In climacteric and postmenopausal women, low serum levels of 17β-estradiol (E₂) often result in symptoms such as hot flashes or degenerative processes such as osteoporosis. This has prompted women to receive hormone replacement therapy (HRT) to prevent these ageing-associated symptoms or diseases (Burger 2003). Because estrogens alone stimulate endometrial proliferation, which may result in cancer, they have to be given in combination with progestins (Albertazzi and Sharma 2005). This has resulted in massive consumption of estrogens in combination with progestins. The recent Women’s Health Initiative and the British Million Women Study have shown doubts on the exclusively beneficial effect of HRT, particularly because a higher incidence of mammary cancers and arteriosclerotic complications such as heart attacks and strokes were reported (Beral 2003; Rossouw et al. 2002). This has resulted in a search for HRT alternatives and plant-derived, so-called phytoestrogens.

The estrogenic activity of GEN is known, and it is widely advertised as a phytoestrogen useful in alleviating climacteric complaints and other postmenopausal disorders. Knowledge of effects of long-term administration of GEN in laboratory animals is scarce, and effects in the uterus and mammary gland after long-term administration have not been studied. The uterus and mammary gland are known to be negatively influenced by estrogens used in hormone therapy.

**OBJECTIVES:** We administered two doses of GEN [mean daily uptake 5.4 (low) or 54 mg/kg (high) body weight (bw)] orally over a period of 3 months to ovariectomized (ovx) rats and compared the effects with a treatment with two doses of 17β-estradiol [E₂: 0.17 (low) or 0.7 mg/kg bw (high)]. Mammary glands, vaginae, and uteri were investigated morphologically and immunohistochemically. We quantified the expression of proliferating cell nuclear antigen (PCNA) and progesterone receptor (PR) in the mammary gland.

**RESULTS:** In rats treated with either of the E₂ doses or the high GEN dose, we found increased uterine weight, and histologic analysis showed estrogen-induced features in the uteri. In vaginae, either E₂ dose or GEN high induced hyperplastic epithelium compared with the atrophic controls. In the mammary gland, E₂ (either dose) or GEN increased proliferation and PR expression. Serum levels of luteinizing hormone were decreased by E₂ (both doses) but not by GEN.

**CONCLUSIONS:** In summary, E₂ and GEN share many effects in the studied organs, particularly in the vagina, uterus, and mammary gland but not in the hypothalamo/pituitary unit.

**KEY WORDS:** estradiol, genistein, mammary gland, uterus, vagina. *Environ Health Perspect* 115(suppl 1):62–68 (2007). doi:10.1289/ehp.9367 available via [http://dx.doi.org/](http://dx.doi.org/) [Online 8 June 2007].
oral administration of two doses of GEN in ovx rats and compared them with those induced by E2 treatment. In the present study, tissue samples were analyzed histologically and immunocytochemically. E2-induced effects on body weight and in uterus and vagina are well known (Albertazzi and Sharma 2005; Asarian and Geary 2006; Heinemann and Reid 2005) andallow comparison with effects of test substances. E2-induced effects in the mammary gland have not been well described and therefore we defined our parameters; thus, this study provides not only information on effects of long-term oral administration of GEN but also quantitative data on the effects of E2 in this tissue. E2 stimulates mammary gland proliferation by a mechanism that involves ER-α (Tekmal et al. 2005); this has been associated with the increased risk of cancer development because DNA damage might occur during replication (Krebs et al. 2004). Therefore, we performed proliferation-specific analyses and determined expression of the progesterone receptor (PR), which is stimulated in the mammary gland after E2 exposure (Tekmal et al. 2005). Cells expressing both proliferating cell nuclear antigen (PCNA) protein and PR protein were quantified after immunohistochemical staining.

Materials and Methods

Animals and chemical exposures. Permission for this study was given by the Bezirksregierung (Az. 509.42502/01-36.03), which precludes inhumane treatment and painful experimentation. Sprague-Dawley rats were fed soy-free food during and after breeding, and body weights of each animal were recorded before and at termination of the experiment. Bilateral ovx was performed under isoflurane anesthesia (Tekmal et al. 2005). Cells expressing both proliferating cell nuclear antigen (PCNA) protein and PR protein were quantified after immunohistochemical staining.

