

# Plasma oestrogen fractions in postmenopausal women receiving hormone replacement therapy: influence of route of administration and cigarette smoking

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## Abstract

The aim of this study was to determine the impact of the administration route and cigarette smoking on plasma oestrogen levels during oral and parenteral oestrogen replacement therapy (ERT). Fourteen healthy postmenopausal women (six smokers and eight non-smokers) were recruited for a prospective, randomised, crossover study at a private outpatient medical centre in Oslo, Norway. All patients were randomised to receive cyclic therapy with oestradiol and norethisterone orally or by the transdermal route each for a 6-month period. Plasma levels of oestrone (Oe<sub>1</sub>), oestradiol (Oe<sub>2</sub>) and oestrone sulphate (Oe<sub>1</sub>S) were determined using highly sensitive RIA methods before and during hormone replacement therapy given by the oral and transdermal route. Comparing smokers and non-smokers, plasma levels of Oe<sub>1</sub>, Oe<sub>2</sub> and Oe<sub>1</sub>S were all

found to be 40–70% lower in smokers compared with non-smokers when ERT was given orally (Oe<sub>1</sub>S,  $P < 0.05$ ; Oe<sub>1</sub> and Oe<sub>2</sub>,  $P < 0.01$  for both). Oe<sub>2</sub> given orally caused a higher Oe<sub>1</sub>S/Oe<sub>2</sub> ratio but also a higher Oe<sub>1</sub>/Oe<sub>2</sub> ratio compared with parenteral therapy in smokers (40.2 versus 7.0,  $P < 0.01$ ; and 3.2 versus 0.8,  $P < 0.05$  respectively). No significant differences in these parameters in the different test-situations were seen in non-smokers. Except for a lower level of Oe<sub>1</sub>S in smokers (non-significant), no difference in plasma oestrogen levels between smokers and non-smokers was observed during parenteral therapy. In conclusion, cigarette smoking has been shown to have major impact on plasma oestrogen levels during oral but not during parenteral Oe<sub>2</sub> replacement.

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## Introduction

Oestrogen replacement therapy (ERT) is known to improve quality of life and to prevent severe diseases such as osteoporosis (Kiel *et al.* 1987) and cardiovascular morbidity (Nabulsi *et al.* 1993) in postmenopausal women. Traditionally administered orally, oestrogens may now be given by the transdermal route.

Due to substantial first pass metabolism (Longcope *et al.* 1985), oral oestrogens have to be administered in high doses to achieve acceptable plasma levels. Thus, oral ERT is known to influence hepatic synthesis and secretion of several endogenous compounds (Geola *et al.* 1980). While some of these effects, like increasing levels of high-density lipoproteins, might be beneficial (Lobo 1991), changes in plasma levels of clotting factors probably explain an increased risk of intravascular clotting during ERT (Meade 1982). Therefore, much effort has been made to develop alternative routes of oestrogen administration

that bypass the liver such as vaginal and transdermal delivery systems (Laufer *et al.* 1983, Mandel *et al.* 1983).

While several studies have compared plasma levels of oestradiol (Oe<sub>2</sub>) and oestrone (Oe<sub>1</sub>) in patients treated with parenteral or oral oestrogens (Chetkowski *et al.* 1986, Lignieres *et al.* 1986), little is known about plasma levels of oestrone sulphate (Oe<sub>1</sub>S) in patients receiving ERT. Oe<sub>1</sub>S is the main circulating oestrogen in postmenopausal women, and recent evidence has focused on plasma Oe<sub>1</sub>S as an important source to tissue oestrogens in postmenopausal women (Santner *et al.* 1984). Circulating Oe<sub>1</sub>S is synthesised from plasma Oe<sub>1</sub> and Oe<sub>2</sub>, and between 50 and 90% of both oestrogens is converted into Oe<sub>1</sub>S (Ruder *et al.* 1972, L nning *et al.* 1987, 1989). Liver tissue contains high concentrations of sulphotransferase (Ruder *et al.* 1972), necessary to synthesise Oe<sub>1</sub>S, and only about 10–20% of Oe<sub>2</sub> administered orally reaches the systemic circulation unmetabolised (Longcope *et al.* 1985).

