Systemic Estradiol Levels with Low-Dose Vaginal Estrogens May Differ by Dose and by Product

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Introduction

- Low-dose vaginal estrogens are approved to treat the signs and moderate to severe symptoms (eg, dyspareunia) of vulvar and vaginal atrophy (VVA). However, surveys show that many menopausal women are hesitant to use vaginal estrogens because of the potential of systemic absorption,[1,2] even though local estrogens minimize systemic absorption.[3,4]
- Systemic absorption of estradiol (E2) with low-dose and ultra-low-dose vaginal estrogens is very low, but varies by assay detection type and product and/or dose
- Measuring serum E2 levels can be challenging as different assays detect different levels of serum E2 based on different cross-reactivities and specificities.[5] More specific assays (ie, gas or liquid chromatography/mass spectroscopy [GC or LC/MS/MS]) have less cross-reactivity with other steroids and typically yield lower values than less specific assays with more cross-reactivities (ie, radioimmunoassay [RIA] or enzyme-linked immunosorbent assay [ELISA]).[5,6]
- Two head-to-head comparative studies[7,8] show relatively higher serum E2 levels using RIA compared with GC/MS/MS; basal E2 levels for untreated, postmenopausal women have been historically reported as a range of 3 to 30 pg/mL from studies that detected E2 levels using RIAs (as reviewed)[5]; highly specific assays using LC/MS/MS or GC/MS/MS methodologies quantify systemic E2 as low as 0.5 pg/mL to 3 pg/mL[3,9-16]
- Positioning of the product, higher in the vagina (with an applicator) or lower in the vagina (without an applicator), may also influence E2 absorption with vaginal estrogens, based on the vascularity of the vagina[17]
- Despite minimal systemic absorption of estradiol with vaginal estrogens, approved vaginal estrogen therapies are required to include a boxed warning section ("black box") similar to systemic estrogen therapies

Objective

To review systemic E2 levels with various low-dose and ultra-low-dose vaginal estrogens measured by various methods, and evaluate basal levels of E2 reported in normal, untreated postmenopausal woman

Methods

- A review of E2 absorption with vaginal estrogens was published in 2015.[5] We have updated data of that review by searching for relevant studies of lower-dose products reported since that publication. PubMed was searched in July 2018 for the past 5 years for pharmacokinetic studies that examined systemic E2 levels in postmenopausal women using combinations of keywords that included estradiol, estrogen(s), dehydroepiandrosterone (DHEA), prasterone, vaginal, and pharmacokinetic. General information on E2 assay development was also sought.
- Studies of low-dose vaginal estrogens (25 µg E2 or 0.3 mg conjugated equine estrogens) and ultra-low-dose vaginal estrogens (≤10 µg estradiol) were included based on current availability and use of these products.
- Studies were excluded if E2 levels were measured in women taking aromatase inhibitors
- Mean E2 levels (at baseline and at various time points with treatment or placebo [or in untreated women]), E2 area under the curve (AUC), and maximum E2 concentrations (C_{max}) were extracted from tables or text of retrieved articles; if not reported in text or table, E2 levels were estimated from figures illustrating the data. Any E2 levels reported as pmol/L were converted to pg/mL.
- E2 levels from the studies were summarized by detection assay type, including GC/MS/MS, LC/MS/MS, bioassay, RIA, and ELISA, and then by dose

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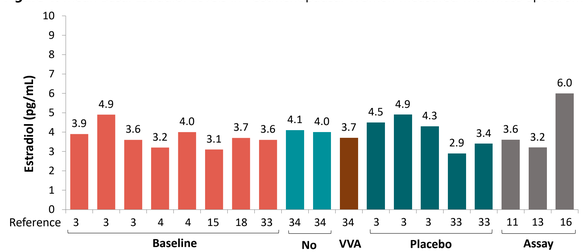
Results

