

Unconventional Estrogens: Estriol, Biest, and Triest

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In recent years, the seemingly rock-solid list of benefits of estrogen has been assailed over and over again. These kinds of challenges are not new in the history of hormones in the United States, but the rate at which information reaches consumers has accelerated tremendously. Patients get the bad news from medical journals as they read their morning newspapers, and physicians in their offices are often times unaware and unprepared for the onslaught of anxious calls that hit them with tsunami-like force. Even if the journal has arrived ahead of the wave, the issue is often unopened, still in its plastic mailer hidden beneath a pile of laboratory reports, insurance forms, and bills.

The presumed benefits of hormones derive from a body of literature that is fraught with bias. Concerns and challenges to the use of exogenous estrogens began to arise in the second half of this past century, when concern about the safety of oral contraceptives surfaced. After the Nelson Hearings in Congress in 1968 and 1969, which centered

on reports of increased rates of "vascular" disease in oral contraceptive users, estrogens were deemed dangerous for women at high risk. Texts and prescribing guidelines listed a number of high-risk conditions that contraindicated the use of sex steroids. The list included all women at risk for myocardial infarction, stroke, migraine, diabetes, obesity, hypertension, renal disease, metabolic disorders, and more. A family history of these disorders was also cited as a potential contraindication to the use of estrogen.¹ So, for almost all of the past half-century, only the healthiest women—white, upper middle class, educated, insured—have been allowed the luxury of using menopausal hormones, whereas women at risk were denied estrogen, directly by virtue of being truly at high risk and indirectly by being poor with limited access to health care. We selectively prescribed hormones to women who have also been determined by careful analysis to have better health indicators—lower cholesterol levels, higher levels of fitness, and lower blood pressure—than nonusers did before they start using hormones.¹

Most of the data supporting the claims regarding health benefits of estrogen reside in observational studies, not randomized con-

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trolled trials (Table 1). The presumed benefits may be weaker than we have been led to believe. The benefits of estrogen to bone have been challenged. One study suggests that if hormone replacement therapy (HRT) is used for less than 9 years, bone mass by age 80 was only 3% higher in HRT users than in nonusers.¹ Clearly, at this time, the evidence for estrogen as prevention for cardiovascular disease resides only in observation studies, highly contaminated with selection bias. Moreover, recent studies have unexpectedly found that women with coronary vascular disease, as seen in the Heart and Estrogen/Progestin Replacement Study² trial and in women with cerebrovascular disease, actually may exhibit worsened outcomes compared with non-users. Moreover, the American Health Association in July 2001 revised its recommendations regarding the use of estrogen, stating that estrogen should not be advocated for the treatment of preexistent cardiovascular disease and that it probably has little role in cardio prevention.³ Breast cancer also represents a challenging Gordian knot for biostatisticians, epidemiologists, and clinicians. Current consensus appears that the baseline risk

TABLE 2. Progestin Side Effects

- Bleeding—withdrawal, over-progesteronization
- Premenstrual syndrome—mood changes and other impairments
- Breast pain
- Headache
- Appetite change—anabolic effect
- Bloating—decreased gastrointestinal motility
- Attenuation of lipid improvements and vasomotion benefits of estrogen

of two cases per 100 women in 10 years has increased to three cases per 100 after 10 years of estrogen exposure, and lately, this fact has been hammered repeatedly with the statistical bludgeon recounting this risk as a 50% increase. This is a truth, but a truth phrased in the most provocative way possible. Fifty percent excites much greater anxiety and elicits much great media attention than one additional case per 1,000 women-years of exposure to HRT. As health professionals debate relative risks, absolute risks, and confidence intervals (CI), almost every women's health and breast cancer advocacy group, fueled by books like Barbara Seaman's *The Doctors' Case Against the Pill*⁴ and *The Controversy in Women's Hormones*⁵ and Dr. Susan Love's *Hormone Book*⁶, tells women that estrogen is a principle cause for the increased incidence and prevalence of breast cancer in the past 25 years.

Endometrial cancer risks imposed by unopposed estrogen are well known. Gynecologists and other health professionals have come to believe that the use of progestins provides almost absolute protection from this risk. But, large numbers of women are not using their progestins as directed, often skipping or lowering doses to try to lessen progestin-related side effects (Table 2). The protection doctors assume to be present oft times is severely compromised by poor adherence and compliance with the progestin arm of HRT. Moreover, whereas doctors tend to view the malignancies induced by estrogen therapy as "good cancers"—diagnosed at early stage, evidencing slow growth, and attaining high cure rates—

TABLE 1. Evidence-Based Benefits of Estrogen Replacement Therapy

Decreased RR	Level of Evidence	Strength of Recommendation
Vasomotor symptoms	I	A
Genitourinary symptoms	II-2	A
CV treatment/prevention	I/II-2	D/B
Hip and vertebral fracture	II-2	A
Alzheimer disease	II-2	B
Colorectal cancer	II-2	B
Tooth loss	II-2	B
Breast cancer mortality	II-2	B
Death at age younger than 80	II-2	B

* RR = relative risk.

women do not view any cancer as a "good" cancer.

Natural Estrogens

The discontent and discomfort provoked by the current HRT offerings have sent women searching for new options. Estimates are that only 10–25% of eligible menopausal women use conventional HRT, 30–50% of women given prescriptions for HRT never have them filled, and 40–60% of women who started using HRT have stopped.^{7,8} The unsettled and unsettling controversies surrounding HRT have not completely dissuaded women from using hormones. However, women are looking for alternatives to conventional estrogens that provide the benefits of pharmaceutical-grade hormones, but with fewer attendant side effects and risks, ie, a "kinder and gentler" estrogen. Advocates for the "forgotten estrogen," estriol, are willing to give them what they want, the myth of a risk-free estrogen.