Cigarette smoking is suggested to have anti-oestrogenic effects in women. The mechanisms for these observations are poorly understood, as no study has revealed any significant difference in plasma levels of  $Oe_1$  or  $Oe_2$  between smoking and non-smoking women. Smoking is known to enhance certain P450 dependent mixed function oxygenases (Conney 1967), some of which also metabolise oestrogens (Bolt 1979). However, plasma  $Oe_1$  and  $Oe_2$  are known to have high clearance rates approximating hepatic plasma flow (Longcope & Williams 1974); therefore, enhancement of enzymes engaged in liver metabolism of plasma oestrogens is expected to have little influence on  $Oe_1$  and  $Oe_2$  clearance rates in postmenopausal women (Wilkinson & Shand 1975, Lønning & Kvinnsland 1988) and to have only minor influence on endogenous  $Oe_1$  and  $Oe_2$  plasma levels and oestrogen levels following parenteral administration.

Based on the considerations above, this study was designed to test three hypotheses. Our first hypothesis was that  $Oe_2$  administered orally, due to excessive first pass metabolism, may produce higher plasma  $Oe_1S$  levels compared with  $Oe_2$  given parenterally. Secondly, we postulated smoking to lower plasma oestrogen levels following oral administration of  $Oe_2$ . Thirdly, because  $Oe_1S$ , contrary to  $Oe_1$  and  $Oe_2$ , is a so-called 'low extracted compound' with a plasma clearance rate much lower than hepatic plasma flow (about 3–6 l/h) (Longcope 1972), enzyme induction (smoking) may be expected to lower plasma levels of  $Oe_1S$  also in postmenopausal women receiving parenteral or no ERT.

## Materials and Methods

### Human subjects

Seventeen postmenopausal women who were to receive ERT due to clinical symptoms of oestrogen deprivation were enrolled. Fourteen patients completed both treatment periods and were included for analysis. Median age, weight, height and body mass index were 49.5 years (range 43.0–63.0), 68 kg (range 44–85), 1.67 m (range 1.56–1.78) and 24.0 kg/m<sup>2</sup> (range 17.6–30.1) respectively.

All patients had gonadotrophin levels in the postmenopausal range with absence of menstrual bleeding for 4 to 240 months. Cervical cytology showed regular cells in all patients prior to ERT. None of the patients suffered from diabetes mellitus, liver or renal diseases. One non-smoker received treatment with ergotamine-tartrate for migraine, while all remaining patients received the study drug only. Any other hormone therapy was terminated at least 6 weeks before inclusion in the study.

Six patients smoked 10–20 cigarettes daily; the other patients were non-smokers. None of the patients had a record of alcohol abuse. All patients gave their written

informed consent, and the study was approved by the regional ethical committee.

### Study procedures

Patients were randomised to receive cyclical therapy with  $Oe_2$  and norethisterone orally (Trisekvens Novo, Novartis, Basle, Switzerland: 17- $\beta$ - $Oe_2$  2 mg days 1–22 and 1 mg days 23–28, norethisterone 1 mg days 13–22) or by the transdermal route (Estracomb, Copenhagen, Denmark  $Oe_2$  50  $\mu$ g/24 h days 1–28, norethisterone 250  $\mu$ g/24 h days 15–28). The progesterone treatments during oral and parenteral therapy were considered to be equal. Each patient was treated with the assigned treatment for 6 months where after they were switched to the other regimen without any wash-out period. Total time of the investigation period was 12 months. Blood samples were obtained before treatment and after 3, 6, 9 and 12 months on treatment. According to protocol, samples were to be drawn during the second week of the first half of the cycle when patients were treated with  $Oe_2$  only in both regimens. All blood samples were obtained in heparinised vials between 0800 and 1000 h after an overnight fast. While on oral treatment, all patients took their last medication before 2000 h in the evening to ensure a free interval of at least 12 h prior to blood sampling on the following day. Plasma was separated by centrifugation and stored at  $-20^\circ\text{C}$  until time of analysis.

