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

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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 luteinized 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 E2 dehydrogenase in the glandular epithelium, thereby increasing local conversion of E2 to the less potent estrone (E_1) (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).

Abbreviations: BMI, Body mass index; CEE, conjugated equine estrogen; CI, confidence interval; EC, endometrial cancer; EH, endometrial hyperplasia; E_1, estrone; FMP, final menstrual period; HRT, hormone replacement therapy; LMP, last menstrual period; McrE, micronized E2; MPA, medroxyprogesterone acetate; OR, odds ratio.

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 Ferenzcy *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 Ferenzcy *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 G_1, G_2, 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 thimidine 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 G_0.

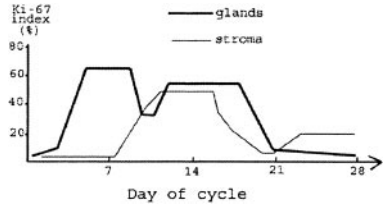

FIG. 1. Proliferation of the glandular and stromal epithelium during the normal menstrual cycle as measured by the Ki-67 proliferative index. [Adapted from Ferenczy *et al.* (31), Jurgensen *et al.* (36), and Dahmoun *et al.* (40).]

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 E_1 sulfate, and increases in E2 levels are a result of the increase in the E_1 sulfate pool (52). The contribution of E_1 to endometrial proliferation in this setting has not been documented but is likely to be significant given E_1 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 E_1 add to the E2-induced endometrial proliferation during treatment with both doses. No E_1 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 $\pm$ 0.14 per week) but was significantly less in women taking 0.5 mg McrE (0.08 mm $\pm$ 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 E_1 (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 E_1 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 E_1 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 E_1 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 E_1 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 E_1 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 E_1, 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 E_1 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_1 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 E_1/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 estriol and epiestriol metabolites) (113, 114). These estriol and epiestriol metabolites have estrogen potency comparable with E_1 (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 predisposes 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 progestin 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

Premenopause	Menopause
⇔ OR ↓ free E2 and E_1	↑ Free E2 and E_1
↓ SHBG	↓ SHBG
↓ Progesterone	
↑ Leptin	↑ Leptin
• ↓ progesterone production	
• interference in ovulation	
↑ Insulin	↑ Insulin
• ↑ IGF-I	• ↑ IGF-I
• ↓ IGF-BP's	• ↓ IGF-BP's
• ↓ PR binding	• ↓ PR binding
Increase D-ring metabolism	
→ Active estriol and epiestriol metabolites	

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 D-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 D-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 eight 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 E_1 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 E_1 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 E_1 levels and an increase in SHBG levels in postmenopausal women on a high isoflavone diet (2.0 mg/kg·d 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 E_1 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 uterotropic effect of steroidal 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 supplements 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 antiuterotropic 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 antiuterotropic 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 perimeno-

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, 211), 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

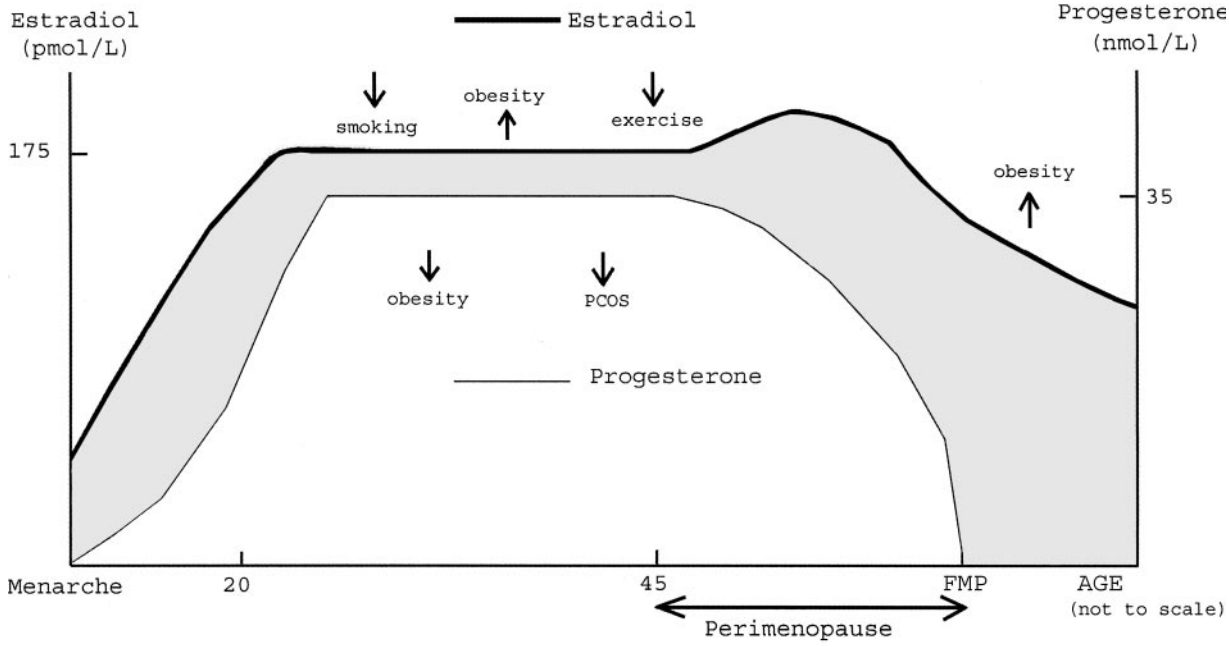

FIG. 2. Representation of the lifetime exposure risk for EC. The risk for EC is proportional to age and the accumulative exposure to estrogen unopposed by progesterone. The *shaded area* represents this accumulative exposure. The top border of the *shaded area* represents the mean follicular phase E2 level, whereas the bottom border represents the mean luteal phase level of progesterone level. The perimenopause is a time when the degree and accumulation of exposure are increased. Exposure risk is increased by polycystic ovary syndrome (PCOS) and obesity (during the premenopause and menopause) and decreased by smoking and exercise.

