*Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.*

# Estetrol

**Evidence Summary**

Estetrol is effective as an oral contraceptive and appears promising for menopausal hormone therapy and certain types of breast or prostate cancer. Neuroprotective benefit is less validated.

**Neuroprotective Benefit:** Neuroprotective potential of estetrol is currently unknown as the only evidence so far is in a model of neonatal hypoxic-ischemic encephalopathy.

**Aging and related health concerns:** Small studies suggest potential benefits for treating certain types of breast cancer or prostate cancer, but larger, longer-term studies are needed.

**Safety:** While potentially safer than estradiol, estetrol increases endometrial thickness and can cause abnormal uterine bleeding. It also rarely causes venous thromboembolism. In men, estetrol increases nipple tenderness and decreases libido.

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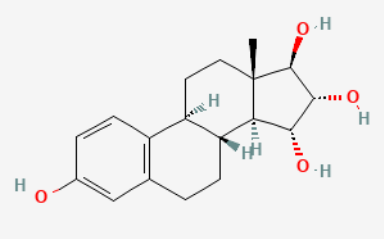
|---|---|---|
| **Availability**: Rx as oral contraceptive, in combination with drospirenone; not available by itself | **Dose**: The oral contraceptive Nextstellis contains 14.2 mg of estetrol and 3 mg of drospirenone, taken once daily for 24 days followed by 4 days of inert tablets. | **Chemical formula:** $C_{18}H_{24}O_4$<br>**MW**: 304.4<br>  <br>Source: PubChem |
| **Half-life**: 24-32 hours | **BBB**: penetrance not documented | |
| **Clinical trials**: Two phase III studies have tested a combined oral contraceptive containing estetrol and drospirenone, enrolling 1,553 and 1,864 women. | **Observational studies**: none to date | |

**What is it?** Estetrol is a type of estrogens produced exclusively by the fetal liver and it is detected from the 9th week of gestation in maternal urine and from the 20th week in the maternal plasma (Gallez et al., 2021). Estrogens impact many organs in the body, including the brain, breast, ovary, endometrium, prostate, colon, liver, bone, skin, and others. Estrogens are protective against many age-related diseases, such as osteoporosis, cardiovascular diseases, obesity, insulin resistance, and neurodegenerative diseases (Gerard et al., 2022).

Each estrogen has different pharmacologic and physiologic effects. Like other estrogens, estetrol binds to estrogen receptors ERα and ERβ. There are two distinct pathways associated with estrogen receptor activation: the nuclear/genomic pathway and the non-genomic/extra-nuclear/membrane-initiated steroid signaling (MISS) pathway. Estetrol's effects on genomic and non-genomic estrogen receptor pathways vary depending on the tissue and dose and are different from estradiol, the predominant estrogen in premenopausal women. Estetrol is an agonist of the genomic ERα pathway, but activation of the MISS pathway is tissue-dependent. Estradiol, on the other hand, activates both genomic and MISS pathways. Estetrol also can antagonize the effects induced by estradiol (Gerard et al., 2022). For example, estetrol can prevent the activation of the MISS pathway induced by estradiol in the endothelium and in human endocrine-sensitive ER-positive breast cancer cells (Gallez et al., 2021).

Estetrol is approved as the estrogen component in a combined oral contraceptive with drospirenone, a progestogen, in the US, Europe, Canada, and Australia. Estetrol is also under clinical development for the treatment of menopausal symptoms and certain types of breast and prostate cancer.

**Neuroprotective Benefit:** Neuroprotective potential of estetrol is currently unclear as the evidence is limited to in vitro and neonatal rat model studies, the evidence of which is mixed.

*Types of evidence:*

- A few laboratory studies
- 2 reviews

***Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function***: None available.

***Human research to suggest benefits to patients with dementia***: None available.

***Mechanisms of action for neuroprotection identified from laboratory and clinical research***:
In a rat model of neonatal hypoxic-ischemic encephalopathy, estetrol pretreatment (5 or 10 mg/kg/day, i.p., alone or in combination with progesterone and/or estradiol of different doses, for 3 days) or post-insult treatment (at 7 days post-insult) failed to consistently improve outcomes (Tskitishvili et al., 2016). For pretreatment, sham was compared with 12 different treatment groups of different hormone combinations/doses (estetrol alone, estetrol + estradiol, estetrol + progesterone, or estetrol + estradiol + progesterone), and a significant improvement in body weight was found in 1 out of 12 treatment/dosage groups. For the post-insult treatment study, significant data were found in 1 out of 12 treatment groups for brain weight. In primary hippocampal neuronal cell culture, high doses of estetrol with progesterone and/or estradiol upregulated cell survival. In a similar study of neonatal rats that underwent hypoxia-ischemia, estetrol pretreatment increased myelin basic protein immunostaining (Tskitishvili et al., 2017).

