HORM CANC (2010) 1:2–10
DOI 10.1007/s12672-009-0003-0

Circulating Sex Steroids and Breast Cancer Risk in Premenopausal Women

Susan E. Hankinson · A. Heather Eliassen

Received: 20 October 2009 / Accepted: 17 December 2009 / Published online: 9 February 2010

Abstract Evidence from both laboratory and epidemiologic studies indicate a key role of hormones in the etiology of breast cancer. In epidemiologic studies, indirect data, including the consistent associations observed between reproductive factors and breast cancer risk, support an important contribution of hormones to risk. Recently, the associations between circulating hormones in premenopausal women and subsequent risk of breast cancer have been evaluated. To date, both positive and null associations have been observed for estrogens and inverse and null associations for progesterone with breast cancer risk. For estrogens, the relationships may vary by menstrual cycle phase (e.g., follicular versus luteal phase), although this requires confirmation. Few studies have evaluated estrogen metabolites in relation to breast cancer risk; hence, no conclusions can yet be drawn. Findings for the largely adrenal-derived dehydroepiandrosterone (DHEA) and DHEA sulfate also are inconsistent and may vary by age. However, relatively consistent positive associations have been observed between testosterone (or free testosterone) levels and breast cancer risk; these associations are of similar magnitude to those confirmed among postmenopausal women. In this review, we summarize current evidence and identify gaps and inconsistencies that need to be addressed in future studies of sex steroids and premenopausal breast cancer risk.

S. E. Hankinson (✉) · A. H. Eliassen
Channing Laboratory, Department of Medicine,
Harvard Medical School and Brigham and Women's Hospital,
181 Longwood Avenue,
Boston, MA 02115, USA
e-mail: sue.hankinson@channing.harvard.edu

S. E. Hankinson · A. H. Eliassen
Department of Epidemiology, Harvard School of Public Health,
Boston, MA 02115, USA

Keywords Prospective · Estrogens · Androgens · Progesterone · Breast cancer · Premenopausal

Over the last decade, the association between circulating sex steroid concentrations, both estrogens and androgens, and risk of breast cancer in postmenopausal women has become well established. Postmenopausal women in the top 25% of estrogen or androgen levels have a 2- to 3-fold higher risk of being diagnosed with breast cancer than women in the lowest 25% of levels [1–3]. Because of these consistent associations, research is now being directed towards determining if circulating hormone levels could be used clinically to help determine a postmenopausal woman's individual risk of breast cancer and could help direct the frequency of mammographic screening or use of chemopreventive agents. However, what is the status of research on circulating sex steroid levels and breast cancer risk in premenopausal women? Are similar positive associations observed? An overview of our current understanding of these relationships is provided in the current review.

The critical role of hormones in the etiology of premenopausal breast cancer is well confirmed from both laboratory and human data [4–7]. Evidence from epidemiologic studies supporting a hormonal etiology includes the consistent associations observed between several hormonally related risk factors and breast cancer (Table 1) [4, 8]. Nulliparous women and women having a late age at first birth (e.g., >35 years) have a higher breast cancer risk than parous women or those with an early age at first birth, respectively. An early age at menarche, and therefore earlier exposure to reproductive hormones, is associated with an increased risk of breast cancer. Further, in premenopausal women, obesity is associated with lower breast cancer risk,

Table 1 Established hormone-related risk factors for premenopausal breast cancer

Risk factor	Higher risk group
Age at menarche	Early age at menarche
Age at first birth	Later age at first birth
Parity	Nulliparous or fewer children[a]
Premenopausal body mass index (kg/m^2)	Low body mass index
Childhood body size	Small body size/lean
Breast feeding	No breast feeding (or shorter durations)
Mammographic density	Higher density

[a] Parity infers a short term (<1 decade) increase in risk, followed by a long-term decrease in risk

References: Hankinson SE et al. 2004 4. *Breast Cancer Res:* 6(5): 213–218; de Waard F, Thijssen JH. 2005 8. *J Steroid Biochem Mol Biol:* 97(5): 451–458

perhaps due to increased anovulatory cycles. Also, tamoxifen, a selective estrogen receptor modulator, reduces breast cancer incidence [9, 10] and is used in breast cancer treatment [11].