### Assays

Plasma levels of  $Oe_1$ ,  $Oe_2$  and  $Oe_1S$  were measured as described elsewhere (Lønning *et al.* 1995) while plasma levels of sex hormone binding globulin (SHBG) were measured using a commercial RIA kit provided by Orion Diagnostica, Espoo, Finland. The biochemical evaluation of the smoking status was done by cotinine measurements in plasma samples (Muranaka *et al.* 1988) using a modified RIA as published previously (Waage *et al.* 1992).

### Data analysis

Previous work in our laboratory revealed plasma oestrogen levels in postmenopausal women to be well fitted to a log normal distribution (Lønning *et al.* 1995). Thus, plasma hormone levels and the ratios between the different oestrogens obtained before and during ERT are given as their geometric mean values with 95% confidence intervals of the means. The mean value of the two blood samples drawn on each treatment (after 3 and 6 or 9 and 12 months respectively) was used for data analysis. The Mann–Whitney test was used to investigate any difference between smokers and non-smokers in the different test-situations and to compare plasma oestrogen levels during oral and parenteral ERT. Considering the ratios between the different plasma oestrogens before and during

treatment with the two regimens, these were compared in the three test situations within each patient group using the Friedman test.

## Results

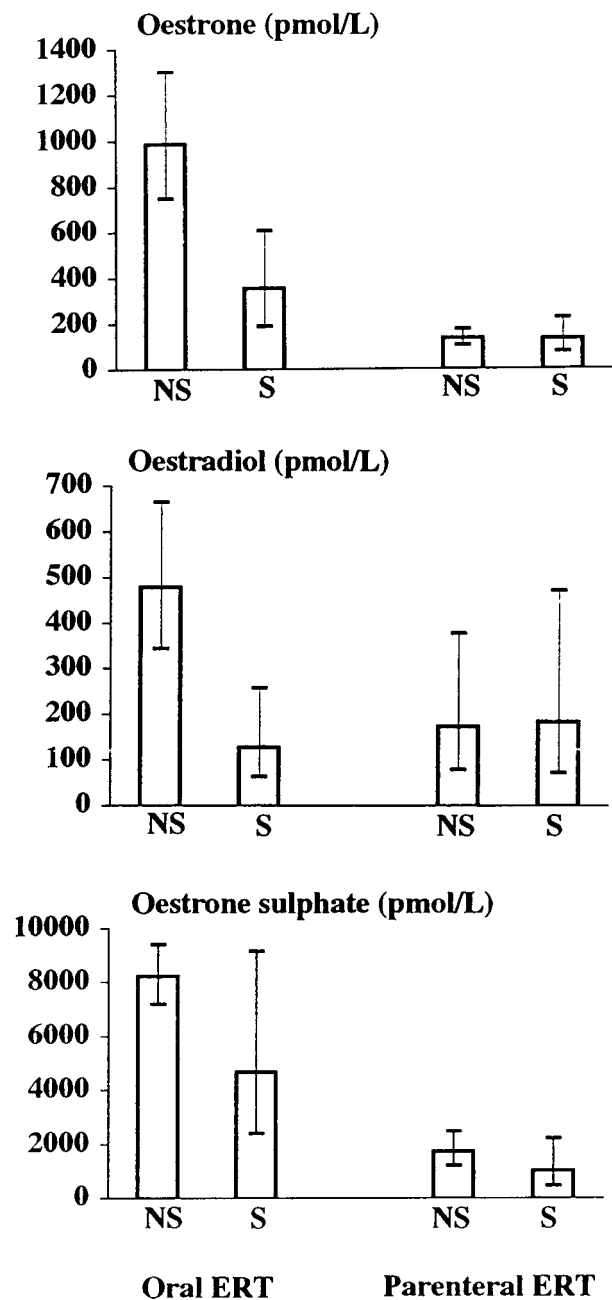
Plasma oestrogen levels obtained before and during oral and parenteral ERT are illustrated in Fig. 1 and given in detail in Table 1, while changes in plasma oestrogen ratios are summarised in Table 2. Concerning pretreatment oestrogen levels, no significant difference comparing smokers and non-smokers could be established, although the mean plasma levels of all oestrogens measured were somewhat lower in smokers.