Animals were fed these diets for 3 months and housed under standardized conditions (lights on 12 hr from 0600 hours to 1800 hours; room temperature, 23°C; 5–6 animals per cage) with free access to water and food. We estimated intake of the test substances from the mean food intake in each cage divided by the number of animals per cage. The daily food intake per animal was as follows: control, 18.7 g; E2 low, 15.4 g; E2 high, 14.9 g; GEN low, 18.8 g; and GEN high, 15.5 g. On the basis of food intake, we estimated that the daily exposure to free E2 or GEN per animal (in milligrams per kilograms body weight) was as follows: E2 low, 0.17; E2 high, 0.7; GEN low, 5.4; GEN high, 53.

Tissue samples. Animals were sacrificed by decapitation under CO2 anesthesia between 0900 and 1200 hours; blood was collected from the trunk, and serum was stored at −20°C until analysis of E2 by radioimmunoassay (3rd Generation RIA Kit; DSL GmbH, Sinsheim, Germany). Sera of the GEN-treated animals were extracted using the SEP-PAK cartridges solid-phase method according to the standard protocol suggested by the manufacturer (Waters, Eschborn, Germany), and ethanol-redissolved aliquots were subjected to HPLC separation and UV-detection as described above.

The uteri were removed and weighed, and the fifth mammary glands and vaginae were collected. All samples were fixed in 10% buffered formalin for 48 hr. Uteri were cut such that three cross-sections per area could be prepared at proximal, medial, and distal areas. Mammary glands were cut to obtain sections from the nipple through the fat pad toward the abdominal muscles. Vaginae were prepared for longitudinal sections. All samples were embedded in paraffin, and 3-μm thick sections were cut, mounted, and stained with hematoxylin and eosin (H&E) for microscopic analysis.

Immunohistochemical analysis. We performed immunohistochemistry in mammary gland sections to identify PCNA and PR. After antigen retrieval using microwave radiation and citrate buffer (Ezaki 2000), we immunolabeled tissue sections with a monoclonal antibody for PCNA (PC-10, sc 56; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or a polyclonal antibody for PR (C19, sc 538; Santa Cruz Biotechnology). The tissue sections were then immunostained using peroxide and diaminobenzidine (EnVision Detection System, Peroxidase/DAB, rabbit/mouse, K5007; Dako Cytomation, Hamburg, Germany). Sections were then stained with H&E. We quantified the number of PCNA- and PR-immunoreactive cells in alveolar and terminal buds of the mammary gland on one slide for each of the 11–12 animals per group; 400–600 cells were counted per slide. The number of PCNA- or PR-expressing cells was expressed as the percentage of the total number of mammary gland cells.

Morphologic analyses. For morphologic analyses of uterus, we recorded known E2-induced features including shape and polarity.

Food was soy-free pelleted chow supplemented with potato proteins (D-59494; SMW Spezialdiäten GmbH, Soest, Germany). Immediately after ovx, animals in four of the groups received the same chow with added E2 [0.17 or 0.7 mg/kg body weight (bw)] or GEN (5.4 or 54 mg/kg bw).

E2 was given as estradiol benzoate (catalog no. 8515, purity of 98.5%; Sigma, Munich, Germany). Genistein (catalog no. SG 20030618, purity of 98.5%) was a commercial product of Chemos (Regensburg, Germany). We checked the purity of the isoflavone by HPLC and ultraviolet (UV) detection at 260 nm. The HPLC conditions were gradient elution with 70% A (water containing 0.085% o-phosphoric acid), 30% B (acetonitril) for 0 min; 25% A, 75% B for 15 min; and a 250 × 4 mm C18 reverse-phase column with a flow rate of 1 mL/min.

Animals were fed these diets for 3 months and housed under standardized conditions (lights on 12 hr from 0600 hours to 1800 hours; room temperature, 23°C; 5–6 animals per cage) with free access to water and food. We estimated intake of the test substances from the mean food intake in each cage divided by the number of animals per cage. The daily food intake per animal was as follows: controls, 18.7 g; E2 low, 15.4 g; E2 high, 14.9 g; GEN low, 18.8 g; and GEN high, 15.5 g. On the basis of food intake, we estimated that the daily exposure to free E2 or GEN per animal (in milligrams per kilograms body weight) was as follows: E2 low, 0.17; E2 high, 0.7; GEN low, 5.4; GEN high, 53.

