

Endometrium protection and acceptability of nasally administered continuously combined hormone therapy: a multicentre, multinational, double-blind trial in post-menopausal women evaluating three regimens of 17 β -estradiol and norethisterone when compared with an orally administered 17 β -estradiol norethisterone regimen

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BACKGROUND: To determine the optimal daily dose of intranasal hormone therapy (HT) in order to achieve adequate endometrial protection.

METHODS: Multicentre, double-blind/double-dummy randomized controlled trial (Phase III) was conducted in 1741 women to compare a daily intranasal dose of 350 μ g 17 β -estradiol (E2) together with 50, 175 or 550 μ g norethisterone (NET) with the oral administration of 2 mg E2 and 1 mg NET, over a period of 52 weeks. An endometrial biopsy was performed at the end of HT use.

RESULTS: Most women (73–86%) had an ‘atrophic and/or inactive’ endometrium. Lower doses of NET were associated with a higher incidence of ‘proliferative’ endometrium. The incidence of vaginal bleeding decreased with time. During the last 4 months of the study, 88.1% of women using the highest dose of NET were in amenorrhoea when compared with 71.7% using the oral comparator (difference 16.5%; 95% confidence interval: 10.9–22.0%) ($P < 0.001$). Premature discontinuation rates were in the range of 12–17% for the three nasal regimens and 22% for the oral comparator.

CONCLUSIONS: HT using a fixed intranasal dose of 350 μ g E2 combined with 550 μ g NET is a safe regimen, in relation to 1 year endometrial safety. This regimen is associated with less vaginal bleeding when compared with an oral comparator using 2 mg E2 and 1 mg NET.

Key words: hormone therapy / menopause / intranasal / estradiol / norethisterone

Introduction

Following the publication of the ‘Women’s Health Initiative’ (WHI) and the results of other large trials, regulating agencies have released

guidelines redefining prescription attitudes of hormone (replacement) therapy (HT) (Rossouw *et al.*, 2002; Beral *et al.*, 2003; Chlebowski *et al.*, 2003; EMEA, 2003; FDA, 2003). As a result, HT use has decreased dramatically (Ena and Rozenberg, 2003). In view of these

results, the need for safe alternative regimens that are different from the most used regimen, i.e. conjugated estrogens (CEE) combined with medroxyprogesterone acetate (MPA), has also emerged. Indeed, the principal limitation of the WHI trial is that in women with an intact uterus, it evaluated only one fixed regimen, i.e. CEE combined with MPA, and in hysterectomized women, it only used CEE (Rossouw et al., 2002). It has been shown that regimens using different estrogens, progestins, routes of administration or dosages may have different effects (Grady et al., 1995; O'Connell, 1995; Grodstein et al., 2000; Sturdee et al., 2000; Rossouw et al., 2002; Grodstein et al., 2003; The Women's Health Initiative Steering Committee, 2004; Fournier et al., 2005). Transdermal and nasal routes may offer a number of advantages, such as avoiding the gastrointestinal and hepatic first-pass (De Lignières et al., 1986; Hermens et al., 1991; Frenkel et al., 1994; Devissaguet et al., 1999; Garnero et al., 1999; Studd et al., 1999; Lopes et al., 2000). For instance, observational studies have reported no increase in C-reactive protein serum level (a marker of inflammation and a surrogate marker of cardiovascular morbidity) using non-oral HT, whereas this is known to occur using an oral route (Post et al., 2002; Skouby et al., 2002; Strandberg et al., 2003; Vongpatanasin et al., 2003; Hemelaar et al., 2006). And more recently, Hemelaar et al. (2008a) observed in a randomized controlled trial (RCT) that during intranasal E2/NET (175/275 µg) therapy, changes in the coagulatory and fibrinolytic markers were to some extent less than those observed during oral therapy (17βE2 1 mg/NET acetate 0.5 mg). Furthermore, in a French cohort study, oral HT but not non-oral HT was associated with a 2- to 3-fold increased risk of thrombo-embolic disease (Scarabin et al., 2003; Fournier et al., 2005; Straczek et al., 2005; Canonico et al., 2006). Some authors suggest, therefore, that the prudent clinical decision is to select the method that has epidemiological support (Hemelaar et al., 2008b). Still, there is a need for more data about safety issues regarding other regimens such as non-oral routes of administration (Skouby et al., 2005).