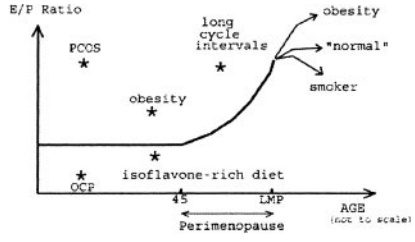

FIG. 3. Diagram of the influence of factors on the estrogen to progesterone (E/P) ratio and consequent risk of EC. The E/P ratio increases in obesity and during prolonged intermenstrual intervals. PCOS, Polycystic ovary syndrome; OCP, oral contraceptive pill.

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). Figures 2 and 3 diagrammatically represents the theoretical increased risk of EC being directly proportional to duration of cyclical estrogen exposure and the amount of time that estrogens are inadequately opposed by progesterone.

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.

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References

1. **Goodman MT, Wilkins LR, Hankin JH, Lyu L, Wu AH** 1997 Association of soy and fiber consumption with the risk of endometrial cancer. Am J Epidemiol 146:294–306
2. **Lenton EA, Landgren BM, Sextron L, Harper R** 1984 Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. Br J Obstet Gynaecol 9:681–684
3. **Ferin M** 2000 The hypothalamic-hypophyseal-ovarian axis and the menstrual cycle. In: Sciarra JJ, ed. Gynecology and obstetrics, vol 5, revised edition. Chicago: Lippincott Williams & Wilkins; 1–15
4. **Liu JH, Yen SSC** 1983 Induction of the midcycle gonadotrophin surge by ovarian steroids in women: a critical evaluation. J Clin Endocrinol Metab 57:979–983
5. **Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Bremner WJ** 1984

Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. J Clin Endocrinol Metab 58:378–383
6. **Soules MR, Clifton DK, Steiner RA, Cohen NL, Bremner WJ** 1988 The corpus luteum: determinants of progesterone secretion in the normal menstrual cycle. Obstet Gynecol 71:659–667
7. **McNeely MJ, Soules MR** 1988 The diagnosis of luteal phase deficiency: a critical review. Fertil Steril 50:1–15
8. **Lenton EA, Landgren BM, Sextron L** 1984 Normal variation in the length of the the luteal phase of the menstrual cycle: identification of the short luteal phase. Br J Obstet Gynaecol 91:685–689
9. **Soules MR, Clifton DK, Bremner WJ, Steiner RA** 1987 Corpus luteum insufficiency induced by a rapid gonadotropin-releasing hormone-induced gonadotropin secretion pattern in the follicular phase. J Clin Endocrinol Metab 65:457–464
10. **Soules MR, Clifton DK, Cohen NL, Bremner WJ, Steiner RA** 1989 Luteal phase deficiency: abnormal gonadotropin and progesterone secretion patterns. J Clin Endocrinol Metab 69:813–820
11. **Daya S, Ward S, Burrows E** 1988 Progesterone profiles in luteal phase defect cycles and outcome of progesterone treatment in patients with recurrent spontaneous abortion. Am J Obstet Gynecol 158:225–232
12. **Landgren BM, Unden AL, Diczfalusy E** 1980 Hormonal profile of the cycle in 68 normally menstruating women. Acta Endocrinol 84:89–98
13. **Wathan NC, Perry L, Lilford RJ, Chard T** 1984 Interpretation of single progesterone measurements in diagnosis of anovulation and defective luteal phase: observations on analysis of the normal range. Br Med J 288:7–9
14. **Gibson M** 1990 Clinical evaluation of luteal function. Semin Reprod Endocrinol 8:130–141
15. **Longcope C, Franz C, Morello C, Baker R, Johnston CC** 1986 Steroid and gonadotrophin levels in women during the peri-menopausal years. Maturitas 8:189–196
16. **Shideler SE, DeVane GW, Kalra PS, Benirschke K, Lasley BL** 1989 Ovarian-pituitary hormone interactions during the perimenopause. Maturitas 11: 331–339
17. **Brown JB, Keller R, Matthew GD** 1959 Preliminary observations on urinary oestrogens excretion in certain gynaecological diseases. Br J Obstet Gynaecol 66:177–211
18. **Leavitt WW, Takeda A** 1986 Hormonal regulation of estrogen and progestin receptors in decidual cells. Biol Reprod 35:475–484
19. **Takeda A, Leavitt WW** 1986 Progestin induced down regulation of nuclear estrogen receptor in uterine decidual cells: analysis of receptor synthesis and turnover by the the density-shift method. Biochem Biophys Res Commun 135:98–104
20. **Tseng L, Gurpide E** 1975 Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology 97:825–833
21. **Scublinsky A, Marin C, Gurpide E** 1976 Localization of estradiol 17-β dehydrogenase in the human endometrium. J Steroid Biochem 7:745–747
22. **King RJB, Townsend PT, Whitehead MI** 1981 The role of estradiol dehydrogenase in mediating progestin effects on endometrium from postmenopausal women receiving estrogens and progestins. J Steroid Biochem 14: 235–238
23. **Rotello RC, Lieberman RC, Lepoff RB, Gerschenson LE** 1992 Characterization of uterine eptithelium apoptotic cell death kinetics and regulation by progesterone. Am J Pathol 140:449–456
24. **Terakawa N, Kigawa J, Taketani Y, Yoshikawa H, Yajima A, Noda K** 1997 The behavior of endometrial hyperplasia: a prospective study. J Obstet Gynecol Res 23:223–230
25. **Kurman RJ, Kaminski PF, Norris HJ** 1985 The behavior of endometrial hyperplasia: a long term study of "untreated" hyperplasia in 170 patients. Cancer 56:402–412
26. **Schroder R** 1954 Endometrial hyperplasia in relation to genital function. Am J Obstet Gynecol 68:294–309
27. **Fraser IS, Baird DT** 1972 Endometrial cystic glandular hyperplasia in adolescent girls. J Obstet Gynaecol 79:1009–1015
28. **Sieberg R, Nillson CG, Stenman UH, Widholm O** 1986 Endocrinologic features of oligomennorrhea in adolescent girls. Fertil Steril 46:852–857
29. **van Hooff MH, Voorhorst FJ, Kapstein MBH, Hirasing RA, Koppenaal C, Schoemaker J** 2000 Polycystic ovary syndrome in adolescents and the relationship with menstrual cycle patterns, luteinizing hormone, androgens and insulin. Fertil Steril 74:49–54
30. **Key TJA, Pike MC** 1988 The dose-effect relationship between unopposed estrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. Br J Cancer 57:205–212
31. **Ferenczy A, Bertrand G, Gelfand MM** 1979 Proilferation kinetics of human endometrium during the normal menstrual cycle. Am J Obstet Gynecol 133: 859–867
32. **Linden MD, Torres FX, Kubus J, Zarbo RJ** 1993 Clinical application of morphologic and immunocytochemical assessments of cell proliferation. Am J Clin Pathol 97:S4–S13
33. **Gerdes J, Schwab U, Lemke H, Stein H** 1983 Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31:13–20
34. **Salmi A, Heikkila S, Rutanen EM** 1998 Cellular localization of *c-Jun* messender RNA and protein and their relation to proliferation marker Ki-67 in the human endometrium. J Clin Endocrinol Metab 83:1788–1796