Increasingly, women are asking for compounded estrogens, particularly estriol and so-called triest and biest preparations, which combine estriol with both estradiol and estrone or with just estradiol, respectively. The popularity of these products is fostered by a number of factors. Women are being given the naïve and simplistic impression that estriol can provide all the benefits of hormone replacement without the attendant risks of neoplasm and venous thrombosis. Proponents of estriol capitalize on the fears of women, promising not only a decreased risk compared with conventional estrogens, but protections against the effects of other estrogens. With great American entrepreneurial zeal, producers of estriol and the compounded, blended estrogens command high prices for this "wonderful" alternative. Many promoters of estriol are as naïve as many patients, and they do not understand that the data linking estriol to lowered risks of endometrial and breast cancer are an associative phenomenon, with estriol being a marker of, not necessarily a cause of,

lower rates of these diseases. Most of the remainder of this review centers on the uses and abuses of the data on estriol and its espoused role in hormone replacement.

Comparative Estrogenology

Estriol is the third major estrogen produced by human females. Estradiol, estrone, and estriol serve different functions in women and different functions at different times in women's reproductive life cycles. Like almost all estrogens used for HRT, except for conjugated equine estrogens (CEE), estriol is produced from plant sterol molecule. In fact, most steroids used in HRT, estrogens, and progestins are derived from diosgenin, a plant sterol. Through biotransformation in a pharmaceutical laboratory, diosgenin is first reconfigured into progesterone, then used as the source molecule for production of androgens, estrogens, and progestins like the C19 nortestosterone in oral contraceptives. The precursor molecule is extracted from high-yield yam or soy plants. The term "plant-derived" or "natural" in advertising parlance can be assigned to any steroid that started as a plant precursor. Therefore, compounded products, commercial progesterone, equine estrogens synthesized in the laboratory, such as those used in esterified estrogen products, and even norethindrone acetate and the other progestins in oral contraceptives could be termed "plant derived" or "natural." Manufacturers put pictures of yams and soy plants in their ads, implying that the estrogens are manufactured by the green plant, not the other kind of plant: a chemical plant! The maker of CEE, Premarin (Wyeth Ayerst, Philadelphia, PA), has even resorted to adding a tag line on the package that reads, "estrogens obtained exclusively from natural sources." Truly, pregnant mare's urine is a natural output.

The principal estrogen before menopause is 17 β estradiol and is produced by the pre-ovulatory follicle and, after ovulation, by the corpus luteum. After secretion by the ovary, some estradiol is bound by sex hormone-binding globulin, whereas unbound estro-

diol enters cells, binds estrogen receptors, and interacts in the nucleus to modulate transcription of estrogen-responsive genes. Estradiol is converted to estrone in the liver and other tissues by 17β estradiol dehydrogenase. Estrone is further metabolized in the liver to estrone sulfate, a water-soluble estrogen that constitutes the circulating estrogen in highest concentration. Estrone provides a reservoir for estradiol production. In postmenopausal women, peripheral conversion of androstenedione in adipose tissue yields mostly estrone. In women of reproductive age, estradiol levels vary throughout the monthly cycle. During the early follicular phase, estradiol levels are low, between 40 and 80 pg/ml. Later in the follicular phase, at midcycle around ovulation, estradiol levels are at their highest, at approximately 250 pg/ml, favoring the development of a rich endometrial lining suitable for implantation of a fertilized ovum. During the luteal phase, estradiol levels gradually decrease to approximately 100 pg/ml and further decrease to approximately 40 pg/ml by the beginning of menstruation. Mean daily circulating estradiol level is 100 pg/ml. Serum levels of estradiol for HRT arbitrarily match those seen in the early follicular phase.

The potency of different estrogen preparations for use in estrogen replacement can be evaluated in a number of ways. Clinically,

potency may be assessed by evaluation of maturation of vaginal epithelium. Gonadotropin suppression or induction of hepatic protein synthesis provide additional bioassays for assessing the relative potency of estrogen replacement preparations. Figure 1 compares the biologic potency of two oral estrogen preparations, CEE (0.625 mg) and micronized 17β estradiol (1 mg), across several parameters. These two doses are equivalent in their ability to suppress follicle-stimulating hormones, but different with regard to effects on corticotropin-binding globulin, sex hormone-binding globulin, and angiotensinogen levels (Fig. 1). These differences may define different tissue effects and differential clinical effects, but overall, in terms of the major indications for HRT, the outcomes are similar. Whereas almost all long-term studies of estrogen in the United States in the past 40 years have been performed with CEE, most reproductive endocrinologists will agree that other forms of estrogen, including estrone and estradiol, offer comparable treatment of vasomotor symptoms, retardation of bone loss, and reversal of urogenital atrophy. Also, different types and doses of oral estrogens yield remarkably similar levels of estradiol.⁹ This self-same logic, however, also suggests that the attendant risks of estrogen treatment, that is breast cancer risk

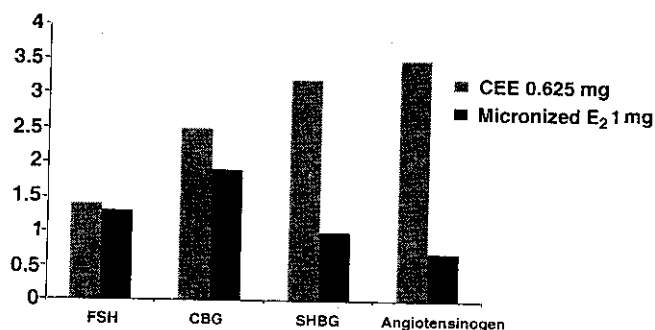


FIG. 1. Relative biological potency of oral estrogen preparations. To compare biologic potency of conjugated equine estrogens and micronized 17β estradiol, four specific parameters of estrogenicity were measured. These included changes in serum follicle-stimulating hormone, corticotrophin-binding globulin, sex hormone-binding globulin, and angiotensinogen. From Mashchak CA, Lobo RA, Dozono-Takano R, et al. Comparison of pharmacodynamic properties of various estrogen formulations. *Am J Obstet Gynecol.* 1982;144:511-518.