Basal Systemic E2 Levels in Normal, Untreated Postmenopausal Women

- Mean basal levels of estradiol were 3.1–4.9 pg/mL using GC/MS/MS,[3,4,15,18] 1.0–3.0 pg/mL using a bioassay,[19] undectable–14 pg/mL using RIA,[20-31] and 7.6 pg/mL using ELISA[32] (**Table 1**)
- **Figure 1** shows mean basal systemic E2 levels measured with GC/MS/MS or LC/MS/MS in postmenopausal women to help establish a more appropriate postmenopausal E2 range
  - At baseline, prior to vaginal therapy administration (3.1–4.9 pg/mL)[3,4,15,18,33]
  - Untreated with or without VVA (3.7–4.1 pg/mL)[34,35]
  - Treated with placebo (2.9–4.5 pg/mL)[3,33]
  - In assay validation studies (3.2–6.0 pg/mL)[11,13,16]
- One study in postmenopausal women found 1.3 pg/mL in women >5 years postmenopausal and 4.9 pg/mL in women <5 years postmenopausal[36]

Figure 1. Mean Basal Estradiol Levels in Postmenopausal Women Measured with Mass Spectrometry



Baseline is in postmenopausal women (PMW) with VVA in trials at baseline prior to vaginal estrogens[3,4,15,18] or dehydroepiandrosterone (DHEA)[33]; **No VVA** is PMW without VVA in various clinical trials[34,35]; **VVA** is PMW with VVA from trials of vaginal DHEA[34]; **Placebo** is PMW given placebo in vaginal therapy trials[3,33]; **Assay** is PMW whose E2 levels were measured in assay validation studies[11,13,16]

Systemic E2 Levels with Vaginal Estrogen Therapies

- As with basal E2 levels, E2 levels with vaginal estrogens were generally lower when measured by LC/MS/MS or GC/MS/MS versus RIA (**Table 1**)
- The extent of E2 systemic absorption reflected the doses ranging from 4 µg to 25 µg of E2 as demonstrated by the C_{max}, C_{avgr} and $AUC_{0\text{-}24}$ (within assay type; **Table 1**)
  - In the only head-to-head study, significantly lower E2 systemic absorption was observed with 10 µg and 25 µg softgel capsules versus tablets of the same doses[37]
  - Mean E2 levels with 4 µg and 10 µg E2 capsules were similar to those at baseline or with placebo at days 1 and 14[3]

Factors That Influence Estradiol Absorption with Vaginal Estrogens

- Four studies found peak and/or average E2 levels higher with early measures on the first day of treatment versus 14 days to 12 weeks after treatment.[3,4,23,31] Higher initial E2 absorption early in a study may be due to women having a thinner vaginal epithelium (atrophic) prior to treatment compared with a thicker vaginal epithelium with estrogenization.
- Lower E2 systemic absorption was observed with capsules inserted without an applicator (lower in the vagina [1-2 inches or up to the second digital joint]) versus tablets of the same doses inserted with an applicator (higher in the vagina [as far as comfortably possible, or until half of the applicator is inside, whichever is less]) in a head-to-head study[37]

Table 1. Mean Systemic E2 Levels at Baseline and Throughout Vaginal Estrogen Studies by Assay Type and Dose

