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THE ENDOGENOUS CONCENTRATION OF ESTRADIOL AND ESTRONE IN NORMAL HUMAN POSTMENOPAUSAL ENDOMETRIUM

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Summary—The endogenous estrone (E_1) and estradiol (E_2) levels (pg/g tissue) were measured in 54 postmenopausal, atrophic endometria and compared with the E_1 and E_2 levels in plasma (pg/ml). The results from the tissue levels of both steroids showed large variations and there was no significant correlation with their plasma levels. The mean E_2 concentration in tissue was 420 pg/g, 50 times higher than in plasma and the E_1 concentration of 270 pg/g was 9 times higher. The E_2/E_1 ratio in tissue of 1.6, was higher than the corresponding E_2/E_1 ratio in plasma, being 0.3. We conclude that normal postmenopausal atrophic endometria contain relatively high concentrations of estradiol and somewhat lower estrone levels. These tissue levels do not lead to histological effects.

INTRODUCTION

Epidemiological studies have identified a number of risk factors for endometrial cancer. Several of these factors are clearly related to the endogenous production of estrogens; in addition the use of exogenous estrogens in postmenopausal women is associated with an increased risk for endometrial cancer [1–4].

Obesity, considered to be the most important risk factor for endometrial pathology, is thought to act through its contribution to the peripheral conversion of androstenedione to estrone [5–6]. Especially in the postmenopause, the resulting continuous exposure of target tissues to the unopposed action of estrogens may be a causal factor in the development of endometrial cancer [7–9].

However, recent studies have shown that the production rate and the peripheral blood levels of estrone (E_1) and estradiol (E_2) are very similar in patients with this cancer, as compared to controls matched for body weight [10–12].

Although the predominant circulating estrogen in postmenopausal women is estrone, Tseng and Gurpide did show that the estrogen tightly bound to the nuclei of superfused human endometrium was not estrone but estradiol [13].

In a previous *in vivo* study we have shown that after a constant infusion of labelled E_1 for 12 h to postmenopausal women, there is a higher E_2/E_1 ratio in endometrial tissue as compared with peripheral plasma. In the cell nucleus only E_2 was accumulated even after an infusion of E_1 [14–15].

We therefore considered the possibility that tissue levels of estrogens may be different from the plasma levels. As far as we know no data are available about endogenous E_1 and E_2 values in postmenopausal endometrium.

Therefore a study was initiated to measure tissue estrogens in normal postmenopausal endometrium.

MATERIALS AND METHODS

Patients

Normal atrophic endometrial tissue was obtained from fifty-four women, aged 47–76 years, who had their last menstrual period at least 1 year before. Years since menopause (Y.M.P.) varied between 1–30 yr, the range of bodyweight between 45–100 kg. To quantitate overweight for each patient the Quetelet index was calculated, as bodyweight (in kilograms) divided by the square of height (in meters). The percent ideal bodyweight was calculated from the ideal weight, obtained from the Metropolitan Life Insurance Company tables. The data are given in Table 1. None of the participating patients had ever taken exogenous estrogens.

Tissue

Normal human atrophic postmenopausal endometrium was obtained at hysterectomy performed on the indication of myomata uteri or prolapsus uteri, or at a diagnostic curettage for postmenopausal bleeding. Operations were performed under general anaesthesia using standard procedures. Immediately after abdominal or vaginal hysterectomy the endometrium was scraped from the uterine cavity with a curette. The uterus and remaining endometrium was subjected to routine histological examination. Endo-

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Table 1. Plasma levels, years since menopause and weight

	N	Mean Value	±SD	Range
E_1	38	30.9 pg/ml	16.9	1–69
E_2	38	15.5	23.9	1–117
Y.M.P.	52	9.5 yr	8	1–30
	38	10.9	9.2	1–30
Body weight	53	70.9 kg	11.4	45–100
	38	70.7	12	45–100
Quetelet index	53	26.8	4.2	18.3–37.6
	38	27.0	4.4	18.3–37.6
% Ideal weight	53	129.7	20.3	89–183.5
	38	130.9	21.6	89–183.5

Plasma estrone and estradiol, years since menopause (Y.M.P.), body weight, Quetelet index and % ideal weight in a group of healthy postmenopausal women.

metrial tissue obtained at curettage was divided into two portions, one for steroid determinations, the other part for histological examination. The endometrial tissues for our study were stored at −70°C until analysis.

