Endometrial Cancer: Hormonal Factors, the Perimenopausal “Window of Risk,” and Isoflavones

G. E. HALE, C. L. HUGHES, AND J. M. CLINE

Center for Women’s Health (G.E.H.), Cedars-Sinai Medical Center, Los Angeles, California 90048; Department of Medical and Scientific Services, Quintiles Inc. (C.L.H.), Research Triangle Park, North Carolina 27709-3979; Department of Obstetrics and Gynecology (C.L.H.), Duke University Medical Center, Durham, North Carolina 27710; and Department of Pathology (J.M.C.), Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

Often, the risk factors for endometrial cancer (EC) are discussed only in terms of their propensity to be associated with estrogen excess, and the importance of inadequate progesterone opposition has received less attention. In this review, the dietary and lifestyle factors that are associated with an increased risk of EC are explored with respect to their effects not only on excessive estrogen levels but also diminished progesterone levels. In addition, legume consumption, which has been shown to have a protective effect on the risk of EC (1), is discussed with respect to the possible antiestrogenic effect of isoflavone compounds on the endometrium. Finally, the menopausal transition also known as the perimenopause, which has recently been characterized as a period of estrogen dominance, is highlighted as a possible “window of risk” for the development of EC.

The normal menstrual cycle reflects the refined balance between the proliferative actions of estrogen and the antiestrogenic and secretory transforming actions of progesterone on the endometrium. Proliferation of the endometrium occurs unopposed by progesterone during the follicular phase of the cycle, which lasts about 13–14 d in younger women and from 10–11 d in older women (2). In the late follicular phase and within an hour of the LH surge, there is a small preovulatory rise in progesterone, probably produced by lutetinized granulosa cells within the dominant follicle (3, 4). Thereafter, continued progesterone production is dependent on the corpus luteum, which seems in turn to be dependent on LH (5, 6). Progesterone secretion dominates during the luteal phase, which is normally between 13 and 15 d in length (7, 8). It has been difficult to define what constitutes a normal progesterone level during the luteal phase (7, 9, 10), owing to the characteristic pulsatile pattern of secretion and wide intersubject variation (6, 11). Levels between 6 and 90 nmol/liter during the midluteal phase have been reported as normal (7, 11, 12), and those consistently less than 9–15 nmol/liter have been reported as indicating luteinization failure (13, 14). In the absence of pregnancy, the corpus luteum maintains progesterone output for 10–12 d, reaching maximum levels about 5–6 d after the ovulation (11). Twelve to 15 d after ovulation, progesterone and estrogen levels fall and continue to fall over the remainder of the cycle.

To avoid excessive proliferation of the endometrium in this cyclical process, adequate duration and levels of progesterone are important. A normal functioning corpus luteum is a prerequisite for normal progesterone production, which is in turn dependent on successful ovulation. During anovulatory menstrual cycles, where there is inadequate development of the corpus luteum, estrogen is unopposed by progesterone, sometimes for prolonged periods of time (15–17). Progesterone opposes the proliferative actions of estrogen by decreasing the expression of ERs via an increase in the rate of ER breakdown and a decrease in the rate of ER synthesis (18, 19). Progesterone has also been shown to increase the activity of 17b-hydroxysteroid dehydrogenase in the glandular epithelium, thereby increasing local conversion of E2 to the less potent estrone (E1) (20–23). Luteal phase levels of estrogen and progesterone together cause the formation of mature secretory epithelium and stromal decidualization necessary for implantation. It has been clearly demonstrated that a lack of cyclical progesterone results in excessive growth of the endometrium, which can lead to endometrial hyperplasia (EH). Over time, if this hormonal imbalance is not corrected, simple EH may develop into complex hyperplasia (24, 25). This lesion, although still reversible in most cases (24), can progress to complex atypical hyperplasia (25). Atypical hyperplasia has a 23–25% risk of progressing to endometrial adenocarcinoma (25).

One of the earliest reports of EH being associated with inadequate progesterone levels was published in 1954. Schroder (26) described 3295 cases of cystic glandular hyperplasia, of which there were 34 corresponding pairs of ovaries available for histological examination. None of the 34 pairs of ovaries showed signs of corpus luteum activity (26).

The incidence of cystic glandular hyperplasia has been shown to peak during adolescence and also between the ages of 40 and 50 yr when anovulatory cycles are more likely to occur (17, 26, 27). Chronic anovulatory cycles characteristic of polycystic ovary syndrome also predispose women with this syndrome to EH (28, 29).
Endometrial proliferation and the threshold theory

Key and Pike (30) have suggested the phenomenon of a threshold level of E2 at which endometrial proliferation is triggered and above which there is no further increase in proliferative activity. This hypothesis was based on an elegant study by Ferenczy et al. (31). In this ex vivo study, autoradiographic analyses were used to quantify radiothymidine-labeled nuclei of endometrial tissues (exposed to tritiated thymidine in culture) from normal cycling women at different stages of the menstrual cycle. Tritiated thymidine labeling highlights cells that are in active S phase. Using the thymidine labeling values of Ferenczy et al. (31) from the upper functionalis layer data plus data from studies of hormonal levels throughout the normal menstrual cycle, Key and Pike (30) hypothesized a threshold level of E2 for endometrial proliferation during the follicular phase. They estimated this level to be around 180 pmol/liter, at which proliferation is switched on and above which there is no further increase in proliferation (30). Key and Pike (30) hypothesized that endometrial proliferation in the upper functionalis layer reaches a plateau by d 5–7 of the cycle and remains at this level until d 19 of the cycle. On d 19, about 2–3 d after the rise in progesterone, the mitotic rate falls dramatically. This fall is more dramatic in the glandular epithelium than in the stroma where it appears to increase slightly throughout the secretory phase (Fig. 1). This differential change in proliferative activity in the glandular and stromal compartments may be partially explained by the absence of E2 dehydrogenase activity in the stroma (21, 22).

