

From the Department of Clinical Sciences, Obstetrics and Gynecology,  
Umeå University, Umeå, Sweden

# Hormone replacement therapy and effects on mood

Inger Björn



Umeå 2003



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Umeå University, Umeå, Sweden

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som med vederbörligt tillstånd av Rektorsämbetet vid Umeå Universitet  
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**Inger Björn**



**Umeå 2003**

Fakultetsopponent:  
Professor Bo von Schoultz  
Institutionen för Kvinnor och Barns hälsa,  
Karolinska sjukhuset, Stockholm

# ABSTRACT

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**Background:** During the past 5 decades, hormone replacement therapy (HRT) has been used, and appreciated for its beneficial effects, by millions of women in their menopause. As treatment for climacteric symptoms, estrogen is outstanding, and effects on hot flushes, vaginal dryness, and insomnia have been widely documented. The increased risks of venous thrombosis and breast cancer, however, restrict the use of estrogen.

Estrogen treatment in women with a remaining uterus includes a progestin, added to protect the endometrium from hyperplasia and malignancies. The long-standing clinical impression, that progestin addition negatively influences mood, has been discussed in previous studies. Mood deterioration is, however, not mortal, although mood is important to the wellbeing and daily functioning of women treated with hormones. Studies of the mental side effects of HRT add to our understanding of steroid effects in the brain.

**Aims and methods:** In our studies, we aimed to establish to what extent negative side effects cause women to discontinue HRT, and find out which drug compounds lead to mood deterioration. The questions asked were whether the type and dose of progestin and the estrogen dose during the progestin addition influence the mood and physical symptoms during sequential HRT.

Compliance with HRT and reasons for discontinuing the therapy were evaluated in a retrospective longitudinal follow-up study. Treatment effects were studied in three randomized, double-blind, cross-over trials. During continuous estrogen treatment, effects of sequential addition of a progestin were studied by comparing two different progestins, medroxyprogesterone acetate (MPA) and norethisterone acetate (NETA), comparing different doses of the same progestin, MPA, and comparing two doses of estrogen during addition of the same dose of MPA. The main outcome measure was the daily rating on mood and physical symptoms kept by the participants throughout the studies. The clinical trials were carried out at three gynecological centers in northern Sweden.

**Results and conclusions:** Besides fear of cancer and a wish to determine whether climacteric symptoms had meanwhile disappeared, negative side effects was the most common reason for discontinuing HRT. Tension in the breasts, weight gain, a depressed mood, abdominal bloating, and irritability were the most important side effects seen both in women who continued HRT and in women who had discontinued the therapy.

In our clinical trials, we showed that addition of a progestin to estrogen treatment induces cyclic mood swings characterized by tension, irritability, and depression, as well as increased breast tension, bloatedness, and hot flushes. Women with a history of premenstrual syndrome (PMS) appeared to be more sensitive to the progestin addition and responded with lower mood scores compared with women without previous PMS. In our studies, MPA provoked depressed mood to a lesser extent than did NETA. Surprisingly, the higher dose of MPA (20 mg) enhanced the mood, compared with 10 mg, when added to estrogen treatment. In women continuously treated with 3 mg estradiol, mood and physical symptoms worsened during the progestin addition, as compared with treatment with 2 mg estradiol. The negative side effects seen during sequential HRT have much in common with symptoms seen in the premenstrual dysphoric disorder (PMDD), which is a psychoneuroendocrine disorder with psychiatric expression. Explanations for treatment effects on mood are likely to be found in drug interactions with neurotransmitter systems of the brain.

**Key words:** adverse effects, estradiol, hormone replacement therapy (HRT), mood, negative side effects, progestins.



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*Till den som söker kunskap  
och strävar efter visdom*

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**Key words:** adverse effects, estradiol, hormone replacement therapy (HRT), mood, negative side effects, progestins.