In earlier experiments [15] histological examination of samples obtained in this way, revealed that, tissues referred to as endometrium are contaminated with about 15% myometrium. The amount of endometrial tissue ranged between 0.01 and 0.28 g per patient. In this study only tissues were used with the histopathological diagnosis of atrophic endometrium.

Plasma

Heparinized venous blood samples were taken from thirty-nine patients just before anaesthesia to determine endogenous levels of E_1 and E_2. After centrifugation to separate cells and plasma, the plasma was stored at −20°C until analysis.

Plasma estrogens

Plasma E_2 and E_1 were measured by radioimmunoassays, using highly specific antisera raised in rabbits against their 6-carboxymethyloxime-derivates coupled to bovine serum albumin. After double extraction of the steroids from plasma with freshly distilled ether, the extracts were purified by column chromatography on Sephadex-LH-20 and analysed. E_2 and E_1 were eluted with toluene–methanol (92:8, v/v) and collected in separate fractions. Details of these assays have been described earlier [16].

Tissue estrogens

In postmenopausal atrophic endometria estrone and estradiol were measured with a method according to that described by van Landeghem *et al.* [17]. In brief the following method was used: the frozen tissue was chilled in liquid nitrogen at −196°C and subsequently pulverized with a Micro-Dismembrator (Braun, Melsungen, W. Germany). After pulverization the powder obtained was homogenized in 1.8 ml phosfate-buffer and [^{3}H]E_1 and [^{3}H]E_2 were added to enable correction for recovery. The extraction was done twice with ethanol–acetone (1:1, v/v), the first time with 10 ml and the second time with 5 ml, both times followed by centrifugation (10′, 2,500 *g*). The pellet obtained was used to measure DNA-content according to Burton [18]. The combined supernatants were concentrated to 1.5 ml under a stream of nitrogen and 1 ml of phosfate-buffer was added. Next extraction was done with 10 ml of freshly distilled ether and the extract was taken to dryness at 45°C under a stream of nitrogen. The residue was subjected to chromatography on LH-20 column as for the plasma estrogens.

The following data were measured: DNA per pellet, E_1 and E_2 in the supernatant and the following data were calculated: ratio of E_1 and E_2 to DNA and concentration of E_1 and E_2 per gram tissue. From the 54 tissues, 3 DNA concentrations are not available and from one portion the E_2 determination failed.

Statistical analysis

The data were analysed by means of Spearman's rank correlation test (r_s). Differences between two groups of data were tested by using Student *t*-test.

If estrone or estradiol in tissue were below the level of sensitivity of the assays, we have used 5 pg/g or 2 pg/mg DNA for our calculations. If these estrogens were not detectable in plasma (< 1 pg/ml), we have used 1 pg/ml for our calculations.

The results on estrogen levels in tissue amounts of less than 0.05 g, were equally distributed over the total range of observations and therefore these results have been included in our calculations.

The levels of E_1 and E_2 in tissue showed large variations, therefore in all figures results are expressed on a log-scale.

RESULTS

Plasma estrogens

Our findings on the plasma estrogen levels are given in Table 1, together with the data on body-

Table 2. Correlation coefficients between estrogen plasma levels and weight and years since menopause

	Estrone	Estradiol
Body weight	$r_s = +0.21$	$r_s + 0.10$
Quetelet index	$r_s = +0.26$	$r_s = +0.15$
% Ideal weight	$r_s = +0.27$*	$r_s = +0.13$
Y.M.P.	$r_s = +0.05$	$r_s = -0.09$

Spearman's rank correlation coefficients of the patients characteristics and the measured E_1 and E_2 concentration in plasma ($n = 38$). *$P = 0.1$, all other P values > 0.1.