We found treatment with  $Oe_2$  given orally (2 mg daily) to cause significant higher plasma levels of all oestrogens compared with 50  $\mu$ g daily given by the parenteral route (Table 1 and Fig. 1). The ratio of  $Oe_1S$  to  $Oe_2$  increased during treatment with oral  $Oe_2$ , while it decreased during parenteral ERT compared with the pretreatment ratio (this change, however, was significant in smokers only,  $P < 0.01$ ). The ratio of  $Oe_1S$  to  $Oe_1$  increased to the same extent during both oral and parenteral ERT in the total patient group; however, the ratio differed significantly between smokers and non-smokers in both treatment situations, with a higher ratio in smokers during oral therapy but a higher ratio in non-smokers during parenteral therapy ( $P < 0.05$  comparing the three test-situations among smokers as well as non-smokers; Table 2).

While no significant difference in plasma levels of  $Oe_1$ ,  $Oe_2$  and  $Oe_1S$  could be established between smokers and non-smokers before initiation of ERT, cigarette smoking caused major changes in plasma hormone levels during oral ERT. In smokers, the mean plasma levels of  $Oe_1$ ,  $Oe_2$  and  $Oe_1S$  reached 37% ( $P < 0.01$ ), 27% ( $P < 0.01$ ) and 57% ( $P < 0.05$ ) of the mean plasma levels found in non-smoking women during oral ERT respectively. In addition, the ratio of  $Oe_1S$  to  $Oe_1$  and  $Oe_1S$  to  $Oe_2$  was higher in smokers compared with non-smokers (Table 2;  $P < 0.05$  and  $P < 0.01$  respectively comparing smokers to non-smokers), suggesting a more substantial effect of smoking on plasma  $Oe_1$  and  $Oe_2$  compared with  $Oe_1S$  during oral ERT.

No difference in plasma levels of  $Oe_1$  and  $Oe_2$  between smokers and non-smokers was seen during parenteral treatment. However, we found a non-significant lowering of plasma  $Oe_1S$  levels in smokers (1034 pM) compared with non-smokers (1744 pM;  $P = 0.16$ ).

Oral  $Oe_2$  increased plasma SHBG values in both smokers and non-smokers (Table 3). The plasma SHBG level increased from pretreatment levels of 64.4 nmol/l (95% c.i. 41.4–100.2) in smokers and 41.6 nmol/l (95% c.i. 29.2–59.3) in non-smokers (64.9% of the value obtained for smokers, 95% c.i. 39.3–106.2%) to



**Figure 1** Plasma levels of oestrone ( $Oe_1$ ), oestradiol ( $Oe_2$ ) and oestrone sulphate ( $Oe_1S$ ) during treatment with oral and parenteral ERT in the subgroups of smokers (S) and non-smokers (NS). All oestrogen values are expressed as geometric means with 95% confidence intervals of the mean.

88.7 nmol/l (95% c.i. 63.4–124.1) and 88.0 nmol/l (95% c.i. 58.7–132.0) during oral treatment respectively (non-smokers  $P < 0.05$ ; smokers n.s.). No change in plasma SHBG was observed during parenteral ERT.

