

# Endogenous Estrogens and Breast Cancer Risk: The Case for Prospective Cohort Studies

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It is generally agreed that estrogens, and possibly androgens, are important in the etiology of breast cancer, but no consensus exists as to the precise estrogenic or androgenic environment that characterizes risk, or the exogenous factors that influence the hormonal milieu. Nearly all the epidemiological studies conducted in the 1970s and 1980s were hospital-based case-control studies in which specimen sampling was performed well after the clinical appearance of the disease. Early prospective cohort studies also had limitations in their small sample sizes or short follow-up periods. However, more recent case-control studies nested within large cohorts, such as the New York University Women's Health Study and the Ormoni e Dieta nell'Eziologia dei Tumori study in Italy, are generating new data indicating that increased levels of estrone, estradiol and bioavailable estradiol, as well as their androgenic precursors, may be associated with a 4- to 6-fold increase in the risk of postmenopausal breast cancer. Further new evidence, which complements and expands the observations from the latter studies, shows that women with the thickest bone density, which may be a surrogate for cumulated exposure to hormones, experience severalfold increased risk of subsequent breast cancer as compared to women with thin bones. These data suggests that endogenous sex hormones are a key factor in the etiology of postmenopausal breast cancer. New prospective cohort studies should be conducted to examine the role of endogenous sex hormones in blood and urine samples obtained early in the natural history of breast cancer jointly with an assessment of bone density and of other important risk factors, such as mammographic density, physical activity, body weight, and markers of individual susceptibility, which may confer increased risk through an effect on the metabolism of endogenous hormones or through specific metabolic responses to Western lifestyle and diet. — *Environ Health Perspect* 105(Suppl 3):587-592 (1997)

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## Endogenous Estrogens and Breast Cancer Risk

Because of their physiologic stimulatory actions on mammary glands, estrogens, especially estrone and estradiol, have long been linked to the promotion and growth of breast cancer (1). Animal studies have shown repeatedly that estrogens are able to induce and promote mammary tumors and that the removal of the ovaries, or the

administration of antiestrogenic drugs, achieves the opposite effect (2,3). Substantial indirect evidence (4,5) supports an etiologic role for estrogens in human breast cancer. For example, it has long been known that reproductive factors, such as delayed age at first full-term birth, increase a woman's risk for breast cancer, and that bilateral oophorectomy at a young age confers lasting protection against breast cancer (6). The hormonal environment typical of premenopausal women, characterized by high levels of estradiol, progesterone, and gonadotropins, has been suggested (7) as the key to understanding why, in all populations, the incidence of breast cancer increases much more steeply among premenopausal women than among postmenopausal women (8,9).

Epidemiological research seeking direct evidence on the role of endogenous estrogens in breast cancer has produced conflicting and disappointing results (10). In the late 1960s and 1970s, a number of

hospital-based case-control studies of the relationship between urinary and circulating estrogens and breast cancer (11-17) generated enthusiasm and were followed by a wave of similar efforts (7), most notably at the Harvard School of Public Health (12,18-21). Such efforts produced inconsistent results. Most reports found no association (22-24), although quite a few observed a modest, positive relationship with estradiol (13,21,25-30).

Renewed expectations followed the 1981 publication of a report by Siiteri and colleagues (31) suggesting that only the free and albumin-bound fractions of estradiol, rather than the fraction bound to sex hormone-binding globulin (SHBG), are relevant to breast cancer. The hypothesis was based on observations that 35 to 65% of estradiol and 50 to 75% of testosterone circulate bind to SHBG (a glycoprotein secreted by the liver), from which they dissociate very slowly (32). Approximately 0.5 to 2% of the steroids circulate unbound (free) and the rest bind to albumin. The prevailing opinion concerning the role of SHBG is that binding reduces the availability of estradiol to the cells and that the free hormone (including the fraction that continuously dissociates from binding with albumin) diffuses freely into the cytoplasm and thus is immediately available for biologic action (33).

