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Plasma estriol and its conjugates following oral and vaginal administration of estriol to postmenopausal women: Correlations with gonadotropin levels

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A study was designed to compare the metabolic fate and the biologic effects of 4 mg of estriol (E_3) administered either orally or vaginally to six postmenopausal women. Blood samples were collected every hour for 6 hours and five different estriol fractions as well as gonadotropins were measured. Vaginal E_3 administration resulted in a decline of 45% in luteinizing hormone (LH) levels and 17% in follicle-stimulating hormone (FSH) levels at 6 hours after treatment ($p < 0.05$). In contrast, the administration of 4 mg of E_3 orally did not produce a decline of LH and FSH, despite the fact that the serum levels of E_3-3-sulfate, E_3-3-sulfate-16-glucosiduronate, estriol-3-glucosiduronate, and estriol-16-glucosiduronate were all fourfold to 24-fold higher after oral administration than after vaginal estriol administration. However, since the levels of unconjugated E_3 were higher after the vaginal than after the oral administration of estriol, we conclude that only unconjugated E_3 suppresses gonadotropins. (AM. J. OBSTET. GYNECOL. 138:1137, 1980.)

IN AN EARLIER REPORT from one of our laboratories, we have shown that estrogens administered vaginally are absorbed systemically and are biologically active.[1] We subsequently extended these studies and found that 0.5 mg of estriol (E_3) inserted vaginally in postmenopausal women caused the same minimal decrease in the concentration of serum luteinizing hormone (LH) as 8 mg given orally.[2] Therefore, it can be predicted that administration of 4 mg of E_3 orally will have little or no effect on LH values while the administration of an equivalent dose vaginally will have a dramatic effect. To determine why the route of E_3 application was critical for determining its biologic effect, we quantified and identified the various forms of E_3 found in the serum after the oral and vaginal administration of 4 mg of E_3.

Subjects and methods

Six hypogonadal women visiting the menopause clinic, after signing informed consent, volunteered to participate in this study. Two women (Numbers 3 and 5), aged 50 and 65, were postmenopausal and four (aged 35, 51, 62, and 64) had previously undergone oophorectomy for benign gynecologic diseases. None had received any drugs or hormones for at least 2 months prior to entering the study and all had vasomotor symptoms.

Each patient was studied twice, once after receiving 4 mg of E_3 orally and again, 4 weeks later, after having 4 mg of E_3 dispensed in 2 ml of saline, placed in the vagina. Each study was always begun at 8:00 to 9:00 AM

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Table I. Concentration of FSH in serum of subjects following oral (O) and vaginal (V) administration of E_3

	Subject 1		*Subject 2*		*Subject 3*		*Subject 4*		*Subject 5*		*Subject 6*		*Mean ± SE*	
Hour	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*
Baseline	112	104	166	>200	>200	>200	122	184	44	72	88	88	120.3 ± 22.4	141.3 ± 24.3
1	−11	+8	−18	0	−36	−18	+80	−11	+9	−5	−36	−16	−2.2 ± 17.6	−7.2 ± 4.0
2	−18	+2	−13	+16	−28	−22	+80	−7	−2	−20	−46	+11	−4.8 ± 17.7	−3.2 ± 6.4
3	−30	−40	−1	−22	−18	0	+80	−17	−2	−8	−36	−16	−1.6 ± 17.0	−17.4 ± 5.3*
4	−41	−12	−18	−28	−22	−18	+80	−22	−16	−20	−36	−16	−9.1 ± 18.0	−19.2 ± 2.3*
5	−25	−8	−13	−28	−30	−24	+80	−11	+9	0	−11	−16	1.3 ± 16.4	−14.4 ± 4.2*
6	0	−17	−11	−32	−12	−12	+80	0	−9	−8	−30	−11	2.8 ± 15.6	−13.5 ± 4.4*

Values are in milli–International Units per milliliter for baseline followed by percent change from baseline for 6 succeeding hours.