Table 3. Estrogen tissue and plasma concentrations

	N	Mean ±SD log pg/g	Mean pg/g	N	Mean ±SD log pg/mg DNA	Mean pg/mg DNA	N	Mean ±SD log pg/ml	Mean pg/ml
E_1	54	2.43 ± 0.92	270	51	2.06 ± 0.93	115	39	1.43 ± 0.31	26.9
E_2	53	2.62 ± 0.75	417	50	2.33 ± 0.60	214	39	0.88 ± 0.48	7.6

The mean (±SD) tissue concentrations of E_1 and E_2 expressed as pg per gram tissue or as pg per mg DNA, were calculated after log transformation. These data were converted back to untransformed mean-values and are also given in this table. For comparison the mean concentrations ±SD of E_1 and E_2 in plasma were added.

weight, Quetelet index, % ideal weight and years after menopause (Y.M.P.).

In view of the fact that we had only 39 plasma samples available and because from one of the patients the bodyweight was unknown, the calculations were done with the data from 38 patients. The data obtained in this group of 38 patients are shown separately in Table 1. The correlation coefficients between the parameters given in Table 1 are summarized in Table 2.

These data show that there are no significant correlation coefficients. The highest correlation coefficients are found between the E_1 level in plasma and either % ideal weight as well as Quetelet index, resp. +0.27 and +0.26. Also no correlation coefficients are found between the E_2 level in plasma and bodyweight, Quetelet index or % ideal weight. No correlation was found for plasma levels of both estrogens and Y.M.P.

Tissue estrogens

The amount of DNA in the tissue ranged from 0.2 to 8.4 mg/g, the mean value being 3.2 mg/g ±2.1 (SD). The levels of E_1 and E_2 in tissue showed large variations. The tissue level for E_1 ranged from 5–7,784 pg/mg DNA and from 5–11,479 pg/g tissue. The tissue level for E_2 ranged from 5–3,649 pg/mg DNA and from 5–12,670 pg/g tissue. Because of these large variations the calculations were done after log-transformation. The mean concentrations and standard deviations, calculated after log-transformation for E_1 and E_2 in endometrium and in plasma are given in Table 3. The calculated mean-values on log-basis, were converted back to untransformed mean-values to reduce the influence of extreme data, and also given in Table 3. The mean E_1 concentration in all the available tissue was calculated at 270 pg/g tissue and 115 pg/mg DNA, for E_2 at 417 pg/g tissue and 214 pg/mg DNA. The estrogen

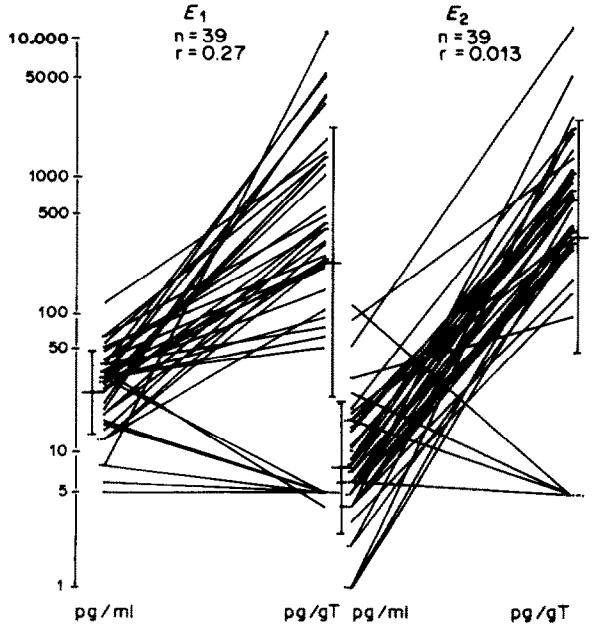

Fig. 1. The tissue E_1 and E_2 levels (in pg/gT) in relation to their plasma levels (in pg/ml) for each ($n = 39$) individual separately. The mean values (±SD) for both variables are indicated by a vertical line at the left and right side in the figure.