Because of the inconvenience of handling fresh tissue for thymidine labeling or bromodeoxyuridine labeling techniques, immunocytochemical identification of the Ki-67 antigen has become a more popular method of measuring proliferation in normal and abnormal tissue since its development in 1983 (32, 33). Many investigators have studied endometrial proliferative patterns in the normal and abnormal menstrual cycle using the Ki-56 antigen (34–42). The Ki-67 antigen is a nuclear protein present in proliferating cells including G1, G2, S, and mitosis and is absent in quiescent or resting cells (43). Its gene has now been sequenced, but its function in cellular proliferation remains unknown (43). In the human endometrium, marked differences in Ki-67 expression have been observed between the glandular and stromal layers. Glandular Ki-67 expression increases markedly during the early proliferative phase and decreases dramatically to almost zero between the early and midsecretory phase (34, 35, 38, 39). Jurgensen et al. (36) studied the endometrial Ki-67 expression (using the Ki-S3 monoclonal antibody that measures a formalin-resistant epitope of the Ki-67 antigen) in 111 women with adhesive tubal disease being investigated for infertility. Ki-67 expression (calculated as a percentage of 1000 cells counted under the light microscope) in the glandular epithelium rose steeply from 5% on d 5 of the cycle to 62% on d 10 (36). After this there was a short-lived 50% fall, then rise with ovulation (d 13), followed by a dramatic fall by d 19 of the cycle. Ki-67 expression ceased completely after d 21. Ki-67 expression in the surface epithelium increased earlier and more gradually than the glandular epithelium, and there was also a lower plateau and more gradual fall from the time of ovulation to d 20 (Fig. 1). In the stroma, Ki-67 expression rose steeply after d 8, peaking shortly after ovulation (d 14), then falling less steeply by d 18. After d 20, there was another gradual rise until d 28–29 (Fig. 1). Dahmoun et al. (40) demonstrated this same difference between late secretory phase Ki-67 expression in the glandular and stromal epithelial cells. Although other studies showed similar patterns in glandular and stromal Ki-67 expression throughout the menstrual cycle, only Jurgensen et al. (36) and Dahmoun et al. (40) timed biopsies according to the day of cycle. Other studies classified the timing of biopsies broadly into early, mid, and late proliferative and secretory phases (34, 35, 38, 39). Although these studies demonstrated a similar pattern to the findings by Jurgensen et al. (36), the data could not be pooled or compared because of the different methods by which the Ki-67 expression was quantified. Three studies subjectively measured Ki-67 expression by counting the percentage of cells stained (expressed as a percentage) (38, 39, 41); two studies used automated computer-assisted image analysis systems to quantify Ki-67 staining (35, 37); and one study used a subjective 3-point scale of weak, moderate, and strong staining (34). The data of Jurgensen et al. (36) are consistent with that of Ferenczy et al. (31) of thymidine labeling of glandular epithelium, except that there is a later decline in glandular Ki-67 expression compared with thymidine labeling. This could be explained by the fact that thymidine more closely reflects DNA synthesis because it measures cells in the S phase only, whereas Ki-67 is expressed in all phases of the cell cycle, except G0.

These Ki-67 data do suggest that, at least in the glandular epithelium of the human endometrium, proliferation is characterized by an abrupt increase and abrupt decrease. This could indicate that a threshold-like mechanism is involved in endometrial proliferation as Key and Pike (30) proposed, but the possibility that the absolute level of E2 above the threshold is important remains. For example, absolute levels of estrogen may influence the level and duration of progesterone required for progesterone to exert its normal physiological antiproliferative effect or perhaps expression of the PR. The expression of PR type A is constant throughout the menstrual cycle, and PR type B is expressed in response to increasing levels of E2 with rapid disappearance in the late secretory phase (44). Both isoforms play a role in protection of overproliferation of the endometrium, but basal E2 levels may affect the PR type A whereas E2 peaks may affect the PR type B (45).
Endometrial proliferation and exogenous estrogens

The association between unopposed exogenous estrogen therapy in postmenopausal women and the development of type 1 EC was originally suggested in the early 1970s when a 20–35% increase in incidence of EC was observed in Western Caucasian women using estrogen therapy (46). The increase in risk has been shown to decrease gradually over time after cessation of therapy but “ever use” of low- or high-dose unopposed estrogen therapy are associated with an increased risk of EC (47–49). This risk seems to rise with increasing doses of estrogen used. Grady et al. (47) reviewed 14 case control studies that stratified estrogen dose and relative risk of EC. Eleven of these studies demonstrated a rise in relative risk with increasing doses of conjugated equine estrogen (CEE) (47). Weiderpass et al. (49) found a 4-fold increase in risk of EC after 5 yr of use of the low-dose regimens (of four different estrogen formulations) but an 8-fold increase with 5 yr use of the high-dose regimens. The corresponding increments in relative risk per year of use were 12% and 18%, respectively. Ever use of unopposed low-dose conjugated estrogens (0.3 mg CEE) was associated with a relative risk of EC of 5.4 [95% confidence interval (CI), 2.9–29], which is comparable with that seen in a study by Cushing et al. (50). Although the 0.3-mg and 0.625-mg doses of CEE have been shown to correspond to serum E2 levels of between 75 and 110 pmol/liter and 145 and 185 pmol/liter, respectively, there is a substantial proportion of equilin and hydro-equilin compounds not measured in routine assays that are likely to augment endometrial proliferation (51). Moreover, up to 50% CEE consists of E1 sulfate, and increases in E2 levels are a result of the increase in the E1 sulfate pool (52). The contribution of E1 to endometrial proliferation in this setting has not been documented but is likely to be significant given E1 is at least half as potent as E2 in this tissue (52). Genant et al. (53) studied the effect of increasing doses of unopposed esterified estrogen on the risk of EH. Four hundred postmenopausal women were prescribed 0.3, 0.635, or 1.25 mg esterified estrogen over a 2-yr period. At the conclusion of 1 yr, 1 of 60 women in the 0.3-mg group had EH compared with 12 of 59 women in the 0.625-mg group and 26 of 60 in the 1.25-mg group (53). At 2 yr, the incidences were 1 of 60, 17 of 59, and 32 of 60, respectively. The very low incidence of EH in the 0.3-mg group is of interest because the mean E2 levels were 105, 89, and 95 pmol/liter (at 12, 18, and 24 months, respectively), all below the theoretical threshold of Key and Pike (30) for endometrial proliferation (53). The 0.625- and 1.25-mg doses resulted in E2 levels around 110 and 165 pmol/liter, respectively. Only the highest dose resulted in E2 levels near the theoretical threshold, but it is likely that the increased levels of E1 add to the E2-induced endometrial proliferation during treatment with both doses. No E1 levels were measured in this study (53).