This review will focus primarily on data from prospective studies. In these studies, hormone levels are measured prior to breast cancer diagnosis and hence are less susceptible to bias than are retrospective case-control studies. In retrospective case-control studies, hormone levels in the women with breast cancer may be influenced either by the tumor or its treatment and hence may not reflect prediagnostic levels. First, we discuss several methodologic issues that are relevant in interpreting the results of these prospective studies.

Methodologic Issues

In most epidemiologic studies, for both logistic and cost reasons, usually, only a single blood sample can be collected per study subject. Hence, an important issue is whether a single blood hormone measure can reasonably reflect long-term hormone levels in premenopausal women (as long-term hormone levels are the exposure of primary interest in etiologic studies of breast cancer). To address this, several studies have evaluated the correlation in hormone levels from samples collected repeatedly over a several-year period from premenopausal women.

For testosterone measured over a 1–3 year period, intraclass correlation coefficients (ICC; the ratio of the between person variation/[the between + within person variation]) ranged from 0.56–0.73 [12–14], for both timed (e.g., follicular or luteal phase) and untimed samples. For dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), androgens derived primarily from the adrenals, the ICC over the same period of time were even higher, ranging from 0.81–0.95 [12, 14].

Estrogens, particularly estradiol, vary substantially over the menstrual cycle such that a single blood sample would not well represent an entire menstrual cycle. However, several studies have assessed how well a single hormone measurement from a specific phase of the menstrual cycle (i.e., follicular or luteal) might be representative of long-term levels in that phase. In the only large study to date (n= 113 women), the ICC over a 2–3 year period for estradiol was 0.38 in the follicular phase and 0.45 in the luteal phase; the luteal phase ICC increased to 0.51 when samples collected in an anovulatory cycle were excluded. The ICC for estrone was similar, while the ICC for estrone sulfate was higher (0.60–0.69). When women who are anovulatory in at least one cycle or missed their luteal phase are not excluded from these types of analysis, the ICC can be very low (e.g., <0.1[12, 15]) depending on the prevalence of these two factors. Notably, the ICC over time for estradiol when combining follicular and luteal samples (i.e., disregarding cycle phase) was just 0.02 [14] indicating that samples untimed in the menstrual cycle would not be able to distinguish between woman differences in hormone levels and hence are not usable for epidemiologic studies. Progesterone also varies substantially over the menstrual cycle. The ICC for luteal phase progesterone has been reported at 0.29 [14] to 0.54 [15] over a 1–3 year period.

Using a single blood sample in epidemiologic studies to represent long-term hormone levels undoubtedly results in some attenuation of relative risk estimates. The level of reproducibility noted above for androgens and estrone sulfate, and to a lesser extent estradiol, estrone, and progesterone, is similar to that of blood pressure or serum cholesterol (where ICCs over a several-year period are 0.6–0.7), parameters that are reasonably measured and consistent predictors of disease in epidemiologic studies [16]. For assessment of estrogen levels, given the poor ICCs for estrogens when menstrual timing is disregarded, it is critical to account for timing within the menstrual cycle either in the study design or data analysis. In contrast, the androgens vary much less markedly over the menstrual cycle (relative to the between woman variation in levels), and hence, timing the blood collection is not required [14]. Given the lower ICC for estradiol and progesterone, correction of relative risks for measurement error would provide additional insight into the underlying true association between hormones and risk, as risk estimates for hormones with lower ICCs are likely underestimated [17].