35. **Wahab M, Thompson J, Al-Azzawi F** 1999 The distribution of endometrial leukocytes and their proliferation markers in trimegesterone-treated postmenopausal women compared to the endometrium of the natural cycle: a dose ranging study. Hum Reprod 14:1201–1206
36. **Jurgensen A, Mettler L, Volkov N, Parwaresch R** 1996 Proliferative activity of the endometrium throughout the menstrual cycle in infertile women with and without endometriosis. Fertil Steril 66:369–375
37. **Charpin C, Andrac L, Habib MC, Vacheret H, Lavaut MN, Xerri L, Toga M** 1989 Immunocytochemical assays in human endometrial carcinomas: A multiparametric computerized analysis and comparison with non-malignant changes. Gynaecol Oncol 33:9–22
38. **Pickartz H, Beckmann R, Fleige B, Gerdes J, Stein H** 1990 Steroid receptors and proliferative activity in non-neoplastic endometria. Virchows Arch Pathol Anat 417:163–171
39. **Matsumoto Y, Iwasaka T, Yamasaki F, Sugimori H** 1999 Apoptosis and Ki-67 expression in adenomyotic lesions and in the corresponding eutopic endometrium. Obstet Gynecol 94:71–77
40. **Dahmoun A, Boman K, Cajander S, Westin P, Backstrom T** 1999 Apoptosis, proliferation and sex hormone Receptors in supoerficial parts of the human endometrium at the end of the secretory phase. J Clin Endocrinol Metab 84:1737–1743
41. **Darj E, Axelsson O, Nilsson G, Nilsson S, Risberg B** 1995 Ki-67 Immunostaining of endometrial biopsies with special reference to hormone replacemnt therapy. Gynecol Obstet Invest 39:120–124
42. **Hachisuga T, Hideshima T, Kawarabayashi T, Aguchi F, Emoto M, Shirakusa T** 1999 Expression of steroid receptors, Ki-67 and epidermal growth factor receptor in Tamoxifen-treated endometrium. Int J Gynecol Pathol 18: 297–303
43. **Scholzen T, Gerdes J** 2000 The Ki-67 protein: from the known to the unknown. J Cell Physiol 182:311–322
44. **Fiel PD, Clarke CL, Satyaswaroop PG** 1988 Progestin-mediated changes in progesterone receptor forms in the normal human endometrium. Endocrinology 123:2506–2513
45. **Kastner P, Krust A, Turcotte B, Stropp** 1990 Two distinct estrogen regulated promotors generate the transcripts of two functionally different human progesterone forms A and B. EMBO J 9:1603–1614
46. **Shapiro S, Kaufman DW, Slone D, Rosenberg L, Miettinen OS, Stolley PD, Rosenshein NB, Watring WG, Leavitt T Jr, Knapp RC** 1980 Recent and past use of conjugated estrogens in relation to adenocarcinoma of the endometrium. N Engl J Med 303:485–489
47. **Grady D, Gebretsadik T, Kerlikowske K** 1995 Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 85:304–312
48. **Herrington LJ, Weiss NS, Abbara S** 1993 Postmenopausal unopposed estrogens: characteristics of use in relation to the risk of endometrial carcinoma. Ann Epidemiol 3:308–318
49. **Weiderpass E, Adami HO, Baron JA, Magnusson C, Bergstrom R, Lindgren A, Correia N, Persson I** 1999 Risk of endometrial cancer following estrogen replacement with and without progestins. J Natl Cancer Inst 91:1131–1137
50. **Cushing KL, Weiss NS, Voigt LF, McKnight B, Beresford SAA** 1998 Risk of endometrial cancer in relation to use of low-dose, unopposed estrogens. Obstet Gynecol 91:35–39
51. **O'Connell MB** 1995 Pharmacokinetic and pharmacologic variation between different estrogen products. J Clin Pharmacol 35:18S–24S
52. **Stanczyk FZ** 1998 Structure-function relationships and metabolism of estrogens and progesterones. In: Fraser IS, Jansen RPS, Lobo RA, Whitehead MI, eds. Estrogens and progestogens in clinical practice. London: Churchill Livingston; 27–39
53. **Genant HK, Lucas J, Weiss S, Akin M, Emkey R, McNaney-Flint H, Downs R, Mortola J, Watts N, Yang HM, Banav N, Brennan JJ, Nolan J** 1997 Low-dose esterified estrogen therapy. Arch Inter Med 157:2609–2615
54. **Ettinger B, Mainton L, Upmalis DH, Citron JT, VanGessel A** 1996 Comparison of endometrial growth produced by unopposed conjugated estrogens or by micronized estradiol in postmenopausal women. Am J Obstet Gynecol 176:112–117
55. **Naessen T, Bergland L, Ulmsten U** 1997 Bone loss in elderly women prevented by ultra-low doses of parenteral 17 β-estradiol. Am J Obstet Gynecol 177:155–119
56. **Smith P, Heimer G, Lindskog M, Ulmsten U** 1996 Oestradiol-releasing vaginal ring for treatment of urogenital atrophy. Maturitas 16:145–154
57. **Bakos O, Smith P, Heimer G, Ulmsten U** 1994 Transvaginal sonography of the internal genital organs in postmenopausal women on low-dose estrogen treatment. Ultrasound Obstet Gynecol 4:326–329
58. **Ettinger B, Pressman A, Sklarin P, Bauer DC, Cauley JA, Cummings SR** 1998 Associations between low levels of estradiol, bone density and fractures among elderly women: the study of osteoporotic fractures. J Clin Endocrinol Metab 83:S178
59. **Cummings SR, Browner WS, Bauer DC** 1998 Endogenous hormones and the risk of hip and vertabral fractures among elderly women: Study of Osteoporotic Fractures Research Group. N Engl J Med 339:733–738
60. **Persson I, Adami HO, Bergvist L** 1989 Risk of endometrial cancer after