Based on this limited data, it is not possible to extrapolate these findings into potential neuroprotective/cognitive benefits in humans.

***APOE4 interactions***: Unknown.

**Aging and related health concerns:** Small studies suggest potential benefits for treating certain types of breast cancer or prostate cancer, but larger, longer-term studies are needed.

*Types of evidence:*

- 3 randomized controlled trials, 1 in advanced prostate cancer, 1 in early-stage breast cancer, and 1 dose-finding study
- 2 open-label dose-finding clinical studies
- A few laboratory studies
- 2 reviews

***Breast cancer***: MIXED FINDINGS BUT POTENTIAL BENEFIT IN CANCER PATIENTS BASED ON BIOMARKERS In a prospective randomized controlled trial of 15 postmenopausal and 15 premenopausal women with ER-positive early-stage breast cancer, estetrol treatment (20 mg once daily) for 14 ± 2 days significantly increased the number of apoptotic cells in the tumor tissue compared to placebo, whereas Ki67 expression (indicating cell proliferation) remained unchanged (Singer et al., 2014). IGF-1 is a mitogenic and anti-apoptotic molecule that stimulates the growth of normal and malignant breast epithelium (Hamelers and Steenbergh, 2003). With estetrol treatment, serum IGF-1 levels decreased significantly in both pre- and post-menopausal women (Singer et al., 2014). Estetrol also significantly increased levels of sex-hormone-binding globulin (SHBG), thereby reducing the concentrations of bioavailable estradiol. Follicle-stimulating hormone (FSH) levels decreased in postmenopausal women only and luteinizing hormone (LH) levels remained unchanged. Bioavailable testosterone significantly decreased with estetrol treatment in both pre- and post-menopausal women, though no significant changes were seen in the levels of total testosterone and androstenedione. Estetrol treatment led to a significant decrease in intratumoral epithelial ERα expression and a trend was found towards an increased expression of ERβ. Progesterone receptor expression was not affected by estetrol treatment.

In an open-label phase 1b/2a dose-escalation study of 12 postmenopausal women with locally advanced and/or metastatic ER+/HER2- breast cancer resistant to anti-estrogens, treatment with estetrol (20, 40, or 60 mg per day, orally) for 12 weeks resulted in 5 out of 9 patients showing objective anti-tumor effects and 6 out of 9 patients reported improvement in wellbeing (Schmidt et al., 2021). Of the remaining 3 patients, 2 of them withdrew in phase 1b due to disease progression and non-compliance, and 1 patient discontinued during phase 2a due to disease progression after 9.5 weeks of estetrol treatment; she passed away 2 weeks later. Also, the patient who achieved complete response and the patient who had the highest increase in tumor diameter were both taking the 20 mg estetrol dose. All 3 patients on the highest estetrol dose (60 mg) showed progressive tumor growth. The 5 patients showing

an anti-tumor effect continued with estetrol treatment beyond the 12-week study and tumor assessment after 24 weeks of treatment showed stable disease in 4 out of 4 patients. As the study was small with a diverse set of outcomes across subjects, further research is needed to delineate which patient populations could benefit from estetrol treatment.

In cell culture models of breast cancer (MCF-7 cells), estetrol induced apoptosis in long-term estrogen-deprived breast cancer cells, but stimulated growth of non-estrogen-deprived breast cancer cells at concentrations from $10^{-11}$ to $10^{-8}$ M (Yue et al., 2019). However at a lower dose ($10^{-12}$ M), estetrol induced apoptosis in non-estrogen-deprived breast cancer cells. Thus, effects of estetrol on breast cancer may depend on the dose, what other estrogens are present in the environment, and whether the cancer has been estrogen-deprived. Also in breast cancer cells (MCF-7 cells), estetrol stimulated the growth of hormone-dependent breast cancer only at doses exceeding the therapeutic dose for menopausal therapy (Gerard et al., 2015). Estetrol also exerts anti-tumor activity by antagonizing the effects of estradiol.