Table 1. Initial and final body weights (grams) of E2- and GEN-treated ovx rats (mean ± SEM).

| Treatment       | Time point | Control | E2 low | E2 high | GEN low | GEN high |
|-----------------|------------|---------|--------|---------|---------|---------|
| Initial bw      |            | 256 ± 5.5| 268 ± 5.8| 261 ± 3.1| 264 ± 6.6| 250 ± 3.8|
| Final bw        |            | 341 ± 8.1*| 348 ± 8.3***| 348 ± 5.2**| 354 ± 8.1*| 293 ± 4.3***|

*p < 0.05 compared with initial control weight. **p < 0.5 compared with final control weight.

Figure 1. Serum levels (mean ± SEM) of E2 (A) and LH (B) in ovx rats after 3 months of treatment. Note the dose-dependent reduction of LH by E2 and the lacking effect of GEN.

*p > 0.01 compared with control.

Figure 2. Uterine weights (mean ± SEM) of ovx rats after 3 months of treatment with E2 or GEN. Note the stimulating effects of both E2 doses and the GEN high. The dashed line indicates the average uterine wet weight for an intact female rat.

*p < 0.01 compared with control.
of the lamina propria cells, epithelial mitotic figures and necrosis, determination of hypertrophy and hyperplasia of glands and endometrial epithelium, and pathologic features such as squamous metaplasia and pyometra. We also measured the thickness of endometrium and myometrium. The parameters of estrogenic effects in the vaginae were number of epithelial cell layers and their cornification. In the mammary glands, we determined the degree of luminal formation and the presence or absence of secretion. Quantification of these parameters was performed on three slides per animal; at least 44 slides per group were evaluated.

Statistical analyses. We set the number of PCNA and PR positive cells per slide in relation to the total number of alveolar and terminal bud epithelial cells. The percentages of related values were used to calculate means. Similarly, we used individual serum hormone levels and uterine weights to calculate means and to perform statistical analysis of the data by analysis of variance followed by Newman-Keuls post hoc test. Morphologic features were statistically analyzed with contingency tables compared with controls. All analyses were performed with GraphPad PRISM 4 software (GraphPad Software, Inc., San Diego, CA, USA). Data are presented as mean ± SEM.

Results

Animal weights. Table 1 presents body weights of animals before ovx and on the day of termination of the experiments. The E2 low and GEN high animals gained significantly less weight and the E2 high gained no weight compared with the control animals. Serum E2 levels in controls and E2-treated animals are shown in Figure 1A. Serum E2 concentrations were barely detectable in control animals, whereas they were in physiologic range in the E2 low group and at supraphysiologic levels in the E2 high group. Serum luteinizing hormone (LH) levels were high in control animals, decreased dose-dependently in the E2 groups, and not reduced significantly in the GEN groups (Figure 1B).

Uterus. Both E2 doses and GEN high significantly increased uterine weight. With E2 (both doses), uterine weight was around 5-fold that of controls (Figure 2), and weights were in the range observed in intact adult female rats (441.1 ± 18.7 mg in previous experiments using the same rat strain). GEN high increased uterine weights only around 2-fold (Figure 2). Figure 3 shows microscopic preparations of representative uteri from one animal per treatment group. The morphologic findings in uteri of all animals were quantified and are presented in Table 2. In control uterus we observed atrophy affecting all structures (Figure 3A). The endometrium was composed of cuboidal inactive cells, and the connective tissue was an unorganized lax syncytium of round nuclei. No mitotic activity was detected in epithelial cells. E2 low (Figure 3B) induced estrogenic features such as large cytoplasm in endometrial cells, vacuolar degeneration, and necrosis; we also observed spindle lamina propria cells intermingled with areas mimicking proestrus of a normal cycle (smaller nucleus:cytoplasm ratio, less degeneration, and necrosis). Few pathologic findings, such as hypertrophic and hyperplastic glands, were detected. Mitotic activity was present in endometrial cells in most animals at various degrees.