*$P < 0.05$ (significant as compared to baseline values).

Table II. Concentration of LH in serum of subjects following oral (O) and vaginal (V) administration of E_3

	Subject 1		*Subject 2*		*Subject 3*		*Subject 4*		*Subject 5*		*Subject 6*		*Mean ± SE*	
Hour	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*
Baseline	29	36	60	84	41	96	70	88	47	50	26	27	45.5 ± 7.0	63.5 ± 12.0
1	−14	−17	−70	−24	+80	−24	0	−1	−38	−4	−12	+4	−9.0 ± 20.4	−14.8 ± 4.9
2	+3	−39	−26	−52	+71	−21	+3	−8	−16	−16	−23	−23	2.0 ± 22.2	−26.5 ± 6.6*
3	−17	−42	−32	−56	+100	−41	+3	−31	−40	−44	−45	−38	5.5 ± 22.2	−42.0 ± 3.4*
4	+66	−58	−37	−38	+70	−42	−25	−42	−32	−44	−46	−19	−0.3 ± 21.8	−40.5 ± 5.1*
5	+34	−39	−25	−31	+107	−41	−15	−52	−40	−46	−23	−36	11.0 ± 22.2	−40.8 ± 3.0*
6	+86	−66	−30	−48	+114	−62	−28	−30	−38	−46	−62	−38	16.8 ± 29.0	−48.3 ± 5.6*

Values are in milli–International Units per milliliter for baseline followed by percent change from baseline for 6 succeeding hours.

*$P < 0.05$.

after overnight fasting. Samples for determination of baseline blood levels were drawn through an intravenous heparinized catheter twice before the drug administration and again every hour for 6 hours. Serum was separated and stored at −20° C until analyzed.

LH and follicle-stimulating hormone (FSH) were measured by radioimmunoassay.[3] The various E_3 fractions were measured as follows.

For unconjugated E_3 2 ml of serum was extracted twice with 3 ml of ether. ^{3}H-E_3 (500 cpm) was added to the extracts, which were then evaporated. The extracts were dissolved in 3 ml of water and extracted first with benzene-hexane (2 × 3 ml), then with ether (2 × 3 ml). The ether was evaporated, dissolved in benzene-methanol (85-15), and transferred to a column (10 by 0.7 cm) of Sephadex LH-20 suspended in the same solvent.[4] The E_3 was eluted with benzene-methanol (85-15) in the 4 to 6 ml cut. The solvent was evaporated, 1 ml of ethanol was added to the samples, and 0.2 ml was taken for estimation of recovery. Appropriate aliquots, usually 0.2 ml, were submitted to radioimmunoassay as described.[5]

The residual serum which remained following the extraction of the unconjugated estriol with ether, was assayed for the four major conjugates of estriol, namely, estriol-3-sulfate-16-glucosiduronate* (E_3-SG),[1] estriol-3-sulfate† (E_3-3S), estriol-16-glucosiduronate (E_3-16G), and estriol-3-glucosiduronate‡ (E_3-3G) as previously described.[6] The aliquots submitted to radioimmunoassay were adjusted so that the counts per minute fell on the appropriate portion of the standard curve. The coefficient of variation of these methods was less than 12%.

Statistical analysis. The FSH and LH levels measured at each hour after the E_3 administration were compared to those before treatment (baseline) and the percent change from baseline was calculated. The mean levels during the oral and vaginal routes were compared by means of the paired t test.[7]

Results

The effects of oral and vaginal E_3 administration on serum FSH and LH levels are shown respectively in Tables I and II and in Fig. 1. The vaginal administration of 4 mg of E_3 was associated 3 to 6 hours later with a fall of 13.5% ± 4.4% (SEM) to 19% ± 2.3% (SEM)

*17β-Hydroxyestra-1,3,5(10)-trien-3-*yl*-sulfate-16α-yl-β-D-glucopyranosiduronate.

†16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-*yl*-sulfate.