FIG. 2. Tissue/plasma gradients. From Thijssen JH, Wiegerninck MA, Donker GH, et al. Uptake and metabolism of oestradiol in human target tissues. *J Steroid Biochem.* 1984;20(4B):955-958.

TISSUE /PLASMA GRADIENTS			
	Endometrium	Myometrium	Vaginal
Estradiol	33 X	20 X	10 X
Estriol	37 X	19 X	11 X

and enhanced thrombotic risk, also are comparable with estrogens other than CEE.

Recent estimates of risk reduction¹¹ suggest current users of estrogen replacement therapy have an odds ratio of 0.35 (95% CI: 0.24-0.53) for hip fracture, and former users have an odds ratio of 0.76 (95% CI: 0.57-1.01). The protective effects of estrogen against osteoporotic fractures have been principally demonstrated in studies using CEE, with bone mineral loss being commonly used as a surrogate marker for long-term fracture risk. The dose range for preventing bone loss with CEE is between 0.3 and 0.625 mg. Oral micronized 17 β estradiol 0.5 mg arrests bone mineral loss in 60% of women, whereas the remaining 40% may require higher doses of 1 to 2 mg daily. Transdermal estradiol products also have been approved to prevent osteoporosis at a dose of 25 μ g per day.

The optimal dose of estradiol, or any estrogen for that matter, depends on the biological outcome of interest. Clearly, the best dose for bones is the highest dose, because there appears to be a stochastic response, with increasing doses producing increasing positive bone responses. Similarly, high-density lipoprotein cholesterol also increases steadily with increasing doses. However, when considering "bad" outcomes, the lowest dose would appear to be the safest dose. With regard to endometrial proliferation, breast cancer, triglyceride levels, and coagulation profiles, administration of the lowest dose possible is desirable to minimize risk. The optimal dose for cardio-

vascular protection (if it exists at all) has yet to be determined for any estrogen. In summary, the best dose would be one with maximal benefit and minimal risk, perhaps the smallest effective dose for the longest period of time. Currently, the optimal therapeutic range for serum 17 β estradiol levels is thought to be between 60 and 80 pg/ml, with acceptable levels between 40 and 120 pg/ml. As studies continue and understanding of dosing requirements increases, these values may need to be redefined for different populations, different health outcomes, different degrees of symptomatology, and differences in physiology over the course of time as women progressively age.

Estriol

While pharmaceutical marketers and advertisers compete to win our hearts, minds, and hands writing the prescriptions, alternative estrogen products are being promoted as "safer" options for menopausal women. Estriol is being offered as the estrogen of choice. Estriol is said to hold a lesser potential risk than estradiol or estrone, particularly in regard to the increases in endometrial and breast cancer associated with estrogen replacement therapy use. At the least, advocates insist that estriol decreases the risk of these cancers. Some promoters even tell women that estriol can block the neoplastic-inducing effects of estradiol and estrone. A typical example is presented here, taken from Community.Drug.com¹²:

"Estriol (E₃) plus progesterone: Estriol (E₃) is the estrogen that is made in large

quantities during pregnancy. Both estriol and progesterone, which exist in much greater quantities than the other sex hormones in pregnancy, have potential protective properties against the production of cancerous cells. Estradiol is the most stimulating to the breast (causes cell division) and estriol the least.

For a dose equal to 0.6 to 1.25 mg of Premarin, you would require 2 to 5 mg of estriol. Some advantages of estriol are:

- Better than estradiol to treat urinary tract infections
- Most beneficial to the vagina, cervix, and vulva (for vaginal dryness treatment)
- Benefits of other estrogens without the risks
- Estriol leaves the body more quickly than estradiol and estrone
- Estriol is breast-tissue protective, not proliferative"

Alternative women's health advocates elected estriol as the natural estrogen of choice for menopause, based on information derived from animal models, observational studies on endogenous estriol in selected populations, and limited human clinical trials. No long-term interventional studies comparing estriol with other estrogens regarding long-term risks exist, and shorter therapeutic trials of estriol do not support these claims.

Estriol is the principle estrogen produced by the placenta. Reasoning dictates that during gestation, high levels of estriol protect estrogen-sensitive tissues from the neoplastic-inducing effects of the other human estrogens. Enthusiasts promoting estriol extrapolate that exogenous administration will have a salutary effect, somehow mimicking the action of endogenous estriol. The role of endogenous estriol or exogenous estriol as an active estrogen is highly speculative.

Observational studies have found that women with early age during first-term pregnancy excrete more estriol than do nulliparous women. Asian females excrete more estriol than do their American counter-

parts, and when Asians migrate eastward to European or Americanized areas, becoming increasingly acculturated into Western lifestyles, estriol excretion decreases while, inversely, breast cancer rates increase.¹³ It appears that high estriol excretion is a marker of lower breast cancer risk in premenopausal women. In postmenopausal women, the story is quite different. As serum estriol, estrone, and estradiol increase, urinary excretion of each these estrogens increases, and breast cancer risk increases. High estriol levels in postmenopausal women appear to be a marker of high endogenous estrogen activity and increased breast cancer risk.^{14,15}

During the 1970s, research by Lemon et al sought to establish estriol as a potential preventive of and treatment for breast cancer. Using female Sprague Dawley rats, breast tumors were induced by ingestion of 7,12 dimethylbenza, anthracene, or procarbazine, which are known carcinogens.¹⁶ The animals in this exemplar study were premedicated with subcutaneously implanted pellets with 5 to 7 mg normal saline plus 1–20% estriol 48 hours before exposure to the carcinogen. Rats that were pretreated had markedly reduced rates of tumor induction. Moreover, rats treated after induction evidenced tumor regression. Estriol also produced remissions in human breast cancer patients,¹⁷ a phenomenon that can be seen with a number of estrogens. Diethylstilbestrol,¹⁸ estrone, and estradiol all have been used successfully to induce short-term remissions. Chemotherapeutic adjuvant therapy later was shown to produce even better outcomes.¹⁹ In 1978, Follinstad published a commentary in *JAMA* entitled "Estriol, the Forgotten Estrogen?"²⁰ He implies in this treatise that low levels of estriol after menopause somehow foment the "villainy" of estrone and estradiol, thus inducing high rates of breast cancer in postmenopausal women. Citing published and unpublished data from Lemon et al, he reported a 37% induction of remission or arrest of growth of metastatic lesions in women with advanced breast cancer. Later studies failed to confirm

the hope that estriol would be an effective breast cancer therapy.