Ettinger et al. (54) observed increasing proliferation of the endometrium (measured by transvaginal ultrasound) with increasing doses of CEE or micronized E2 (McrE) in a 24-wk prospective study. Endometrial thickness measurements were taken in 87 postmenopausal women taking 0.5 mg or 1.0 mg McrE or 0.625 mg CEE. Endometrial thickness measurements were taken at 6, 12, and 24 wk of treatment. Endometrial growth as expressed in millimeters of growth per week was progressive over time. Endometrial growth was similar in the 0.625 mg CEE and 1.0 mg McrE groups (0.19 mm ± 0.14 per week) but was significantly less in women taking 0.5 mg McrE (0.08 mm ± 0.16 per week). The mean E2 concentrations correlated with endometrial thickness and were 200 and 315 pmol/liter in the 0.5-mg and 1.0-mg groups, respectively. Both these levels are above the theoretical threshold of Key and Pike (30) for endometrial proliferation of 145–200 pmol/liter. The E2 level in the CEE group was only 160 pmol/liter, but it is likely that there was an additional proliferative effect by both E1 (levels were >450 pmol/liter in this study) and equilin estrogens that are currently not measured in routine assays (54).

Transvaginal preparations of estrogen have been investigated in terms of their ability to cause endometrial proliferation. In a study of postmenopausal women using vaginal estrogen rings releasing 5–10 μg/24 h, there was no significant rise in E2 or E1 levels and no significant increase in endometrial thickness as measured by transvaginal ultrasonography after 6 months of treatment (55). In another study of 222 menopausal women using a similar E2 dose vaginal ring, there was a significant rise in E1 levels from 670 pmol/liter before treatment to 980 pmol/liter at 24 months. There was no increase in endometrial thickness at 6, 12, or 24 months as measured by transvaginal ultrasound. Two women had a positive progestin challenge test at 24 months (56). In a third study using transvaginal rings (releasing 5–10 μg/24 h), no increase in endometrial thickness was detected in 30 menopausal women after 6 months of administration (57). Despite these negative ultrasound findings, additional studies on the effect of ultra-low dose E2 preparations on endometrial proliferative markers should be performed before their absolute safety can be publicized. This is particularly important because ultra-low dose preparations of exogenous estrogen are becoming popular given their ability to decrease menopausal bone loss (55, 58, 59).

Endometrial proliferation and progestin doses

In the model of combined estrogen and progestin hormone replacement therapy (HRT), the dose of progestin needed to adequately oppose the proliferative effect of estrogen on the endometrium seems to be influenced by the dose of estrogen (60–63). Gibbons et al. (64) examined the expression of cytosolic ER in endometrial samples taken from postmenopausal women given increasing doses of medroxyprogesterone acetate (MPA). Three groups of women (13 in total) were given 0.3, 0.625, or 1.25 mg CEE for four treatment cycles (separated by a 4-wk rest), with 0, 2, 5, or 10 mg MPA on d 15–25 of a 28-d cycle (64). All MPA doses decreased ER expression to baseline, except with the 1.25-mg CEE dose. The highest dose of CEE required a dose of at least 5 mg MPA to return ER expression to baseline levels. These results suggest that higher progestin doses are needed to counteract higher doses of estrogen in terms of down-regulation of the ER. Woodruff et al. (62) showed that 2 of about 270 women developed EH after 12 months on 0.625 mg CEE and 2.5 mg MPA daily and 1 of 277 developed it after 12 months on 0.625 daily CEE with 5 mg for 14 d of the cycle (62). No EH was
seen in the women (>500 subjects) who took the same dose of CEE with either 5 mg MPA daily or 10 mg MPA for 14 d of the cycle (62). In another study of continuous combined therapy with 17β-estradiol and dydrogesterone, the minimum daily dose required to protect the endometrium was 5 mg (65). A proliferative endometrium was found after 12 months of therapy in 7% and 15% of women using the 1- and 2-mg doses, respectively (65).

The length of time that progesterone is administered is also likely to be important in protecting the endometrium. One would expect that any less than the normal luteal phase levels and duration of progestin would predispose to the effects of unopposed proliferation. The proliferative phase shortens with age from about 14 d in women between 20 and 25 yr to as short as 10 d in women between 45 and 50 yr (2). In contrast, the luteal phase is more consistent and lasts between 13 and 15 d, and changes in length seem to be independent of age (8). Using the model of a normal 28-d cycle, administration of a progestin from d 12–25 would most closely mimic true physiology. It has been shown that there is about a 3-d delay between appearance of progesterone in the circulation and its full antiproliferative effect on the endometrium (66). Administration of a progestin from d 12 onward, therefore, will result in the full antiproliferative effect by d 15–17. This is close to the physiological situation demonstrated in both DNA and Ki-67 proliferative studies (31, 36). In addition, it has been shown that endometrial glandular mitotic rates are significantly reduced after 9 or more days of progesterone therapy (200 mg micronized progesterone daily) with the maximum decrease in mitotic rate achieved with the use of 11 d of a progestin (67). That inadequate duration of progestin administration can predispose the endometrium to EH is supported by the findings of a 2-fold increase in risk of EC in young women using the sequential oral contraceptive pill (primarily Oracon) (68, 69).

Sequential preparations were based on a 21–23-d regimen of an estrogen with the addition of a progestin for only 7 d, leaving an abnormally long time of unopposed estrogen during the proliferative phase and also during the treatment break of 5–7 d.