With E2 high (Figure 3C) treatment, all uterine structures were hypertrophic and hyperplastic. Although differing in intensity, endometria were hypertrophic and hyperplastic...
in 10 of 11 animals (in 1 animal endometrium could not be analyzed due to severe pyometra) with squamous metaplasia and anaplastic signs such as hyperchromatic nuclei with dispersed chromatin and prominent nucleoli. We observed various mitotic figures in endometrial epithelium and, within these, bizarre mitotic figures were apparent. Pyometra also developed in 5 of 11 animals. Some of these effects in the E2 high group are shown in Figure 3F (a bizarre mitotic cell and keratinized cells). The treatment with GEN low (Figure 3D) induced negligible effects: Lamina propria cells were found in some areas in 6 of 12 animals but epithelial structures in endometrial epithelium remained unaltered, without hyperplastic/ hypertrophic changes or mitotic activity. In GEN high animals (Figure 3E), endometrial cells were stimulated but no pathologic signs were detected. Endometrial mitotic activity was found abundantly in 1 of 12, rarely in 2 of 12, and absent in 9 of 12 animals.

Vagina. Figure 4 shows microscopic preparations of representative vaginae from one animal per treatment group; the morphologic findings in vaginae of all animals were quantified and are presented in Table 3. Atrophic vaginal epithelium was observed in control animals (Figure 4A); in 12 of 12 rats, only two to three cell layers were present, and these were composed of flattened cells with no cornification.

The E2 low animals (Figure 4B) displayed a typical squamous multilayered epithelium. Approximately 10 cell layers with cornification were observed in all 11 samples. Almost the same changes were seen with E2 high (Figure 4C) as with E2 low: All 11 animals had around 10–15 cell layers with cornification. We found no clear differences between controls and GEN animals (Figure 4D). In GEN animals, epithelium thickness seemed slightly augmented in some areas, the number of cell layers did not differ from controls (two to three on average), and no cornification was found. An incipient cytoplasmatic vacuolization of epithelial cells was observed in 5 of 12 rats. The treatment with GEN high (Figure 4E) increased epithelial thickness and also the number of cell layers (5–10 layers). Cornification was found only in 1 of 12 animals, and cytoplasmatic vacuolization was clearly present in all samples.

Mammary gland. Figure 5 shows microscopic preparations of representative mammary glands from one animal per treatment group, and quantitative data obtained from glands of all animals are shown in Table 3. In controls (Figure 5A) all epithelial structures appeared atrophic. Deep in the fat pad, we observed scarce clusters of densely packed terminal structures, many of which did not show luminal formation. Treatment with E2 low (Figure 5B) induced formation of small lumina, but no secretory material was detected. Terminal epithelial structures were abundant after treatment with E2 high (Figure 5C).

Table 2. Summary of physiologic and pathologic findings in uteri of ovx rats after treatment with E2 or GEN.

| Treatment | Uterine weight (mg) | Spindle-shaped lamina propria cells | Endometrial epithelium with mitosis | Hyperplastic/hypertrophic glands | Squamous metaplasia | Cystic glands | Pyometra |
|-----------|-------------------|----------------------------------|-----------------------------------|-------------------------------|-------------------|--------------|---------|
| Control   | 91.6              | 0/12                             | 0/12                              | 0/12                          | 0/12              | 0/12         | 0/12    |
| E2 low    | 478.4*            | 11/11                            | 11/11                             | 0/12                          | 0/12              | 0/12         | 0/12    |
| E2 high   | 548.5*            | 10/10                            | 10/11                             | 10/10                         | 9/11              | 4/10         | 5/11    |
| GEN low   | 87.92             | 6/12                             | 0/12                              | 0/12                          | 0/12              | 0/12         | 0/12    |
| GEN high  | 196.8*            | 12/12                            | 12/12                             | 12/12                         | 12/12             | 12/12        | 12/12   |

*p < 0.05 vs. control.

Figure 4. Photomicrographs of representative vaginal sections from one animal per treatment group. (A) Control: atrophic vaginal epithelium is composed of a few layers of flattened cells. (B) E2 low: vaginal epithelium is hypertrophic and hyperplastic, with cornification in the upper layers. (C) E2 high: stronger hypertrophy and hyperplasia of epithelium are present; compared with E2 low, there are more cell layers and a higher degree of cornification. (D) GEN low: atrophic vaginal epithelium and the thickness and number of layers resemble that of control animals, but some areas have an incipient cytoplasmatic vacuolization (arrowheads). (E) GEN high: vaginal epithelium is increased (5–10 cells per layer on average), and large cytoplasmatic vacuoles are present in the upper layers. Bar = 80 µm.
Luminal formation was present in all animals, and secretion was seen in 9 of 11 animals. Both GEN treatments (Figure 5D, E) were only slightly different from controls: No secretion was induced, and incipient luminal formation was present in one-half of the animals of both groups. An overview of all collected data, including their significance, is presented in Table 4.