Siiteri's initial paper (31) reporting that free estradiol was elevated in postmenopausal breast cancer was followed by a number of similar case-control studies (21,26,30,34-42). With some exceptions, (36,39,42) these studies appeared to confirm Siiteri's initial observations, but their overall impact was modest when it became evident that the observed association was not of sufficient strength to explain much of breast cancer epidemiology. Enthusiasm for the hypothesis was further dampened by a number of case-control studies of SHBG, some reporting weaker than predicted protective associations (34,40,43,44), and many failing to observe any (21,29,30,39,42,45).

To date, only a handful of investigators have examined the role of endogenous estrogens prospectively. In the mid-1950s, Bulbrook and colleagues (46) pioneered the effort by initiating a prospective cohort study of 5000 women in the British island of Guernsey, which eventually led to the identification of 27 cases of breast cancer. Initially, they reported no differences in

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Abbreviation used: SHBG, sex hormone-binding globulin.

urinary estrogens between cases and noncases, but later showed that serum levels of free estradiol were considerably higher among the cases than the controls in the same population (38). More recently, Wysowski and colleagues (47) conducted a case-control study nested in a prospective cohort study of 11,009 women in Washington County, Maryland, which was assembled in 1974 (48). In a small study group that included 17 premenopausal and 39 postmenopausal cases, they reported no change in breast cancer risk associated with serum levels of estrone, total estradiol, estriol, and progesterone among premenopausal and postmenopausal subjects. These results were later questioned on technical grounds (49). Later a small prospective cohort study in California (the Rancho Bernardo study) reported no association between breast cancer and serum levels of estrone, estradiol, and SHBG in 442 middle-age women, with 15 postmenopausal breast cancer cases (50).

The current chapter of the epidemiology of breast cancer concerned with the role of reproductive hormones could have ended in the early 1990s with the conclusion that, contrary to expectations, endogenous estrogens measured in blood and urine do not reflect breast cancer risk. Moreover, because epidemiologic evidence did not suggest evident associations, launching new epidemiologic studies on the topic would have appeared futile, or even wasteful.

Despite the lack of supporting evidence, a few additional studies, which had been designed specifically to address the role of endogenous hormones in breast cancer, were already underway. The results of four of these studies were published in rapid sequence between late 1994 and early 1996. First, a new case-control study, nested in the Washington County cohort and based on a set of 10 to 12 premenopausal and 29 postmenopausal cases who had not been part of previous analyses, reported a 4-fold increase in breast cancer risk associated with upper-tertile total and free estradiol levels in the postmenopausal group (51). Subsequently, the preliminary results of a cohort of more than 14,000 women in New York (the New York University Women's Health Study), which had been assembled and followed up since 1985, were reported (52). In a postmenopausal case-control study nested within this cohort (130 cases, 271 controls), estrone, total estradiol, and free estradiol were related to a 3- to 6-fold

increase in breast cancer risk among women who were sampled 2 years or more before cancer diagnosis; SHBG, as estimated by the percentage of estradiol bound to the protein, appeared strongly protective (53). Soon afterward, Berrino and colleagues (54) reported the results of a prospective cohort study in northern Italy (the Ormoni e Dieta nell'Eziologia dei Tumori study), with a design similar to the New York Women's Health Study Cohort. In a case-control study nested in this cohort of 10,000 (24 breast cancer cases out of 4040 postmenopausal subjects), they reported a 5.5-fold increase in breast cancer risk in the upper tertile of serum estradiol, as well as a strong protective effect of SHBG, and a strong association with serum testosterone. Dorgan et al. (55) reported the result of a case-control study (71 cases, 133 controls) nested within the Columbia, Missouri, Breast Cancer Serum Bank, a cohort of 3375 postmenopausal women enrolled between 1977 and 1989. In this study, women in the highest quartile of bioavailable (non-SHBG bound) estradiol and testosterone had a 5- to 6-fold increase in risk of breast cancer. Key and colleagues recently reported an update analysis of urine samples from 1000 participants of the Guernsey Island cohort, including 69 confirmed breast cancer cases (56). No associations were evident with premenopausal estrogens, but among women who were postmenopausal at the time of urine collection, there were evident trends of increasing risk of breast cancer with increasing excretion of estradiol and total estrogens.

