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WHITE PAPERS

Estrogen HRT in Menopause

Best Practices for Selecting and Monitoring Estrogen Replacement Therapy in Menopause and Beyond

PRECISION ANALYTICAL INC.
CREATORS OF THE DUTCHTEST®

INTRODUCTION

SUMMARY

Menopause is a dynamic process defined by permanent loss of ovarian follicular function. It also involves extensive restructuring of complex regulatory pathways throughout the entire female body. In other words, the characteristic decline in estrogen levels in menopause extends far beyond the cessation of fertility, affecting aging trajectories of many organ systems. Moreover, this endocrine chapter is often hallmarked with disruptive symptoms..

Estrogen is more than a reproductive hormone–it is a systemic modulator with extraordinary integrative powers. Estrogen leverages a repertoire of receptors and signaling pathways to mechanistically connect feedback loops and axes, required for survival at the cellular, tissue, organ, and ultimately whole-body levels. Disruption of estrogen homeostasis during menopause contributes to profound shifts towards metabolically compromised phenotypes in cardiovascular, musculoskeletal, genitourinary, neurological, immune, and, of course, reproductive systems. With that, menopause often presents itself with symptoms that can severely impact the quality of life of affected individuals and lead to long-term health consequences.

Some symptoms can manifest during perimenopause (for example, hot flashes and night sweats), the transitional period of about 2-8 years, leading up to menopause. Others (like genitourinary symptoms) may emerge later and worsen with time and without treatment [1]. Symptom presentation and their intensity tend to vary from person to person, but these generally include [2,3]:

Vasomotor symptoms (~75-80% women experience these)

- Hot flashes
- Night sweats
- Palpitations
- Migraines

Genitourinary symptoms (~50-75% women experience these) [4]

- Vaginal dryness
- Burning
- Itching
- Irritation
- Pain during intercourse
- Urinary urgency and frequency

Neurological symptoms [5]

- Depression
- Anxiety
- Insomnia
- Mood swings
- Anger
- Irritability
- Forgetfulness

Musculoskeletal effects of menopause [6]

- Gradual loss of muscle mass, function, and strength
- Bone loss

Cardiovascular effects of menopause [7]

- Hypertension
- Central adiposity
- Insulin resistance
- Pro-atherogenic lipid profile
- Decreased endothelial function
- Heart rate variability

Although menopause denotes cessation of reproductive function, it simultaneously elicits broader health ramifications, necessitating introduction of therapies that can address the multifaceted aspects of overall well-being affected by hormonal changes. Experts agree that systemic hormone replacement therapy (HRT) remains the most effective option for symptom management in menopause [8]. Restoration of estrogen with estrogen replacement therapy (ERT) prevents or reverses many symptoms, provides protective effects for many organs systems, and offers longevity of clinical solutions [9].

A thorough understanding of the benefits and risks associated with ERT is important when individualizing treatment options for menopausal patients. To this day, personalizing and optimizing HRT continues to be an unmet need in women's health and a key issue in precision medicine.

The goal of this white paper is to summarize published clinical evidence with regard to individualizing ERT. Types of estrogen therapies, doses, formulations, routes of administration, and duration of use will be discussed in the context of maximizing benefits and minimizing risks associated with HRT in menopause.