This report describes the results of a 52-week double-blind study comparing a continuous daily regimen (S21405) of intranasal 17β-estradiol (350 µg) combined with norethisterone (50, 175 or 550 µg) with a reference oral daily regimen of 17β-estradiol (2 mg) and norethisterone (1 mg) in a large sample. This report will focus on the endometrial effects and safety issues.

Materials and Methods

Design

This was a multicentre, multinational, double-blind and double-dummy RCT (Phase III) with a reference product and conducted in five parallel groups.

Patients and protocol

Healthy, non-hysterectomized women more than 2 years after menopause and between 40 and 75 years old, and without contraindications for the use of HT were included. The patients were recruited from various countries: Belgium, Brazil, Denmark, France, Germany, Hungary, Poland, Russia, Spain, Switzerland and UK. In addition, inclusion criteria comprised: serum E2 lower than 30 pg/ml, follicle-stimulating hormone higher than 30 mIU/ml, a normal cervical smear and a normal mammography within the last 12 months, a BMI less than 32, no abnormal uterine bleeding,

no cervical pathology (stenosis and distortion) precluding endometrial biopsy, a normal transvaginal ultrasound, an endometrial biopsy without hyperplasia or polyps, and normal blood tests for lipids, liver enzymes, kidney function, glucose and thyroid-stimulating hormone. Previous use of oral percutaneous or transdermal hormone replacement therapy (HRT) till the selection visit was authorized, but implants could not have been used during the last 3 years before entering the study, and intrauterine devices had to be removed at the selection visit.

Exclusion criteria were: any ear–nose–throat disease or treatment that might interfere with intranasal drug administration, or treatment liable to interfere with coagulation enzyme inducers or systemic vasoconstrictors.

The sample size should allow a precise estimate of the incidence of hyperplasia or serious adverse endometrial effects. Considering incidences of 1–2% for women treated with currently marketed combined HRT regimens, 300 patients treated for 1 year are necessary to estimate the incidence within a 95% confidence interval (CI) where the upper limit does not exceed 2%. Assuming a drop-out rate of 20 and 8% non-assessable biopsies, 400 patients had to be included in each treated group in order to obtain 300 assessable biopsies per group at the end of the study (PEPI Trial, 1996). The study was initiated on 27 June 2001 and completed on 16 June 2003. Then, because the development programme of the combined spray was discontinued, following the WHI study, the analysis was restricted to the main objective, especially as far as safety is concerned. A flow chart of patient recruitment and distribution per analysis set is presented in Fig. 1. Out of 2295 women screened to participate, 2016 were included after the run-in period of 1–6 weeks, but only 2012 were retained because of the protocol, and only 2007 effectively took at least one dose of the study drug. The latter group was considered the 'safety set of patients'. All safety analyses, gynaecological acceptability (vaginal bleeding pattern and mastalgia), local nasal acceptability, adverse events and biology were performed in this set of patients. Endometrial histology was assessed in all of the randomized patients who received at least one dose of treatment and who had at least one endometrial biopsy post-baseline performed at most 30 days after the last study treatment administration. This group of patients was defined as the 'full analysis set (FAS)' of patients ($n = 1741$). The analyses were also repeated in all the 'FAS' patients who had no protocol deviation at baseline or during the study ($n = 1592$). The latter group of patients was defined as the 'per protocol set' (PPS) of patients. No patients were lost to follow-up.

The therapeutic units were allocated by a balanced block randomization with stratification, using a voice response system, called by the investigator at the inclusion visit. Although the main objective was to determine the optimal daily dose of intranasal NET in order to achieve adequate endometrial protection, other end-points, such as the efficacy in preventing bone loss, were evaluated in subgroups of patients. Therefore, a group using a placebo (both for estrogen and progesterone) was also assessed in a smaller number of patients in Denmark. All the patients had given written informed consent, and the protocol had been accepted by the ethical review board of the institutions.