Thus, in the last 2 years, and in sharp contrast with previous data, the hypothesis that in postmenopausal women circulating estrogens are associated with the risk of breast cancer has gained unexpected momentum from new data, showing relative risks that are sufficiently strong to justify additional efforts to exploit their potential preventive implications. If it can be shown conclusively that endogenous estrogens are strongly related to breast cancer risk, the road may be open to investigate the origins of the association and to explore new possibilities for chemopreventive, nutritional and lifestyle interventions. Before launching new efforts, however, thought should be given to why the outcome of epidemiological studies has shifted so dramatically. Because all the recent, positive reports were the product of case-controls nested within prospective cohorts and the older negative

ones were hospital-based case-control studies, it is possible that differences in study design could explain the huge differences in results between early and recent studies.

## Issues in Study Design

### Traditional Case-Control Studies

The majority of early studies that assessed the association between endogenous estrogens in blood and urine and the risk of breast cancer were case-control studies in which breast cancer cases were identified among patients attending medical facilities for diagnosis or treatment. In this study design, assessment of exposure to endogenous hormones is performed among the cases on biological specimens (e.g., peripheral venous blood, urine, or saliva) that are obtained at the time, or sometimes long after, breast cancer has become clinically manifest. Because sampling occurs after the onset of clinical disease, there is uncertainty as to whether exposure truly precedes disease or, in other words, whether exposure and disease occur in the correct temporal sequence—one of the most fundamental prerequisites of observational studies. Thus, the results of these studies are meaningful only if it can be reasonably assumed that the presence of the disease at the clinical stage does not influence hormonal measurements and that the hormonal measurements provide an unbiased and accurate reflection of hormone levels during an appropriate time in the natural history of the disease.

It is not known whether biochemical measurements conducted on samples obtained after clinical diagnosis reflect long-term endogenous hormone levels. However, it is clear that under normal conditions, blood hormone levels are subject to fluctuations, such as circadian, menstrual, and seasonal cycles, and are influenced by physical activity, diet, emotions, trauma, and disease. The possibility of distortion on relative risk estimates consequent to the misclassification of exposure induced by these fluctuations has been recognized. Attempts at reducing their impact have been made by most investigators by restricting biological sample collection to a narrow time frame, such as a few hours of the day, a single season, or a specific phase of the menstrual cycle (but never by repeat sampling, which might have been more effective).

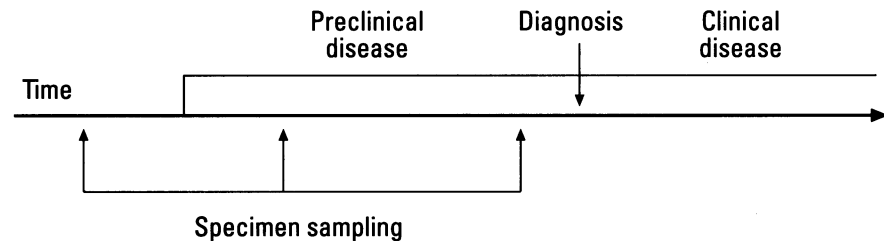
Even if single hormonal measurements were a good reflection of past levels in normal conditions, postdiagnostic sampling

can introduce bias; the disease itself may affect hormonal concentrations among the cases, but not among the nondiseased controls. Clinical cancer is accompanied by localized or systemic responses, such as angiogenesis, necrosis, inflammatory reactions in regional lymph nodes, and metastatic spread, which may be accompanied by (or be the expression of) metabolic or hormonal imbalance. Furthermore, among the patients, but not the controls, the diagnosis of cancer is accompanied by inevitable emotional distress, by changes in diet, and by sudden reductions in the level of physical activity, which can have a profound influence on hormonal concentrations in biological fluids. Obviously, the potential for bias will be greater in studies in which specimen sampling is performed after the start of surgical or medical treatment.

It is not surprising that the results of case-control studies with postdiagnostic sampling have shown inconsistent results. Indeed, in the absence of any information about the validity of the underlying assumptions concerning postdiagnostic measurements, it could not be reasonably excluded that the absence or the presence of an association simply reflects measurement error or bias. Therefore, the results of these studies should be taken very cautiously and with the understanding that their value in assessing associations is limited or at best purely exploratory.