These facts notwithstanding, alternative pharmacies in the United States are compounding estrogen replacement therapy pills, gels, and capsules with estradiol 10%, estrone 10%, and estriol 80% (Fig. 3). Typical formulations contain combinations like estradiol 0.0125 mg, estrone 0.0125 mg, and estriol 1 mg. For this particular formulation, a common dosing regime is two pills twice daily. The aggregate dose of estradiol therefore is 0.5 mg. So, this triest estrogen preparation in fact, is relying on estradiol, which is present in sufficient quantity to provide functional estrogenic benefits. Whereas compounding pharmacies making triest do not provide research on the efficacy of these formulations, anecdotal tests in practice have verified that women using triest most often have a serum estradiol level in a good therapeutic range. Promoters of triest preparations, intuitively or discerningly, have come to realize that estrone is probably superfluous in the mixture; so, many natural estrogen proponents now advise women to use "biest" preparations, compounded with only estradiol and estriol. Again, the journeyman in the duet is estradiol.

Biochemistry of Estriol

Estriol differs vastly from estradiol. Unlike estrone, estriol does not convert to estradiol. Whereas it does offer significant bioactivity, that activity is limited by the fact that it is rapidly metabolized and binds fleetingly to estrogen receptors. When orally administered, in the first liver pass, not unlike estradiol, a great deal is sulfated. But, unlike estradiol, 65% is converted to estrone sulfate, and 80–90% of estriol is conjugated to sulfate and then rapidly excreted. Thus, only 10–20% remains in the circulation and, of the total dose, only 1–2% remains in its native form. Like estradiol, when administered vaginally, the bioavailability of estriol is markedly increased. Like natural progesterone, administration with food greatly enhances absorption. Even when large amounts of estriol are administered, there is no attendant increase in the serum levels of other estrogens. In doses as large as 10 mg, no increases are seen in estrone, estradiol, or their sulfated forms. The only detectable increase is in estriol sulfate, which is not bioactive.²¹ Estimates of the potency of estriol vary greatly, ranging from one-tenth to one-hundredth that of estradiol.²² The estrogen

	Estrone	Estradiol	Estriol
Dr. Wright's Triest	0.125	0.125	1
Dr. Wright 2.5	0.25	0.25	2
Triest 3.75	0.275	0.275	3
Dr. Mark's Triest	0.175	0.175	2.5
NP Lane's Bi Est		0.5	2

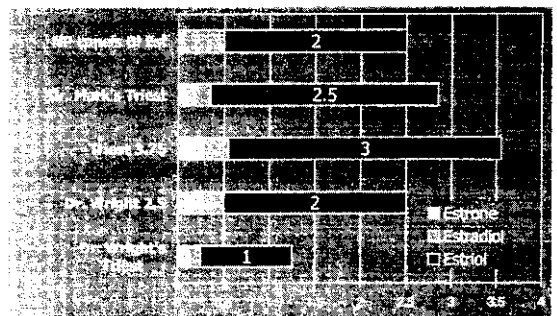


FIG. 3. Comparisons of estrone, estradiol, and estriol.

receptor affinity for estriol is only one-third to one-fifth that for estradiol, and estriol binds weakly to both α and β sites, with affinities of 14% and 21%, respectively, when compared with the affinity of estradiol for the same receptor-binding sites.

Though estriol is much weaker than estradiol at some tissue sites, it appears to have the ability to stimulate endometrial proliferative histologic changes identical to those seen with stronger estrogens.²³ Estrogen and progesterone receptors in the cytosol and estrogen receptors in the nuclear compartment were measured in the endometrium, myometrium, and vagina of 29 postmenopausal women who underwent hysterectomies. Researchers attempted to compare the effects of vaginal estriol (0.5 mg daily) to 17β estradiol (0.05 mg daily) therapy on receptor levels. Overall, they found no clear differences between vaginal estradiol and estriol with regard to the effects on receptor levels in vaginal and uterine tissues. On histologic examination, similar signs of estrogen stimulation of the endometrium were seen after estradiol and estriol.

Melamed et al²⁴ examined the concept that estriol might act as a competitive antagonist to estradiol by looking at the effects of estriol on receptor configuration. They concluded that alone, estriol acts as a weak estrogen, but when acting in concert with estradiol, it appears to act as an antiestrogen. Estriol interferes with estradiol-induced, positive, cooperative binding, with receptor dimerization, and with the binding of hER completely to the estrogen response element. The ability of estriol to act as an antiestrogen is maximal when present in a 10-fold molar concentration in excess of the amount of estradiol. At this concentration, it decreases estradiol binding by only 50%, but it down-regulates estradiol-dependent protein transcription by 85%. These findings lend some support to those who believe that estriol can counteract some of the tissue effects of other estrogens, but clinical studies have not confirmed this supposition. There appears to be no preferential uptake

or metabolism of estriol in human tissue. Thijssen et al²⁵ assessed the tissue/plasma gradients for estriol and estradiol and found that both estrogens exhibit profound uptake by estrogen-sensitive tissues, concentrating in much greater amounts in the tissues than in the plasma (Fig. 2).