Although some conflicting evidence remains on the safest HRT regimens using progestins, most specialists recommend administration of a progestin for 12–14 d in a 28-d cycle (70–72). In the Postmenopausal Estrogen/Progestin Interventions Trial, administration of 10–12 d of 10 mg MPA or 200 mg micronized progesterone successfully prevented EH over a 2-yr intervention period (73). In a case control study, Pike (74) found the adjusted risk of EC after 5 yr of CEE with 7 d of MPA was 2.17 and with 10 or more days of MPA was 1.87. Other studies suggest, however, that there may be a small increase in risk of EH with regimens using less than 12–16 d of a progestin. Gelfand and Ferenczy (75) found that the administration of 5 mg MPA for 11 d with 0.635 mg CEE was associated with EH in 4.4% in 45 women at 6 months. Subsequently, a review of nine studies on a total of 66 cases of EC occurring during combined HRT concluded that a minimum daily dose of 10 mg MPA or 2.5 mg norethindrone acetate was required for 12–14 d of the cycle for full protection from EH (76). In a large Swedish case control study, however, an increased risk of EC was found [odds ratio (OR), 1.6; CI, 1.1–2.4] with the administration of fewer than 16 d of a progestin (49). In this HRT study of 709 cases of EC and 3368 controls, there was an increased risk of EC in postmenopausal women using a progestin for fewer than 16 d of the cycle (OR, 2.9; CI, 1.8–4.6). There was also a reduced risk of EC with use of cyclic combined HRT regimens (OR, 0.2; CI, 0.1–0.8) (49) similar to the protection expected from the combined oral contraceptive pill (77). Despite this, there are several reports of EC occurring in women during cyclic combined therapy (70, 78). Gruber et al. (78) attribute these failures of the progestin to adequately oppose the proliferative effect of estrogen to supraphysiological levels of E2 (>400 pg/ml), but Cormerci et al. (70) attribute them to poor compliance with the progestin and also prior use of unopposed estrogen therapy. Although intuitively logical that the higher the level of E2, the less easily it will be adequately opposed by a given dose of a progestin, current understanding of mechanisms of the antiproliferative action of progesterone make any precise explanation uncertain and speculative (79).

**Obesity and EC risk**

Obesity has consistently been shown to be associated with an increased risk of EC (80). Seventeen of 18 epidemiological studies revealed the frequency of overweight and obesity to be systematically and substantially greater in cases than in controls (81), and of 11 case control studies, all but 3 demonstrated a significant association between EC and severe obesity (weight in the upper 90th percentile) (82). Most theories that have been put forward to explain the increased risk of EC are based on the increased levels of circulating estrogens via the conversion of androstenedione to E1 in adipose tissue (83, 84) and decreased circulating levels of SHBG (84–88).

Studies that have addressed this issue in obese postmenopausal women have reported E1 and E2 levels to be increased (88, 89–91) decreased (92) or no different from normal weight individuals (93–97). The study which found a decrease in serum E1 and E2 included obese premenopausal women between 40 and 45 yr of age with FSH levels of greater than 15 (92). In the study by Vermeulin et al. (96), in which only women greater than 4 yr after their last menstrual period (LMP) were included, mean E2 levels 4–9, 10–19, and more than 20 yr after their last menstrual period were 51, 58, and 33 pmol/liter, respectively. In the study by Potischman et al. (88), women at least 2 yr after the LMP had average E2 levels of 18.7, 22.3, and 38.5 pmol/liter from lowest to highest tertile of body mass index (BMI). E2 levels have also been compared in obese women with and without EC. Austin et al. (98) found that in women with EC, elevated estrogens were found in only women who were very obese (upper quartile BMI). The mean E2 level in cases and controls in this study was 48 pmol/liter compared with 32.5 pmol/liter, respectively (P < 0.0001), and for E1, 135 and 100 pmol/liter (P = 0.004), respectively. The levels of estrogen seen in these studies of obese postmenopausal women are similar to those seen as a result of administration of vaginal ring estrogen preparations in menopause (57). The significance of such small increases in E2 and E1 levels demonstrated in these studies is uncertain.
in terms of ability to cause an increase in endometrial proliferation and perhaps some other feature associated with being obese predisposes women to developing EC.

In premenopausal women, obesity is also a major risk factor for EC (68, 99–103). In a case control study of 111 women with EC, obesity occurred in 43.8% of young women compared with 18% in postmenopausal women (101). In another study, the mean weight of premenopausal women with EC was 198 pounds compared with 173 pounds in women older than 45 yr of age (104). Many of these studies on premenopausal women, however, may have included women with polycystic ovaries, a condition associated with a decrease in SHBG through both an increase in circulating androgens and obesity (105). The resultant increase risk of EH from the increase in circulating free estrogen is also compounded by chronic anovulatory cycles characteristic of women with polycystic ovaries (29).

In contrast to obesity in the menopause, most studies suggest that obesity during the premenopause is associated with a decrease in serum E, or E2. In a study of premenopausal women, those with the lowest BMI tertile had 45% higher mean follicular phase E2 and free E2 concentrations compared with women with the highest BMI tertiles (88). Several other smaller studies have also shown that obese premenopausal women have lower follicular phase E2 levels than those in women of normal weight (106–110). In a study of 1420 women between the ages of 35 and 50, an inverse association was found between BMI and E2 levels (110). This association was found to be significant in African American women but not in Caucasian women (110). Only two studies have shown a nonsignificant rise in circulating estrogens (94, 111). Kopelman et al. (94) found a significant increase in the E1/E2 ratio in massively obese premenopausal women, and Westhoff et al. (111) showed a nonsignificant 14% higher mean cyclical E2 level in 175 obese premenopausal women (P = 0.16). The lower SHBG levels associated with obese premenopausal women compared with normal weight women may account for increased levels of free estrogens and, thus, increased estrogenic action on the endometrium (88, 112). In addition, in obese subjects, there is a shift in E2 metabolism from the catechol pathway (producing two relatively inactive metabolites, 2-hydroxyestrone and 2-methoxyestrone) to D-ring metabolism (producing estradiol and epiestriol metabolites) (113, 114). These estradiol and epiestriol metabolites have estrogen potency comparable with E1 (115).