As described above, in cell culture and animal models, estetrol has shown both pro-apoptotic as well as pro-tumoral effects on breast cancer and estetrol's action on breast cancer is highly complex and not fully understood. Estetrol acts through ERα to promote proliferation and growth of human ER-positive breast cancer cells, patient-derived xenografts from ER-positive breast tumors, and endometrial cancer cells (Gallez et al., 2021). In breast cancer cells, estetrol activates both the genomic ERα and the membrane-initiated steroid signaling pathway. In endocrine-resistant ER-positive breast cancer cells, estetrol induces a distinct ERα-mediated signaling pathway resulting in the unfolded protein response and apoptosis, associated with the pro-apoptotic profile. In endocrine-sensitive breast cancer, estetrol appears to have a neutral effect.

For hormone replacement therapy in postmenopausal women or contraceptive use in younger women, assessing the risk of breast cancer is a long-term effort requiring decades of patient follow-up. However, the effect of estetrol on breast cancer appears to be significantly less than estradiol, the predominant estrogen in premenopausal women.

***Prostate cancer***: POTENTIAL BENEFIT BASED ON BIOMARKERS
In a phase 2 double-blind randomized controlled trial of 62 men with advanced prostate cancer (infiltrating or metastatic castration-sensitive prostate cancer) receiving androgen deprivation therapy, addition of high-dose estetrol (40 mg) for 24 weeks significantly decreased total and free testosterone earlier, significantly suppressed prostate-specific antigen (PSA) at a larger magnitude and earlier

timepoint, and suppressed follicle-stimulating hormone (FSH) levels by 98% compared to 57% in placebo suggesting improved disease control with high-dose estetrol (Bennink et al., 2021). After 24 weeks, the median PSA with high-dose estetrol was 0.05 ng/ml, compared with 0.56 ng/ml with placebo. Estetrol treatment also reduced the incidence of hot flushes (13.5% of patients experienced hot flushes, compared to 60.0% of patients in placebo) and bone turnover parameters. Osteocalcin (bone formation and turnover marker) decreased by 22% and CTX1 (bone resorption marker) decreased by 25% after 24 weeks of estetrol treatment (while osteocalcin increased by 48% and CTX1 increased by 151% in the placebo group). Larger and longer-lasting clinical studies are needed to extend these findings.

***Lipid profile***: MIXED
In a double-blind randomized controlled dose-escalating study of 43 healthy men, estetrol treatment (20, 40, and 60 mg/day, orally) for 4 weeks significantly decreased total cholesterol (by 5-11%) and LDL-cholesterol (by 11-18%), though these changes were not significantly different from the placebo group (Bennink et al., 2018). The placebo group also experienced decreased total cholesterol (by 7%) and LDL-cholesterol (by 8%). With all estetrol doses, there were small increases in HDL-cholesterol ($p<0.05$ at the 20 mg dose). Triglyceride levels were not significantly altered with estetrol.

In an open-label, dose-finding study of 109 healthy women (aged 18-35 years), effects of different oral contraceptive formulations were compared: estetrol (5 or 10 mg) with drospirenone (3 mg), estetrol (5, 10, or 20 mg) with 150 µg levonorgestrel; or 20 µg ethinyl estradiol with 3 mg drospirenone (Mawet et al., 2015). HDL-cholesterol was increased with estetrol + drospirenone (by up to 8.1%) and LDL-cholesterol was increased by up to 6.7% and 8.9% with estetrol + drospirenone and 20 mg estetrol + levonorgestrel treatment. Total cholesterol increased with estetrol + drospirenone (by 4-5%) but decreased in the estetrol + levonorgestrel group (by up to 15.5%). Triglyceride levels decreased by up to −29.7% in the estetrol + levonorgestrel treatment groups, but increased in the estetrol + drospirenone and ethinyl estradiol + drospirenone (by up to 10.0% and 61.2%, respectively).