**Table 1** Plasma levels of oestrone, oestradiol and oestrone sulphate obtained before and during ERT by the oral or parenteral route (geometrical mean values with 95% confidence limits of the mean)

|                           |                          | Before therapy |            | During oral ERT    |             | During parenteral ERT |             |
|---------------------------|--------------------------|----------------|------------|--------------------|-------------|-----------------------|-------------|
| Oestrogen levels (pmol/l) |                          |                |            |                    |             |                       |             |
| Oe <sub>1</sub>           | smokers <sup>c</sup>     | 68             | (38–123)   | 356 <sup>b</sup>   | (190–608)   | 133                   | (78–225)    |
| Oe <sub>1</sub>           | non-smokers <sup>d</sup> | 73             | (42–127)   | 987 <sup>b</sup>   | (750–1301)  | 135                   | (104–175)   |
| Oe <sub>2</sub>           | smokers                  | 23             | (7–76)     | 128.5 <sup>b</sup> | (64–258)    | 184.6                 | (72–473)    |
| Oe <sub>2</sub>           | non-smokers <sup>c</sup> | 46             | (14–144)   | 479.1 <sup>b</sup> | (345–665)   | 173.5                 | (79–379)    |
| Oe <sub>1</sub> S         | smokers <sup>c</sup>     | 417            | (225–771)  | 4684 <sup>a</sup>  | (2401–9145) | 1034                  | (478–2237)  |
| Oe <sub>1</sub> S         | non-smokers <sup>d</sup> | 549            | (225–1342) | 8226 <sup>a</sup>  | (7194–9405) | 1744                  | (1221–2487) |

Oe<sub>1</sub>, oestrone; Oe<sub>2</sub>, oestradiol; Oe<sub>1</sub>S, oestrone sulphate; significant difference between smokers and non-smokers (Mann–Whitney), <sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.01; significantly difference in plasma oestrogen levels comparing oral and parenteral ERT (Mann–Whitney), <sup>c</sup>*P*<0.05, <sup>d</sup>*P*<0.001.

**Table 2** Plasma hormone ratios before and during ERT by the oral or parenteral route (geometrical mean values with 95% confidence limits of the mean)

|                                   |                          | Before therapy |            | During oral ERT   |             | During parenteral ERT |            |
|-----------------------------------|--------------------------|----------------|------------|-------------------|-------------|-----------------------|------------|
| Oestrogen ratios                  |                          |                |            |                   |             |                       |            |
| Oe <sub>1</sub> S/Oe <sub>1</sub> | smokers <sup>c</sup>     | 6.1            | (4.0–9.5)  | 13.6 <sup>a</sup> | (9.2–20.0)  | 8.2 <sup>a</sup>      | (5.9–11.3) |
| Oe <sub>1</sub> S/Oe <sub>1</sub> | non-smokers <sup>c</sup> | 7.5            | (4.4–12.8) | 8.5 <sup>a</sup>  | (6.4–11.4)  | 13.0 <sup>a</sup>     | (8.7–19.4) |
| Oe <sub>1</sub> S/Oe <sub>2</sub> | smokers <sup>d</sup>     | 18.0           | (8.1–40.2) | 40.2 <sup>b</sup> | (28.5–67.9) | 7.0                   | (4.8–10.2) |
| Oe <sub>1</sub> S/Oe <sub>2</sub> | non-smokers              | 12.1           | (5.2–27.7) | 19.1 <sup>b</sup> | (12.2–29.8) | 11.6                  | (5.0–26.7) |
| Oe <sub>1</sub> /Oe <sub>2</sub>  | smokers <sup>c</sup>     | 2.9            | (1.3–6.5)  | 3.2               | (1.5–4.9)   | 0.8                   | (0.6–1.2)  |
| Oe <sub>1</sub> /Oe <sub>2</sub>  | non-smokers              | 1.6            | (0.8–3.1)  | 2.1               | (1.5–3.1)   | 0.9                   | (0.5–1.6)  |

Oe<sub>1</sub>, oestrone; Oe<sub>2</sub>, oestradiol; Oe<sub>1</sub>S, oestrone sulphate; significant difference between smokers and non-smokers (Mann–Whitney), <sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.01; significantly difference in the three test-situations (Friedman test), <sup>c</sup>*P*<0.05, <sup>d</sup>*P*<0.01.