In epidemiologic studies, circulating hormone levels are most often measured because blood samples are relatively easy to obtain and can be readily used for risk screening. Yet, relatively little is known about how circulating levels correlate with exposure in the breast tissue. Only two

studies have presented the correlation between circulating hormone levels and breast tissue levels. In the first, 22 premenopausal women (16 with breast cancer and six with benign breast disease) were included and the correlation between tumor tissue hormone levels and blood hormone levels assessed [18]. The correlation for estrone was 0.52 in the follicular phase and 0.63 in the luteal phase. For testosterone, the correlation was 0.64 in the follicular phase and −0.51 in the luteal phase; why the correlation in the follicular phase was strongly positive and that in the luteal phase strongly inverse is not clear and requires confirmation. Only one study has presented correlations between non-malignant breast tissue levels and blood levels, collected from 13 premenopausal women with breast cancer [19]. The correlation was 0.69 for estradiol, estrone, and estrone sulfate; correlations were not reported separately by menstrual cycle phase. Thus, although data remain scarce, to date results suggest some correlation between circulating levels and tissue levels in premenopausal women.

Sex Steroids and Breast Cancer Risk

Estrogens and Estrogen Metabolites

Mechanistically, estrogens (e.g., estradiol and estrone) contribute to tumor growth by promoting the proliferation of cells with existing mutations or perhaps by increasing the opportunity for mutations [20]. Although most research has focused on the primary (or parent) estrogens, increasing interest has also focused on the metabolites of estrogen. The metabolism of estrone and estradiol occurs through different pathways, including the 2-, 4-, and 16α-hydroxylation pathways, and experimental studies have shown that estrogen metabolites may have differential estrogenic and genotoxic activities [21, 22]. For example, the metabolites in the 4- and 16α-hydroxylation pathways may have higher estrogenic activity than estradiol [23–29] while 2-catechol estrogen metabolites may act as either weak mitogens [30, 31] or inhibitors of proliferation [32, 33]. In addition, DNA damage may vary by metabolite, e.g., the 2-catechol estrogen quinones form stable DNA adducts that are reversible without DNA destruction [34–36], while the 4-catechol estrogen quinones form unstable adducts, leading to depurination and mutation in vitro and in vivo [35, 37–40]. Thus, the specific pattern of estrogen metabolism has been hypothesized to influence a woman's breast cancer risk, either through estrogen receptor-mediated cell proliferation or through genotoxic effects of the metabolites.

Seven prospective studies of circulating estrogens (i.e., estradiol, estrone, and estrone sulfate) in premenopausal women and risk of breast cancer have been published to date. Three of the studies were very small (14–51 cases [41–43]) or did not account for menstrual cycle phase [43]; no significant association with estradiol was noted, although because of study size, precision of the estimates was uniformly low. In two other studies (with 62 and 79 cases) where timing of the blood collection was carefully accounted for, non-significant positive associations were reported (relative risks for the top versus bottom tertile of estradiol levels: 1.7 for both follicular and luteal estradiol levels and risk [44] and 2.0 [95% CI 0.9–4.0] over the entire menstrual cycle [45]).

Two much larger studies have also addressed these associations. In the largest study to date, conducted in the EPIC cohort, with 285 invasive breast cancer cases and 555 controls, a single blood sample was collected per woman, and the day of collection within the menstrual cycle was recorded [46]. Controls were matched to cases on age, study center, time-of-day of collection, and phase of the menstrual cycle at blood collection (in five categories). Comparisons between case and control hormone levels were based on residuals from spline regression models; the residuals indicated how much an individual's hormone level deviated from the predicted hormone levels on that day of the menstrual cycle. Overall, no association was observed for either estradiol or estrone (top to bottom quartile comparison $RR = 1.0[95\%\mathrm{CI} = 0.7 - 1.5]$ for estradiol; Table 2). Of note, because blood samples were collected across the menstrual cycle, the investigators had relatively limited ability to evaluate associations within specific parts of the cycle.