***Bone health***: DECREASED BONE RESORPTION AND BONE FORMATION BIOMARKERS
In a double-blind randomized controlled dose-escalating study of 43 healthy men, estetrol treatment (20, 40, and 60 mg/day, orally) for 4 weeks significantly altered bone turnover markers (Bennink et al., 2018). CTX-1 levels decreased by 20% in the 20 mg-dose group and by 27% in the 40 mg-dose group ($p<0.01$ for both). However, no decrease was observed at the highest dose of estetrol (60 mg). In contrast, in the placebo group, CTX-1 increased by 16%. Osteocalcin levels were not significantly changed or different across groups.

In an open-label, dose-finding study of 109 healthy women (aged 18-35 years) comparing different oral contraceptive formulations, a dose-related decrease was seen for biomarkers of bone resorption (C-telopeptide) and bone formation (osteocalcin) by 22.4% and 16.3%, respectively in estetrol combination groups (Mawet et al., 2015). A numerically greater suppression of bone turnover (-34.9% and -22.3%, respectively) was seen with the ethinyl estradiol/drospirenone groups.

***Metabolic health***: UNKNOWN
In a double-blind randomized controlled dose-escalating study of 43 healthy men, estetrol treatment (20, 40, and 60 mg/day, orally) for 4 weeks did not significantly alter fasting glucose levels (Bennink et al., 2018).

In mice fed a Western diet, resulting in obesity and atheroma, estetrol treatment (1 mg, s.c. pellet every 3 weeks) from 6 to 15 weeks of age reduced weight gain and improved glucose tolerance while preventing steatosis and atherosclerosis (Buscato et al., 2021).

***Liver health***: LIMITED EFFECT ON LIVER FUNCTION
In an open-label, dose-finding study of 109 healthy women (aged 18-35 years) comparing different oral contraceptive formulations, small decreases in liver enzymes (AST and alkaline phosphatase) were observed with estetrol + levonoregestrel and estetrol (5 mg) + drospirenone (by 4.0% and 11.3%, respectively)(Mawet et al., 2015). However, a small increase in AST was observed in the estetrol (10 mg) + drospirenone group (by 2.0%) and a decrease in alkaline phosphatase by 17.6%. Because of the large interindividual variability, no statistically significant differences were found between the pooled estetrol/drospirenone and estetrol/levonorgestrel groups or between the estetrol groups and the ethinyl estradiol/drospirenone comparator in liver enzymes, except for alkaline phosphatase levels, which were significantly lower in the ethinyl estradiol/drospirenone group compared with the estetrol/levonorgestrel group.

**Safety:** While potentially safer than estradiol, estetrol increases endometrial thickness and can cause abnormal uterine bleeding. It also rarely causes venous thromboembolism. In men, estetrol increases nipple tenderness and decreases libido.

*Types of evidence:*

- 4 randomized controlled trials, 1 in postmenopausal women as hormone replacement therapy, 1 in breast cancer, 1 in prostate cancer, and 1 dose-escalation study in healthy men

- 2 open-label phase 3 trials testing a combined oral contraceptives containing estetrol
- 4 open-label studies testing combined oral contraceptives containing estetrol
- 1 open-label phase 1b/2a study in breast cancer patients
- Several laboratory studies
- 2 reviews

Estetrol does not have any active metabolic end products and has minimal first-pass metabolism. Estetrol has a high oral bioavailability and an elimination half-life of 24-32 hours (Gerard et al., 2022).

***Healthy men***:
In a double-blind randomized controlled dose-escalating phase I study of 43 healthy men, estetrol treatment (20, 40, and 60 mg/day, orally) for 4 weeks resulted in significantly decreased total and free testosterone levels in a dose-dependent manner, nipple tenderness occurring in 40% of subjects (12 out of 30), and decreased libido occurring in 30% of subjects (9 out of 30)(Bennink et al., 2018). Total testosterone levels decreased by 28% in the 20 mg group, by 60% in the 40 mg group, and by 76% in the 60 mg group. Free testosterone levels decreased by 43% in the 20 mg group, by 83% in the 40 mg group, and by 84% in the 60 mg group. Mean angiotensinogen levels increased with estetrol treatment by 21%, 75%, and 86% in the 20, 40, and 60 mg groups, respectively. By day 56, mean total testosterone and free testosterone had returned to baseline levels in all dose groups. FSH and estradiol levels significantly decreased with estetrol treatment and were significantly different from placebo. No clinically relevant changes in body weight, vital signs, electrocardiogram results, physical examinations, and safety laboratory parameters were observed. No subject discontinued the study due to an adverse event. More than 90% of the side effects were classified as mild, and there were no serious side effects.