**PCNA and PR immunostaining.** Figure 6A shows representative PCNA-immunostained section of mammary gland from a control animal, and Figure 6B shows a tissue section from an E2 high animal. In mammary gland samples immunostained for PCNA (Figure 6C) or PR (Figure 6D), the number of PCNA- or PR-expressing cells was counted and expressed as a percentage of the total number of cells. E2 stimulated expression of PCNA dose dependently, and GEN also stimulated expression of this proliferation marker (Figure 6C). PR-expressing nuclei were found only in mammary gland cells of animals treated with E2 and GEN (both doses of each; Figure 6D). E2 low was more effective in increasing the number of PR-expressing cells than was E2 high.

**Discussion**

In the present study we compared the effects of two doses of genistein with those of two doses of E2 in ovx rats. We chose these doses on the basis of published data (Diel et al. 2004; Tinwell and Ashby 2004) and compared them with those used in recently published studies (Hertrampf et al. 2006; Michael McClain et al. 2006). The route and the duration of application were chosen because little information is available about the effects of subchronically and orally administered GEN in the uterus and mammary gland tissue of ovx rats (Hertrampf et al. 2006; Michael McClain et al. 2006). Michael McClain et al. (2006) reported that GEN at 500 mg/kg/day (higher than doses used in the present study) had no estrogenic effect in the uterus or the mammary gland; the animals in those experiments however were not ovx. Because we sought an animal model that might be comparable to postmenopausal women, we used ovx rats, which are in line with the Organisation for Economic Co-operation and Development recommendation to test for estrogenicity either in immature or ovx rats (Kanno et al. 2001). Hertrampf et al. (2006) used ovx rats and GEN doses similar to those used in the present study, but studied the effects after only 3 days of oral application. In agreement with our data, they observed estrogenic effects of GEN in the mammary gland (PR expression was increased) and a mild uterotrophic effect of GEN.

GEN is known to bind to both estrogen receptors, with a slight preference for ER-β. Data from ER-α–knock-out mice suggest that the ER-α is the receptor that mediates the typical effects of E2 in the uterus, vagina, and mammary gland.
mammary gland (Couse and Korach 1999; Hewitt and Korach 2003). Most studies have addressed effects of E2 and GEN in these organs after a relatively short duration of application, and reports about longer-lasting effects particularly in the mammary gland are scarce.

In the present study, administration of the two doses of E2 added to the daily food for 3 months resulted in high physiologic serum concentrations of E2 low and in supraphysiologic concentrations of E2 high. Serum LH concentrations were suppressed under both doses, which is typical for E2, whereas neither GEN dose affected LH serum values. Using LH serum levels as a marker of estrogenic effect in the hypothalamus—where the gonadotropin-releasing hormone pulse generator resides—GEN seems to be ineffective in reducing the activity of this brain structure. This may also explain the clinical finding that GEN did not substantially alleviate hot flashes (Geller and Studee 2005). Indeed, in women suffering from climacteric complaints, few placebo-controlled trials yielded significant effects of isoflavones. The placebo educed climacteric complaints by 30–50%, which was similar to the effectiveness of isoflavones (Geller and Studee 2005; Krebs et al. 2004; Phipps et al. 2002). Upon gross morphologic analyses, ovx animals had very low uterine weights; in animals treated with either E2 dose, uterine weight was increased to the range of that of intact animals. GEN high animals had increased uterine weight, whereas GEN low animals did not.

Table 4. Effects of treatment with E2 or GEN on morphologic features of mammary glands in ovx rats.

| Group            | Luminal formation |
|------------------|-------------------|
|                  | Absent | Incipient | Clear | Secretion |
| Control          | 6/12   | 6/12      | 0/12  | 0/12      |
| E2 low           | 0/12   | 0/12      | 11/11 | 0/11      |
| E2 high          | 0/12   | 0/12      | 9/11  | 0/11      |
| GEN low          | 6/12   | 6/12      | 0/12  | 0/12      |
| GEN high         | 6/12   | 6/12      | 0/12  | 0/12      |

*p < 0.05 compared with control.