treatment with estrogens alone or in conjugation with progestagens: results of a prospective study. Br Med J 298:147–153
61. **Voigt LF, Weiss NS, Chu J, Daling JR, McKnight B, van Belle G** 1991 Progestagen supplementation of exogenous estrogens and risk of endometrial cancer. Lancet 338:274–277
62. **Woodruff JD, Pickar JH** 1999 Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone. Am J Obstet Gynecol 170:1213–1223
63. **Casanas-Roux F, Nisolle N, Marbais E, Smets M, Bassil S, Donnez J** 1996 Morphometric, immunohistological and three dimensional evaluation of the endometrium of menopausal women treated by estrogen and Crinone, a new slow-release vaginal progesterone. Hum Reprod 11:357–363
64. **Gibbons WE, Moyer DL, Lobo RA, Roy S, Mishell DR** 1986 Biochemical and histological effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women. Am J Obstet Gynecol 154:456–461
65. **Bergeron C, Ferenczy A** 2001 Endometrial safety of continuous combined-hormone replacement therapy with 17 β-oestradiol (1 or 2 mg) and dydrogesterone. Maturitas 37:191–199
66. **Cameron ST, Critchley HOD, Glasier AF, Williams AR, Baird DT** 1997 Continuous transdermal oestrogen and interrupted progestogen as a novel bleed-free regimen of hormone replacement therapy for postmenopausal women. Br J Obstet Gynaecol 104:1184–1190
67. **Moyer DL, de Lignieres B, Driguez P, Pez JP** 1993 Prevention of endometrial hyperplasia by progesterone during long-term estradiol replacement: influence of bleeding pattern and secretory changes. Fertil Steril 59:992–997
68. **Henderson BE, Casagrande JT, Pike MC, Mack TM, Rosario I, Duke A** 1983 The epidemiology of endometrial cancer in young women. Br J Cancer 47: 749–756
69. **Silverberg SG, Makowski EL** 1975 Endometrial cancer in young women taking oral contraceptive agents. Obstet Gynecol 46:503–506
70. **Comerci JT, Fields AL, Runowicz CD, Goldberg GL** 1997 Continuous low-dose combined hormone replacement therapy and the risk of endometrial cancer. Gynecol Oncol 64:425–430
71. **Pike MC, Peters RK, Cozen W, Probst-Hensch NM, Wan PC, Mack TM** 1997 Estrogen-progestin replacement therapy and endometrial cancer. J Natl Cancer Inst 89:1110–1116
72. **Beresford SAA, Weiss NS, Voigt LF, McKnight B** 1997 Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. Lancet 349:458–461
73. **The Writing Group for the PEPI Trial** 1996 Effects of hormonal replacement therapy on endometrial histology in postmenopausal women. JAMA 275: 370–375
74. **Pike MC** 2000 Progestins and menopause: epidemiological studies of risks of endometrial and breast cancer. Steroids 65:659–664
75. **Gelfand MM, Ferenczy A** 1989 A prospective 1 year study of estroegen and progestin in postmenopausal women: the effects on the endometrium. Obstet Gynecol 74:398–402
76. **Gambrell RD** 1997 Strategies to reduce the incidence of endometrial cancer in postmenopausal women. Am J Obstet Gynecol 177:1196–1207
77. **Henderson BE, Ross RK, Pike MC** 1993 Hormonal chemoprevention of cancer in women. Science 259:633–638
78. **Gruber DM, Wagner G, Kurz C, Sator MO, Huber JC** 1999 Endometrial cancer after combined hormone replacement therapy. Maturitas 31:237–240
79. **Clarke CL, Sutherland RL** 1990 Progestin regulation of cellular proliferation. Endocr Rev 11:266–301
80. **Kelsey JL, LiVolsi VA, Holford TR, Fischer DB, Mostow ED, Schwartz PE, O'Connor T, White C** 1982 A case-control study of cancer of the endometrium. Am J Epidemiol 116:333–342
81. **Parazzini F, La Vecchia C, Bocciolone L, Francheschi S** 1991 The epidemiology of endometrial cancer. Gynecol Oncol 41:1–16
82. **Hill HA, Austin Harland** 1996 Nutrition and endometrial cancer. Cancer Causes Control 7:19–32
83. **MacDonald PC, Edman CD, Hemsell DL, Porter JC, Siiteri PK** 1978 Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. Am J Obstet Gynecol 130:448–455
84. **Folsom AR, Kaye SA, Potter JD, Prineas RJ** 1989 Association of incident carcinoma of the endometrium with body weight and fat distribution in older women: early findings of the Iowa Women's Health Study. Cancer Res 49: 6828–6831
85. **Siiteri PK** 1987 Adipose tissue as a source of hormones. Am J Clin Nutr 45:277–282
86. **Kirschner MA, Samojlik E, Drejka M, Schneider G, Ertel N** 1990 Androgen-estrogen metabolism in women with upper *versus* lower body obesity. J Clin Endocrinol Metab 70:473–479
87. **Nisker JA, Hammond GL, Davidson BJ, Frumar AM, Takaki NK, Judd HL, Siiteri PK** 1980 Serum sex-hormone-binding globulin capacity and the percentage of free estradiol in postmenopausal women with and without endometrial cancer. Am J Obstet Gynecol 138:637–642
88. **Potischman N, Swanson CA, Siiteri PK, Hoover RN** 1996 Reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. J Natl Cancer Inst 88:756–758
89. **Judd HL, Lucas WE, Yen SS** 1976 Serum 17 β-estradiol and estrone levels in postmenopausal women with wnd without endometrial cancer. J Clin Endocrinol Metab 43:272–278
90. **Davidson BJ, Gambone JC, Lagasse LD, Castaldo JW, Hammond GL, Siiteri PK** 1981 Free estradiol in postmenopausal women with and without endometrial cancer. J Clin Endocrinol Metab 52:404–408
91. **Judd HL, Davidson BJ, Frumar AM, Shamonki IM, Lagasse LD, Bollon SC** 1980 Serum androgens and estrogens in postmenopausal women with and without endometrial cancer. Am J Obstet Gynecol 136:859–871
92. **Klinga K, von Holst T, Runnebaun B** 1982 Serum concentrations of FSH, oestradiol, oestrone and androstenedione in normal and obese women. Maturitas 4:9–17
93. **Benjamin F, Deutsch S** 1976 Plasma levels of fractionated estrogens and pituitary hormones in endometrial carcinoma. Am J Obstet Gynecol 126: 638–647
94. **Kopelman PG, Pilkington TRE, White N, Jeffcoate SL** 1980 Abnormal sex steroid secretion and binding in massively obese women. Clin Endocrinol 12:363–369
95. **Poortman J, Thijssen JHH, de Waard F** 1981 Plasma oestrone, oestradiol and androstenedione levels in postmenopausal women: relation to body weight and height. Maturitas 3:65–71
96. **Vermeulen A, Verdonck L** 1978 Sex hormone concentrations in postmenopausal women: relation to obesity, fat mass, age and years postmenopause. Clin Endocrinol 9:59–66
97. **Olson SH, Vena JE, Dorn JP, Marshall JR, Zielezny M, Laughlin R, Graham S** 1997 Exercise, occupational activity and risk of endometrial cancer. Ann Epidemiol 7:46–53
98. **Austin Harland, Austin Jr JM, Partridge EE, Hatch KD, Shingleton HM** 1991 Endometrial cancer, obesity, and body fat distribution. Cancer Res 51:568–572
99. **Le Marchand L, Wilkins LR, Mi MP** 1991 Early-age body size, adult weight gain and endometrial cancer risk. Int J Cancer 48:807–811
100. **Levi F, La Vecchia C, Negri E, Parazzini F, Francheschi S** 1992 Body mass at different ages and subsequent endometrial cancer risk. Int J Cancer 50: 567–571
101. **Gallup DG, Stock RJ** 1984 Adenocarcinoma of the endometrium in women 40 years of age or younger. Obstet Gynecol 64:417–420
102. **Farquar CM, Lethaby A, Sowter M, Verry J, Baranyai J** 1999 An evaluation of risk factors for endometrial hyperplasia in premenopausal women with abnormal menstrual bleeding. Am J Obstet Gynecol 181:525–529
103. **Silverberg SG, Makowski EL, Roche WD** 1977 Endometrial carcinoma in women under 40 years of age. Cancer 39:592–598
104. **Evans-Metcalf ER, Brooks SE, Reale FR, Baker SP** 1998 Profile of women 45 years of age and younger with endometrial cancer. Obstet Gynecol 91: 349–354
105. **Lobo RA, Granger L, Goebelsmann U** 1981 Elevations in unbound serum estradiol as a possible machanism for inappropriate gonadotropin secretion in women with PCO. J Clin Endocrinol Metab 52:156–162
106. **Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, Wang DY** 1997 Re: reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. J Natl Cancer Inst 89:396–398
107. **Zumoff B** 1982 Relationship of obesity to blood estrogens. Cancer Res 42: 3289S–3294S
108. **De Pergola G, Giorgino F, Cospite MR, Giagulli VA** 1993 Relationship between sex hormones and serum lipoprotein and lipoproten A concentrations in obese premenopausal women. Arterioscler Throm 13:675–679
109. **Grenman S, Ronnemaa T, Irjala K, Kaihola HL** 1986 Sex steroid, gonadotrophin, cortisol and prolactin levels in healthy massively obese women: correlation with abdominal fat cell size and weight reduction. J Clin Endocrinol Metab 63:1261
110. **Manson JM, Sammel MD, Freeman EW, Grisso JA** 2001 Racial differences in sex hormone levels in women approaching the transition to menopause. Fertil Steril 75:297–304
111. **Westhoff C, Gentile G, Lee J, Zacur H., Helbig D** 1996 Predictors of ovarian steroid secretion in reproductive-age women. Am J Epidemiol 144:381–388
112. **Pike MC, Spicer DV, Dahmoush L, Press MF** 1993 The role of estrogens and progestins in the epidemiology and prevention of breast cancer. Eur J Clin Oncol 24:29–43
113. **Fishman J, Boyar RM, Hellman L** 1975 Influence of body weight on estradiol metabolism in young women. J Clin Endocrinol Metab 41:989–993
114. **Gruber DM, Huber JC** 2001 Tissue specificity: the clinical importance of steroid metabolites in hormone replacement therapy. Maturitas 37:151–157
115. **Clark JH, Paszko Z, Peck EF** 1977 Nuclear binding and retention of the receptor estrogen complex: relation to the agonistic and antagonistic properties of estriol. Endocrinology 100:91–98
116. **Rogers J, Mitchell GW** 1952 The relation of obesity to menstrual disturbances. N Engl J Med 247:53–55
117. **Hartz AJ, Rupley DC, Rimm AA** 1984 The association of girth measurements with disease in 32,856 women. Am J Epidemiol 119:71–80
118. **Peterson E** 1968 Endometrial cancer in young women. Obstet Gynecol 31: 702–707