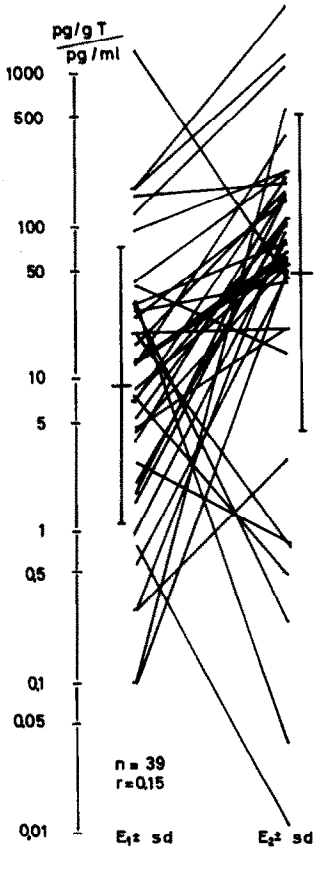

Fig. 2. The ratios between the tissue concentration (in pg/gT) and the plasma concentration (in pg/ml) of E_1 and E_2. The data of the same subject are connected with a line. At the left and the right side the mean ratio (±SD) is indicated by a vertical line.

levels in tissue and plasma from all subjects for whom we had both data ($n = 39$) are shown in Fig. 1. For these 39 subjects the mean E_1 and E_2 concentration in tissue was resp. 240 and 372 pg/g tissue and is serum resp. 26.9 and 7.6 pg/ml.

We found considerable variations in the tissue/plasma ratio (expressed as pg/g and pg/ml, resp.) for both E_1 and E_2. Six subjects had lower tissue levels for E_1 as compared with plasma level and for E_2 five subjects had lower tissue levels. All other levels for estrogens in tissue were much higher than in plasma. There was no significant correlation between individual plasma and tissue levels of E_1 and E_2 (Fig. 1). Figure 2 shows the ratio between the tissue concentration in pg/g and the plasma concentration in pg/ml for E_1 and E_2. Data of the same subject are connected with a line. The mean tissue/plasma ratio for E_2 was significantly higher than for E_1 resp. 50.1 ± 11 (SD) and 9.1 ± 8.1 ($P < 0{,}001$). The mean ratio's and SD are also indicated in Fig. 2. Eight out of the 39 subjects had a higher tissue/plasma ratio for E_1 than for E_2. The ratio for both estrogens are not correlated, $r_s = 0.15$ ($P > 0.1$).

Comparison of the E_2/E_1 ratio in plasma (in pg/ml) and in tissue (in pg/g T), as depicted in Fig. 3, showed that there is a higher ratio in tissue (mean 1.6) than in plasma (mean 0.3).

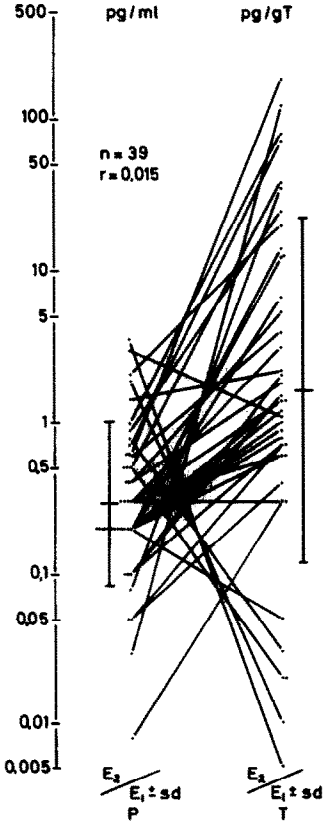

Fig. 3. The E_2/E_1-ratios in plasma (in pg/ml) as compared to the ratios in postmenopausal endometrial tissue (in pg/gT). At the left and the right side the mean ratio (±SD) is indicated by a vertical line.