The main baseline characteristics (mean age, time since menopause, BMI and use of previous HT) did not differ among treatment groups (Table 1).

Criteria for evaluation

Endometrial safety was evaluated by measuring endometrial thickness using an endovaginal ultrasound probe (at selection, after 24 weeks of treatment and at completion after 52 weeks) and by endometrial histology using endometrial biopsies (at selection, after 24 weeks of treatment, when an endometrial thickness of more than 10 mm was measured, and at the end of the study after 52 weeks of treatment).

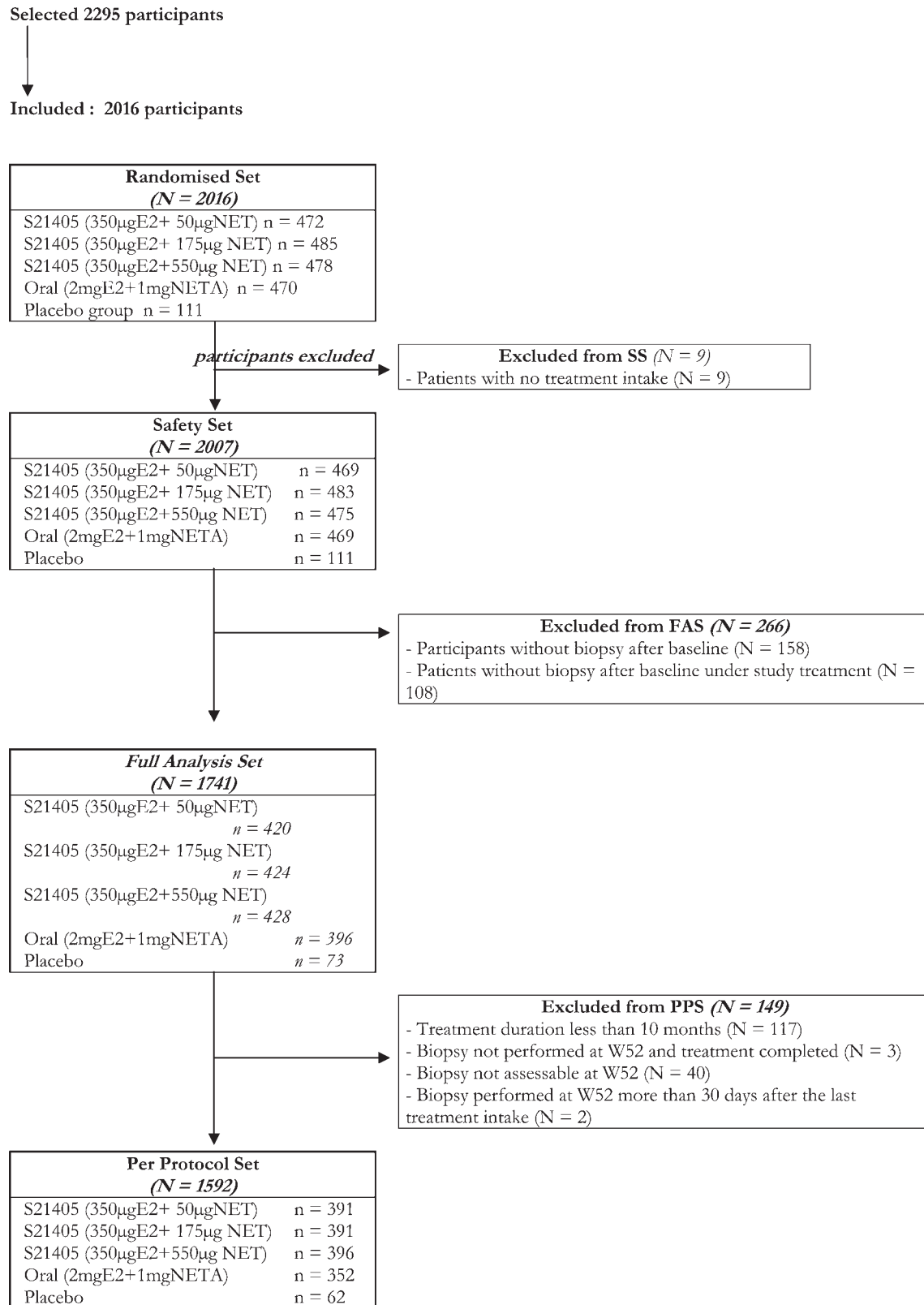


Figure 1 A flow chart of the patient distribution per analysis set for the different used regimens. Out of 2295 selected participants, 2007 took at least one dose of study drug ('safety set of patients'). The endometrial histology was assessed in all the randomized patients who received at least one dose of treatment and who had at least one endometrial biopsy post-baseline performed at most 30 days after the last study treatment administration 'FAS' of patients. The analyses were also repeated in all the patients of 'FAS' who had no protocol deviation at baseline or during the study, the 'PPS' patients.

Table I Summary of the demographic data at baseline, of the randomized participants (safety set of patients)

		S21405 (350 µg E2 + 50 µg NET) (n = 469)	S21405 (350 µg E2 + 175 µg NET) (n = 483)	S21405 (350 µg E2 + 550 µg NET) (n = 475)	Oral (2 mg E2 + 1 mg NETA) (n = 469)	Placebo (n = 111)
Age (years)	Mean (SD)	56.0 (5.4)	56.2 (4.7)	56.4 (4.9)	56.5 (5.3)	56.7 (3.7)
	Min–max	39–72	42–71	43–74	41–72	40–66
BMI ^a (kg/m ²)	Mean (SD)	24.9 (3.3)	25.2 (3.1)	25.1 (3.4)	25.1 (3.3)	24.6 (2.9)
	Min–max	15.9–32	17.9–35.3	16.6–32	17.1–35.1	17.5–32.1
Age at menopause ^a (years)	Mean (SD)	48.7 (4.3)	49.2 (3.8)	49.1 (4.6)	49.1 (4.1)	49.5 (3.8)
	Min–max	28.7–60.3	34.0–59.4	22.4–60.6	31.9–58.1	31.0–56.5
Menopause type (years)	Natural	466 (99.4%)	479 (99.2%)	474 (99.8%)	466 (99.4%)	110 (99.1%)
Previous HRT	Yes	334 (71.2%)	356 (73.7%)	357 (75.2%)	344 (73.3%)	56 (50.4%)

^aAge at menopause and BMI were calculated, respectively, on $n = 454, 462, 449, 445, 106$ and $n = 469, 483, 474, 468, 111$ patients.