***Oral contraceptive use in healthy women***:
For oral contraceptive use, estetrol has been combined with drospirenone, a progestogen.

In a phase 3 open-label trial of 1,553 sexually active women (aged 18-50), a combined oral contraceptive containing estetrol (15 mg) and drospirenone (3 mg) were tested for up to 13 cycles (Gemzell-Danielsson et al., 2022). The most common treatment-related adverse events were abnormal bleeding from the uterus (5.0%), vaginal hemorrhage (4.3%), acne (3.8%), and headache (2.8%). Serious adverse events were reported in 13 women (0.8%), of which 1 was considered treatment-related: a lower extremity venous thromboembolism (VTE). The estetrol/drospirenone was discontinued, the VTE resolved after antithrombotic treatment without sequelae. Overall, 141 women (9.1%) discontinued study participation because of treatment-related adverse events, the most common of which were

abnormal bleeding from the uterus (1.5%), acne (1.3%) and vaginal hemorrhage (1.0%). The frequency of adverse events and of treatment-related adverse events was 50.5 and 28.5%, respectively, of which the majority (63%) were of mild intensity. The mean change in body weight compared with baseline was +0.68 (±3.58) kg and the mean change in body mass index (BMI) was +0.25 (±1.29) kg/m$^2$.

In the other phase 3 open-label trial of 1,864 women (aged 16-50), the same combined oral contraceptive (15 mg estetrol + 3 mg drospirenone) were tested for up to 13 cycles (Creinin et al., 2021). The most frequently reported adverse events were headache (5.0%) and abnormal bleeding from the uterus (4.6%). Overall, 132 women (7.1%) discontinued the study early for an adverse event, most commonly for abnormal bleeding from the uterus (0.9%) and menorrhagia (heavy/prolonged menstrual bleeding; 0.8%). No thromboembolic events occurred in this phase 3 study. The mean change in BMI from baseline was 0.4 ± 1.7 kg/m$^2$. There were 2 (0.1%) participants who had clinically significant elevated potassium levels (normal 3.5–5.0 nmol/L), 1 at Cycle 10 (6.0 nmol/L) and 1 post-Cycle 13 (6.3 nmol/L), though neither participant experienced any clinical sequelae. There were 25 participants (1.3%) who experienced 30 serious adverse events, of which investigators considered 2 as treatment-related: 1 hospitalization for depression not leading to discontinuation and 1 ectopic pregnancy. One death unlikely related to the study drug occurred, involving a prescription drug overdose.

In a randomized open-label study of 82 women, estetrol (15 mg) + drospirenone (3 mg) treatment was compared with ethinyl estradiol (20 µg) + drospirenone (3 mg)(Duijkers et al., 2021). Most frequently reported treatment-related adverse events were breast pain, breast enlargement, headache, emotional liability, nausea, lower abdominal pain, and acne. Frequencies of these adverse events were similar between treatment groups except for breast pain, which was reported by more participants in the estetrol + drospirenone group (11 out of 41) compared to the ethinyl estradiol + drospirenone group (4 out of 41). Most treatment-related adverse events were of mild or moderate intensity, but there were 3 adverse events of severe intensity (gastroenteritis, emotional liability, and stress), all with estetrol + drospirenone treatment.

In a randomized open-label controlled study of 98 women, several different oral contraceptive formulations were compared (Klipping et al., 2021). Estetrol (15 mg) + drospirenone (3 mg) had limited effects on liver proteins, lipid profile, carbohydrate metabolism, cortisol, and gonadotropins. While this combination increased total cortisol levels by 26%, the increase was significantly less with ethinyl estradiol + levonorgestrel (increased by 109%). Liver proteins increased but the effect was less pronounced with estetrol + drospirenone compared with ethinyl estradiol + levonorgestrel or ethinyl estradiol + drospirenone. Estetrol + drospirenone treatment resulted in an increase in triglycerides (by

24.0%), but this was less compared to ethinyl estradiol + levonorgestrel (by 28.0%) and ethinyl estradiol + drospirenone (by 65.5%). Estetrol + drospirenone treatment did not significantly change LDL-C, total cholesterol, HDL-c:LDL-c ratio, and lipoprotein A. Estetrol + drospirenone treatment had no effect on carbohydrate metabolism (fasting insulin and glucose, C-peptide, and HbA1c).