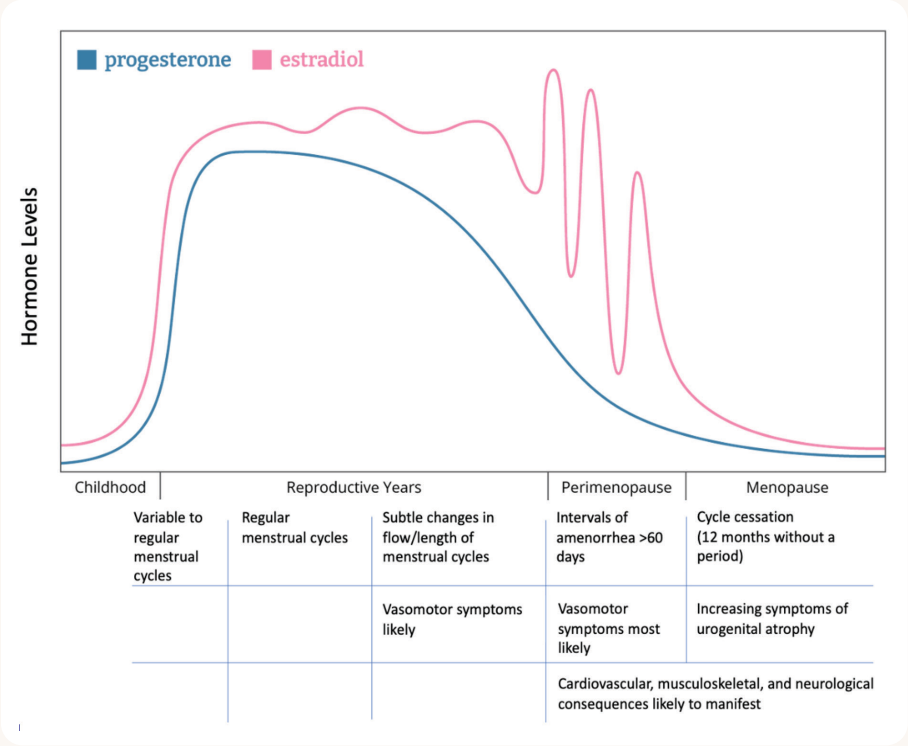

Figure 1: *During reproductive years, the levels of estrogen and progesterone fluctuate on a monthly basis to support ovulation but are generally sustained at normal physiological levels (that are higher than pre-puberty or menopausal levels). In perimenopause, the levels of follicle-stimulating hormone (FSH) increase, progesterone levels gradually decrease, and estrogen levels can fluctuate dramatically, giving rise to irregular cycles (unpredictable or absent ovulation) and bothersome symptoms. Eventually, cycles stop completely. No cycles for 12 consecutive months, low levels of estrogen and progesterone, and high levels of FSH and luteinizing hormone (LH) signal that menopause is complete. The typical age range for the onset of menopause is 45-55 years.*

USEFUL NOMENCLATURE

Estrogen

Estrogen is a term that typically refers to 17b-estradiol (also called E2) due to its physiological relevance, widespread distribution in the body, and predominance during reproductive years.

E2 Displays

E2 displays high affinity for the estrogen receptors, ERa and ERb. E2 regulates the development of secondary female sex characteristics, governs the menstrual cycle, and modulates growth of the endometrial lining from the onset of menstruation to menopause.

Estrone (E1) and Estriol (E3)

Estrone (E1) and estriol (E3) are weaker estrogens with a lower affinity for the estrogen receptors compared to E2. E3 is the main estrogen during pregnancy, secreted by the placenta.

PROGESTOGEN HRT

HRT that includes estrogen in menopausal patients with an intact uterus must also include progestogen therapy, in order to safeguard endometrial health from proliferative estrogen effects. Oral micronized progesterone offers reliable protection of the endometrium against hyperplasia with concomitant estrogen use.

ANDROGEN HRT

Androgens are not central to menopausal HRT but may carry additional benefits for some patients. Vaginal DHEA is approved for vulvovaginal atrophy and dyspareunia. Testosterone can be used in women for female sexual dysfunction.

HORMONE CHANGES IN MENOPAUSE

During reproductive years estrogens are predominantly produced by the ovaries, although they are also synthesized in the adrenal glands and fat tissue in smaller amounts [10]. In perimenopause, ovarian production of E2 can be erratic—high or low, fluctuating dramatically, with an overall downward trajectory to eventually stop all together (Fig. 1).

After menopause, ovaries will continue to generate some androgens, like testosterone; while others, like DHEA and androstenedione, will be produced by the adrenal glands. In the absence of HRT, androgen aromatization in adipose tissue is the primary source of estrogen after menopause (albeit at profoundly diminished levels compared to the pre-menopausal time period) [11]. As it is, menopause can be regarded as a state of relative androgen excess.

ESTROGEN REPLACEMENT THERAPY INDICATIONS

Estrogens are the key hormone replacement target. So menopausal HRT most commonly consists of estrogen, progesterone (needed to protect the endometrium in women with an intact uterus), and often, but not always, androgens like testosterone or DHEA.

Although E2 replacement is not common in perimenopause (before periods have stopped completely), testing and carefully considering symptoms is a good way to determine if E2 is becoming more consistently low and ERT initiation is warranted. (If endogenous E2 is high, adding more estrogen with ERT might make symptoms worse.) Overall, ERT should be considered as first-line support around menopause when significant vasomotor symptoms disrupt daily activities and are not adequately managed by lifestyle changes, provided estrogen is safe based on the patient's medical history.

ERT can come in a variety of forms—some that are FDA-approved prescriptions and others are custom compounded. FDA-approved indications for ERT include treating moderate to severe vasomotor symptoms and vulvovaginal symptoms, such as vaginal atrophy and dyspareunia [12]. Additionally, ERT is now approved for prevention of bone loss and fractures from osteoporosis in postmenopausal women [13].