‡16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-*yl*-β-D-glucosiduronate.

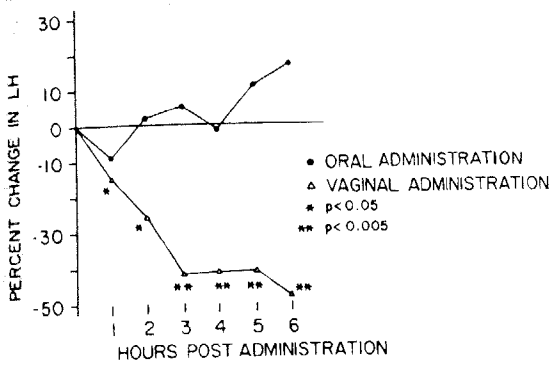

Fig. 1. The percent change in serum LH concentrations over 6 hours following the oral and vaginal administration of 4 mg of E_3.

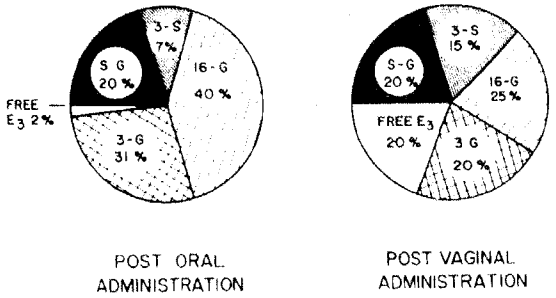

Fig. 2. The distribution in serum of the various conjugates of E_3 as well as unconjugated E_3 (free) after the administration of 4 mg of oral or vaginal E_3. The mean total E_3 concentration during the 6 hours after its administration was considered as 100%, and the mean percentages of unconjugated (free) E_3 and of E_3-3G, E_3-16G, E_3-3S, and E_3-SG are depicted.

($p < 0.05$) in FSH levels and 40.5% ± 5.1% (SEM) to 48.3% ± 5.6% (SEM) ($p < 0.05$) in LH levels. In contrast, the oral E_3 administration had no effect on FSH and LH levels ($p > 0.05$).

To elucidate the reasons for the differences in biologic activity exerted by the vaginal and oral E_3, we have studied the metabolic fate of the administered E_3 in detail. Data on the concentrations of four different E_3 conjugates as well as unconjugated E_3 of one representative patient are presented in Table III. Data on the conjugates of the other five patients were very similar to those of this patient and are not presented. In the oral phase of the study, there was a prompt increase in the concentrations of all four conjugates in the blood. Throughout the 6-hour period E_3-16G and E_3-3G accounted for greater than two thirds of the total E_3 measured. However, a decline in the concentrations of E_3-16G and a reciprocal increase in those of E_3-3G resulted in a predominance of E_3-3G at the later period. The concentration of E_3-SG and E_3-3S also declined in the latter 2 hours of the study, whereas the concentrations of unconjugated E_3 remained extremely low, never exceeding 0.031 ng/ml throughout the 6-hour study period.

The serum E_3 profile after E_3 vaginal administration was quite different. The total concentration of E_3 in serum after its vaginal administration was only about one tenth that observed after its oral administration ($p < 0.05$). The percent contribution of each form of E_3 to the total remained fairly constant throughout the 6-hour period, with E_3-16G predominating (Fig. 2). However, the most striking differences were found in the concentrations of serum unconjugated (free) E_3, which were much higher after vaginal than after oral E_3 treatment.

Since the important differences in the two modes of administration of estriol appear to be in the concentrations of unconjugated E_3, these data are presented in detail in Table IV. Within 1 to 2 hours after vaginal E_3 administration, serum unconjugated E_3 constituted approximately 30% of the total circulating E_3 and its contribution decreased to 15% to 17% in the next 4 hours. In contrast, in three of the patients, during the first 5 hours after oral E_3 treatment, the unconjugated E_3 concentration did not exceed 1% of the total circulating E_3 and its concentration amounted to less than 10% of that measured after vaginal E_3 treatment. Patients 5 and 6 did have elevations of unconjugated estriol at the sixth hour after treatment and this was reflected by a fall in LH.