In an open-label, dose-finding study of 109 healthy women (aged 18-35 years), effects of different oral contraceptive formulations were compared: estetrol (5 or 10 mg) + drospirenone (3 mg), estetrol (5, 10, or 20 mg) + 150 µg levonorgestrel; or 20 µg ethinyl estradiol + 3 mg drospirenone (Mawet et al., 2015). Fifteen subjects discontinued the study during the treatment phase and 9 subjects did not complete the post-treatment cycle. Reasons for discontinuation were adverse events (5 subjects: emotional lability and acute bronchitis, tiredness, increased frequency of headache, mood swings, and decreased libido and headache), intracyclic bleeding (1 subject), personal reasons (1 subject), use of prohibited concomitant medication to treat acute bronchitis (1 subject), incorrect study medication intake (1 subject), withdrawal of consent (1 subject), pregnancy during the post-treatment cycle (1 subject), and inability to adhere to the visit schedule (13 subjects).

In a randomized open-label exploratory study of 101 healthy women, estetrol + drospirenone treatment for 6 cycles showed changes in hemostasis parameters that were smaller than those seen with ethinyl estradiol + levonorgestrel or ethinyl estradiol + drospirenone (Douxfils et al., 2020). For example, median change of endogenous thrombin potential based activated protein C sensitivity resistance (APCr) was increased by 30% with estetrol + drospirenone, but increased by 165% and 219% with ethinyl estradiol + levonorgestrel or ethinyl estradiol + drospirenone groups, respectively. Changes to prothrombin fragment 1+2 increased by 23%, 71%, and 64% in estetrol + drospirenone, ethinyl estradiol + levonorgestrel, and ethinyl estradiol + drospirenone groups, respectively. Thus, the estetrol formulation appears to be a safer option with regards to thrombotic events.

***Menopausal women***:
In a double-blind randomized controlled trial of 257 postmenopausal women with moderate to severe hot flushes, estetrol treatment (2.5, 5, 10, or 15 mg, once daily) for 12 weeks significantly decreased the frequency of hot flushes, but in non-hysterectomized women, endometrial thickness increased during treatment (Gaspard et al., 2020). Endometrial biopsy was performed throughout the study for 34 women (mainly because of abnormal bleeding), but no endometrial hyperplasia was observed in any of these women, and endometrial thickness returned to baseline after progestin therapy. The frequency of women experiencing one or more treatment-emergent adverse events was comparable between the 2.5, 5, and 10 mg estetrol groups (53.2%-57.7%) and slightly higher than in the placebo group (47.3%).

Overall, 21 women (8.2%) experienced one or more severe treatment-emergent adverse events, with frequencies ranging from 6.1% in the 15 mg estetrol group to 10.6% in the 5 mg estetrol group; the frequency in the placebo group was 9.1%. Serious adverse events were reported for 2 women in the 15 mg estetrol group (abnormal uterine bleeding and intervertebral disc protrusion), and for 1 woman in the placebo group (intervertebral disc protrusion). In the woman with abnormal bleeding, transvaginal ultrasound showed an endometrial thickness of 14.3 mm, and study treatment was stopped. However, an endometrial biopsy did not demonstrate endometrial hyperplasia, and the participant had no sequelae and had a normal endometrial thickness (2.7 mm) after progestin treatment at follow-up. During the study, no major changes were observed in vital signs, electrocardiogram parameters, physical/gynecological examinations, and routine laboratory tests (hematology, biochemistry, and urinalysis parameters).

An editorial of this study noted that the minimum efficacy dose for vasomotor symptom relief also carried notable estrogenic effects on the endometrium, with endometrial biopsy required in 13% of participants due to abnormal bleeding (Reame 2020).

