while statistical power has become less of a problem, achieving adequate accuracy and specificity for the estradiol assays remains a challenge [3].

2. Early research on estrogen metabolism

The contribution of estrogen metabolism to the development of breast cancer is much more ambiguous than that of estradiol and estrone, the parent estrogens. The parent estrogens can be irreversibly hydroxylated at the 2-, 4-, or 16-position of the steroid ring (Fig. 1). Reactive catechol estrogen metabolites, metabolites with adjacent hydroxyl groups on the steroid ring, are formed through 2-hydroxylation and 4-hydroxylation, but can be converted to less reactive compounds by methylation. Estradiol, estrone, and estrogen metabolites can exist in conjugated forms, which are covalently linked to glucuronide, sulfate, or glutathione residues, or unconjugated forms. The conjugated forms are believed to be important in bioavailability, specifically estrogen storage, cellular transport, and excretion. Almost always, when circulating estradiol is assayed for an epidemiologic study, only the unconjugated form is measured.

Multiple hypotheses, based on laboratory experiments, exist about the role of specific estrogen metabolites and estrogen metabolism profiles in the etiology of breast cancer [4–6]. Both estrogen receptor-mediated mechanisms involving increased mitosis and proliferation and estrogen receptor-independent mechanisms involving direct DNA damage have been proposed. However, estrogen metabolism remained largely unexplored in epidemiologic studies until recently because no robust analytic methods were available to accurately and reproducibly characterize estrogen metabolism in large population-based studies.

More than 30 years ago, Jack Fishman and Leon Bradlow published one of the first epidemiologic studies of estrogen metabolism and breast cancer [7]. Included were 33 breast cancer cases and 10 controls; all the women were postmenopausal or perimenopausal. Estrogen metabolism was measured retrospectively, after breast cancer diagnosis. A novel *in vivo* radiometric method had been developed in order to measure the total oxidative

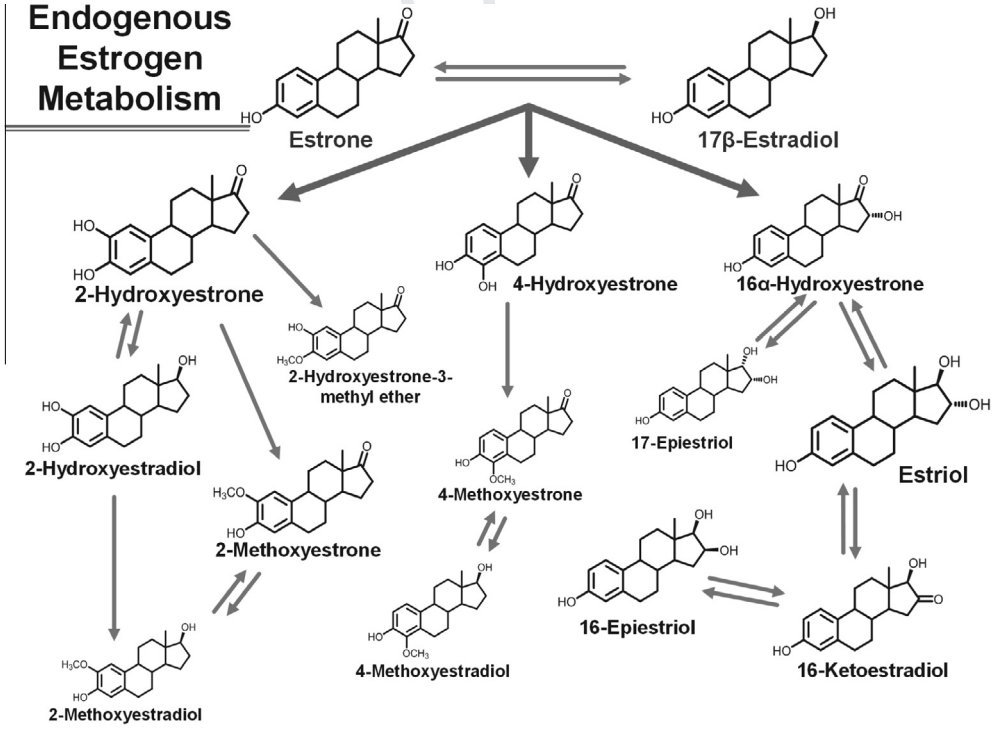

Fig. 1. Estrogen metabolism pathways. The parent estrogens, estrone and estradiol, can be irreversibly hydroxylated at the C-2, C-4, or C-16 positions of the steroid ring. The relative abundance of the estrogen or estrogen metabolite in serum from postmenopausal women is indicated by the relative size of the chemical structure. The structures are for the unconjugated forms of the estrogens and estrogen metabolites.

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