The classification used was the one approved by the WHO: no tissue obtained, tissue insufficient for diagnosis, atrophic and/or inactive endometrium, proliferative endometrium, secretory/progestational endometrium, menstrual type endometrium, polyps, simple hyperplasia, complex hyperplasia, atypical hyperplasia and carcinoma (Mazur, 2005). In order to take into account the interobserver variability, a double reading was carried out in a blinded manner by two independent pathologists.

The specimens were read by a third pathologist in cases where the two pathologists differed on whether hyperplasia was present or on the severity of hyperplasia. For data analyses, the results of two of the three pathologists were considered. Whenever the three pathologists gave readings of different types of hyperplasia, the reading representing the most severe type of pathology was selected. Any slides for which there were discrepancies (other than hyperplasia) between the two readers were simultaneously read by the two experts at a 'consensus meeting'.

Endometrial thickness analyses were performed in the FAS and described according to the following stratification based on an endometrial thickness of: less than or equal to 4 mm, more than 4 mm and no more than 8 mm, or of more than 8 mm.

Other safety measures included the evaluation of local nasal tolerance, gynaecological acceptability (vaginal bleeding and mastalgia), general and gynaecological examination, mammography and biological safety.

Statistical analysis

Due to the low rate of expected endometrial hyperplasia and carcinoma, only descriptive statistics are performed on the main criterion. Descriptive statistics were provided according to the type of parameter (frequency and percentage for qualitative parameter and frequency, mean, standard deviation and range for quantitative or categorical data). Percentages in groups were compared using χ^2 test or Cochran–Mantel–Haenszel test (CMH modified ridit score). Alpha risk was fixed at 0.05.

Results

The distributions of endometrial histological classifications at the beginning of the study were similar for all groups (Table II). The vast majority (73–86%) of women had an 'atrophic and/or inactive' endometrium both at the beginning and at the end of the study (Table III).