ERT also provides numerous additional health benefits beyond those claims that are FDA-approved [14]:

- Reduces cardiovascular disease
- Enhances bone mineral density
- Preserves muscle mass
- Eases joint pain
- Minimizes menopausal weight gain
- Maintains skin elasticity
- Improves sleep
- Alleviates mood disorders and improves mood
- Potentially reduces the risk of dementia
- Enhances overall quality of life

RISK REDUCTION FOR ALL-CAUSE MORTALITY, CARDIOVASCULAR DISEASE, AND DIABETES

Besides the above-mentioned health benefits, initiated at (or near) menopause, ERT reduces all-cause mortality, cardiovascular disease (while other prevention therapies, such as lipid-lowering, fail to do so), and various aging-related diseases with an excellent risk profile [15]. Moreover, menopause (and aging in general) is associated with adverse metabolic profiles and increased risk of diabetes. ERT improves insulin resistance, increases glucose tolerance, and reduces the incidence of diabetes, a major risk factor for cardiovascular disease [16].

RISK REDUCTION FOR BONE FRACTURE

E2 is essential for bone health. Normal bone turnover cycle is impaired by estrogen deficiency in menopause, with increased prevalence of osteoporosis [17]. When adequate estrogen levels are reached through ERT, estrogen effectively prevents bone loss and reduces the risk of hip, vertebral, and other osteoporosis-related fractures with RCT-proven efficacy [18].

IMPROVEMENT IN GENITOURINARY SYNDROME

Estrogen plays a vital role in preserving the structure and function of the urogenital system, which includes producing mucous secretions. When estrogen levels decline, blood flow decreases and the concomitant loss of glycogen and collagen, contribute to thinning of the vaginal epithelium. Genitourinary syndrome of menopause (GSM) describes a broad range of symptoms that affect the genitourinary tract. These symptoms arise from the decrease in natural sex hormone levels during and after menopause. Symptoms of GSM typically worsen over time and are unlikely to improve on their own without treatment, contributing to decreased quality of life in affected individuals [19].

ERT is very effective in managing vulvovaginal symptoms of menopause. One-third of women already receiving systemic HRT may still experience symptoms of GSM which do not resolve from systemic ERT alone, and thus need additional vaginal therapy. For many years, topical vaginal estrogen has been the preferred treatment for addressing vulvovaginal symptoms in postmenopausal women, specifically those associated with menopause [20].

NEUROPROTECTION

Menopause can be regarded as a neuroendocrine transition state, as many menopausal symptoms are neurological in nature. Without intervention, some women may be at an increased risk of developing neurological dysfunction later in life [21]. Research shows that postmenopausal women face a higher risk of age-related dementia compared to men of the same age, suggesting a potential influence of the decline in sex hormones during menopause on this occurrence [22].

Although controversial, estrogen replacement in menopause shows considerable promise with regard to its neuroprotective mechanisms and prevention (or delay) of dementia [23]. More research is necessary to confirm this stipulation, with specific focus on type, formulation, dosage, and duration of HRT.

BREAST CANCER RISK

Synthetic progestins — associated with increased risk of developing breast cancer.

Oral micronized progesterone is safe — does not increase breast cancer risk in the first 5 years of use.

RISK REDUCTION FOR BREAST CANCER

A recurring concern raised in discussion of hormone therapies during menopause pertains to the risk of developing breast cancer. At the initial onset of publication of the Women's Health Initiative (WHI) study, HRT received negative publicity, arising from misinterpretation of the findings.

The study initially reported a higher risk and occurrence of breast cancer among women using conjugated equine estrogens (CEE) combined with a synthetic progestin (medroxyprogesterone acetate, MPA), while no such increase was observed in the group receiving CEE-only treatment [24]. In other words, women on estrogen-only HRT do not have an increased risk of breast cancer. Breast cancer risk is increased, however, only when a synthetic progestin is included in the HRT regimen.

 report that unopposed estrogen does not elevate the risk of breast cancer and may even lower it [25] demonstrating that unopposed estrogens do not heighten the risk of breast cancer, and may, indeed, decrease it [26,27]. Please keep in mind that when estradiol is paired with oral micronized progesterone, as is common for most women on ERT (for endometrial protection), studies show no increased risk of breast cancer for the first 5 years, but data is unclear beyond that timeframe.