Insufficient luteal phase levels of progesterone may be a major contributing factor in the increased risk of EC in premenopausal women. Obesity has been shown to be associated with irregular menstrual periods, amenorrhea, and luteal phase progesterone deficiency via a disruption in ovulation (116, 117). In 1952, Rogers and Mitchell (116) studied 60 women with amenorrhea and 19 women with dysfunctional uterine bleeding. Of the 60 amenorrheic women, 28 were 20% above ideal weight and of the other 19 women, 11 were 20% above ideal weight. In a later study of more than 11,000 women, there was a significant positive correlation between weight and waist girth and irregular menstrual periods (as defined by cycle length >36 d) (117). Whether or not this relationship means that increased body weight pre-disposes to prolonged anovulatory cycles and periods of unopposed estrogen has not been established. Epidemiological studies, however, have confirmed that there is an increased risk of EC in women with prolonged menstrual cycles (118), oligomenorrhea or longer days of flow (119). Increased body weight has also been associated with decreased progesterin levels even in ovulating obese women. Westhoff et al. (111) found an 18% lower mean progesterone concentration (P = 0.003) during the luteal phase in 84 women in the upper half of the weight distribution compared with 83 women in the lower half of the distribution. In addition, Thomas et al. (106) found a 12% lower mean progesterone concentration in women with a BMI in the highest tertile for BMI.

Why obesity may predispose women to insufficient luteal phase progesterone levels is unclear, but recent studies point toward leptin, which is increased in obesity (120), as a possible cause of disruption of both ovulation and steroidogenesis. In human granulosa cells, leptin appears to inhibit both insulin-induced and gonadotropin-induced progesterone production (121). This inhibitory effect has been confirmed in vivo in bovine and murine animal models (122, 123). In the murine model, leptin has also been observed to interfere with ovulation (124). Perhaps increased levels of leptin associated with obesity (120) are somehow adversely affecting luteal phase progesterone output, thus leaving the endometrium relatively less protected than in normal weight individuals.

Table 1 summarizes the factors associated with obesity in premenopausal and postmenopausal women and how they may contribute to an increase in risk of EC.

### Diabetes mellitus and EC risk

A number of case control studies have found about a 2-fold increase in risk of EC in diabetics vs. nondiabetics (119, 125, 126). Among 13 epidemiological studies published between 1958 and 1990, all showed a higher incidence of diabetes in cases than controls but the difference reached statistical significance in only three (81). The percentage of women with EC reporting a history of diabetes ranged from 6–23%. Garnet (127) examined the prevalence of insulin resistance in EC cases and found that 31 of 50 cases and 10 of 50 controls had an impaired glucose tolerance test. Similarly, another study observed significantly more abnormal glucose tolerance tests in women with EC than those without (128). In a case control study of 123 cases and 2291 controls, compared with women...

### TABLE 1. Mechanisms for the increased risk of EC in obesity during the premenopause and menopause

<table>
<thead>
<tr>
<th>Premenopause</th>
<th>Menopause</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>Free E2 and E1</td>
</tr>
<tr>
<td>↓</td>
<td>SHBG</td>
</tr>
<tr>
<td>↓</td>
<td>Progesterone</td>
</tr>
<tr>
<td>↑</td>
<td>Leptin</td>
</tr>
<tr>
<td>↓</td>
<td>progesterone production</td>
</tr>
<tr>
<td>↓</td>
<td>interference in ovulation</td>
</tr>
<tr>
<td>↑</td>
<td>Insulin</td>
</tr>
<tr>
<td>↓</td>
<td>IGF-I</td>
</tr>
<tr>
<td>↓</td>
<td>IGF-BP’s</td>
</tr>
<tr>
<td>↓</td>
<td>PR binding</td>
</tr>
<tr>
<td>Increase D-ring metabolism</td>
<td>→ Active estril and epiestriol metabolites</td>
</tr>
</tbody>
</table>

| Insulin |
| ↓ IGF-I |
| ↓ IGF-BP’s |
| ↓ PR binding |
without diabetes, women with diabetes had an adjusted OR for EC of 1.86 (95% CI, 1.37–2.52) (129). This association, however, was significantly modified by body size (BMI, <29.1), and the authors found that diabetes conferred no additional risk of EC in women who were not overweight or obese. This is in contrast to findings from several other studies, which demonstrate a significant association between diabetes and risk of EC after adjustment for BMI (119, 126). Whether type I vs. type II diabetes is more likely to predispose to EC is unknown, and only one study so far has addressed this. Weiderpass et al. (125) found that the OR for type II diabetes was 1.5 (CI, 1.0–2.1) compared with 13.3 (CI, 3.1–56.4) for type I diabetes.

There has been considerable debate as to how hyperinsulinemia or hyperglycemia influences the risk of EC in diabetic women. Three studies have found higher circulating levels of insulin in nondiabetic women with EC compared with nondiabetic women without EC (128, 130, 131). Troisi et al. (132) explored the relationship between hyperinsulinemia and risk of EC further by measuring C-peptide levels in 165 EC cases and 180 controls. They found that C-peptide was positively correlated with BMI and E2 levels but negatively correlated with SHBG. In the age-adjusted analysis, the highest tertile of C-peptide was associated with an OR for EC of 2.2 (1.3–3.7) (132). This association, however, was eliminated after adjustment for BMI. In contrast, adjustment for C-peptide had little effect on the association between BMI and EC. These findings support the theory that insulin plays a role in increasing endometrial proliferation and it has been postulated that insulin may act as an endometrial mitogen by augmenting the effects of IGFs in the endometrium (133–135) (Table 1). An alteration in endometrial insulin growth factor binding proteins in diabetes mellitus may increase the availability of IGFs to stimulate endometrial proliferation (133, 134). IGFs, especially IGF-I, play a role in mediating estrogen-induced endometrial proliferation via autocrine and paracrine mechanisms (135–137). In addition, insulin has been observed to decrease PR-progesterone binding (138) and attenuate the antiproliferative actions of antiestrogens (79) (Table 1). These findings from molecular studies suggest that clinical studies on the effect of hyperinsulinemia on endometrial proliferation are warranted.