At the end of treatment, the percentage of women with a 'proliferative' endometrium was significantly higher when lower doses of NET

were used (1.4% for 550 µg NET, 3.1% for 175 µg NET and 7.1% for 50 µg NET, respectively) ($P < 0.001$). 'Secretory' endometrium was more frequent in the oral group (10%) in comparison with the intranasal estradiol groups (<1%) (global analysis $P < 0.001$) (Table III). Four cases of hyperplasia occurred, all in the group treated with the lowest dose of NET (350 µg E2 + 50 µg NET), an incidence of 1% (95% CI: 0.3–2.4%). No case of endometrial cancer was diagnosed in any group. The results obtained in the full analyses set were similar to those in the PPS.

Although most of the participants had an endometrial thickness of ≤ 4 mm, women with the lowest (50 µg) dose of NET more often had an endometrial thickness higher than 8 mm (Fig. 2). Analysis of confounding factors showed a relationship between BMI and proliferative or secretory endometrium, the incidence of which was four times higher in women with the highest quartile BMI in comparison with women in the lowest quartile BMI ($P < 0.01$).

About 62% of women experienced at least one bleeding episode during the study: 63.5, 59.4, 56.8, 72.5 or 37.8% of women using 350 µg E2 + 50 µg NET, 350 µg E2 + 175 µg NET, 350 µg E2 + 550 µg NET, oral 2 mg E2 + 1 mg NETA or a placebo, respectively. The incidence of vaginal bleeding decreased with time, and was the lowest for the placebo group and those using the highest dose of NET; 88.1% of women on the highest nasal dose were in amenorrhoea during the last 4 months of treatment when compared with 71.7% of those using the oral comparator (difference 16.5%; 95% CI: 10.9–22.0%) ($P < 0.001$) (Fig. 3).

Finally, premature discontinuation rates were in the range of 12–17% for the three nasal regimens, 22% for the oral comparator and 45% for the placebo group. In the placebo group, about 24% of discontinuations were due to lack of efficacy, and 10% were due to adverse events. In particular, cardiovascular side effects were sparse, the most frequent being hypertension which occurred in 1.5% (350 µg E2 + 50 µg NET), 3.7% (350 µg E2 + 175 µg NET), 2.9% (350 µg E2 + 550 µg NET), 3.0% (2 mg E2 + 1 mg NETA) and 0.9% (placebo) of the women. In the nasal group, discontinuation due to adverse events also occurred in 7–9.5% of the cases and in 17% of the oral comparator group. In all groups, about 4–5% discontinued the treatment for non-medical reasons. Fig. 4 illustrates the proportion of women who completed or prematurely discontinued

Table II Number (%) of histological diagnoses at the beginning of the study; for the various randomized groups in the FAS

	S21405 (350 µg E2 + 50 µg NE) (n = 420)	S21405 (350 µg E2 + 175 µg NET) (n = 424)	S21405 (350 µg E2 + 550 µg NET) (n = 428)	Oral (2 mg E2 + 1 mg NETA) (n = 396)	Placebo (n = 73)
No tissue obtained	2 (0.5)	2 (0.5)	3 (0.7)	2 (0.5)	0 (0.0)
Tissue insufficient for diagnosis	31 (7.4)	42 (9.9)	49 (11.4)	37 (9.3)	6 (8.2)
Atrophic and/or inactive endometrium	318 (75.7)	310 (73.1)	310 (72.4)	295 (74.5)	61 (83.6)
Proliferative endometrium	35 (8.3)	28 (6.6)	29 (6.8)	31 (7.8)	2 (2.7)
Secretory/progestational endometrium	19 (4.5)	27 (6.4)	19 (4.4)	19 (4.8)	3 (4.1)
Menstrual type endometrium	15 (3.6)	14 (3.3)	18 (4.2)	12 (3.0)	1 (1.4)
Hyperplasia simple/complex/atypical	0/0/0	0/1 (0.2)/0	0/0/0	0/0/0	0/0/0

There were no differences in between groups.