119. **Brinton LA, Berman ML, Mortel R, Twiggs LB, Barrett RJ, Wilbanks GD, Lannom L, Hoover RN** 1992 Reproductive, menstrual and medical risk factors for endometrial cancer: results from a case-control study. Am J Obstet Gynecol 167:1317–1325
120. **Conway MG, Johnson D, Kelly A, Griffin D, Smith J, Wallace AM** 2000 Differences in circulating concentrations of total, free, bound leptin relate to gender and body composition in adult humans. Ann Clin Biochem 37:717–723
121. **Brannian JD, Zhao Y, McElroy M** 1999 Leptin inhibits gonadotrophin-stimulated granulosa cell progesterone production by antagonizing insulin action. Hum Reprod 14:1445–1448
122. **Spicer LJ, Chamberlain CS, Francisco CC** 2000 Ovarian action of leptin: effects on insulin-like growth factor-1-stimulated function of granulosa and thecal cells. Endocrine 12:53–59
123. **Barkan D, Jia H, Dantes A, Vardimon L, Amsterdam A, Rubinstein M** 1999 Leptin modulates the glucocorticoid-induced ovarian steroidogenesis. Endocrinology 140:1731–1738
124. **Duggal PS, Van Der Hoek KH, Milner CR, Ryan NK, Armstrong DT, Magoffin DA, Norman RJ** 2000 The *in vivo* and *in vitro* effect of exogenous leptin on ovulation in the rat. Endocrinology 141:1971–1976
125. **Weiderpass E, Persson I, Adami HO, Magnusson C, Lindgren A, Baron JA** 2000 Body size in different periods of life, diabetes mellitus, hypertension and risk of postmenopausal endometrial cancer. Cancer Causes Control 11: 185–192
126. **La Vecchia C, Negri E, Francheschi S** 1994 A case control study of diabetes mellitus and endometrial cancer risk. Br J Cancer 70:950–953
127. **Garnet JD** 1958 Constitutional stigmas associated with endometrial carcinoma. Am J Obstet Gynecol 76:11–19
128. **Rutanen EM, Stenman S, Blum W, Karkkainen T, Lehtovirta P, Stenman UH** 1993 Relationship between carbohydrate metabolism and serum insulin-like growth factor system in postmenopausal women: comparison of endometrial cancer patients with healthy controls. J Clin Endocrinol Metab 77: 199–204
129. **Shoff SM, Newcomb PA** 1998 Diabetes, body size and risk of endometrial cancer. Am J Epidemiol 148:234–240
130. **Brown R** 1974 Carbohydrate metabolism in patients with endometrial cancer. Br J Obstet Gynaecol 81:940–946
131. **Nagamani M, Hannigan EV, van Dinh T, Stuart CA** 1988 Hyperinsulinemia and stromal luteinization in the ovaries of postmenopausal women with endometrial cancer. J Clin Endocrinol Metab 67:144–148
132. **Troisi R, Potischman N, Hoover RN, Siiteri PK, Brinton LA** 1997 Insulin and endometrial cancer. Am J Epidemiol 146:476–482
133. **Rutanen EM, Nyman T, Lehtovirta P, Ammala M, Pekonen F** 1994 Suppressed expression of insulin-like growth factor binding protein-2 mRNA in the endometrium: a molecular mechanism associating endometrial cancer with its risk factors. Int J Cancer 59:307–312
134. **Nagamani M, Stuart CA, Dunhardt PA** 1991 Specific binding sites for insulin and insulin-like growth factor I in human endometrial cancer. Am J Obstet Gynecol 165:1865–1871
135. **Murphy LJ, Ghahary A** 1990 Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation. Endocr Rev 11:443–453
136. **Rutanen EM, Pekonen F, Nyman T, Wahlstrom T** 1993 Insulin-like growth factors and their binding proteins in benign and malignant uterine diseases. Growth Regul 3:72–75
137. **Murphy LJ** 1994 Growth factors and steroid hormone action in endometrial cancer. J Steroid Biochem 48:419–423
138. **Sarup JC, Rao KVS, Fox CF** 1988 Decreased progesterone binding and attenuated progesterone action in cultured human breast cancer cells treated with epidermal growth factor. Cancer Res 48:5071–5078
139. **Moore MA, Park CB, Tsuda H** 1998 Physical exercise: a pillar for cancer. Eur J Cancer Prev 7:177–193
140. **Kramer MM, Wells CL** 1996 Does physical activity reduce risk of estrogen-dependent cancer in women. Med Sci Sports Exerc 28:322–34
141. **Terry P, Baron JA, Weiderpass E, Yuen J, Lichtenstein PNO** 1999 Lifestyle and endometrial cancer risk: a cohort from the Swedish Twin Registry. Int J Cancer 82:38–42
142. **Moradi T, Nyren O, Bergstrom R, Gridley G, Linet M, Wolk A, Dosemeci M, Adami HO** 1998 Risk for endometrial cancer in relation to occupational physical activity: a nationwide cohort study in Sweden. Int J Cancer 76: 665–670
143. **Sturgeon SR, Brinton LA, Berman ML, Mortel R, Twiggs LB, Barrett RJ, Wilbanks GD** 1993 Past and present physical activity and endometrial cancer risk. Br J Cancer 68:584–589
144. **Shu XO, Hatch MC, Zheng W, Gao YT, Brinton LA** 1993 Physical activity and risk of endometrial cancer. Epidemiology 4:342–349
145. **Russell JP, Mitchell D, Musey PI** 1984 The relationship of exercise and anovulatory cycles in female athletes: hormonal and physical characteristics. Obstet Gynecol 63:452–459
146. **Bullen BA, Skrinar GS, Beitins IZ, von Mering G, Turnbull BA, McArthur JW** 1985 Induction of menstrual disorders by strenuous exercise in untrained women. N Engl J Med 312:1349–1353