**Table 3** Plasma levels of sex hormone binding globulin before and during ERT by the oral or parenteral route (geometrical mean values with 95% confidence limits of the mean)

|                                       |                          | Before therapy |              | During oral ERT |              | During parenteral ERT |             |
|---------------------------------------|--------------------------|----------------|--------------|-----------------|--------------|-----------------------|-------------|
| Sex hormone binding globulin (nmol/l) |                          |                |              |                 |              |                       |             |
| SHBG                                  | smokers                  | 64.4           | (41.4–100.2) | 88.7            | (63.4–124.1) | 66.2                  | (43.9–99.7) |
| SHBG                                  | non-smokers <sup>a</sup> | 41.6           | (29.2–59.3)  | 88.0            | (58.7–132.0) | 50.7                  | (35.1–73.1) |

Significant difference in the three test-situations (Friedman test), <sup>a</sup>*P*<0.05.

The measurement of cotinine in plasma samples (five samples for every subject) confirmed the smoking status as reported by the patients. The cut-off value for cotinine in plasma used to discriminate between smokers and non-smokers was 70 ng/ml. The six smokers had cotinine levels in the range from 500 to 1000 ng/ml. While seven non-smokers had cotinine levels <7 ng/ml during the study period, one non-smoker had cotinine plasma values ranging between 70 and 280 ng/ml. This patient denied any cigarette smoking on her own, but informed that she was living in a household with heavy in-door smoking.

## Discussion

ERT is widely used in peri- and postmenopausal women with symptoms related to oestrogen deprivation. Oe<sub>2</sub>

given orally or by the percutaneous route increases plasma levels of Oe<sub>2</sub> as well as Oe<sub>1</sub> (Chetkowski *et al.* 1986, Lignieres *et al.* 1986). However, little is known about the influence of the different forms of ERT on plasma levels of Oe<sub>1</sub>S. While Oe<sub>1</sub>S is biologically inactive on its own, most tissues are known to contain the enzymes necessary to convert Oe<sub>1</sub>S into Oe<sub>1</sub> and Oe<sub>2</sub> (Santner *et al.* 1984). Considering the high plasma levels of circulating Oe<sub>1</sub>S, this steroid conjugate is suggested to be a major source for intra-tissue unconjugated oestrogens in postmenopausal women (Santner *et al.* 1986). Thus, any difference in plasma levels of Oe<sub>1</sub>S related to the route of administration may contribute to the efficacy of the different oestrogen replacement regimens.

In this study, we examined the influence of route of administration and smoking on plasma oestrogens following treatment with ERT given orally and parenterally to

the same patient group, eliminating confounding factors like interindividual response to enzyme inducing agents (Vesell & Page 1969) and variation in life style like dietary habits (Longcope *et al.* 1987, Michnovicz & Bradlow 1990). We hypothesised that ERT given orally would cause a more marked increase in plasma  $Oe_1S$  and the ratio of  $Oe_1S$  to  $Oe_1$  and  $Oe_1S$  to  $Oe_2$  compared with parenteral treatment. What we found was an increase in the ratio of  $Oe_1S$  to  $Oe_2$  during oral ERT compared with pretreatment levels and values obtained during parenteral treatment when evaluating the total group of patients. This finding, however, was due to a difference in smokers only; while the ratio of  $Oe_1S$  to  $Oe_2$  increased significantly during oral treatment compared with pretreatment values in smokers, no significant difference in the ratio of  $Oe_1S$  to  $Oe_2$  in the three test-situations was found among non-smokers. In addition, we found a significant increase in the  $Oe_1S/Oe_1$  and  $Oe_1/Oe_2$  ratio among smokers during oral ERT but no change in these ratios in non-smokers. Our observations are consistent with an enhanced first pass metabolism of  $Oe_2$  but also a preference for hepatic conversion of  $Oe_2$  to  $Oe_1$  as well as  $Oe_1S$  in smokers. We suggest the following explanation for these findings.

Oestrogens are metabolised by P450 dependent mixed function oxygenases (Bolt 1979). Cigarette smoking is known to enhance some of these mixed function oxygenases (Conney 1967) and has been found to stimulate 2-hydroxylation, a major metabolic pathway of oestrogens in man (Michnovicz *et al.* 1986).