Low-density lipoprotein (LDL) oxidation has been associated with an increased risk of cardiovascular disease. Protecting LDL from oxidation represents another mechanism by which estrogens may help minimize cardiovascular risk in premenopausal women. Shwaery et al performed an in vitro study in which plasma LDL was incubated with the three naturally occurring estrogens, estrone, estradiol, and estriol, at physiologic concentrations. The incubated material was then subjected to copper ion-mediated oxidation to assess the degree of oxidative damage. Estrone and 17β estradiol both associated with LDL at a five-fold to eight-fold greater rate than estriol. The only estrogen that conferred oxidation resistance to the LDL, however, was after formation of esters with 17β estradiol. This suggests that only 17β estradiol, not estrone or estriol, has antioxidant activity and protects against LDL oxidation.²⁶ Postmenopausal women with high levels of endogenous estrogens, but predominantly estrone, have a high incidence of cardiovascular disease. In contrast, premenopausal women have higher levels of 17β estradiol and are well protected from cardiovascular disease. This implies that the specific loss of 17β estradiol at menopause, not the loss of estrogens per se, results in increased levels of atherogenic oxidized LDL.

Estriol and the Endometrium

Studies performed with less than adequate doses of estriol give the fallacious impression that estriol protects the endometrium from hyperplasia. When estriol is administered in doses that are comparable with estradiol and, more frequently, to compensate for rapid metabolic clearance, estriol induces endometrial hyperplasia.²⁷ Englund²⁸

asserts that when estriol was "administered in a way that gives prolonged elevation of the blood levels, is able to produce the same effect on the endometrium as other estrogens." In human studies, pretreatment and contemporaneous treatment with estriol does not limit estradiol binding, nor does it block the development of endometrial hyperplasia. Biopsies of the endometrium in women treated with estriol only, estradiol only, and the two in combination showed similar dose-dependent histological proliferative and hyperplastic changes. Padwick et al²⁹ administered doses of estradiol (E₂) or estradiol plus estriol (E₃) to women for a 3-month period. Both therapies provided similar treatment outcomes: similar degrees of vaginal bleeding, psychological symptom profiles, reductions in night sweats, and relief from vaginal dryness. Endometrial hyperplasia was found in 9 of 14 endometrial biopsy samples, a rate of 64%. There appeared to be no mitigation of the tendency toward hyperplasia with dual administration of estradiol plus estriol. In reviewing the available literature on estriol and the endometrium, Doren³⁰ concluded, "... data suggest that use of oral estriol may be associated with endometrial hyperplasia and endometrial carcinoma relatively more often compared to sequential HRT."

Vaginally administered estriol is generally regarded as having a higher safety profile than oral estriol. One study reviewed endometrial biopsy material from 215 subjects who were using vaginal estriol.³¹ The vast majority of biopsies were performed after 6 to 12 months of therapy. Only 13 of the biopsies were performed at 24 months. There were no significant abnormalities, leading the authors to suggest that progestational opposition is not necessary when using estriol via the vaginal route. Nonetheless, the study is severely limited by the fact that few subjects were studied after lengthy use. This is, however, consistent with larger epidemiological studies wherein vaginal estriol appears to yield low levels, if any, of increased risk of endometrial carcinoma. Weidnerpass

et al³² in 1999 investigated the premise that low-potency estrogen formulations like estriol had few, if any, adverse effects for the endometrium. They set out to quantify the level of risk using Sweden's national population-based data registry on endometrial cancer. Seven hundred eighty-nine cases were identified in the registry and were matched with 3,368 control subjects from the general population. They determined that after 5 years, oral estriol 1 to 2 mg per day was associated with a relative risk of endometrial cancer equal to 3.0 (95% CI: 2.0–4.4). The relative risk increased with duration of use, by 8% per year, whereas vaginal use increased risk by 2% per year, which was determined to be less than statistically significant. Review of these findings prompted the New Zealand government to recommend, "If vaginal atrophy is the only indication for hormone replacement therapy, it is worth recommending vaginal treatment as possibly a safer alternative ... to oral replacement."³³ Not unexpectedly, the relative risk of endometrial hyperplasia associated with estriol 1 to 2 mg orally was increased by 8.3-fold (95% CI: 4.0–17.4) compared with never-use. The use of unopposed CEE, in general, has been said to increase the risk of cancer by eight-fold to 20-fold after 10 years of use. It would appear that the order of magnitude of risk associated with estriol is not as great as that seen with other unopposed oral estrogen therapies, but nonetheless, it is substantially greater than nil. Again, in parallel with the other types of estrogens, endometrial neoplasms in estriol users tended to be well differentiated, with limited invasion. The risk regresses rapidly after discontinuation of estriol.

Estriol and the Breast

As stated previously, all impressions of estriol and its role in breast cancer derive from observational studies that have correlated endogenous estriol metabolism with breast cancer risk. There are no interventional stud-

ies using estriol and then comparing rates of breast cancer with other therapeutic estrogens. Estriol is touted as a breast cancer preventive, but in vitro studies contradict these assertions. Estriol binds to breast cancer cells and also appears to partially antagonize the antiestrogenic effect of tamoxifen.³⁴ Estriol is metabolized to 16-hydroxyestrone (16-OHE1), and high endogenous levels of 16-OHE1 have been suggested as a marker of risk for breast cancer. Telang in the *Journal of the National Cancer Institutes* reported that 16 α -hydroxyestrone (16 α -OHE1) appears to initiate transformation in C57/MG mouse mammary epithelial cell lines. The 16 α -OHE1 increased DNA repair synthesis by 55.2%, increased proliferative activity by 23.09, and increased the number of soft agar colonies by 18-fold, all measures being regarded as quantitative endpoints and markers of increased cellular transformation. This phenomenon is not seen with estradiol or estrone.³⁵ Whereas 16-OHE1 and 16-hydroxyestradiol stimulate the growth of human breast cancer cells as much, if not more than, estradiol, the metabolites of estrone and estradiol—2-hydroxyestrone (2-OHE1) and 2-hydroxyestradiols—exhibit

some ability to inhibit hormone-induced cell proliferation seen with estradiol alone when administered concomitantly with estradiol. High concentrations were needed, however, to produce a significant inhibition of hormone-induced cell proliferation.³⁶ The clinical significance of these findings remains unclear. But, these findings suggest that 2-OHE1 and 2-hydroxyestradiols are protective, whereas 16 α -OHE1, the estriol metabolite, is deleterious in terms of breast cancer risk.