ERT CONTRAINDICATIONS

It is important to consider each patient's individual medical history and any potential contraindications prior to starting ERT. Contraindications for transdermal or oral (but not low-dose vaginal) ERT include [28]:

- Breast cancer
- Endometrial cancer
- Uterine cancer
- Untreated endometrial hyperplasia
- Unexplained vaginal bleeding
- Deep vein thrombosis
- Pulmonary embolism
- Blood clotting disorders
- Cirrhosis
- Myocardial infarction (heart attack)
- Stroke (although there is some evidence that stroke risk is improved with ERT)

ROUTES OF ADMINISTRATION OF ESTROGEN REPLACEMENT THERAPY

When it comes to deciding how to start your patients on ERT, you must first consider how this hormone should being administered (orally, topically, vaginally, etc).

NOTE

Estradiol or E2 is the most potent and abundant estrogen in the body before menopause. Therefore, most published research focuses on E2 replacement as part of ERT, and this paper is focused on estradiol replacement.

ERT FORMS & ROUTES OF ADMINISTRATION

ERT forms include conjugated equine estrogens (CEE), synthetic estrogens, and bioidentical estrogens. These can be delivered via the following routes:

- Oral*
- Transdermal/topical*
- Vaginal*
- Sublingual
- Injectable
- Pellet
- Rectal

*FDA-approved routes

TRANSDERMAL E2

Transdermal (topical) ERT is the most commonly prescribed form of HRT in menopausal women to manage vasomotor symptoms, promote vaginal comfort, and ensure bone preservation. Transdermal E2 delivery systems are FDA-approved for the following indications in menopause:

- E2 patch for osteoporosis prevention
- E2 gels for the treatment of vasomotor symptoms (Divigel, Elestrin, and EstroGel) and vulvar and vaginal atrophy (EstroGel only)

Transdermal E2 is metabolized differently than the oral route, which has implications for clinical efficacy, potential side effects, and risk profile. E2 applied to the skin bypasses the GI tract and therefore first pass metabolism in the liver and appears to give rise to more stable serum estradiol levels (without resulting in supraphysiological concentrations in the liver) [29]. By avoiding first-pass metabolism, transdermal E2 delivery elicits a reducing effect on triglyceride levels, coagulation factors, and the risk of gallbladder disease. In contrast to oral therapy, transdermal ERT does not significantly elevate venous thromboembolism (VTE) risk [30]. Transdermal formulations are especially preferred in women presenting with any risk factors for cardiovascular disease, such as hyperlipidemia, diabetes, hypertension, and others[31].

TRANSDERMAL/TOPICAL E2 FORMS

- Patches
- Gels
- Creams
- Sprays (not recommended)

Table 1: FDA Approved E2 Gels ("Low" = lowest recommended or studied dose)

Brand of E2 Gel Product	Dose Level	Daily E2 Dose (mg)	Serum (pg/mL)	Vasomotor Symptoms (VMS)	Bone Mineral Density (BMD)	Vulvovaginal Atrophy (VVA)	References
Estrogel	Ultra-Low	0.27	11.7	Delayed	X	Failed	1
Divigel	Low	0.25	16	Delayed	X	Success	2,3
Elestrin	Low	0.52	9	Delayed	X	Success	4,5
Evamist	Low	1.53	19-23	Delayed	X	X	6,7
Estrasorb	Low	2.9	30	Success	X	X	8,9
Estrogel	Low	0.375	21	Success	X	Success	1
Estrogel	Moderate	0.75	33.5	Success	Delayed	Success	1,10,11,12,13
Divigel	Moderate	0.50	31	Success	X	Success	2,3
Elestrin	Moderate	1.04	32	Success	X	Success	4,5
Evamist	Moderate	3.06	24-32	Success	X	X	6,7
Estrasorb	Moderate	5.7	43	Success	X	X	8,9
Estrogel	High	1.5	65	Success	Success	Success	1,10,11,12,13
Divigel	High	1.0	62	Success	X	Success	2,3
Elestrin	High	1.56	60	Success	X	X	4,5
Evamist	High	4.59	31-40	Success	X	X	6,7
Estrasorb	High	8.6	63	Success	X	X	14
Estrogel	High	3.0	102.9	Success	Success	X	12,15

All products listed above are considered E2 gels except Estrasorb, which is an emulsiɒed product