147. **Schweiger U, Laessle R, Schweiger M, Herrmann F, Riedel W, Pirke K** 1988 Caloric intake, stress and menstrual function in athletes. Fertil Steril 49: 447–450
148. **Chatterton RT, DeLeon-Jones FA, Hudgens GA, Dan AJ** 1984 Lack of effect of initiation of exercise training on incidence of anovulation. Fertil Steril 41:2S
149. **Soules MR, McLachlan RI, Ek M, Dahl KD, Cohen NL, Bremner WJ** 1989 Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. J Clin Endocrinol Metab 69:804–812
150. **La Vecchia C, Francheschi S, Decarli A, Gallus G, Tognoni G** 1984 Risk factors for endometrial cancer at different ages. J Natl Cancer Inst 73:667–671
151. **Villani C, Pucci G, Pietrangeli D, Pace S, Tomao S** 1986 Role of diet in endometrial cancer patients. Eur J Gynecol Oncol 7:139–143
152. **Shu XO, Zheng W, Potischman N, Brinton LA, Hatch MC, Gao YT, Fraumeni Jr JF** 1993 A population based case-contol study of dietary factors and endometrial cancer in Shanghai, People's Republic of China. Am J Epidemiol 137:155–165
153. **Barbone F, Austin H, Partridge EE** 1993 Diet and endometrial cancer: a case-control study. Am J Epidemiol 137:393–403
154. **Potischman N, Swanson CA, Brinton LA, McAdams M, Barrett RJ, Berman ML, Mortel R, Twiggs LB, Wilbanks GD, Hoover RN** 1993 Dietary associations in a case-control study of endometrial cancer. Cancer Causes Control 4:239–250
155. **Levi F, Francheschi S, Negri E, La Vecchia C** 1993 Dietary factors and the risk of endometrial cancer. Cancer 71:3575–3581
156. **Goodman MT, Hankin JH, Wilkens LR, Lyu LC, McDuffie K, Liu LQ, Kolonel LN** 1997 Diet, body size, physical activity and the risk of endometrial cancer. Cancer Res 57:5077–5085
157. **Zheng W, Kushi LH, Potter JD, Sellers TA, Doyle TJ, Bostick RM, Folsom AR** 1995 Dietary intake of energy and animal foods and endometrial cancer incidence: the Iowa Women's Study. Am J Epidemiol 142:388–394
158. **Armstrong B, Brown JB, Clark HT** 1981 Diet and reproductive hormones: a study of vegetarian and non-vegetarian postmenopausal women. J Natl Cancer Inst 67:761–767
159. **Goldin BR, Adlercreutz H, Gorbach SL, Woods MN, Dwyer JT, Conlon T, Bohn E, Gershoff SN** 1986 The relationship between estrogen levels and diets of Caucasian American and Oriental women. Am J Clin Nutr 44:945–953
160. **Shultz TD, Leklem JE** 1983 Nutrient intake and hormonal status of premenopausal vegetarian Seventh Day Adventists and premenopausal nonvegetarians. Nutr Cancer 4:945–953
161. **Prentice R, Thompson DJ, Clifford C, Gorbach SL, Goldin BR, Byar D** 1990 Dietary fat reduction and plasme estradiol concentration in healthy postmenopausal women. J Natl Cancer Inst 82:129–134
162. **Hagerty MA, Howie B, Tan S, Shultz TD** 1988 Effect of a low-fat intakes on the hormonal milieu of premenopausal women. Am J Clin Nutr 47:653–659
163. **Bennett FC, Ingram DM** 1990 Diet and female sex hormone concentrations: an intervention study for the type of fat consumed. Am J Clin Nutr 52:808–812
164. **Katsouyanni K, Boyle P, Trichopuolos D** 1991 Diet and urine estrogens among postmenopausal women. Oncology 48:490–494
165. **London S, Willett WC, Longcope C, McKinlay SM** 1991 Alcohol and dietary factors in relation to serum hormone concentrations in women at climacteric. Am J Clin Nutr 53:166–171
166. **Longcope C, Gorbach S, Goldin B** 1987 The effect of a low fat diet on estrogen metabolism. J Clin Endocrinol Metab 64:1246–1250
167. **Burke TW, Tortolero-Luna G, Malpica A, Baker VV, Whittaker L, Johnson E, Follen Mitchell M** 1996 Endometrial hyperplasia and endometrial cancer. [Review] [176 refs]. Obstet Gynecol Clin North Am 23:411–456
168. **Wynder EL, Fujita Y, Harris RE, Hirayama T, Hiyama T** 1991 Comparative epidemiology of cancer between the United States and Japan: a second look. Cancer 67:746–763
169. **Nagata C, Kabuto M, Kurisu Y, Shimizu H** 1997 Decreased serum estradiol concentration associated with high dietary intake of soy products in premenopausal Japanese women. Nutr Cancer 29:228–233
170. **Cassidy A, Bingham S, Setchell KDR** 1994 Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 60:333–340
171. **Duncan AM, Underhill KEW, Xu X, Lavalleur J, Phipps WR, Kurzer MS** 1999 Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 84:3479–3484
172. **Brzezinski A, Aldercreutz H, Shaoul R, Rösler A, Tanos V, Schenker JG** 1997 Short-term effects of phytoestrogen-rich diet on postmenopausal women. Menopause 4:89–94
173. **Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KD, Weinberg CR, Haney AF, Wilcox AJ, Mclachlan JA** 1995 Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. J Clin Endocrinol Metab 80:1685–1690
174. **Bennetts HW, Underwood EJ, Shier FL** 1946 A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust Vet J 22:2–12
175. **Folman Y, Pope GS** 1966 The interaction in the immature mouse of potent estrogens with coumestrol, genistein and other utero-trophic compounds of low potency. J Endocrinol 34:215–225
176. **Braden AWH, Hart NK, Lamberton JA** 1999 The oestrogenic activity of and metabolism of certain isoflavones in sheep. Aust J Agric Res 18:355–348