Clinical Applications of Estriol

In Europe and other regions, estriol is used as HRT in oral, topical, and intravaginal preparations, often in combination with other estrogens, most commonly only as a short-term topical in treatment of urogenital atrophy before urogenital surgery (Table 3 and Fig. 4 show lists of typical products and prescribing information). Clearly, estriol achieves excellent results as a topical therapeutic. It is regarded by many in Europe as the estrogen of choice for atrophy, and trials of newer vaginal estrogens usually

TABLE 3. Estriol: Branded Products in Europe, South Africa, Australia, and New Zealand

Brand	Form	Manufacturer
Synapause	Estriol succinate 2-mg tablet	Organon
Synapause Forte	Estriol succinate 4-mg tablet	Organon
Synapause	Vaginal cream 0.1%	Organon
Ovestin	1- and 2-mg tablets orally	Organon
	Pessaries 500 μ g (ovula)	Organon
	Vaginal cream 0.1%	Organon
Hormonin	Estriol 0.27 mg/estradiol 0.6 mg/estrone 1.4 mg	Shire/Schering
Ortho Gynest	0.1% cream	Janssen Cilag
Ortho Gynest D	0.5-mg pessary	Janssen Cilag
Cyclo-Menorette	Estradiol 1 mg, Estriol 2 mg, levonorgestrel 0.25 mg	Wyeth
Estrofem	Estradiol 2 mg, Estriol 1-mg tablet	Novo Nordisk
Estrofem Forte	Estradiol 4 mg, Estriol 2-mg tablet	Novo Nordisk
Menoflush		Pharm. Ent.
Trisequens	12 tablets, oestradiol 2 mg, oestriol 1 mg; 10 tablets, oestradiol 2 mg, oestriol 1 mg, norethisterone acetate 1 mg; 6 tablets oestradiol 1 mg, oestriol 0.5 mg	Novo Nordisk
Trisequens Forte	12 tablets, oestradiol 4 mg, oestriol 2 mg; 10 tablets, oestriol 4 mg, oestriol 2 mg, norethisterone acetate 1 mg; 6 tablets, oestradiol 1 mg, oestriol 0.5 mg	Novo Nordisk

Excerpts from product prescribing information from European Estriol Products:

Ovestin Cream - Each gram of cream contains 1 mg estriol.

Therapeutic indications

Atrophy of the lower urogenital tract related to estrogen deficiency, notably

- for the treatment of vaginal complaints such as dyspareunia, dryness and itching.
- for the prevention of recurrent infections of the vagina and lower urinary tract.
- in the management of miction complaints (such as frequency and dysuria) and mild urinary incontinence.
- Pre- and postoperative therapy in postmenopausal women undergoing vaginal surgery.
- A diagnostic aid in case of a doubtful atrophic cervical smear.

Posology and method of administration

- For atrophy of the lower urogenital tract:
1 application per day for the first weeks, followed by a gradual reduction, based on relief of symptoms, until a maintenance dosage (e.g. 1 application twice a week) is reached.
- As pre- and postoperative therapy in postmenopausal women undergoing vaginal surgery:
1 application per day in the 2 weeks before surgery; 1 application twice a week in the 2 weeks after surgery.
- As a diagnostic aid in case of a doubtful atrophic cervical smear:
1 application on alternate days in the week before taking the next smear.

Ovestin Pessaries - Each pessary contains 0,5 mg estriol.

Therapeutic indications

Atrophy of the lower urogenital tract related to estrogen deficiency, notably

- for the treatment of vaginal complaints such as dyspareunia, dryness and itching.
- for the prevention of recurrent infections of the vagina and lower urinary tract.
- in the management of miction complaints (such as frequency and dysuria) and mild urinary incontinence.
- Pre- and postoperative therapy in postmenopausal women undergoing vaginal surgery.
- A diagnostic aid in case of a doubtful atrophic cervical smear.

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1 pessary per day for the first weeks, followed by a gradual reduction, based on relief of symptoms, until a maintenance dosage (e.g. 1 pessary twice a week) is reached.
- As pre- and postoperative therapy in postmenopausal women undergoing vaginal surgery:
1 pessary per day in the 2 weeks before surgery; 1 pessary twice a week in the 2 weeks after surgery.
- As a diagnostic aid in case of a doubtful atrophic cervical smear:
1 pessary on alternate days in the week before taking the next smear.

Ovestin Tablets - Each tablet contains 0,25 mg, 1 mg or 2 mg estriol. for oral use

Therapeutic indications

Atrophy of the lower urogenital tract related to estrogen deficiency, notably
for the treatment of vaginal complaints such as dyspareunia, dryness and itching.
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in the management of miction complaints (such as frequency and dysuria) and mild urinary incontinence.

- Pre- and postoperative therapy in postmenopausal women undergoing vaginal surgery.
- Climacteric complaints such as hot flushes and night sweating.
- A diagnostic aid in case of a doubtful atrophic cervical smear.
- Infertility due to cervical hostility.