Comment

The concept of administering E_3 to patients requiring estrogen replacement stems from two lines of investigation. First, experiments with rats suggest that E_3 is a weak estrogen or even an estrogen antagonist.[8, 9] Second, epidemiologic studies based on assays of urinary E_3 suggest that E_3 may be protective against breast cancer.[10] On close scrutiny, conclusions based on both lines of investigation may be flawed. For example, in the rat chronically treated with E_3, specific estrogen effects normally elicited by estradiol are observed.[11] Moreover, studies have shown that E_3 can stimulate the growth of breast tumor cells maintained in culture.[12] In the light of these apparent contradictions, we feel that further studies on the effects of E_3 should be undertaken.

In this study, we have confirmed that E_3 possesses biologic activity in women. This is apparent from the prompt suppression of LH that followed vaginal E_3 administration. The corresponding decrease in FSH was less impressive, but these differences may be attributed in part to the incidental increase in FSH levels

Table III. Concentrations of different forms of E_3 in serum following the oral and vaginal administration of E_3 in Subject 1

Route of administration	Hour	E_3-SG*		E_3-3S		E_3-16G		E_3-3G		E_3	
		ng/ml	%†	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%
Oral	0‡	0.057	26	0.0	0	0.103	47	0.061	28	<0.01	0.00
	1	2.7	18	1.8	12	6.0	41	4.2	29	0.031	0.1
	2	6.7	27	1.6	7	11.8	48	4.6	19	0.006	0.1
	3	2.4	19	1.7	13	5.4	42	3.4	26	0.00	0
	4	1.3	14	1.3	14	3.5	38	3.1	34	0.005	0.1
	5	1.4	9	1.7	11	5.9	38	6.4	42	0.014	0.1
	6	1.8	10	1.1	6	4.9	28	9.5	55	0.015	0.1
Vaginal	0‡	0.22	81	0.00	0	0.025	9	0.027	10	<0.010	<50
	1	0.24	14	0.29	17	0.61	35	0.17	10	0.46	26
	2	0.25	14	0.40	22	0.53	30	0.19	11	0.42	24
	3	0.22	16	0.33	24	0.43	32	0.16	12	0.23	16
	4	0.16	14	0.26	22	0.38	33	0.15	13	0.21	18
	5	0.22	16	0.32	23	0.44	32	0.21	15	0.21	15
	6	0.17	13	0.29	21	0.52	38	0.18	13	0.20	15

*Abbreviations of E_3 conjugates are in text.

†Percent of total circulating E_3.

‡Baseline concentrations just prior to administration of 4 mg of E_3.

Table IV. Concentrations of unconjugated E_3 in serum following the oral and vaginal administration of E_3 in five subjects