177. **Francis CM, Millington AJ, Bailey ET** 1967 The distribution of oestrogenic isoflavones in the genus Trifolium. Aust J Agric Res 22:663–670
178. **Markiewicz L, Garey J, Aldercreutz H, Gurpide E** 1993 In vitro bioassays of non-steroidal phytoestrogens. J Steroid Biochem Mol Biol 45:399–405
179. **Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA** 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138: 863–870
180. **Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA** 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor α and β. Endocrinology 139:4252–4263
181. **Nagel SC, vom Saal FS, Welshons WV** 1998 The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. Proc Soc Exp Biol Med 217:300–309
182. **Aldercreutz H, Bannwart C, Wahala K** 1993 Inhibition of human aromatase by mammalian lignans and isoflavanoid phytoestrogens. Mol Biol 44:147–153
183. **Akiyama T, Ishida J, Nakagawa S,** 1987 Genistein: a specific inhibitor of tyrosine-specific protein kinase. J Biol Chem 262:5592–5595
184. **Evans BA, Griffiths K, Morton MS** 1995 Inhibition of 5-α reductase in genital skin fibroblasts and prostate tissue. J Endocrinol 147:295–302
185. **Makela S, Poutanen M, Lehtimaki J, Kostian ML, Santti R, Vihko R** 1995 Estrogen specific 17β-hydroxysteroid oxidoreductase type I (EC. 1.1.1.62) as a possible target for the action of phytoestrogens. Proc Soc Exp Biol Med 208:51–59
186. **Wei H, Wei L, Frenkel K, Bowen R, Barnes S** 1993 Inhibition of tumor promoter-induced hydrogen peroxide formation *in vitro* and *in vivo* by genistein. Nutr Cancer 20:1–12
187. **Mousavi Y, Aldercreutz H** 1993 Genistein is an effective stimulator of SHGB production in hepatocarcinoma human liver cells and supresses proliferation of these cells in culture. Steroids 58:301–304
188. **Baber RJ, Templeman C, Morton T, Kelly GE, West L** 1999 Randomized placebo-controlled trial of an isoflavone supplement and menopausal symptoms in women. Climacteric 2:85–92
189. **Upmalis D, Lobo RA, Bradley L, Warren M, Cone FL, Lamia CA** 2000 Vasomotor symptom relief by soy isoflavone extract tablets in postmenopausal women: a multicenter, double-blind, randomized, placebo-controlled study. Menopause 7:236–242
190. **Scambia G, Mango D, Signorile PG, Anselmi RA, Palena C, Gallo D** 2000 Clinical effects of a standardized soy extract in postmenopausal women: a pilot study. Menopause 7:105–111
191. **Hale GE, Hughes CL, Agarwal SK, Robboy SJ, Bievre M** 2001 A double blind placebo controlled trial on the effect of an isoflavone, P-07 on the endometrium. Menopause 8:338–346
192. **Foth D, Cline JM** 1998 Effects of mammalian and plant estrogens on mammary glands and uteri of macaques. Am J Clin Nutr 68(Suppl 6):1413S-01417S
193. **Tansey G, Hughes CL, Cline JM, Krunner A, Schmoltzer S** 1998 Effects of dietary soybean estrogens on the reproductive tract in female rats. Proc Soc Exp Biol Med 217:340–344
194. **Santell RC, Chang YC, Muralee GN, Helferich WG** 1997 Dietary genistein exerts estrogenic effects upon the uterus, mammary gland and the hypothalamic/pituitary axis in rats. J Nutr 127:263–269
195. **Whitehead SA, Lacey M** 2000 Protein tyrosine kinase activity of lavendustin A and the phytoestrogen genistein on progesterone synthesis in culture rat ovarian cells. Fertil Steril 73:613–619
196. **Prior JC** 1998 Perimenopause: the complex endocrinology of the menopausal transition. Endocr Rev 19:397–428
197. **Furuhjelm M** 1966 Urinary excretion of hormones during the climacteric. Acta Obstet Gynecol Scand 45:352–364
198. **Burger HG, Dudley EC, Hopper JL, Groome N, Guthrie JR, Green A, Dennerstein L** 1999 Prospectively measured levels of serum follicle-stimulating hormone, estradiol and dimeric inhibins during the menopausal transition in a population-based cohort of women. J Clin Endocrinol Metab 84:4025–4030
199. **Santoro N, Rosenburg Brown J, Adel T, Skurnick JH** 1996 Characterization of reproductive hormonal dynamics in the perimenopause. J Clin Endocrinol Metab 81:1495–1501
200. **Reyes FI, Winter JSD, Faiman C** 1977 Pituitary ovarian relationships preceding the menopause: a cross-sectional study serum follicle stimulating hormone and luteinizing hormone, prolactin, estradio and progesterone levels. Am J Obstet Gynecol 129:557–564
201. **Ballinger CB, Browning NC, Smith AHW** 1987 Hormone profiles and psychological symptoms in perimenopausal women. Maturitas 9:235–251
202. **Reame NE, Kelch RP, Beitins IZ, Yu M, Zawacki CM, Padmanabhan V** 1996 Age effects on follicle-stimulating hormone and pulsatile luteinizing hormone secretion across the menstrual cycle of premenopausal women. J Clin Endocrinol Metab 81:1512–1518
203. **Brown JB, Harrisson P, Smith MA** 1985 Return of fertility after childbirth and during lactation and changes in the climacteric. J Biosoc Sci 17(Suppl 9):5–27
204. **Rannevik G, Jeppsson S, Johnell O, Bjerre B, Laurell-Borulf Y, Svanberg L** 1995 A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas 21:103–113
205. **Metcalf MG** 1979 Incidence of ovulatory cycles in women approaching the menopause. J Biosoc Sci 11:39–48
206. **Metcalf MG, MacKenzie JA** 1985 Menstrual cycle and exposure to estrogens unopposed by progesterone: relevance to studies on breast cancer incidence. J Endocrinol 104:137–141
207. **Lee SJ, Lenton EA, Sextron L, Cooke ID** 1988 The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone. Hum Reprod 3:851–855
208. **Sauer MV, Miles RA, Dahmoush L, Paulson RJ, Press MF, Moyer DL** 1993 Evaluating the effect of age on endometrial responsiveness to hormone replacement therapy: a histologic ultrasonographic and tissue receptor analysis. J Assist Reprod Genet 10:47–52
209. **Noci I, Borri P, Scarselli G, Chieffi O, Bucciantini S, Biagiotti R, Paglierani M, Moncini D, Taddei G** 1996 Morphological and functional aspects of the endometrium of asymptomatic post-menopausal women: does the endometrium really age? Hum Reprod 11:2246–2250
210. **Leone M, Costantini C, Gallo G, Voci A, Massajoli M, Messeni Leone M, de Cecco L** 1993 Role of growth factors in the human endometrium during aging. Maturitas 16:31–38
211. **Palmieri D, Watson JM, Rinehart CA** 1999 Age-related expression of PEDF/EPC-1 in human endometrial stromal fibroblasts: implications for interactive senescence. Exp Cell Res 247:142–147
212. **Koshiyama M, Yoshida M, Takemura M, Yura Y, Matsushita K, Hayashi M, Tauchi K, Konishi I, Mori T** 1996 Immunohistochemical analysis of distribution of estrogen and progesterone receptors in the postmenoapausal endometrium. Acta Obstet Gynecol Scand 75:702–706
213. **Ali IU** 2000 Gatekeeper for endometrium: the *PTEN* tunor suppressor gene. J Natl Cancer Inst 92:861–863
214. **Mutter GL, Ince TA, Baak JPA, Kust GA, Zhou XP, Eng C** 2001 Molecular identification of latent precancers in histologically normal endometrium. Cancer Res 61:4311–4314
215. **Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, Weng LP, Eng C** 2000 Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 92:924–930
216. **Maxwell GL, Risinger JI, Gumbs C, Shaw H, Bentley RC, Barrett JC, Berchuck A, Futreal PA** 1998 Mutation of the *PTEN* tumor suppressor gene in endometrial hyperplasia. Cancer Res 58:2500–2503
217. **Yoshida A, Harada T, Kitazawa T, Yoshida T, Kinoshita M, Maita K** 1996 Effects of age on endometrial carcinogenesis induced by concurrent oral administration of ethylenethiourea and sodium nitrite in mice. Exp Toxicol Pathol 48:289–298