Endometrial epithelial cells were also affected by E2 and GEN. During the course of an estrous cycle, E2 induces mitotic replication of cells and induces epithelial cells to adopt a tall cylindrical shape. Hence, in E2-treated animals, the epithelial cells depict such cylindrical shape with a high cytoplasmic:nuclear ratio. We observed changes like these mainly in the E2 low and GEN high animals. Under supraphysiologic doses of E2, we noted some pathologic features. Cells from epithelial glands were hypertrophic and hyperplastic, and the endometrial epithelium was multilayered and often developed squamous metaplasia. We did not observe such pathologic features in the GEN-treated animals. E2 also widens the lumen of the uterine cervix, which under chronic conditions allows bacterial ascension that may result in development of pyometra. Consequently, we found pyometras in some animals treated with E2 high. The morphologic changes induced by E2 or GEN in uterus are similar, differing only in degree. These results suggest that GEN has similar although weaker effects than E2 in the rat uterus.

Vaginal epithelium also subject to estrogenic action in that E2 causes proliferation and cornification. In the present study, we observed increased vaginal epithelial height, number of layers, and cornification in all samples from both E2 groups, with a stronger effect in the E2 high group. GEN low increased epithelial height only slightly; GEN high dose, however, increased numbers of layers comparable to E2 high. A different feature, namely, cytoplasmatic vacuolization, was detected in the GEN animals at incipient degrees in the low-dose animals and clearly in the high-dose animals. Cytoplasmatic vacuolization was not observed in any of the E2-treated animals. Hence, the morphologic changes induced by GEN in vaginal epithelium are not identical with those exerted by E2, suggesting that the mechanisms by which this phytosterogen and its gut metabolites (e.g., equol) affect vaginal epithelium are different from those mediating the effects of E2.

Mammary gland tissue was scarce in the ovx control rats, and the few detectable terminal structures were collapsed. E2 low induced luminal formation but no secretion, whereas in the mammary gland of E2 high animals, ample tissue and signs of secretion were observed. GEN did not have marked effects on mammary gland morphology; half of the animals in each GEN dose group had an incipient luminal formation. PCNA is a nuclear protein that is expressed in proliferating cells during the S phase of the cell cycle and is a useful tool in mammary cancer prognosis research (Kurki et al. 1986; Schimmelpenning et al. 1993). It can be visualized by immunohistochemistry; this allows the estimation of the percentage of
proliferating cells in relation to the total number of cells. In deep terminal mammary structure, epithelial proliferation was increased by both E2 doses; PCNA analysis also indicates that GEN has a stimulatory effect on mammary gland epithelial cells. Stimulation of mammary cell proliferation is an increased risk factor for the development of cancer (Gadducci et al. 2005).

Stimulation of PR in mammary epithelial cells is another typical effect of E2, which we observed in the animals treated with both E2 doses and GEN high; however, we counted significantly fewer PR-expressing cells in the E2 high tissues compared with the E2 low tissues. The reason the E2 high did not stimulate PR receptor as profoundly as did E2 low remains obscure, particularly in view of the profound stimulation of mammary duct tissue with luminal formation and secreting activity by the E2 high.

In summary, we found that GEN had similar but milder effects in the uterus than E2, without showing any selective ER modulator properties. Also, mammary gland proliferation was increased under both E2 and GEN. In the hypothalamic and vagina GEN exerted different effects compared with E2. LH release was unaltered, suggesting that GEN did not exert the estrogenic negative feedback on GnRH in the rat hypothalamus. The clearest finding that GEN does not act exactly the same as E2 was found in the vaginal epithelium, where GEN high induced a different feature: cyttoplasmic vacuolization.

**References**

Albertazzi P, Sharma S. 2005. Urogenital effects of selective estrogen receptor modulators: a systematic review. Climacteric 8(3):214–220.

Alfred CD, Alfred KA, Ju YH, Clausen LM, Doerge DR, Schantz SL, et al. 2004. Dietary genistein results in larger MRN-induced, estrogen-dependent mammary tumors following ovariec-
tomy of Sprague-Dawley rats. Cancer Genet Cytogenet 152(2):211–218.

Aslan Y, Geary N. 2006. Modulation of appetite by gonadal steroid: a comparison of different compounds. Micron 31(6):639–649.

Boue SM, Zieske TG, Kohler S, Burrow ME, Elliott S, Carter-Beral V. 2003. Breast cancer and hormone-replacement therapy during the Women's Health Initiative era. Report a a meeting held in Funchal, Madeira, February 24–25, 2003. Climacteric 8(3):214–220.