In the second large prospective study [47], conducted within the Nurses' Health Study II (NHSII), both early follicular (days 3–5) and mid-luteal (estimated 7–9 days prior to next cycle) samples were requested from each woman. Timing of the luteal sample collection was by backward dating from the onset of the next menstrual cycle. The analysis included 197 cases (in situ and invasive combined) with 394 controls matched on age, menopausal status at diagnosis, ethnicity, luteal day, date and time of blood draw, and fasting status. Follicular, but not luteal, total and free estradiol was significantly associated with breast cancer risk (top to bottom quartile comparison $RR = 2.1[95\%\mathrm{CI} = 1.1 - 4.1]$ for follicular total estradiol; Table 2). Associations were stronger among the 89 estrogen receptor positive (ER+)/progesterone receptor positive (PR+) cases (similar comparison $RR = 2.7[95\%\mathrm{CI} = 1.2 - 6.0]$ for follicular total estradiol), supporting the biologic hypothesis that estrogens stimulate ER + tumors. No association was observed with either estrone or estrone sulfate (in either the follicular or luteal phase of the cycle).

Interestingly, in this study, follicular, and not luteal, estradiol levels were positively associated with risk. Although these initial findings require confirmation, several possible reasons exist for these differences. A greater

Table 2 Circulating levels of estradiol, testosterone, and DHEAS in relation to premenopausal breast cancer risk

RR (95% CI) by category of circulating hormone levels[a, b]						
Study	Cases/controls	1 (low)	2	3	4 (high)	*p* trend
Estradiol						
Rosenberg et al., 1994 [45]	79/306	1.0	1.8 (1.0–3.7)	2.0 (0.9–4.0)		0.18
Kaaks et al. 2005 [46]	285/555	1.0	1.0 (0.6–1.5)	1.0 (0.7–1.6)	1.0 (0.7–1.5)	0.89
Eliassen et al. 2006 [47]						
Follicular	185/368	1.0	2.0 (1.1–3.6)	1.7 (1.0–3.2)	2.1 (1.1–4.1)	0.08
Luteal	175/349	1.0	1.2 (0.7–2.3)	1.8 (1.0–3.3)	1.0 (0.5–1.9)	0.99
Testosterone						
Micheli et al. 2004 [63]	40/108	1.0	1.1 (0.4–3.0)	2.2 (0.6–7.6)		0.28
Kaaks et al. 2005 [46]	370/726	1.0	1.4 (1.0–2.1)	1.4 (0.9–2.0)	1.7 (1.2–2.6)	0.01
Eliassen et al. 2006 [47]						
Follicular	190/374	1.0	1.3 (0.8–2.2)	1.4 (0.8–2.3)	1.3 (0.8–2.4)	0.35
Luteal	192/390	1.0	1.3 (08–2.3)	1.4 (0.8–2.3)	1.6 (0.9–2.8)	0.10
DHEAS						
Helzlsouer et al. 1992 [65]	15/29	1.0	1.4 (0.7–6.4)	1.2 (0.2–6.3)		0.90
Micheli et al. 2004 [63]	65/260	1.0	0.9 (0.3–2.6)	1.2 (0.4–3.3)		0.76
Page et al. 2004 [66]	302/591[c]	1.0	1.3 (0.8–1.9)	1.3 (0.9–2.0)	1.1 (0.7–1.7)	0.83
	101/197[c]	1.0	1.5 (0.7–3.4)	1.5 (0.7–3.4)	1.5 (0.7–3.3)	0.62
Kaaks et al. 2005 [46]	370/726	1.0	1.4 (1.0–2.0)	1.0 (0.7–1.5)	1.5 (1.0–2.1)	0.10
Tworoger et al. 2006 [67]	208/421	1.0	0.7 (0.4–1.2)	1.1 (0.6–1.8)	1.3 (0.8–2.1)	0.08

[a] Studies included in Table 2 provided relative risks (*RR*) within categories of hormone levels in the original publication. Other studies (four for estradiol, two for testosterone) provided either no *RR*, *RR* for 1-log-unit increase in hormone levels, or *RR* for levels above vs. below median; these studies are discussed in the text