Assay	Dose	Treatment	n	Study duration	Mean BL (pg/mL)	Mean C_{max} (pg/mL)	Mean C_{avg} or level reported (pg/mL)	Mean $AUC_{0\text{-}24}$ (pg · h/mL)	Reference
GC/MS/MS	25 µg	E2 softgel capsule/Placebo	18/17	12 wks	3.6 (E2)/4.5 (P)	Day 1: 29.8 (E2); 6.6 (P)/Day 14: 15.7 (E2); 5.5 (P)	Day 1: 9.1 (E2); 4.9 (P)/Day 14: 7.1 (E2); 4.3 (P)	Day 1: 217.4 (E2); 116.6 (P)/Day 14: 171.6 (E2); 104.2 (P)	Archer et al 2017[3]
LC/MS/MS	25 µg	E2 softgel capsule (single dose)	36	1 d	NR	31.0	NR (9.1)	218.7	Data on file[37a]
		E2 tablet (single dose)	36	1 d	NR	59.8	NR (22.7)	545.4	Data on file[37a]
GC/MS	25 µg	E2 tablet	27	83 d	4.03	Day 1: 43/Day 14: 25/Day 83: 18	Day 1: 19.8/Day 14: 18.3/Day 83: 9.4	Day 1: 476.1/Day 14: 438.9 Day 83: 225.9	Eugster-Hausmann et al 2010[4]
GC/MS	25 µg	E2 tablet	10	7 d	3.12	21.43	16.7	401	Labrie et al 2009[15]
GC/MS/MS	10 µg	E2 softgel capsule/Placebo	19/17	12 wks	4.9	Day 1: 10.9 (E); 6.6 (P)/Day 14: 7.3 (E) 5.5 (P)	Day 1: 5.8 (E) 4.9 (P)/Day 14: 4.6 (E) 4.3 (P)	Day 1: 138.2 (E) 116.6 (P)/Day 14: 110.1 (E) 104.2 (P)	Archer et al 2017[3]
LC/MS/MS	10 µg	E2 softgel capsule (single dose)	35	1 d	NR	21.2	NR (7.4)	178.2	Data on file[37a]
		E2 tablet (single dose)	35	1 d	NR	32.5	NR (14.8)	355.2	Data on file[37a]
GC/MS	10 µg	E2 tablet	29	83 d	3.15	Day 1: 24/Day 14: 8/Day 83: 7.5	Day 1: 9.4 /Day 14: 6.6/Day 83: 4.6	Day 1: 225.35/Day 14: 157.47/Day 83: 111.41	Eugster-Hausmann et al 2010[4]
GC/MS/MS	4 µg	E2 softgel capsule/Placebo	18/17	12 wks	3.9	Day 1: 6.5 (E); 6.6 (P)/Day 14: 4.8 (E); 5.5 (P)	Day 1: 3.9 (E); 4.9 (P)/Day 14: 3.6 (E); 4.3 (P)	Day 1: 91.7 (E); 116.6 (P)/Day 14: 87.2 (E); 104.2 (P)	Archer et al 2017[3]
GC/MS/MS	0.3 mg	CEE cream	24	7 d	3.7	12.8	NR (9.6)	231	Dorr et al 2010[18]
Bioassay	10 µg	E2 cream[b]	7	12 wks	2.0 (E2)/1-3 (U)	Initial: 3.1/Week 3: 3.8/Week 12: 5.7	NR	NR	Santen et al 2002[19]
RIA	25 µg	E2 tablet	59	48 wks	4.1	NR	Weeks 24 & 48: 9.79	NR	Weisberg et al 2005[20]
RIA	25 µg	E2 tablet	19	12 wks	7.0	Day 1: 51/Week 12: 49	Day 1: 22/Week 12: 23	Day 1: 538/Week 12: 563	Notelovitz et al 2002[21]
RIA	25 µg	E2 tablet	6	12 wks	7.5	20.4	Week 2: NR (15.8)/Week 12: NR (16.4) 13.6 (after last tablet)	Week 2: 380.0/Week 12: 392.5	Nilsson and Heimer 1995[22]
RIA	25 µg	E2 tablet	24	14 d	9.5	Day 1: 42.8/Day 14: 21.8	Day 14: 16 (24h)	NR	Nilsson and Heimer 1992[23]
RIA	25 µg	E2 tablet (once weekly)	17	52 wks	10.5	NR	Week 2: 10.1/Week 12: 9.9/Week 52: 10.8	NR	Mettler and Olsen 1991[24]
RIA	25 µg	E2 tablet (twice weekly)	34	52 wks	9.6	NR	Week 2: 9.3/Week 12: 9.6/Week 52: 11.3	NR	Mettler and Olsen 1991[24]
RIA	25 µg	E2 tablet	80	24 wks	NR (≤30)[c]	NR	Week 24: 5% of women >49[e]	NR	Rioux et al 2000[25]
RIA	10 µg	E2 tablet	23	12 wks	7.6	Day 1: 35/Week 12: 22	Day 1: 15/Week 12: 11	Day 1: 349/Week 12: 264	Notelovitz et al 2002[21]
RIA	10 µg	E2 tablet	24	14 d	9.5	Day 1: 24.5/Day 14: 15.0	Day 14: 13, hour 24	NR	Nilsson and Heimer 1992[23]
RIA	10 µg	E2 tablet	336	52 wks	5.2	NR	Week 52: 6.05	NR	Ulrich et al 2010[26]
RIA	0.3 mg	CEE cream	20	4 wks	8 (with placebo)	NR	13	NR	Mandel et al 1983[27]
RIA	7.5 µg/d	E2 ring/Untreated	27/27	12 mos	3.7 (E)/4.2 (U)	NR	4.20 (E)/4.09 (U)	NR	Naessen et al 2002[28]
RIA	7.5 µg/d	E2 ring/Untreated	20/10	6 mos	4.4 (E)/ 3.5 (U)	NR	6 mos: 7.9 (E); 3.0 (U)	NR	Naessen et al 1997[29]
RIA	8.0 µg/d	E2 ring	126	48 wks	4.4	NR	Week 24: 13.3/Week 48: 5.5	NR	Weisberg et al 2005[20]
RIA	9.0 µg/d	E2 ring	222	24 wks	<5.5 (UD)	NR	Within postmenopausal range[d]	NR	Smith et al 1993[30]
RIA	6.6 µg/d	E2 ring	11	84 d	<12.3 (UD)	Median at 2h: 64.5 (range 29.4-158.8)	Median Day 2: 19.1/Days 7, 14, 28, 84: <12.3 (UD)	NR	Holmgren et al 1989[31]
RIA	20.2 µg/d	E2 ring	11	84 d	<12.3 (UD)	Median at 2h: 96.4 (range 72.7-231.8)	Median Day 2: 51.8/Days 7, 14, 28, 84: <12.3 (UD)	NR	Holmgren et al 1989[31]
ELISA	25 µg	E2 tablet	27	12 wks	7.6	NR	8.9	NR	Manonai et al 2001[32]
IC	25 µg	E2 tablet/Placebo	828/784	12 mos	15.7 (E)/14.2 (P)	NR	4 mos: 17.3 (E); 15.1 (P)/12 mos: 15.5 (E); 13.8 (P)	NR	Simunic et al 2003[38]
NR	25 µg	E2 tablet	96	24 wks	<2.7	NR	<2.7[e]	NR	Dugal et al 2000[39]