**Exercise and EC risk**

The role of exercise in protection of EC is remains unclear. Ten of 11 case control studies suggest that moderate exercise is associated with a reduction in risk of EC (139, 140). In one large Swedish Twin Registry case control study, there was a marked decrease in risk of EC in women who exercised regularly, independent of weight and parity (141). In another Swedish case control study, however, the decrease in risk of EC was confined to women with regular occupational exercise rather than recreational exercise (142). A United States case control study found no decrease in EC risk with increasing occupational exercise but did find a decrease with exercise in early adulthood (97). This study also demonstrated no difference between moderate and very intense exercise in terms of protection from EC (97). Similar associations with active lifestyle and protection from EC were found in three other studies from the United States and China (143, 144). There are no data to suggest that women who exercise have different levels of circulating estrogens, but there is evidence of a shift away from O-ring to catechol E2 metabolism in exercising individuals (145). Both 2-hydroxyestrone and 2-methoxyestrone produced via the catechol metabolic pathway have virtually no estrogenic activity compared with the estriol metabolites produced via O-ring metabolism, despite their relatively high affinity for the ER (only about 20–50% weaker than E2) (115).

Exercise may, in some cases, adversely affect luteal phase progesterone production via a disruption of ovulatory function. Vigorous and intense running exercise, in previously untrained women, has been reported to induce shortening of the luteal phase, and if accompanied by pronounced weight loss, anovulation (146, 147). Exercise involving less intense exercise (daily cycling) that is not accompanied by weight loss, however, does not seem to be associated with menstrual cycle changes (148, 149). Moderate exercise may therefore decrease the risk of EC through the association with other healthy factors such as normal weight and healthy diet.

**Diet, isoflavones, and EC risk**

The role of dietary factors in the development of EC has been of interest for decades, especially in view of the large difference in incidence of EC between women living in Western and Asian countries. Seven case control studies and one prospective study examining the role of diet in EC were published between 1986 and 1997 (150–157). All studies found that consumption of whole grains, fresh fruit, and fresh vegetables was associated with a decreased risk of EC. There has subsequently been interest in whether vegetarian diets have the ability to favorably alter hormonal profiles in women. Lower urinary excretion of estriol (158) and lower circulating levels of E1 and E2 (159, 160) have been found to be associated with a vegetarian diet in some studies but not others (161–164). The association between a high fiber diet and a decrease in serum E2 levels has been investigated and confirmed in some studies (165) but not others (161, 165). There is some evidence, however, that E2 metabolism can be influenced by dietary fat intake. It has been found that conversion to less active catechol-metabolites via 2-hydroxylase oxidation can be increased when the percentage of calories consumed is decreased by 25% (166).

Asian women living in Asia have one tenth the risk of EC compared with Caucasian women living in the West (167, 168). As a result, there has been considerable interest in the Asian diet as a possible protective factor. Not only is the Asian diet uniformly higher in fiber and plant foods and lower in fat than the Western diet, it also includes a large portion of “the pulses” as a major source of dietary protein. Goodman et al. (1) performed a large case control study of a multiethnic population in Hawaii and were the first investigators to include the consumption of the pulses (tofu and other soy products) in their dietary analysis. The authors found that a high consumption of tofu and other soybean products was associated with a decreased risk of EC (OR, 0.45; CI, 0.26–0.83). This association was inverse for each of the ethnic groups examined and was independent of the
other risk factors identified in this population. Consistent with this, Nagata et al. (169) found that in a study of 50 young regularly cycling Asian women, the intake of soy products was inversely correlated with serum E1 and E2 levels.

Soy foods are a rich source of isoflavones. Isoflavones are diphenolic nonsteroidal estrogen-like compounds that can have hormonal effects on the human physiology when ingested in large amounts (169). They have been shown to have an estrogen lowering effect in some short-term studies in Caucasian postmenopausal women (170). Duncan et al. (171) found a significant decrease in serum E2 levels and an increase in SHBG levels in postmenopausal women on a high isoflavone diet (2.0 mg/kgd in the form of a soy powder), and a nonsignificant decrease in serum E2 levels. Brzezinski et al. (172) also found a significant rise in SHBG levels in a 12-wk study of postmenopausal women on an isoflavone-rich diet. Nonsignificant decreases in serum E2 and E1 were seen in another study of 97 postmenopausal women on a diet containing 165 mg isoflavones per day for 4 wk (173). In contrast to these findings in postmenopausal women, however, one study of six premenopausal women found that 4 wk of 45 mg isoflavones per day was associated with a significant increase in follicular phase E2 levels (170).

Isoflavones interact with the mammalian ER and can appear to have both estrogen-agonist and estrogen-antagonist effects on mammalian physiology, depending on the tissue involved and the amounts circulating. As early as 1946, isoflavones were found to have profound effects on mammalian reproductive physiology. Bennetts et al. (174) reported widespread infertility in sheep grazing on isoflavone-rich subterranean clover pasture in Western Australia. In 1966, Folman and Pope (175) showed that high doses of sc administered genistein (an isoflavone) had significant proliferative effects on the uteri of the rats. They found, however, that this effect was significantly less than steroidal estrogen and that when given in high doses actually appeared to decrease the uterotropin effect of steroid estrogen given simultaneously (175). The estrogenicity of these compounds derived from both red clover and soybean (176, 177) has recently been assessed using ER-affinity human cell culture bioassays (178). Relative binding affinities for the ER-α compared with E2 (value of 1.0) were found to be coumestrol (0.202), genistein (0.084), equol (0.061), daidzein (0.013), and biochanin A (<0.006) (178). Although these compounds have lower affinities than steroidal estrogen, they circulate in the plasma at very much higher concentrations than steroidal estrogen (179), and their physiological potency can be significant (180). In addition, the biological activity of both genistein and daidzein has been reported to be 5- to 10-fold higher when measured in human serum (181). The physiological response secondary to the ER-isoflavone interaction is also complex and subject to multiple influences. Isoflavones have multiple non-ER-mediated physiological effects that are likely to contribute to antiestrogenic effects, and these include inhibition of aromatase (182), inhibition of tyrosine protein kinase (183), inhibition of α reductase (184–186), and increased SHBG synthesis (187).