Posology and method of administration

- For atrophy of the lower urogenital tract:
4-8 mg per day for the first weeks, followed by a gradual reduction, based on relief of symptoms, until a maintenance dosage (e.g. 1-2 mg per day) is reached.
- As pre- and postoperative therapy in postmenopausal women undergoing vaginal surgery:
4-8 mg per day in the 2 weeks before surgery; 1-2 mg per day in the 2 weeks after surgery.
- For climacteric complaints such as hot flushes and night sweating:
4-8 mg per day during the first weeks, followed by a gradual reduction. For maintenance therapy the lowest effective dosage should be used.
- As a diagnostic aid in case of a doubtful atrophic cervical smear:
2-4 mg per day for 7 days before taking the next smear.
- For infertility due to cervical hostility: In general 1-2 mg per day on days 6-15 of the menstrual cycle. However, for some patients dosages as low as 0,25 mg per day are sufficient, whereas others may need up to 8 mg per day. Therefore, the dosage should be increased each month until an optimal effect on the cervical mucus is obtained.

FIG. 4. Excerpts from prescribing information from European estriol products. Accessed at: http://www.organon.com/home_index.html?MedicalProfessional.

compare the new agent with estriol as the current treatment of choice. Two hundred fifty-one postmenopausal women reporting at least one bothersome lower urinary tract symptom were treated with an estradiol-releasing ring or with estriol pessaries 0.5 mg every other day for 24 weeks.³⁷ The ring and pessaries were equally effective in reducing urgency, frequency, nocturia, dysuria, stress incontinence, and urge incontinence. Dysuria was alleviated in 76% versus 67%. This difference is not statistically significant. Patient satisfaction was much higher for those using the ring. Sixty percent rated the ring as excellent whereas only 14% rated pessaries as excellent ($P < 0.0001$). A similar study of vaginal tablets again used estriol "vagitories" as the control treatment. Ninety-six postmenopausal women with symptoms of atrophic vaginitis were treated for 24 weeks with either estradiol tablets or estriol ovules. Both medications were used daily for 2 weeks and then twice weekly thereafter.³⁸ Only three women in the tablet group (6%) reported urinary leakage; none required sanitary protection. In contrast, 31 (65%) of the ovule users reported leakage and 14 (29%) required sanitary pads. Patients rated the tablets superior in terms of hygiene and ease of use. The tablets increased endometrial thickness more than the estriol ovules—1.1 mm versus 0.5 mm—during the first 2 weeks of the study, but returned to baseline levels when the use was reduced to twice weekly. Studies of this sort and new drug developments may be leading to the obsolescence of estriol for its most common indication.

Note that pharmaceutical companies in Europe, South Africa, and Australia produce and market oral HRT preparations containing both estradiol and estriol, though newer formulations have discontinued the estriol component. Trisequens (Novo Nordisk A/S, Copenhagen, Denmark), an estradiol plus estriol preparation still marketed in some parts of the world, has been reformulated in Europe. The newest "domestic" version made by Novo Nordisk in Netherlands only

contains estradiol. There has been recognition that the estriol has not added value in the HRT mix. Hart³⁹ conducted a long-term trial of continuous combined hormone replacement, initially randomizing subjects to 2 mg 17 β estradiol and 1 mg norethisterone (norethindrone) acetate administered once daily with or without 1 mg estriol. No differences were observed during the initial 1-year trial between the groups with or without estriol. When the project was extended to a 9-year open-label study, estriol was eliminated from the treatment protocol, and all women were administered estradiol alone.

Estriol's clinical performance has been less than stunning. Estriol does show good effects in the treatment of vasomotor symptoms, sleep disturbances, night sweats, and other "vegetative" symptoms of menopause. Yang⁴⁰ (Taiwan) reported that estriol was effective in alleviating symptoms of the climacteric, but it did not prevent bone loss. Takahashi⁴¹ reported the experience of 68 postmenopausal Japanese women administered estriol, 2 mg per day, daily for 12 months. Oral estriol therapy improved maturation index and greatly decreased hot flushes, night sweats, and insomnia. Whereas estriol did evidence an impact on the hypothalamus by lowered serum follicle-stimulating hormone and luteinizing hormone concentrations, no changes were noted in lipids, bone mineral density (BMD), liver function, or blood pressures. Vaginal bleeding occurred in 14.3% of the women with an intact uterus. Similarly, Minaguchi⁴² reported statistically significant decrease in Kupperman's Index with estriol 2 mg daily. The same study, in a Japanese population, demonstrated an increase in BMD of 1.79% in 50 weeks in individuals preselected to have a baseline density more than 10% less than peak levels.

The studies of the efficacy of estriol in treatment of osteoporosis have shown mixed results. Itio⁴³ studied 64 healthy, early menopausal women and treated them for 24 months, either with estradiol plus estriol 2.0 mg plus medroxyprogesterone acetate 2.5

mg ($n = 15$), CEE 0.625 mg plus 2.5 mg medroxyprogesterone acetate ($n = 19$), 1.0 μ g 1- α -hydroxyvitamin D3 daily ($n = 13$), or 1.8 g calcium lactate containing 250 mg of elemental calcium daily ($n = 17$). Bone mineral density at the third lumbar vertebra was measured with quantitative computed tomography, and bone markers in serum including osteocalcin and total alkaline phosphatase, urinary calcium-to-creatinine ratio, and hydroxyproline-to-creatinine ratio were evaluated at baseline and every 6 months for the 2 years of the study. The nonestrogen groups lost 12–14% of BMD during the 24-month study period. The estriol treatment group lost $-4.1 (\pm 4.8\%)$, and the CEE group had less than 1% loss ($-0.9 [\pm 3.2\%]$). Serum markers of bone turnover decreased or remained unchanged in the estradiol plus estriol and CEE groups, but increased in other arms. Estriol produced less uterine bleeding than CEE. Estriol incurred 2.4 (± 4.2) days of bleeding versus 13.1 (± 14.8) days per person per year ($P < 0.001$) for CEE. The authors felt that the outcomes seen with estriol and CEE were comparable; however, if the study period were more prolonged, the 2% loss per year seen with estriol might well prove to be significantly worse than that seen with CEE.