$AUC_{0\text{-}24}$=area under the curve over 24 hours; BL=baseline; C_{avg}=average concentration; C_{max}=maximum concentration; CEE=conjugated equine estrogens; d=days; E2=estradiol; ELISA= enzyme-linked immunosorbent assay; IC=immunochemical (Ortho-Clinical Diagnostics US); mos=months; NR=not reported (for average E2 levels NR, $AUC_{0\text{-}24}$ [if reported] was divided by 24); RIA=radioimmunoassay; U=untreated; UD=undetectable (near detection limit); wks=weeks.

[a]From same study reported by Pickar et al[10]
[b]Diluted Estrace given daily during first 3 weeks then twice weekly for 9 weeks[24]
[c]Reported percentage of women who had levels > postmenopausal range (>49 pg/mL); basal level of ≤30 pg/mL based on inclusion criteria
[d]E2 levels were in postmenopausal range (27.2-68.1 pg/mL) and below limit of detection (5.5 pg/mL) before and during (time not specified) treatment[15]
[e]Blood E2 levels were within the normal range for postmenopausal women (<2.7 pmol/L) after 24 weeks of treatment[19]

Conclusions

- Consistent with our previous review,[5] systemic E2 levels in postmenopausal women from studies of vaginal estrogens vary by assay and estrogen dose
  - Systemic E2 levels in postmenopausal women with various low-dose and ultra-low--dose vaginal estrogen therapies vary by assay and estrogen dose and product
  - Lower levels of E2 absorption were found with LC/MS/MS and GC/MS/MS versus RIA; more sensitive MS-based methods should be used to detect low E2 levels in postmenopausal women
- Basal levels of E2 absorption range from 2.9 to 4.9 pg/mL in postmenopausal women, with or without VVA, untreated or before treatment, when measured using mass spectrometry
- In studies with ultra-low–doses of 4 µg and 10 µg of an estradiol softgel vaginal capsule insert, mean peak levels of E2 due to absorption are similar to basal E2 levels and E2 levels with placebo, while those in separate studies with a 10 µg estradiol vaginal tablet insert are relatively higher (all measured by GC/MS/MS)
- Differences in the physical characteristics of their formulations, as well as positioning in the vagina, may affect E2 absorption with vaginal estrogens

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