From these data it is justified to conclude that postmenopausal atrophic endometrium contains more E_2 than E_1. Even when the plasma-level of E_2 is lower than for E_1, the tissue is able to build up a higher gradient for E_2 than for E_1.

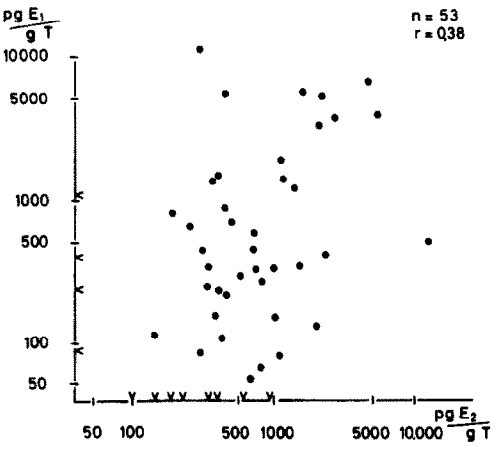

Fig. 4. The tissue E_1 levels (in pg/gT) plotted against the tissue E_2 levels (in pg/gT) for 53 patients. The symbol < or v at the y-axis and the x-axis indicates a value below the sensitivity of the assay used.

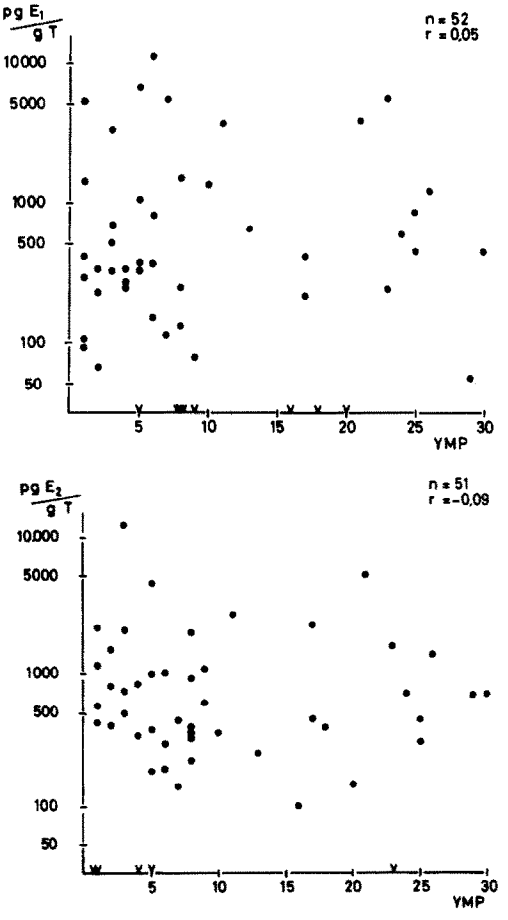

Fig. 5 a and b. Years since menopause (Y.M.P.) in relation with the tissue levels for E_1 and E_2 (in pg/gT); resp. The symbol v at the x-axis indicates a value below the sensitivity of the assay used.

In Fig. 4 the tissue E_1 levels were plotted against the tissue E_2 levels (both in pg/g tissue) for 53 subjects. With an increasing E_1 tissue level we see an increasing E_2 tissue level. ($r_s = 0.38$, $P < 0.01$). The Spearman's rank correlation coefficient between tissue level of E_1 (pg/mg DNA) against E_2 (in pg/mg DNA) is 0.32 ($n = 50$, $P < 0.05$). (Data not shown).

In Fig. 5a and b the years since menopause are plotted against the tissue E_1 and E_2 level (pg/g tissue), resp. There is no obvious fall in tissue E_1 or E_2 level with time elapsed since menopause.