### Prospective Cohort Studies

The alternative approach used to assess the relationship between endogenous hormones and breast cancer is conduction of case-control studies nested within a prospective cohort. In this type of study, the assessment of exposure is performed on biological samples collected from all or most of the cohort members prior to clinical disease onset and stored for future use (Figure 1). Rather than measuring biochemical markers on specimens from all members of the cohort, which would be prohibitively expensive, only breast cancer cases and controls drawn from among the nondisease members of the cohort are considered. This approach provides unbiased results and only a negligible loss of statistical power, as compared to a full cohort analysis (57,58). Samples from the cases are collected prior to the clinical detection of cancer so that exposure and clinical disease follow in the appropriate temporal sequence. In addition to being free from postdiagnostic sampling problems, nested case-control studies offer the unique advantage that cases and controls are drawn



**Figure 1.** Sampling of biological specimens in case-control studies nested within a prospective cohort.

from the same source population and are highly internally comparable.

Prediagnostic sampling and high comparability between cases and controls make case-control studies nested within prospective cohorts the ideal study design to assess the etiologic role of metabolic factors in chronic disease, particularly endogenous hormones in breast cancer. Few such studies have been conducted for logistical and financial reasons. A large case-control study with postdiagnostic sampling can be completed rapidly (i.e., a few years), presents limited organizational complexities, and is usually relatively inexpensive. A medium-size case-control study nested within a cohort, unless it exploits existing resources, requires a period of time sufficiently long for cases to accrue, could be logistically complex and (because the underlying cohort is large) usually requires a substantial budget.

It is undeniable that cohort studies take a long time to complete, are complex and expensive, and carry the danger that, after several years of data collection, the hypotheses justifying the original efforts are superseded by new research developments, or that the laboratory methods originally proposed become obsolete. On the other hand, it is true also that the long time lag between biological sampling and the occurrence of clinical disease represents the most fundamental strength of these studies. Unless biomarkers are developed that would allow us to estimate past exposures with sufficient degree of reliability, the best chance to study the role of endogenous hormones in relation to cancer is by measuring hormones as early as possible during the various stages of the natural history of the disease, a goal that can be achieved only through well designed and long-lived prospective cohort studies.

A further advantage of the nested case-control approach over traditional hospital-based case-control studies is the need to bank biological specimens from the whole cohort. Even though the bank is organized to fulfill the requirements of

specific hypotheses, ultimately, only a very small fraction of the total number of specimens banked will be used to test the study's original hypotheses. Most of the remainder will be available for additional investigations. Thus, prospective cohort studies with biological banking provide resources of great efficiency that would remain available for scientific inquiries long after the completion of the initial study. This unique advantage, however, must be openly recognized at the very beginning so that the cohort and its biological bank are designed to take full advantage of it. The design of such studies should take into account issues such as *a*) obtaining appropriate informed consent from individuals for future reference, *b*) the timing of sampling in relation to physiological factors (e.g., pregnancy, ovariectomy, menstrual cycle, menopause) and external events (e.g., recent meals, medication use, recreational drug use, physical activity), *c*) the tight standardization of procedures for the collection, preparation, and handling of biological specimens, and *d*) considerations for long-term storage of specimens, such as storage temperature, type of specimens in storage, volume and number of aliquots, defrosting, and the likelihood of accidents. All these factors may significantly affect the efficiency of prospective cohort studies in conducting future studies on many disease outcomes. They may mask differences in biomarker levels between individuals or within the same individual at different points in time, or may affect the ability to control for confounding, or to assess effect modification (e.g., through markers of genetic susceptibility).

### Future Perspectives

Emerging epidemiological evidence shows that increased blood levels of major sex steroid hormones (androgens and estrogens), play an important role in the etiology of breast cancer in postmenopausal women. Recent data also show that women with the thickest bone density, which can be taken as a surrogate for cumulative, lifetime exposure to endogenous hormones,

experience a 3-fold increased risk of breast cancer as compared to women with thin bones (59). These findings complement and expand the observations based on direct measurement of endogenous hormones and are consistent with data relating reduced risk of breast and endometrial cancers to the occurrence of bone fractures in the forearm (60) and hip (61,62).