At least four human studies examining the proliferative effect of isoflavones on the endometrium (171, 188–190) have shown that use of a high isoflavone diet or isoflavone supple-

ments in postmenopausal women does not increase endometrial thickness when measured by transvaginal ultrasound. Hale et al. (191) studied the effect of a 3-month course of a 33 mg red clover isoflavone supplement on the Ki-67 proliferative index in endometrial Pipelle specimens taken between d 8 and 11 of the menstrual cycle. In this study of 30 late reproductive aged and perimenopausal women, there was no difference in the endometrial Ki-67 index or endometrial thickness between P-07 and placebo groups (191). Other studies investigating the possible antiuterotrophic effects of soy isoflavones have been in animals. Foth and Cline (192) studied four groups of ovariectomized adult macaque monkeys fed either no hormone treatment (0), oral E2 (E), oral soy protein isolate (soy), or both E2 and the soy protein (E + soy). After 6 months of treatment, histopathological assessment of both mammary and endometrial sections were performed. There was a significant decrease in the Ki-67 proliferative index in the E + soy group compared with the E group (192). There was, however, no significant difference between endometrial thickness in the E and the E + soy group. Tansey et al. (193) investigated the possible antiuterotrophic properties of isoflavone administration in rats. They found that administration of isoflavone-rich soy protein plus steroidal estrogen caused a significant reduction in uterine luminal epithelial height and uterine lactoferrin expression compared with steroidal estrogen alone. There was, however, no change in uterine weight or uterine proliferation as measured by immunohistochemical staining for proliferating cell nuclear antigen. When isoflavone-rich soy protein was administered without estrogen, the high dose (118 mg isoflavones per 1800 calories) but not the low dose (11.8 mg isoflavones per 1800 calories) caused a nonsignificant increase in uterine weight (193). In another study using the murine model, dietary genistein was administered at doses of 125, 375, and 750 μg/g feed. The two higher doses caused significant increases in uterine weight when administered together with 17β-estradiol (194), and none of the three doses of genistein were shown to reverse the E2-induced increase in uterine weight. No other markers of uterine or endometrial proliferation were measured. In another rat model study, genistein was shown to cause a dose-dependent inhibition of progesterone production from cultured ovarian cells (195) and at high doses, an inhibition of progesterone production from granulosa cells. The inhibitory effect of genistein on steroidogenesis in this study appeared to be independent of cytokines and growth factors (195).

The perimenopausal “window of risk”

Given that increased or supraphysiological doses of estrogen may increase the requirement of progesterone to adequately oppose the proliferative actions of estrogen on the endometrium, the perimenopausal transition could represent a special “window of risk” for unopposed estrogen action (Fig. 2). Both erratic and elevated levels of E2 observed in perimenopausal women are likely to be a result of elevated FSH levels and increase in follicular recruitment characteristic of late reproductive age and the menopausal transition (196). There have been a number of studies confirming increased estrogen levels and excretion during the perimenopausal window.
pause. In a Swedish study, urinary estrogen excretion was measured throughout a single menstrual cycle in 53 regularly cycling women between the ages of 15 and 50 (197). There was a significant positive correlation between estrogen excretion and increasing reproductive age. In an analysis of 12 studies that measured serum E2 levels during the perimenopause, the average follicular phase (d 4–7 of menstrual cycle) E2 level from 415 perimenopausal women was 224.9 compared with 174.7 pmol/liter in 292 premenopausal controls (196). One study alone from Melbourne, Australia, with 277 women, demonstrated this same follicular phase E2 elevation in perimenopausal women (198). The study demonstrated an average follicular phase serum E2 level of 226 pmol/liter in perimenopausal aged women with new onset cycle irregularities compared with 173 pmol/liter in premenopausal women (198). Santoro et al. (199) studied six regularly cycling women 47 yr and older and compared them with 11 regularly cycling women between 19 and 39. Significantly increased estrogen excretions in both the follicular and premenstrual phases of the menstrual cycle were found in the older compared with younger women (199). Combined data from three other studies that measured the difference between perimenopausal and premenopausal luteal phase E2 levels revealed significantly higher premenstrual E2 levels in perimenopausal women compared with premenopausal controls (371 pmol/liter vs. 304 pmol/liter) (200–202).

Most longitudinal studies of the perimenopause also demonstrate elevated serum levels or increased excretion of estrogens during the menopausal transition. One of the longest studies performed was that by Brown (203), who followed two women through 6–7 yr of the menopausal transition reporting on menstrual flow and urinary hormone excretion patterns. The mean urinary estrogen excretion from weekly urine samples were 44.1 and 30.8 µg per 24 h compared with the expected excretion of 26.1 µg per 24 h in premenopausal women. In another longitudinal study of 152 women, levels of 400 pmol/liter or greater were found in women 6 months before their final menstrual period (FMP) (204). Although the timing of the blood samples according to the menstrual cycle was not recorded, E2 levels often remained elevated at more than 150 pmol/liter until 2 yr after the FMP (204). Longcope et al. (15) also observed that E2 levels were maintained at an average 293 pmol/liter and 165 pmol/liter, 6 and 12 months after the FMP, respectively. Finally, Shideler et al. (16) closely monitored urinary estrogen excretion in five perimenopausal women over four menstrual cycles and found that although higher than normal estrogen excretion levels were not found, there was a sustained release of proliferative phase levels of E2 (>350 pmol/liter) during prolonged intermenstrual intervals.