In a study of a percutaneous estradiol gel and its effects on BMD, women were administered oral estriol 2 mg per day as a control.⁴⁴ The study is an exception in one regard: it included measures of BMD of the proximal femur in addition to the more common measure of BMD of the lumbar spine. Women were studied every 3 months by dual-energy x-ray absorptiometry for 2 years. In the 21 patients using estriol, both sites lost BMD. The percutaneous estradiol-treated group gained 1.2 per year in the lumbar spine, but had no significant change at proximal femur. Estriol was judged to be a treatment failure. This study, performed in Europe, contradicts the outcomes seen in studies performed in Asian women.

Interestingly, the dose of oral estriol recommended in Europe, Australia, and South

Africa is often much greater than the doses used in Asia. European pharmaceutical estriol tablets contain 2 or 4 mg, double the doses used in Japan. Perhaps, because of their body mass, previous BMD levels, or some other variable, European women appear to be less responsive to estriol than Asian women. Remember that women with the worst BMD levels have the greatest response to estrogen, and they may even respond to extremely low levels. Naessen⁴⁵ found that women older than age 60 with no previous estrogen treatment gained 2.1% in the BMD of the forearm in 6 months, versus a 2.7% decrease in controls, whereas being treated with a vaginal estrogen ring provided just 7.5 μ g per day. Markers of bone turnover decreased significantly. Alkaline phosphatase decreased 8%, bone-specific alkaline phosphatase decreased 14%, and osteocalcin decreased 9%. Clearly, minuscule doses of estrogen can be remarkably effective in individuals with severe hypoestrogenemia.

The lipid effects of estriol differ from other estrogens and support the concept that estriol is weaker. Itoi et al performed a 2-year trial of estriol 2 mg plus medroxyprogesterone acetate 2.5 mg versus CEE 0.625 mg plus medroxyprogesterone acetate 2.5 mg versus placebo, and found that total cholesterol decreased in the estriol group, and the CEE group evidenced no change. Placebo controls demonstrated a 5.4% increase in total cholesterol. High-density lipoprotein cholesterol increased 10.7% in the CEE and 3.8% in the estradiol plus estriol groups, but decreased in the controls by 3.6%. Low-density lipoprotein cholesterol was decreased 11.4% in the CEE group, remained unchanged in the estradiol plus estriol group, and increased 11.8% in controls. Triglycerides decreased a modest 6.7% in the estradiol plus estriol subjects, remained stable in controls, and, as expected, increased 17.6% in the CEE users. Thus, the overall profiling of estriol indicates minimal impact on lipids.⁴⁶ The authors suggest that estriol might be a good choice for women

prone to hypertriglyceridemia. That conclusion seems reasonable.

Conclusions

When examining estriol as a menopausal intervention, one may view the glass as either half empty or half full. Head,⁴⁷ in her review of estriol, concluded:

"While conventional hormone replacement therapy provides certain benefits, it is not without significant risks. Estriol has been found to provide some of the protection without the risks associated with stronger estrogens. Depending upon the situation, estriol may exert either agonistic or antagonistic effects on estrogen. Estriol appears to be effective at controlling symptoms of menopause, including hot flashes, insomnia, vaginal dryness, and frequent urinary tract infections. Results of research on its bone-density-maintaining effects have been contradictory, with the most promising results coming from Japanese studies. Estriol's effect on cardiac risk factors has also been somewhat equivocal; however, unlike conventional estrogen prescriptions, it does not seem to contribute to hypertension. Although estriol appears to be much safer than estrone or estradiol, its continuous use in high doses may have a stimulatory effect on both breast and endometrial tissue."

This is a generous and upbeat view of estriol. Since 1998 (when that statement was published), the new information has surfaced regarding the endometrial risks of estriol. Moreover, the work performed in Japan may not be applicable to other populations. In his excellent study in 2000, Takahashi concluded, "Estriol is a safe and effective alternative for relieving climacteric symptoms in postmenopausal Japanese women."⁴² Emphasis in that statement should be on "Japanese." Perhaps that author is subtly suggesting that the results cannot be extrapolated to other populations. I would also insert a similar cautionary note.

The most that can be said is that estriol is no worse than the currently available formu-

lations using estrone and estradiol in terms of overt risks. It appears to offer good symptom control. But, it is clearly no better than estradiol or estrone. Triest and biest preparations do contain sufficient amounts of estradiol to provide all the therapeutic benefits of conventional HRT, and patients using them clearly will have good symptom relief. Unconventional, compounded estrogens do not appear to be worth the extra cost and effort they exact, but they do represent an additional therapeutic option for women who cannot tolerate conventional prescription estrogens.

Summary

The use of estriol alone or with other estrogen does not provide documented safety, security, or protection for the breast or endometrium.

There is no clinical or epidemiological documentation for the claim that estriol will protect against breast cancer. Estriol clearly has the potential to up-regulate estrogen receptors in the endometrium, and to stimulate the endometrium. Estriol is associated with an increased risk of endometrial cancer and endometrial hyperplasia when administered orally in doses from 1 to 2 mg per day. It does not "block" the endometrial proliferative effects of other estrogens. Oral estriol, used any longer than short-term and not unlike other estrogens, should be used in combination with progestational therapy to obviate the risk of hyperplasia and carcinoma associated with the use of unopposed estrogens. The bone effects of estriol appear to be milder than those of estradiol or estrone. Estriol has minimal lipid effects. Whereas estriol does not improve total cholesterol, high-density lipoprotein, or LDL, it does not increase triglycerides like other oral estrogens, and it may be a safer alternative for women prone to hypertriglyceridemia. Transdermal estradiol is also a safer option for women with this type of lipid disorder.

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