In general, about 80–90% of orally given  $Oe_2$  is first pass metabolised in the splanchnic tissue (Longcope *et al.* 1985), leaving only about 10–20% for the systemic circulation as unmetabolised  $Oe_2$ . In contrast,  $Oe_2$  given by the transdermal route as well as endogenous oestrogens are directly passed into the circulation. If enhancement of liver enzymes due to cigarette smoking increased the extraction rate of  $Oe_2$ , e.g. from 80 to 90% or 90 to 95%, that would have little influence on the total plasma clearance rate of endogenous  $Oe_2$  or  $Oe_2$  administered parenterally. On the other hand, it may reduce the amount of  $Oe_2$  escaping first-pass metabolism following oral administration from 20 to 10% or 10 to 5% respectively, thus having a profound effect on plasma  $Oe_2$  levels after oral administration (Wilkinson & Shand 1975, Longcope *et al.* 1985, Lønning & Kvinnsland 1988).

The finding that smoking caused less suppression of plasma  $Oe_1S$  levels compared with  $Oe_1$  and  $Oe_2$  levels during oral therapy, but tended to suppress plasma  $Oe_1S$  during parenteral therapy, may seem contradictory. Considering  $Oe_1S$  as a 'low extracted compound' this may explain an influence of smoking on parenteral  $Oe_1S$  contrary to  $Oe_1$  and  $Oe_2$  (Wilkinson & Shand 1975), while sulphation may probably slow down and protect metabolism of  $Oe_1S$  compared with  $Oe_1$  and  $Oe_2$  following oral administration. Other explanations may also be

considered. Oestrogens undergo enterohepatic cycling (Bolt 1979), and drugs and food constituents are known to influence intestinal hydrolysis of biliary oestrogen conjugates and absorption (Dada & Martins 1983). However, while an influence of smoking on enterohepatic cycling should have a similar influence on endogenous and exogenous oestrogen disposition, any direct influence of smoking on intestinal absorption of  $Oe_2$  cannot be ruled out.

There are additional mechanisms by which smoking may exert antioestrogen effects. Thus, nicotine has been shown to act as an aromatase inhibitor (Barbieri *et al.* 1986), substantiated by the observation of a reduced oestrogen/androgen ratio in smokers compared with non-smokers (Longcope & Johnston 1988, Law *et al.* 1997). While this may explain a difference in endogenous oestrogen levels between smokers and non-smokers and a reduced risk for oestrogen dependent malignancies like breast or endometrial cancer (Doll *et al.* 1980, Lesko *et al.* 1985), but increased risk for symptomatic osteoporosis (Daniell 1976) in smokers, it may not explain our observation of decreased oestrogen plasma levels in smokers following oral ERT.

Oestrogens in high concentrations are known to stimulate the synthesis of SHBG in hepatocytes (Anderson 1974), and the substantial increase during oral but not parenteral ERT may reflect a more profound oestrogen stimulation of the liver cells during oral therapy. Apart from sex hormones, agents inducing mixed function oxidases are known to elevate hormone binding globulins like SHBG (Toone *et al.* 1980). A balance between these influences could explain why smokers had somewhat higher plasma SHBG levels prior to ERT than non-smokers but that the two groups experienced similar levels during oral ERT.

In conclusion, our results suggest oral and transdermal ERT to cause different ratios of plasma oestrogens in smokers, suggesting plasma  $Oe_1S$  as well as  $Oe_1$  and  $Oe_2$  measurements to be necessary whenever different treatment regimens are compared in scientific studies in this patient group. The finding that cigarette smoking decreased all plasma oestrogen levels during oral ERT by 40–70%, but caused only minor changes in plasma  $Oe_1S$  during parenteral ERT, emphasises the need to consider smoking habits when prescribing ERT. In view of the number of patients involved in this study, our findings have to be considered as preliminary and should be confirmed in a larger group of patients.

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