Table 2: FDA Approved E2 Patches

Brand of E2 Patch	Total E2 in patch (mg)	Daily E2 Dose (mg)	Serum (pg/mL)	Vasomotor Symptoms (VMS)	Bone Mineral Density (BMD)	Vulvovaginal Atrophy (VVA)	References
Menostar	1	0.014	8.6,13.7	Success	Success	Success	16,17,18
Alora	0.77	0.025	24.5	X	Success	X	19
Climara	2	0.025	22	Success	Success	X	20
Vivelle-Dot	0.39	0.025	X	X	Success	X	21
Vivelle-Dot	0.585	0.0375	34	Success	Success	X	21
Esclim	5	0.025	15.5	Success	X	X	22
Estraderm	X	0.025	X	X	Success	X	23
Alora	1.5	0.05	64	Success	Success	Success	19
Menorest	4.4	0.05	48.5	X	X	X	24
Climara	3.8	0.05	41	Success	Success	X	20,24
Vivelle-Dot	0.78	0.05	57	Success	Success	X	21
Esclim	10	0.05	26.3	Success	X	X	22
Estraderm	4	0.05	32	Success	X	X	23
Alora	3.1	0.1	98	Success	Success	Success	19
Climara	7.6	0.1	87	Success	Success	X	20
Vivelle-Dot	1.56	0.1	89	Success	Success	X	21
Esclim	20	0.1	51.4	Success	X	X	22
Estraderm	8	0.1	74	Success	X	X	23

PRECAUTIONS WITH TD APPLICATION

- Patches may cause skin irritation because they stay in one spot for several days. This can be mitigated by switching brands.
- Exposure to household members is a concern with gels, creams, emulsions, sprays, and is best for people without children in the house. Applying to areas covered with clothing helps prevent spread to other people.
- Exposure can be controlled with good practices: wash hands thoroughly after application. Apply 2-3 hours or more before close contact with others.

Various transdermal ERT preparations exhibit distinct pharmacokinetics, resulting in varying metabolic pathways and absorption rates, consequently necessitating different effective doses. This means that dosing is not interchangeable from one type of transdermal or topical therapy to another.

Patches are lower maintenance and carry less risk of transfer to household members. Dosing options for patches are straightforward, but slightly more limited than with gels and creams. Available patch doses are 0.025, 0.035, 0.05, 0.075, and 0.1 mg for either twice weekly or once weekly applications. Patch dosing represents the amount of estradiol absorbed by the body each day. It's interesting to call out that even 0.014mg patches have demonstrated efficacy in clinical studies.

Common dosing ranges are similar with when you compare gels and compounded creams to one another, but cream results show lower systemic exposure to estradiol than with gels. If switching from a gel to a cream, dosing may need to be higher for a cream preparation. In contrast to patches, cream and gel dosing represent the amount of estradiol contained in the preparation. Keep in mind that on only about 10% of the estradiol in the preparation will (and may not necessarily be absorbed).

ORAL E2

Oral ERT is easy, convenient, and non-invasive, with options that sometimes include an effective dose of progestin (synthetic) or progesterone (bioidentical) in a one pill regimen. Oral ERT is approved for reduction of vasomotor symptoms and protection against bone loss; and overall improves the quality of life for postmenopausal women.

Oral E2 undergoes extensive liver (first-pass) metabolism, so dosing of oral formulations is typically higher than transdermal preparations. In this scenario, E2 concentrations will be high in the hepatic portal vein, but not necessarily in general circulation. During first-pass metabolism, E2 is converted to E1, making E1 the predominant hormone detected in the bloodstream with this delivery [32].

Dose ranges depend on the brand and formulation and are typically administered daily at:

- Oral CEEs: 0.3-1.25 mg
- Oral 17β-E2 (bioidentical E2): 0.5-2 mg
- Oral Ethinyl Estradiol (synthetic): 2.5-5 mcg

The risks associated with oral estrogens primarily arise from their initial metabolism in the liver, resulting in heightened production of coagulation factors and various inflammatory markers, along with increased levels of triglycerides, sex hormone-binding globulin, thyroxine binding globulin, corticosteroid binding globulin, high-density lipoproteins (HDL cholesterol), and biliary cholesterol saturation [33]. Additionally, oral ERT use is linked to a greater risk of gallstone formation and venous thromboembolism (and therefore stroke). So, the oral route of administration may be an option in patients at low risk of venous thromboembolism or gallbladder disease and who prefer the convenience of a pill.

Pills regimens carry minimal risk of exposure to household members, regardless of the dose. Side effects of oral ERT may include abdominal cramping, anxiety, and/or bloating.

It's important to call out that oral estrogen has been shown to be very safe for most women. While integrative clinicians tend to recommend transdermal estrogen applications, which have an even better safety profile, oral estrogen is a safe alternative for most women who prefer it.