Table III Number (%) of histological diagnoses at the end of the study; for the various randomized groups in the FAS

	S21405 (350 µg E2 + 50 µg NE) (n = 420)	S21405 (350 µg E2 + 175 µg NET) (n = 422)	S21405 (350 µg E2 + 550 µg NET) (n = 427)	Oral (2 mg E2 + 1 mg NETA) (n = 396)	Placebo (n = 73)
No tissue obtained	3 (0.7)	1 (0.2)	3 (0.7)	1 (0.3)	0 (0.0)
Tissue insufficient for diagnosis	46 (11.0)	53 (12.6)	48 (11.2)	50 (12.6)	9 (12.3)
Atrophic and/or inactive endometrium	322 (76.7)	347 (82.2)	367 (85.9)	290 (73.2)	61 (83.6)
Proliferative endometrium	30 (7.1)	13 (3.1)	6 (1.4)	4 (1.0)	2 (2.7)
Secretory/progestational endometrium	4 (1.0)	4 (0.9)	3 (0.7)	41 (10.4)	0 (0.0)
Menstrual type endometrium	11 (2.6)	4 (0.9)	0 (0.0)	10 (2.5)	1 (1.4)
Hyperplasia simple/complex/atypical	2 (0.5)/2 (0.5)/0	0/0/0	0/0/0	0/0/0	0/0/0

There were significantly more proliferative endometria in the low dose (50 µg NET) in between groups ($P < 0.001$).

(due to lack of efficacy, adverse event or non-medical reasons) according to the HT used.

Discussion

The primary objective of this study was to determine the daily dose of intranasal NET (50, 175 or 550 µg) that should be co-administered with a fixed daily dose of 350 µg intranasal E2 to achieve adequate endometrial protection. In this large, randomized, double-blind study, the rate of endometrial hyperplasia was low with only four cases of endometrial hyperplasia (two simple and two complex

cases without atypia), all in the group treated with the lowest dose of NET (50 µg), i.e. an incidence of 1%. Endometrial cancers were not observed, but this is not unexpected since in the Million Women Study, for instance, the overall incidence of endometrial cancer was only 3 per 1000 over a 5-year period (Beral *et al.*, 2005).

The rate of 'no tissue' was very low, <1%, and the rate of 'tissue insufficient for diagnosis' was around 12%, and the majority of women had an endometrial thickness of <4 mm. The majority of women who had been treated with an active regimen had, as expected, an endometrium that was considered to be 'atrophic and/or inactive'. Still, a dose-effect of NET was observed: women

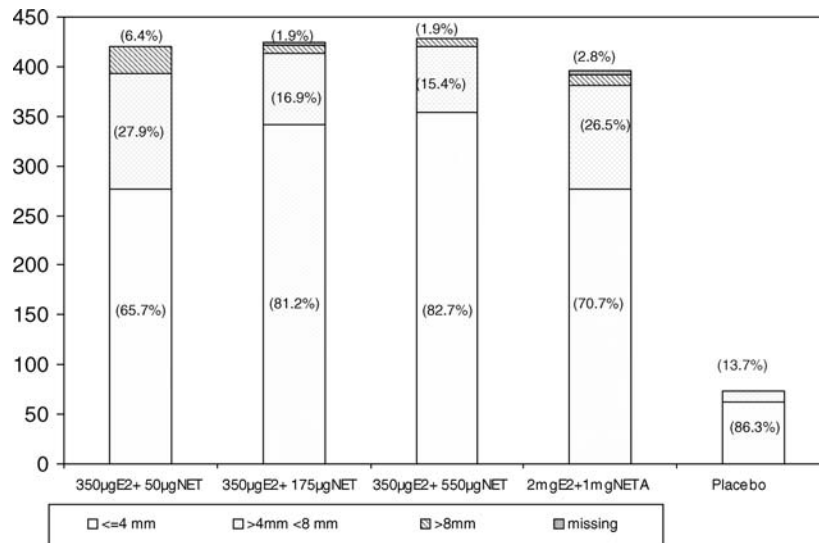


Figure 2 Number of patients (%) with various endometrial thicknesses in relation to the used HT.