Given the increased cycle irregularity and increased incidence of anovulatory cycles (205), the perimenopause also represents a time of low and irregular levels of progesterone. The theoretical increase in risk of unopposed estrogen during the perimenopause becomes greater with the development of prolonged intermenstrual periods. Metcalf and McKenzie (206) examined urinary progesterone excretion (by measuring pregnanediol 3-glucuronide in the urine) in women who were experiencing new onset menstrual irregularities. These women excreted significantly less pregnanediol 3-glucuronide than women of the same age with regular ovulatory cycles (206). They also studied the estrogen to progesterone excretion ratio in relation to menstrual cycle length. Excretion estrogen to pregnanediol 3-glucuronide ratios of greater than 100 occurred in 7% of cycles 18–35 d in length and in 47% of cycles 50–260 d in length (206). Not surprisingly, a prolonged high ratio of greater than 100 occurred 30 times more often in perimenopausal women than premenopausal women (206). Prolonged intermenstrual periods with sustained E2 levels (350–400 pmol/liter) and low progesterone levels (<16 nmol/liter) were also demonstrated in the longitudinal study by Shideler et al. (16). This supports the likelihood that any increase in risk of unopposed estrogen is more likely with the development of prolonged anovulatory cycles. Whether progesterone levels in regularly cycling and ovulating perimenopausal women are lower than younger women, is not clear. Lee et al. did not show any decrease in serum progesterone levels with age in a cross-sectional study of regularly cycling women aged between 24 and 50 yr of age (207). Reame et al. (202), however, demonstrated significantly higher average progesterone levels in 20- to 29-yr-old women compared with 45- to 50-yr-old women (25.2 nmol/liter vs. 11.9 nmol/liter). There was, however, no difference in the average progesterone levels between women aged 34–39, those aged 40–44, and those aged 45–50 (200). Overall, the hormonal environment during the perimenopause seems to favor inadequately opposed endometrial proliferation, particularly with the onset of irregular cycles.

In addition to being exposed to inadequately opposed estrogen, the perimenopausal endometrium is unique in that it has been exposed to at least 350 proliferative cycles. Added to this, there may be as yet unknown age-related changes that may compromise its ability to respond to proliferative stimuli. Although there do not seem to be any obvious age-related changes in the endometrium with respect to morphological appearance (208, 209), growth factors (210), or hormone receptor content (212), detailed studies on the perimenopausal endometrium are lacking. Changes in expression of the PTEN tumor suppressor gene may be an example of one of these age-related or exposure-related changes in the endometrium. The PTEN tumor suppressor gene is the term used for a phosphate TEN’sin homologue found on chromosome 10 (10q23), which plays a key role in the regulation of cellular proliferation (213). Loss of expression of PTEN associated with abnormal proliferation of endometrium (213) has been found to be associated with a mutation of the gene in over 80% of cases (214). Patchy clonal outgrowths of PTEN-depleted epithelium have frequently been found in persistent proliferative endometrium (214), hyperplastic endometrium (215), and EC (213). It is also equally as likely to occur in EH without atypia as EH with atypia, suggesting that PTEN mutation is an early event in the carcinogenic process of EC (216). Moreover, there is a suggestion that loss of PTEN expression is related to age and possibly duration of exposure to estrogen, particularly unopposed estrogen (214). Other age-related changes in the endometrium have been investigated in animal studies. When rats divided into three age-groups (3, 6, and 12 months) are exposed to an endometrial carcinogenic stimulus, the middle and older age
groups had significantly higher incidences of EH and EC than the younger age group (217). These results suggest that age-related changes may increase the probability of an adverse response by the endometrium to proliferative stimuli, and this issue is worth pursuing in human studies (217).

Conclusions

There are a number of lifestyle factors that are associated with an increased risk of EC that can be modified by women. Protective modifications include maintaining BMI at 28 or below, consuming a diet rich in vegetables and fiber, and participating in a program of regular moderate exercise. In addition, if there is either a family history of or a predisposition to diabetes mellitus, a glucose tolerance assessment is prudent and compliance with appropriate dietary and medical management is indicated. The role of a high isoflavone diet or isoflavone supplementation in decreasing the risk of EC is suggested by the published studies but is not established, and more research into the physiological effects of these compounds on the endometrium is warranted. Finally, although the perimenopause has not been previously received attention as a “window of risk” for EC, we are convinced that this is a period of time when supportive cyclical (or possibly continuous) progestin or progesterone therapy is particularly warranted to offset the effects of intervals of physiologically increased unopposed estrogen that occur during this phase of life. Consideration of this preventative opportunity is particularly important in women who already have one of more other risk factors for EC.

Acknowledgments

Received February 2, 2001. Accepted September 13, 2001.
Address all correspondence and requests for reprints to: Claude L. Hughes, M.D., Ph.D., Quintiles Inc., P.O. Box 13979, Research Triangle Park, North Carolina 27709-3979. E-mail: claude.hughes@quintiles.com.

References

35. Persson I, Adami HO, Bergvist L 1989 Risk of endometrial cancer after...
65. Bergeron C, Ferenczy A 2001 Endometrial safety of continuous combined hormone replacement therapy with 17β-estradiol (1 or 2 mg) and hydrogesterone. Maturitas 37:191–199
70. The Writing Group for the PEPI Trial 1996 Effects of hormonal replacement therapy on endometrial histology in postmenopausal women. JAMA 275: 370–375
96. Lobo RA, Granger L, Goebelmann U 1981 Elevations in unbound serum estradiol as a possible mechanism for inappropriate gonadotropin secretion in women with PCO. J Clin Endocrinol Metab 52:156–162
101. Manson JM, Sammel MD, Freeman EW, Grasso JA 2001 Racial differences in sex hormone levels in women approaching the transition to menopause. Fertil Steril 75:297–304


187. Mousavi Y, Aldercreutz H 1993 Genistein is an effective stimulator of SHBG production in hepatocarcinoma human liver cells and suppresses proliferation of these cells in culture. Steroids 58:301–304.