DISCUSSION

In this study we investigated the levels of E_1 and E_2 in atrophic endometrial tissue from postmenopausal women in comparison with the corresponding plasma levels.

In our group of normal women, the mean E_1 level of 33.2 pg/ml ($n = 39$) in plasma is slightly higher than that reported by Poortman *et al.* (24.2 pg/ml) [19]. Also our mean E_2 level of 15.1 pg/ml is somewhat higher. Our data are comparable to those reported by Judd *et al.* [10].

We found no significant correlation between estrogen levels in plasma and weight or Quetelet index or % ideal weight, in contrast to the findings published by Poortman and Judd. Their correlations were not very strong and therefore relatively small differences in the estrogen estimations at these very low levels can lead to loss of correlation between bodyweight and estrogen levels. On the other hand also Klinga *et al.* [20], were unable to find a correlation between E_1 levels and overweight and found only a weak correlation coefficient with weight ($r = 0.31$). In agreement with Poortman and Judd we did not find a correlation between plasma estrogens and years after the menopause.

In a recent study [14–15] the estrogen uptake by target tissues was studied in postmenopausal women after a long term (12 h) infusion of tritium-labelled estrogens.

A large gradient between tissue and plasma levels for endometrium, myometrium and vagina was found, the gradient being higher for E_2 and E_3 than for E_1 in all three tissues.

Estrogens are thought to be involved in endometrial disorders and they can exert their biological effect only after entrance into the cell. Therefore we wanted to measure these hormones at the tissue level. To our knowledge this is the first study in which quantitative data are given on endogenous estrogens in postmenopausal endometria, no comparable data are available.

The levels for E_1 and E_2 in the atrophic endometrial tissues showed a very large range and the tissue levels were much higher than the corresponding plasma levels. There was no significant correlation between plasma and tissue E_1 or E_2 levels. As we found no correlation between estrogen plasma levels and Y.M.P., in addition we also could not find a correlation between estrogen tissue levels and Y.M.P.

The mean E_2 level in tissue was higher than for E_1, a slight but significant correlation ($r_s = 0.38$, $N = 53$) was found between these levels. This correlation is in accordance with the results of Wiegerinck *et al.* [15] who postulated an intracellular conversion of E_2 to E_1 in endometria of postmenopausal women.

As the E_2 level in serum is lower than for E_1, the tissue/plasma ratio of E_2 is higher than the ratio for E_1. Our mean ratio's were 50 and 9.1 resp. Wiegerinck *et al.*, found an approx 30 times higher E_2 concentration in endometrium than in plasma and the corresponding E_1 tissue to plasma ratio was 7 for postmenopausal women using a different experimental approach [15].

Therefore, the accumulation of E_2 in atrophic endometria was a consistent finding. The differences in gradient between our results and those of others can possibly be attributed to different experimental approaches. The higher E_2 concentration in the cell can be explained by the longer intracellular and nuclear accumulation after uptake. The contribution of local endometrial aromatisation, as observed by Tseng [21], is as yet unclear.

Although the cells were clearly very active in building up a large gradient to plasma of mainly estradiol, histologically the endometrial tissues were classified as atrophic. So the high intracellular concentrations of these estrogens had no histological effect. It must be kept in mind that our determinations were done on total tissue, because too little tissue per patient was available to allow determinations in the subcellular fractions.

In view of the results of Wiegerinck *et al.* [15] in subcellular distribution of the infused estradiol it is to be expected that also the endogenous estradiol is mainly present in the nuclear compartment of the cells. Therefore it is difficult to understand why the high E_2 concentrations in the presence of estradiol receptors do not lead to histological effects. It seems that either still higher E_2 concentrations are necessary to elicit effects, at least in the absence of progesterone or another possibility might be the necessity of unknown factors for histological results.

In conclusion: normal postmenopausal atrophic endometria contain relatively high concentrations of estradiol and somewhat lower estrone levels. These tissue levels do not lead to histologically demonstrable effects.

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