ORAL E2 FORMS

- CEEs
- Micronized 17β-E2 (bioidentical)
- Ethinyl Estradiol (synthetic)

VAGINAL E2

FDA-approved low-dose vaginal estrogen therapy is available to treat GSM in multiple forms. Vaginal ERT primarily addresses postmenopausal vulvovaginal changes locally without causing a systemic increase in estrogen. levels beyond what is typical for postmenopausal women. Another popular choice is to use a vaginal E3 preparation for local effect, which also has a good safety profile. The only vaginal estrogen products utilized for treating vasomotor symptoms (in addition to GSM) with systemic absorption is estrogen acetate vaginal rings, known as Femring. These vaginal applications (intended for systemic effect) deliver far higher doses than low-dose vaginal estrogen products. Progesterone HRT is advised for some (e.g., Femring), but is not necessary for all low-dose vaginal estrogen formulations, given minimal absorption and low risk of endometrial cancer.

VAGINAL E2 FORMS

- Creams
- Rings
- Tables
- Capsules

OTHER FORMS OF ERT

Less common forms of ERT include compounded oral, sublingual, transdermal, vaginal, rectal, and pellets, typically available from compounding pharmacies. These less common forms of ERT offer alternatives for individuals who may not tolerate or prefer more traditional E2 products. However, many of these E2 delivery systems have not been as thoroughly evaluated for safety and efficacy, and therefore it is not possible to ascertain if the benefits of these estrogen delivery methods outweigh the risks.

GUIDELINES ON MONITORING ERT

Current guidelines by the North American Menopause Society (NAMS) do not recommend routine hormone testing of menopausal women on HRT [34]. Although testing is not standard of care for ERT in females, testing is part of the course of treatment in testosterone replacement in males and with thyroid hormone therapy (and many others) [35]. This discrepancy could be attributed to the relatively low risk of serious side effects with commonly prescribed FDA-approved doses of ERT in women, and may also be due to the fact that serum, the testing medium typically employed by their physician population, is not an effective means to monitor estrogen therapy [36].

Hormone testing for monitoring ERT offers unique benefits to the patient by providing valuable insights into optimizing dosage and type of therapy to achieve the most favorable treatment outcomes.

HORMONE TESTING MAXIMIZES BENEFITS OF ERT

While it's reassuring to know that the safety profile of ERT offers a favorable benefit-to-risk ratio, ERT monitoring offers an opportunity to personalize treatment and therefore increase the likelihood of therapeutic success. Without monitoring, the one-size-fits-all prescribing misses the specific needs, characteristics, and preferences of individual patients [37]. Hormone testing, on the other hand, enables providers to practice a more holistic and patient-centered approach by tailoring ERT to maximize benefits, reduce side effects, and increase patient satisfaction.

In order for monitoring (of estrogen replacement therapy) to be useful, testing should be able to approximate daily exposure to estrogen and also correlate with clinical outcomes. With serum, a single timepoint fails to capture the up and down pattern observed with transdermal creams and gels.

The precision medicine-oriented approach prioritizes monitoring to optimize hormone prescriptions—a practice we endorse due to its considerable advantages, especially for patients on transdermal ERT. Specifically, monitoring of ERT helps address important questions:

1. Will the prescribed dose optimize treatment outcomes?

Dosage recommendations based on guidelines are usually structured around symptom management—a given ERT dose should provide enough estrogen to suppress disturbing

vasomotor symptoms (e.g., hot flashes). The issue with this approach is that the dosage of estrogen needed to address hot flashes might not be high enough to protect bone or cardiovascular health. This is because estrogen levels required to ensure bone health might be higher than what is needed for a particular woman's hot flash remediation. This is why, after initiating ERT, it is important to implement regular hormone testing to verify that the patient is within the optimal therapeutic range. Typically, healthcare providers strive to achieve therapeutic estrogen levels close to or into premenopausal luteal levels .

2. Does the testing methodology accurately represent the estrogen exposure over 24 hours?

Serum testing is conducted at a single time point and fails to represent the up and down pharmacokinetic pattern observed when estrogen is applied. DUTCH 4-point urine testing allows for an accurate estimation of estrogen exposure.