[b] Hormone data presented in quartile or tertile categories depending on the study

[c] 302/591 subjects premenopausal at blood collection; 101/197 subjects premenopausal at both blood collection and cancer diagnosis

proportion of early follicular estradiol levels (vs. luteal levels) derives from non-ovarian sources [47–49] and thus may better reflect estrogen levels at the breast tissue. Another possibility is that the follicular phase might be a more relevant period of exposure, given that women in later premenopause, as were included in this study, have longer follicular and slightly shorter luteal phases [50, 51]. Alternatively, estradiol might be more important in the low-progesterone environment of the follicular phase. Breast tissue concentrates estradiol to a greater degree at lower circulating levels, such as in the follicular phase [52, 53]. In addition, apoptosis in lobuloalveolar cells may be higher in the luteal versus the follicular phase [54], such that the proliferative effects of high estrogen levels in the luteal phase may be offset by increased apoptosis.

To date, there have been only three prospective studies to evaluate estrogen metabolites and breast cancer risk among premenopausal women, and these studies only have included the 2-hydroxyestrone and 16α-hydroxyestrone metabolites. Two studies of fewer than 70 cases each examined the 2-hydroxyestrone/16α-hydroxyestrone ratio in urine [55, 56]. In the Guernsey III cohort, with cases and controls matched on menstrual cycle phase (follicular or luteal), a non-significant reduced risk of breast cancer was observed among women in the highest vs. lowest tertile of the 2-hydroxyestrone/16α-hydroxyestrone ratio, $RR(95\%\mathrm{CI}) = 0.75(0.35 - 1.62)$ [55]. In the Italian ORDET study, with urine samples collected in the luteal phase of the menstrual cycle (days 20–24), a non-significant inverse association was reported for the 2-hydroxyestrone/16α-hydroxyestrone ratio, *RR* (95% CI) for top vs. bottom quintile=0.55 (0.23–1.32) [56].

The most recent and largest study of estrogen metabolites and breast cancer in premenopausal women, in the NYU study, assessed serum 2-hydroxyestrone and 16α-hydroxyestrone [57]. With 377 cases matched to controls on day and phase of the menstrual cycle, no significant associations were found overall with either metabolite or the 2-hydroxyestrone/16α-hydroxyestrone ratio. However, when examining ER + and ER− cases separately, a non-significant increased risk of ER+ breast cancer was observed with higher 2-hydroxyestrone/16α-hydroxyestrone ratios, *RR* (95% CI) for top vs. bottom quartile= 2.15 (0.88–5.27).

To date, no studies have evaluated the role of other estrogen metabolites in the etiology of breast cancer in premenopausal women. It is possible that other metabolites

are important either individually, e.g., 4-hydroxy estrogens or methylated catechols, or with respect to the particular pattern of metabolism within individuals, e.g., greater proportion of methylated vs. catechol metabolites. A high-performance liquid chromatography–tandem mass spectrometry ($LC–MS^2$) assay was developed recently to measure concurrently 15 estrogens and estrogen metabolites in urine with high sensitivity, specificity, accuracy, and reproducibility [58, 59]. Reproducibility of these estrogen metabolites within woman over time suggests that the metabolites are suitable for epidemiologic study (e.g., ICC for luteal phase catechols, methylated catechols, and the catechol/methylated catechol ratio were 0.72, 0.61, and 0.60, respectively) [60]. In addition, the correlation between the parent estrogens and their metabolites was fairly low (e.g., estrone and estradiol correlations with other individual estrogen metabolites was ≤0.52), suggesting that these estrogen metabolites may provide additional information that may be important in the etiology of breast cancer.

Androgens

Androgens have been hypothesized to increase breast cancer risk either directly, by increasing cellular growth and proliferation, or indirectly, by their conversion to estrogen [5]. In experimental studies, androgens either increase or decrease cell proliferation, depending upon the model system [5]. The effect of adrenal androgens, such as dehydroepiandrosterone (DHEA) or its sulfate, on breast cancer risk has further been hypothesized to depend on estrogen levels, such that before menopause, DHEA/S exhibit anti-estrogenic effects, but after menopause they are weakly estrogenic [61, 62].