***Breast cancer patients***:
In an open-label phase 1b/2a dose-escalation study of 12 postmenopausal women with locally advanced and/or metastatic ER+/HER2- breast cancer resistant to anti-estrogens, treatment with estetrol (20, 40, or 60 mg per day, orally) for 12 weeks did not lead to dose-limiting toxicity and all 3 doses were well-tolerated (Schmidt et al., 2021). At the data cut-off date for this paper (November 2020), one patient was still continuing treatment with 20 mg estetrol (35 months of treatment), and this patient experienced a total of 3 episodes of vaginal bleeding, but no endometrial abnormalities were found. The other 5 patients discontinued after 13-48 weeks of treatment due to progression of the breast cancer disease. In total, 31 treatment-emergent adverse events were reported by 8 patients, mainly of mild or moderate intensity, of which 6 events were considered possibly related to estetrol treatment: dry skin, pruritis and endometrial hyperplasia in one patient given 20 mg estetrol, fatigue and 4 days of vaginal bleeding resolving spontaneously in a second patient on 20 mg estetrol, and regurgitation in a third patient on 40 mg estetrol. No drug-related serious adverse events were reported.

In a prospective randomized controlled trial of 15 postmenopausal and 15 premenopausal women with ER-positive early-stage breast cancer, estetrol treatment (20 mg once daily) for 14 ± 2 days resulted in 67 adverse events, but all were minor and short-lasting, and there were no serious side effects (Singer et al., 2014). Estetrol treatment in post-menopausal women resulted in a trend towards an increased

endometrial thickness (p=0.056) and proliferation. In premenopausal women, interpretation of endometrial thickness was confounded by menstrual cycle changes.

***Prostate cancer patients***:
In a phase 2 double-blind randomized controlled trial of 62 men with advanced prostate cancer (infiltrating or metastatic castration-sensitive prostate cancer) receiving androgen deprivation therapy, addition of high-dose estetrol (40 mg) for 24 weeks caused nipple sensitivity in 34% of patients and gynecomastia (enlarged breast tissue) in 17% of patients (Bennink et al., 2021). No deaths or estetrol-related serious cardiovascular adverse events occurred at 24 weeks. There were seven cardiovascular adverse events, all considered not related to study treatment by the investigators and the independent safety officer: 5 out of 41 (12.2%) patients on estetrol and 2 out of 21 (9.5%) patients on placebo. Four cardiovascular adverse events were reported as serious: 2 (5%) with estetrol (arrhythmia and atrioventricular block) and 2 (10%) with placebo (coronary artery disease and anemia with cardiac failure). There were 3 adverse events in the estetrol group that led to discontinuation of treatment (hypersensitivity reaction, depression, and peripheral edema), all considered not related to estetrol. There were no observations of general safety problems or clinically relevant changes of lipids and hemostasis parameters.

***Preclinical findings***:
In a study in mice, estetrol treatment (0.3 or 3 mg/kg/day) showed endometrial proliferation, suggesting that a progestogen should be combined to protect non-hysterectomized women from hyperplasia and cancer (Gallez et al., 2021).

***Drug interactions***: The oral contraceptive that contains estetrol is known to interact with many drugs, including aprepitant, bosentan, phenobarbital and other barbiturates, lamotrigine, St. John's wort, antibiotics (clarithromycin, rifabutin, rifampin, rifapentine, and telithromycin), antifungal medicine (fluconazole, friseofulvin, itraconazole, ketoconazole, and voriconazole), antiviral medicine (boceprevir, efavirenz, etravirine, indinavir, nelfinavir, nevirapine, ritonavir, and telaprevir), cancer medicine (apalutaminde, enzalutaminde, mitotane), diuretics (amiloride, eplerenone, and spironolactone), seizure medicine (carbamazepine, felbamate, oxcarbazepine, phenytoin, and topiramate), steroids (dexamethaxone and prednisone), and others (Drugs.com).

**Sources and dosing:** Estetrol is available as a prescription oral contraceptive, in combination with drospirenone. It is not sold or marketed by itself except in research grade material. The oral

contraceptive, Nextstellis, contains 14.2 mg of estetrol and 3 mg of drospirenone, taken once daily for 24 days followed by 4 days of inert tablets.

**Research underway:** Based on ClinicalTrials.gov, there are 6 ongoing clinical trials testing estetrol as of June 2022. Two are testing estetrol for menopausal symptoms, 1 is testing it for COVID-19, 1 is examining its effect on prolonged QTc interval, and 1 is a safety study.

**Search terms:**
Pubmed, Google: estetrol

Websites visited for estetrol:

- Clinicaltrials.gov
- NIH RePORTER
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com
- WebMD.com (0)
- PubChem
- DrugBank.ca
- Cafepharma (0)
- Pharmapro.com (0)