Dave B, Eason RR, Titi SR, Gong Y, Velarde MG, Badger TM, et al. 2005. The soy isoflavone genistein promotes apoptosis in mammary epithelial cells by inducing the tumor suppressor PTEN. Cancer Genet Cytogenet 165(2):359–370.

DeClerck F, Gadducci A, Biglia N, Sismondi P, Genazzani AR. 2005. Breast cancer, apoptosis and metalloproteinases: a review of experimental and clinical evidence on epigenotheme,

Dutta M, Bostwick DJ, Brown J. 2005. Anticancer activities of pomegranate extracts and genistein in human breast cancer cells. J Med Food 8(4):469–475.

Ezaki T. 2000. Antigen retrieval on formaldehyde-fixed paraffin sections: its potential drawbacks and optimization for double immunostaining. Micron 31(6):639–648.

Fadlalla AS, Strakova Z. 2002. Endometrial function: cell-spe-
cific changes in the uterine environment. Mol Cell Endocrinol 166(2):143–147.

Gadducci A, Biglia N, Sismondi P, Genazzani AR. 2005. Breast cancer, and reproductive tract. Steroids 63(10):498–504.

Heinemann C, Reif G. 2005. Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. Can J Microbiol 51(9):777–781.

Hertogens F, Schimmelpennin H, Eriksson ET, Franzen B, Zetterberg A, Auer G. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93(12):5925–5930.

Kanno J, Onyon L, Haseman J, Fenner-Crisp P, Ashby J, Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. 1998. Interaction of estrogenic and nuclear receptors: a comparison of different compounds and estrogen receptor beta (ER- 

Kijkuokool P, Parhar IS, Malaivijitnond S. 2005. Genistein enhances N-nitrosomethylurea-induced rat mammary tumorigenesis. Cancer Lett 242(1):53–59.

Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. 1998. Interaction of estrogenic and nuclear receptors: a comparison of different compounds and estrogen receptor beta (ER-

Lamartiniere CA. 2002. Timing of exposure and mammary cancer epidemiology. Cancer Lett 191(3):209–219.

Leiden B, Edgren S, Yang X, Kim A, Ordóñez-Ercan D, Mason T, et al. 2005. Low-dose phytoestrogens abrogate tamoxifen-associated mammary tumor promotion. Cancer Res 65(3):879–886.

McClain R, Volz E, Davidovich A, Planknisch F, Edwards JA, Bausch J. 2006. Acute, subchronic and chronic safety studies with genistein in rats. Food Chem Toxicol 44(1):56–80.

Mosselman S, Poilan J, Dijkema R. 1996. ER beta: identifica-
tion and characterization of a novel human estrogen receptor. FEBS Lett 392(1):49–53.

Mueller SI, Simon S, Chae K, Metzler M, Korach KS. 2004. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor 

Nelson RH, Moore DO, Banu WJ, Mezli O, Shay NF. 2005. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. Review. J Nutr Biochem 16(6):321–330.

Oxomol and reproductive tract. Steroids 63(10):498–504.

Phipps WR, Dunagan AK, Kurzer MS. 2002. Isoflavones and post-

Pittelkow M, Labbie C, Labrie F. 2000. Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. J Endocrinol 165(2):299–310.

Pickett ML, Moore DD, Banu WJ, Mezli O, Shay NF. 2005. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. Review. J Nutr Biochem 16(6):321–330.

Potter JL, Maguire SM, Quaglione J, Miller RR. 1997. Expression of oestrogen receptor alpha (ER alpha) in multiple rat tissues visualised by immunohistochem-

Ricketts ML, Moore DD, Banu WJ, Mezli O, Shay NF. 2005. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. Review. J Nutr Biochem 16(6):321–330.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: princi-

Schimmelpennin H, Eriksson ET, Franzen B, Zetterberg A, Auer G. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93(12):5925–5930.

Shughrue PJ, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.

Sirton DR, Arnoldo A, Johnson SK. 2005. Phytoestrogens: end of a tale? Ann Med 37(6):423–438.

Takwia KM, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.

Takwia KM, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.

Takwia KM, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.

Takwia KM, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.

Takwia KM, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.

Takwia KM, Lane MV, Scrimo PJ, Merchanthaler I. 1998. Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids 63(10):498–504.