Testosterone

As with estrogens, few prospective studies have evaluated the association between circulating testosterone and risk of breast cancer in premenopausal women. Of the five prospective studies published to date, three were small with 17 [41], 40–65 [63] (depending on the analysis), and 65 [44] cases, respectively, and again, confidence intervals were wide. In two of the studies [41, 44], no association was observed while in the third [63], significant positive associations were observed for free testosterone (top vs. bottom tertile $RR = 2.9[95\%\text{CI} = 1.1 - 7.3]$) and non-significant positive associations observed for total testosterone (Table 2).

In the large EPIC cohort, with 370 invasive breast cancer cases and 726 controls, significant positive associations were observed between circulating levels of testosterone and risk of breast cancer [46]. The *RR*s (95% CI) with increasing testosterone level (in quartile categories) were 1.0, 1.4 (1.0–2.1), 1.4 (0.9–2.0), and 1.7 (1.2–2.6) (p trend=0.01; Table 2).

In the NHSII, with 197 cases (including both in situ and invasive disease) and 394 controls, modest, but not statistically significant, positive associations were observed for testosterone (in both the follicular and luteal phase); the associations, particularly for follicular testosterone, did not appear entirely linear [47]. The associations were stronger and statistically significant when restricting to invasive (i.e., using a comparable case group to that in the EPIC study) or ER+/PR+tumors. In the luteal phase, for invasive cancers, relative risks increased with increasing quartile category of levels (*RR* from lowest to highest quartile [95% CI] 1.0, 1.6, 1.3, 2.0 [1.1–3.6]; p trend=0.05); similar findings were observed among the subset of invasive ER+/PR+tumors (comparable *RR*s 1.0, 2.3, 1.4, 2.9 [1.4–6.0]; p trend=0.02). Findings for free testosterone generally mirrored those for total testosterone. Thus, cumulatively, studies are quite consistent in finding a significant positive association between testosterone levels and risk of invasive breast cancer in premenopausal women.

Dehydroepiandrosterone and DHEA Sulfate

A positive association between DHEA/S and breast cancer risk has been observed consistently in postmenopausal women [1–3, 64]. Among premenopausal women, five prospective studies have been published to date. Two of the studies were small, with 15 and 40 cases, and no significant associations were noted [63, 65]. Among premenopausal women in the Nurses' Health Study, no association was observed overall for either DHEA or DHEAS [66]. Among the subset of women who were premenopausal at both blood collection and at diagnosis, a non-significant positive association was reported (top vs. bottom quartile $RR = 1.5(95\%\text{CI} = 0.7 - 3.3)$; Table 2). In the large EPIC cohort, a modest but statistically significant positive association was observed comparing the top 20% of DHEAS levels to the bottom 20% (*RR*=1.5; 95% CI 1.0–2.1) although the trend over hormone categories was not statistically significant (p trend=0.10)[46]. In the only other large study (NHSII), no overall association was observed with DHEA/S—relative risks comparing top vs. bottom quartiles were 1.0 (DHEA) and 1.3 (DHEAS) and not statistically significant [67]. The associations were somewhat stronger among ER+ cases only (top vs. bottom quartile for $\text{DHEA} = 1.6[95\%\text{CI} = 0.9 - 2.8]$ and $\text{DHEAS} = 1.9[95\%\text{CI} = 1.1 - 3.2]$), although, in this analysis, only 75–80% of the women were premenopausal. A significant interaction was noted with age (p for heterogeneity <0.05). Specifically, a non-significant inverse association was observed among premenopausal women <45 years old at blood collection and significant positive associations among those ≥45 years for both hormones (e.g., top vs. bottom quartile *RR* (95% CI) for

DHEAS: $< 45\text{yrs} = 0.6(0.3 - 1.3)$; $\geq 45\text{yrs} = 2.5(1.1 - 5.5)$). This variation in associations by age was not observed in the EPIC cohort however. The data linking either DHEA to DHEAS to breast cancer risk in premenopausal women has not been consistent, and overall, no substantial association has been observed.