Increased levels of circulating hormones may be the result of an overall increase in ovarian or adrenal secretion occurring or persisting after menopause. In high-risk populations, women tend to experience menarche at a younger age, menopause at an older age and reach higher adult body height and weight than in low-risk populations (63,64). These factors, which have been associated with increased breast cancer risk in all populations (65), suggest that the Western lifestyle influences cancer risk early in life. Thus, biochemical measurements of endogenous hormones could be used as biological markers of metabolic dysregulation induced by a lifetime exposure to the hypercaloric diet and sedentary lifestyle that are typical of Western populations. Kaaks (66) argued that nutritionally induced hyperinsulinemia and insulin resistance are the fundamental metabolic changes at the root of the pathologic processes leading to breast cancer. This hypothesis offers a model for a physiologic link between lifetime exposure to overnutrition, excessive body weight and low physical activity, the development of chronic alterations in the endocrine secretion of steroid hormones, especially ovarian androgens, and reduced production of SHBG by the liver. In high-risk populations, nutritionally induced endocrine dysregulation would begin early in life so that the key to understanding breast cancer etiology would be in the metabolic and hormonal alterations present in childhood and in pre- or peripubertal years, which continue throughout a woman's life.

A number of observations, including large differences in incidence rates between populations (67) and studies of women

migrating from low-risk to high-risk areas (68–70) suggest that diet is probably the single most important factor in breast cancer etiology. This may hold true even though analytical epidemiological studies have failed to reveal specific patterns of nutrition that are associated with the disease (71). The lack of a convincing association between diet and breast cancer can be ascribed in large part to methodological problems, e.g., the inadequacy of dietary assessment and the difficulty of measuring small differences among individuals living in the same geographical area who share similar nutritional habits (72). It has been suggested (66) that the Western lifestyle would induce hormonal dysregulation that is dependent more on individual susceptibility to specific physiologic responses to overnutrition than on comparatively small differences in habitual diet, especially if dietary assessment covered only a short period of time during adult life. Thus, in light of the limitations of dietary assessment, it seems that the role of Western lifestyle in breast cancer would be better understood with knowledge of the precise metabolic and hormonal responses induced by overnutrition and lack of physical activity rather than with the study of dietary factors per se.

In summary, evidence is rapidly emerging in support of a key role for endogenous sex hormones in breast cancer etiology. Hormonal metabolite concentrations in body fluids may be used as biological markers of long-term dysregulation induced by exposure to overnutrition and lack of physical activity—probably the most important risk factors for breast cancer in all populations. Unfortunately, it took an inordinately long time to recognize the key role of endogenous hormones. Additionally, the etiologic relationship between endogenous hormones and disease could not be convincingly addressed in epidemiologic studies in which hormonal measurements were made well after the disease had surfaced clinically. In light of the success of more recent efforts, we now have the

elements for an effective effort aimed at reducing the uncertainties surrounding the etiology of breast cancer.

New prospective cohort studies will offer the advantage of allowing the measurement of metabolic and hormonal markers in the early stages of the disease, or even before the beginning of the disease itself. These studies may elucidate the role of the major endogenous reproductive hormones in breast cancer and their relationship to nutritional and metabolic risk factors. Bone density, breast density, anthropometry, and physical activity, as well as genetic markers of individual susceptibility (which may confer increased risk through an effect on endogenous hormones or through specific physiologic and metabolic responses to overnutrition), may need to be considered. These studies will focus primarily on hormonal and metabolic imbalances associated with breast cancer in adult life. However, their results should create new opportunities to relate metabolic biomarkers with lifestyle determinants earlier in life, thus providing the necessary knowledge to design effective strategies for breast cancer prevention.

The new studies will not be rapid or easy to conduct. The best investment of our modest resources during the next decade would be to elucidate the role of endogenous sex hormones in breast cancer. Such an investment of time is necessary and unavoidable; it would be difficult, if not impossible, to implement effective measures for the primary prevention of breast cancer without a sufficient epidemiological knowledge of nutritionally induced hormonal imbalances. Even though time requirements would be substantial, this effort would create tremendous opportunities for additional research, e.g., exploration of the association of breast cancer with exposure to xenobiotics in the environment or in the diet, the role of specific nutritional factors measurable through biomarkers, and other types of chronic diseases affecting women.

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