3. What is the individual estrogen metabolism pattern for each specific patient on ERT?

Understanding a patient's biochemical propensity toward forming certain types of estrogen metabolites is key to optimizing their ERT regimen. Conducting an initial baseline evaluation can assist the practitioner in understanding a patient's estrogen detoxification pathways. For instance, is estrogen being metabolized into potentially carcinogenic 4-hydroxy estrogen catechols, thereby raising the risk of breast cancer? Alternatively, is it being metabolized into proliferative 16-hydroxyestrone (16-OH-E1) compounds, which may contribute to symptoms such as heavy bleeding, breast tenderness, and fibroid growth?

MONITORING WITH SERUM

Serum testing is regarded as the "gold standard" method for assessing endogenous (not on therapy) sex steroid levels. Even on therapy, many providers prefer to test hormones in serum when possible, considering ease of access and cost to the patient. Serum may be a viable option for testing different types of biomarkers within the body, but measuring E2 levels in women on ERT may present challenges not anticipated with other analytes.

One obstacle and possibly a rationale for why routine monitoring is not part of the current guidelines for transdermal/topical ERT, is the rapid fluctuation of E2 in serum with certain estrogen therapies. In a 2002 study, Jarvinen and colleagues reported that E2 patch and gel applications gave rise to highly variable serum levels within and between subjects tested [38].

This outcome was reasonable because estradiol in a patch or gel is applied, absorbed, and metabolized, resulting in potential variability over time, contingent on the timing of the last application. Using a patch for delivery ensures a more consistent and continuous exposure of the skin to the hormone, leading to a steadier serum estradiol level over time. For this reason, serum may be a suitable method to monitor E2 in women on a steady-state dose such as patches but would be difficult for topical applications with periodic (ie daily) applications such as gels and creams.

There are notably fewer peer-reviewed publications scrutinizing the benefits and risks of compounded estrogen formulations, compared to FDA-approved gels and patches. In 2013, one of the only studies measuring serum levels after compounded topical estrogen cream application showed a similar time-dependent, fluctuating pattern of serum E2 [39]. This finding further supports the unreliable nature of serum measurements with cream or gel application—the time that the patient applies a cream or gel drastically influences the levels of hormone expected in serum.

It was recently shown that serum measurements may be erratic and unpredictable when women take oral E2 (different formulations and dosing). The authors concluded in this study that that serum E2 levels are not directly proportional to the estrogen dose used [40].

The most meaningful information to the provider is the patient's daily exposure to estrogen with the use of ERT. These insights would be represented more accurately by the area under the curve from a specific measurement. This is not something that can be estimated based upon only a single time point of a serum draw. Multiple serum tests on the same day and on the same patient may yield significantly different results, prompting consideration of which test result best reflects E2 exposure. A single serum test captures circulating levels at a single moment in time and may exhibit values lower or higher than average for a given E2 dose, depending on the timing of blood collection. This variability is influenced by both inter- and intra-individual differences in pharmacokinetics and route of administration.

So unfortunately this means that it may be easy to overdose a patient using the single-measurement serum testing if the provider is unaware of this potentially rapid rise-and-fall of hormone serum levels. Serum provides accurate measurements and could serve as a suitable monitoring method provided that the fluctuations in hormone levels are taken into consideration or minimized, as is the case with patch application. The potential for overdosing increases if providers target serum levels higher than the 20-60 pg/mL levels (reported to correlate with clinical improvement).

Baseline postmenopausal E2 level: < 10 pg/mL

Goal Range for "on-therapy" E2 Levels in Serum: 20-60 pg/mL (some providers may aim for as high as 100 pg/mL in serum, but studies do not demonstrate that is necessary)

MONITORING WITH DUTCH

To overcome the limitations of serum monitoring, testing hormones continuously over a 24-hour period captures a more accurate picture of daily "on therapy" values, offering comprehensive insights into whether results levels are within the optimal range or not. To further simplify the process of sample collection, dried urine testing provides a significant advantage with its convenient and straightforward at-home collection method. The levels of E2 in urine generally parallel clinical outcomes the way that serum measures do with transdermal E2 gels (DUTCH data), creams (DUTCH data), and patches (DUTCH and published data). (Another advantage of the DUTCH test is that includes valuable insights about estrogen metabolites.)

Recently published clinical literature has introduced dried urine-based monitoring for topical ERT as a viable method to assess hormone levels and treatment efficacy. The study evaluated urinary estrogen levels in cycling females, postmenopausal females not on therapy, and postmenopausal females on various doses of ERT and observed expected dose-proportional changes in estrogen exposure in the “on-therapy” groups [41].

In another, similar study, estrogen exposure from transdermal patches was evaluated in dried urine, demonstrating a dose-dependent increase in urinary E2, other estrogen metabolites and total urinary estrogen [42].