Route of administration	Hour	Subject 2		Subject 3		Subject 4		Subject 5		Subject 6		Mean ± SE	
		pg/ml	%*	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%
Oral	0†	0	<0.1	19	22	0	<0.1	0	<0.1	11	6.5	5.0 ± 3.3	4.8 ± 3.6
	1	19	<0.1	29	<0.1	30	<0.1	6	<0.1	98	0.3	35.5 ± 13.1	0.1 ± 0.03
	2	7	<0.1	6	<0.1	0	<0.1	5	<0.1	95	0.2	19.8 ± 15.1	0.1 ± 0.01
	3	8	<0.1	9	<0.1	16	<0.1	6	<0.1	58	0.2	16.2 ± 8.6	0.1 ± 0.01
	4	17	<0.1	19	<0.1	19	<0.1	2	<0.1	140	0.7	33.7 ± 21.5	0.2 ± 0.1
	5	9	<0.1	56	<0.1	52	<0.1	124	0.5	373	3.2	104.7 ± 56.0	0.7 ± 0.5
	6	0	<0.1	10	<0.1	21	<0.1	113	0.4	1,860	10.8	336.5 ± 305.2	1.9 ± 1.7
Vaginal	0†	0		8	1.6	0		0		0		3.0 ± 1.9	0.3 ± 0.2
	1	143	17	839	27	248	51	263	11	921	52	47.9 ± 133.9	30.6 ± 7.0
	2	260	19	806	20	430	40	474	14	1,730	58	686.6 ± 221.1	29.2 ± 6.8
	3	323	19	534	13	668	40	234	8	322	21	385.2 ± 72.3	19.5 ± 4.5
	4	292	20	546	18	391	28	193	7	230	15	310.3 ± 35.6	17.7 ± 2.7
	5	206	13	483	17	320	24	317	12	215	17	291.8 ± 43.9	16.3 ± 1.7
	6	173	13	555	20	215	19	242	8	173	13	259.7 ± 60.0	14.6 ± 1.8

The mean represents the unconjugated E_3 for all six patients.

*Contribution to the calculated total E_3. The data for individual conjugates are not shown since they were similar to those those in Table III.

†Baseline concentrations just prior to administration of 4 mg of E_3.

observed in one patient (No. 4) as well as to the longer half life of FSH (3 hours) compared to that of LH (30 minutes).[13] Thus, studies of longer duration would be required to fully assess the influence of E_3 on serum FSH in postmenopausal women.

The acute administration of vaginal but not oral E_3 elicited gonadotropin suppression, and this difference may be attributed to the different routes of E_3 transport and metabolism. While the absorption of E_3 was rapid after oral or vaginal E_3 treatment, the total plasma concentration of E_3 in blood was higher after oral than after vaginal E_3 administration (15 to 64 versus 1.0 to 3.0 ng/ml). This may be only partially explained by possible loss of some of the material from the vagina. Moreover, after oral E_3 administration, the main circulating E_3 conjugates were E_3-16G and E_3-3G. Since the intestines are particularly rich in 3- and 16-glucuronyl transferase activities, it appears that the bulk of ingested E_3 was converted to the biological inactive estriol glucosiduronates prior to transport to the blood.[14] The findings of only minute amounts of unconjugated and presumably biologically active E_3 after

its oral administration may explain the lack of effect on gonadotropins. A notable exception was Subject No. 6. The level of unconjugated E_3 in this patient rose after ingestion of E_3 and at 6 hours after its administration the level reached 1,860 pg/ml, explaining the suppression of the serum LH and FSH concentration. Her medical history included no abnormality that could explain this unusual profile.

In contrast, the vaginal administration of E_3 resulted in levels of unconjugated E_3 which were comparable to those of the various E_3 conjugates (Fig. 2). Thus, vaginal insertion of E_3 permits the absorption of the presumably active form of the hormone into the blood for transport to target tissues prior to its inactivation via conjugation (at the 3 and 16 positions) by enzymes residing in the enterohepatic system.[14] It should be noted that because the metabolic clearance rate from blood of glucoconjugates is much faster than that of sulfoconjugates, the plasma concentrations of the latter in blood were disproportionately higher.[15] In all likelihood, only small amounts of the absorbed E_3 were sulfoconjugated, whereas the bulk was glucoconjugated.

In summary, we have demonstrated in a controlled study that E_3 is a biologically potent estrogen. We further demonstrated that, depending on the route of E_3 administration, one will find different metabolites in the plasma. However, it appears that the biologic activity of E_3 as measured by suppression of gonadotropins is due mainly to the levels of unconjugated E_3 and not to the levels of the various conjugates. Because of the very effective conjugation of orally absorbed E_3, it appears that larger doses of E_3 would have to be given orally than vaginally to achieve an equivalent effect. Whether estrogen replacement therapy with E_3 would be safer than or as effective as other estrogen preparations remains to be established.

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