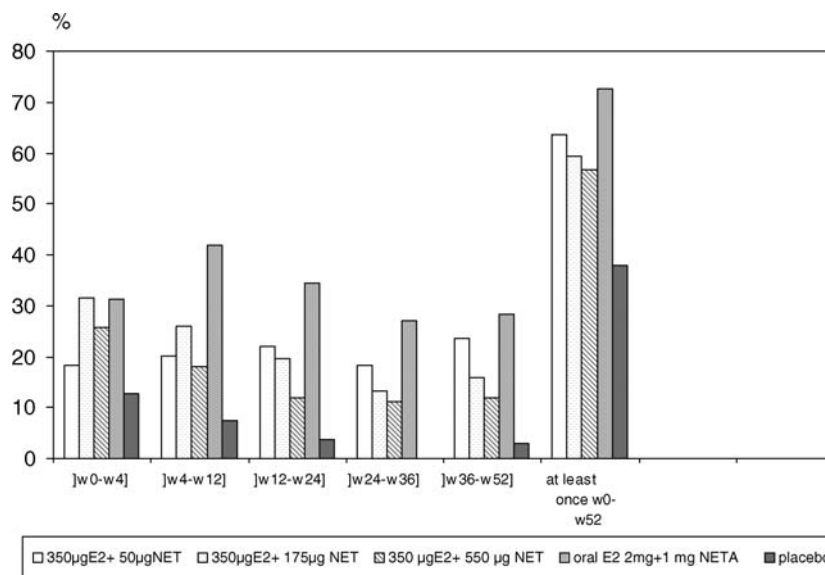


Figure 3 Proportion of patients (%) with at least one bleeding episode in relation to time and to the used HT.

who were treated with the lowest nasal dose of NET had the highest prevalence of 'proliferative' endometria, and higher rates of 'secretory' endometria were observed in women using the oral comparator. This latter effect probably relates to the higher estradiol dose in the oral comparator (2 mg) compared with the lower dose of intranasal estradiol (350 µg) or that pulsed E2 leads to a more limited proliferative effect than does continuous E2 in the uterus. Indeed, it has been shown in ovariectomized Wistar rats treated with IV E2 (to mimic pulse therapy) that proliferating cell nuclear antigen expression in the uterine epithelium (used as a marker of proliferation) is significantly induced after continuous administration but only slightly after pulsed E2 (Diel et al., 2005).

It is well established that long-lasting unopposed estrogen exposure leads to endometrial hyperplasia, which increases the chance of development of atypical hyperplasia and eventually type-I endometrial cancer. The exact molecular basis of this process is still not known. It is, however, well established that there is an association between loss of PTEN function and endometrial cancer, and that progestins may promote involution of PTEN-null endometrial glands, which is consistent with the beneficial effect of progestins in treating hyperplasia (Zheng et al., 2004). Many studies have shown that the length and the doses of the administered progestins play a major role (Sturdee et al., 2000; Lethaby et al., 2004). For instance, continuous norethindrone acetate at doses as low as 0.1 mg combined with 1 mg E2