Progesterone

Progesterone has strong influences on breast physiology. It has been hypothesized to either decrease breast cancer risk, by mitigating the estrogen-induced proliferation in breast epithelial cells [68, 69], or increase risk due to the higher breast cell proliferation in the luteal phase when progesterone levels are the highest [70]. Depending on the model system, evidence from animal and in vitro studies supports each of these hypotheses [71, 72]. In epidemiologic studies of postmenopausal hormone use, breast cancer risk is well confirmed to increase more with use of postmenopausal estrogen plus progestin than with use of postmenopausal estrogens alone [73–76].

To date, only six prospective studies have examined circulating progesterone levels and breast cancer risk in premenopausal women, with four of the six studies including 65 or fewer cases [41, 42, 44, 63]. Non-significant inverse associations were observed in three of the smaller studies [41, 44, 63], and a non-significant positive association was observed in the fourth [42].

In the large EPIC cohort study, with 285 cases and 555 controls, a significant inverse association was observed between progesterone levels (residuals from spline regression model) and breast cancer risk (top to bottom quartile comparison $RR = 0.6[95\%\text{CI}0.4 - 1.0]$) [46]. This association was driven by women with samples drawn in the luteal phase and was only apparent among cases and controls matched by forward dating, not among those matched by the more accurate backward dating approach. In the second large study, utilizing backward dating with 197 cases and 394 controls, no association was observed between luteal progesterone levels and risk [47]. Given the limited number of studies to date and the complexities of measuring progesterone during the menstrual cycle (and resultant lower ICC observed as noted above), the relationship of circulating progesterone levels to breast cancer risk remains unknown.

Conclusion

In contrast to the rapidly accumulating data in postmenopausal women, relatively few studies on circulating sex steroids levels and breast cancer have been conducted in premenopausal women. This is largely due to the variation in hormone levels, particularly estradiol and progesterone levels, over the menstrual cycle thus making epidemiologic studies (that routinely depend on collecting a single blood sample from each study subject) particularly complex. However, several studies have shown these methods to be feasible and valid, although nesting in substudies that allow measurement error correction of relative risk estimates is desirable.

The only consistent finding to date is a positive association between testosterone levels and risk of invasive breast cancer in premenopausal women. The magnitude of the association is similar to that observed in postmenopausal women—with an approximately two-fold higher risk in women in the top (versus bottom) 25% of hormone levels. Of note, the measurement of testosterone in premenopausal women is relatively straightforward given the consistent levels of this hormone across the menstrual cycle. The associations between estrogen and progesterone levels in premenopausal women and breast cancer risk have not been consistent, and further assessments are needed. The complexity of measuring estrogens in different phases of the menstrual cycle may have contributed to the inconsistencies in the literature. In the only study to detect a significant association with estradiol, follicular, but not luteal, levels were associated with risk; thus, further assessments stratified by menstrual cycle phase are needed. Most prior studies have not accounted in their analysis for menstrual cycle length in conjunction with the hormone measurement; evaluation of this additional aspect of the premenopausal hormone exposure could provide additional insights into these relationships. Data addressing the associations of estrogen metabolites with risk are particularly sparse, although with the development of new sensitive and specific assay methods, further studies should be completed in the near future. Thus, although the premenopausal hormonal milieu plays an important role in breast cancer etiology, evidence regarding circulating hormone levels is neither as plentiful nor as consistent as among postmenopausal women; hence, further research, with particular attention paid to menstrual cycle timing, is needed to elucidate these relationships.

Acknowledgments This study is supported by National Institutes of Health grant R01 CA67262.

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