In both of these studies, clinically effective doses of ERT (based upon previously published clinical outcome studies) resulted in urinary levels of E2 between the postmenopausal (not on therapy) and mid-luteal ranges (in cycling females). This may represent a suitable therapeutic target for women on ERT. As with serum testing, when providers target the middle of the luteal range, they may use excessive doses of estradiol. If, however, careful consideration is given to the levels needed for clinical success, appropriate dosing and absorption can be affirmed using urine testing.

A very recent study compared E2 exposure from compounded estrogen creams to previously published data on patches and gels [43]. The study reported that estrogen exposure from compounded creams also increased in a dose-dependent manner, but that the systemic estrogen exposure from creams was lower than that of gels and patches. Dried urine was shown to be an effective method for assessing estrogen exposure across all types of transdermal estrogen replacement therapy.

Measuring E2 in urine with the published 4-point collection (4 urine samples are collected within a 24-hour period) for topical routes of administration of estrogen therapies may help to average out the daily fluctuation patterns that are seen in serum after the application of creams and gels. The aggregate clinical data suggest that an “on therapy” serum E2 level of about 20-60 pg/mL improves clinical outcomes of vasomotor symptoms and bone mineral density. This correlates with an approximate dried urine value of about 0.7-1.8 ng/mg using the test methodology in the recently published studies using dried urine.

Baseline postmenopausal E2 range: 0.2-0.7 ng/mg

Goal Range for “on-therapy” E2 Levels in DUTCH: 0.7-1.8 ng/mg (for bone support)

MONITORING WITH SALIVA

Providers are often surprised to learn there is very little data to evaluate saliva as an appropriate way to monitor estrogen replacement therapy. As it is, there is limited research available that evaluates E2 levels in saliva with concomitant use of topical estrogen gel . In a single study, an estrogen gel formulation was compared to an experimental estrogen gel with nanoparticle delivery. The authors reported that application of a 0.75 mg dose of a standard estrogen gel resulted in salivary levels over 2000 pg/mL.

Saliva labs in our industry report expected luteal range values in cycling females to be well below 10 pg/mL and baseline postmenopausal levels in this study were less than 1

pg/mL. This would imply that a 0.75 mg dose of estrogen gel, which is a moderate dose per guidelines and clinical studies, results in almost 100-fold higher estradiol levels than the premenopausal luteal range. This level of estrogen exposure is certainly excessive—far beyond what is necessary—and does not correspond with published clinical studies indicating that a 0.75 mg dose of estrogen gel is effective (without causing an estrogen overdose).

If you were to assume that saliva results represented tissue exposure and rely on those results to adjust your dose of estradiol for a patient, you would likely choose substantially lower doses of ERT. This is what we observe in integrative practice. Providers who use saliva to monitor (and make clinical decisions) in essence, "microdose" estradiol to patients.

Said another way, relying on the elevated results found in saliva, and presuming that these levels more accurately reflect the clinical situation, renders providers who utilize saliva measurements to choose substantially lower doses of ERT. This is why saliva testing should not be used with creams or gels—the exaggerated values do not appear to correlate to any studied clinical outcomes. And the doses often selected based upon saliva results do not often reach doses which have been shown to be clinically effective, for example, for bone protection. This puts patients significantly at risk, or at best they do not appreciate the full benefits offered by ERT.

NOTE

> *The phenomenon of supraphysiological saliva levels using moderate doses of transdermal estrogen products is true only of gels and creams. Saliva values for patients on estradiol patches are more in alignment with serum and urine values.*

There is little data to rely on to understand whether saliva could serve as a viable means of monitoring topical ERT. As it is, currently, the data that has been published points clearly fails to support its use.

CONCLUDING REMARKS

In the realm of integrative and functional medicine, practitioners frequently find themselves responsible for evaluating scientific evidence to guide their treatment decisions. This applies to both therapeutic interventions and diagnostic procedures. However, when it comes to hormone testing and monitoring, this task can be particularly challenging. Much of the available education on this topic comes directly from laboratories, which may present claims without sufficient empirical support or rely on data that have not undergone rigorous peer review.

Regarding the monitoring of E2 levels in women undergoing ERT, serum measurement is considered suitable for steady-state applications like patches. For treatments involving gels, creams, and patches, urine monitoring through a 4-point dried urine collection method should be utilized, provided that validated protocols are followed. Lastly, the use of saliva for hormone monitoring lacks peer-reviewed data, and the existing studies do not align with clinical outcomes data. Saliva should not be used to monitor estrogen replacement therapy.

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