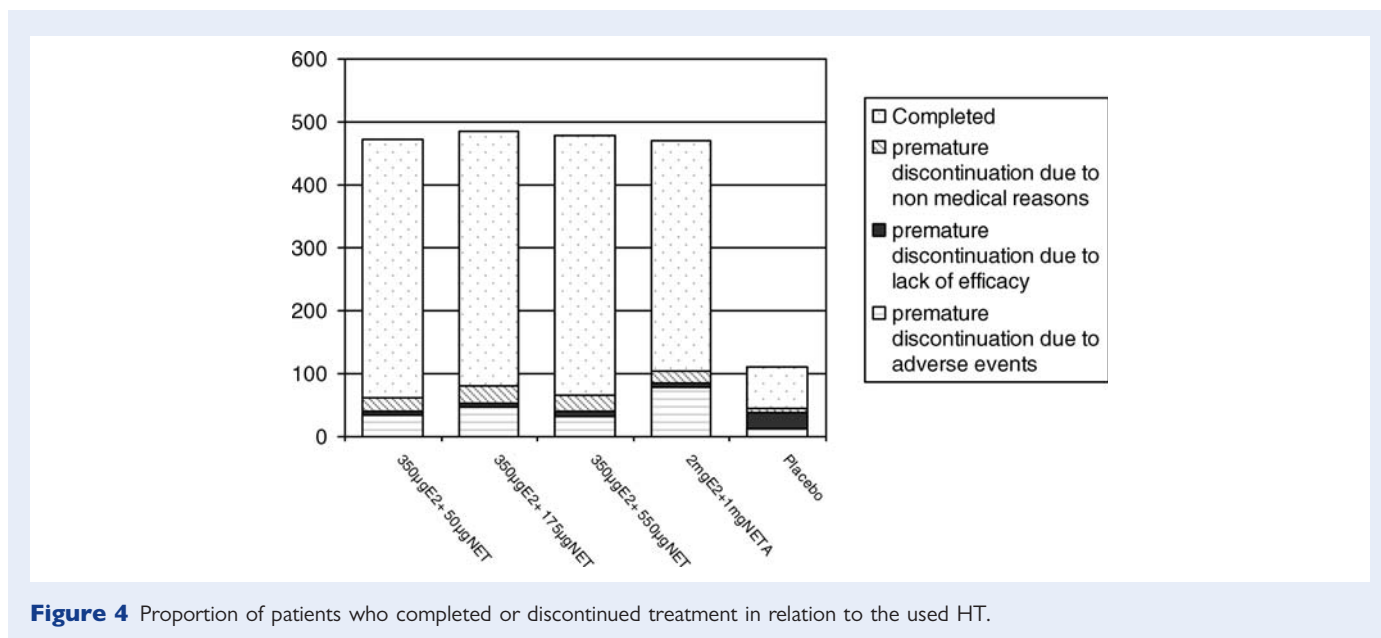


Figure 4 Proportion of patients who completed or discontinued treatment in relation to the used HT.

effectively negated the risk of endometrial hyperplasia, at least for the first year of therapy (Kurman *et al.*, 2000).

Optimally, the lowest effective dose of a progestin ensuring endometrial safety should be chosen, since progestins may be associated with negative effects on breast cancer risk and on cardiovascular morbidity (Skouby *et al.*, 2005). Thus, reducing the dose of progestogen to that which adequately opposes endometrial stimulation of the specific estrogen dose and minimizes unexpected bleeding is clinically justifiable.

In this study, we observed that an optimal histological profile was reached using 550 µg NET in addition to the 350 µg E2 in nasal spray. Indeed, about 86% of women had an atrophic endometrium (while 11% had insufficient tissue for diagnosis). The latter dose was also associated with the lowest bleeding profile (except placebo) and discontinuation rate. Although very obese women were excluded from this trial, increased prevalence of proliferative or secretory endometrium was associated with higher BMI. It is well established that obese women have higher endogenous estrogen exposure that is associated with increased endometrial pathology (Rose, 1996; Sit *et al.*, 2004). Similarly, many studies have also found that HT use in obese women is associated with increased endometrial pathology or thickness (Rose, 1996; Sit *et al.*, 2004): obesity causes higher circulating concentrations of bio-available estrogens from extraglandular conversion of androgens. This change stimulates endometrial-cell proliferation, inhibits apoptosis and promotes angiogenesis. A BMI above 25 kg/m² doubles a woman's risk of endometrial cancer, and a BMI above 30 kg/m² triples the risk (Rose *et al.*, 1996; Beral *et al.*, 2005).

The conclusions that can be drawn from this study present some limitations: the rather short length of follow-up, the absence of long-term evaluation of breast cancer or cardiovascular risk, and the selected population of patients with a low risk profile for endometrial pathology: for instance, women with severe obesity, diabetes or a history of abnormal bleeding were excluded, as were women who had developed hyperplasia or other severe side effects when using HRT previous to the trial.

To summarize, in this large prospective study evaluating three fixed regimens of intranasal estradiol and NET, we were able to document that the dose of 350 µg E2 combined with 550 µg NET is a safe regimen, in relation to short-term endometrial safety, and that it is associated with less vaginal bleeding when compared with an oral comparator using 2 mg of estradiol and 1 mg of NET.

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