# ORIGINAL PAPERS

- I. Björn I and Bäckström T. Drug related negative side effects is a common reason for poor compliance in hormone replacement therapy.  
Maturitas 1999, 32:77-86
- II. Björn I, Bixo M, Strandberg Nöjd K, Nyberg S, and Bäckström T. Negative mood changes during hormone replacement therapy: A comparison between two progestogens.  
American Journal of Obstetrics and Gynecology  
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- III. Björn I, Bixo M, Strandberg Nöjd K, Collberg P, Nyberg S, Sundström-Poromaa I, and Bäckström T. The impact of different doses of medroxyprogesterone acetate on mood symptoms in sequential hormonal therapy.  
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- IV. Björn I, Sundström-Poromaa I, Bixo M, Nyberg S, Bäckström G, and Bäckström T. Increase of estrogen dose deteriorates mood during progestin phase in sequential hormonal therapy.  
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# ABBREVIATIONS

ANOVA	analysis of variance
BMI	body mass index
CD	cyclicity diagnoser
CEE	conjugated equine estrogen
CNS	central nervous system
DNA	deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
E2	estradiol 2
ER	estrogen receptor
ER- $\alpha$	estrogen receptor $\alpha$
ER- $\beta$	estrogen receptor $\beta$
ERT	estrogen replacement therapy
FSH	follicle-stimulating hormone
GABA	gamma aminobutyric acid
GnRH	gonadotropin-releasing hormone
5-HIAA	5-hydroxyindole acetic acid
HRT	hormone replacement therapy
5-HT	5-hydroxytryptamine
LH	luteinizing hormone
LNG	levonorgestrel
MAO	monoamine oxidase
MEIA	microparticle enzyme immunoassay
MPA	medroxyprogesterone acetate
mRNA	messenger ribonucleic acid
NETA	norethisterone acetate
NMDA	N-methyl-D-aspartate
OC	oral contraceptive
PMDD	premenstrual dysphoric disorder
PMS	premenstrual syndrome
PR	progesterone receptor
PRIME-MD	primary care evaluation of mental disorders
RR	relative risk
SHBG	sex hormone-binding globulin
SIR	standard incidence ratio
SSRI	selective serotonin reuptake inhibitor
TPH	tryptophan hydroxylase
VAS	visual analog scale

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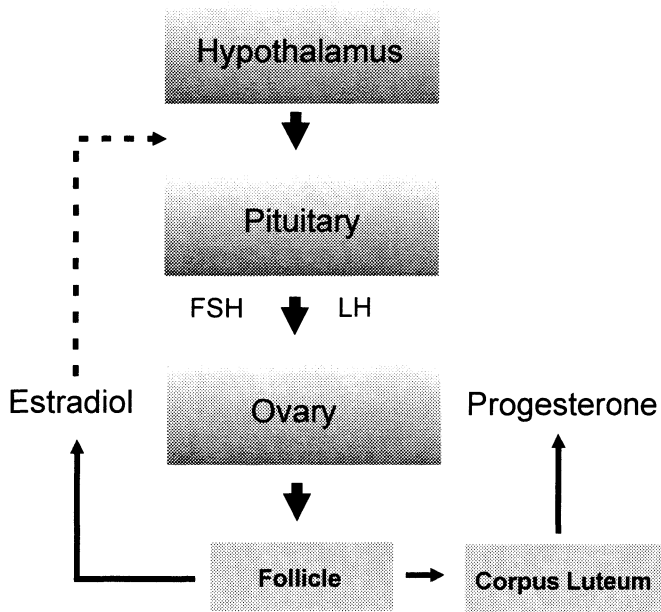
# INTRODUCTION

The mean age for natural female menopause in Western, white women is 51.3 years, according to the Massachusetts Women's Health Study (McKinlay et al 1992). Menopause is defined as the permanent cessation of menstruation resulting from loss of ovarian follicular activity. Smoking, low body weight, and hysterectomy affect the timing of menopause, with current smokers, for example, entering menopause 1.5–2.0 years earlier than nonsmokers (McKinlay et al 1992). The perimenopausal transition is estimated to last for nearly 4 years (McKinlay et al 1992). In the early perimenopause, irregularity in the menstrual cycle is observed, along with rising levels of pituitary follicle-stimulating hormone (FSH) (Hee et al 1993). At the same time, there is a decrease in levels of ovarian epithelial peptides, and inhibin (MacNaughton et al 1992). Secretion of FSH is partly controlled by the hypothalamic gonadotropin-releasing hormone (GnRH), but is also subject to negative feedback of ovarian estradiol, progesterone, and the inhibins (Burger 1999).

In the late perimenopause, levels of serum estradiol start to decline (Shifren & Schiff 2000). At the time of the menopause, few follicles remain in the ovary. Already 3 months after the menopause, estradiol levels have dramatically decreased, and at 12 months past menopause, they are at their nadir (Guthrie et al 1996). Women who are 3–12 months postmenopausal have a mean serum estradiol concentration of approximately 80 pmol/l and in women who are more than 12 months postmenopausal, serum concentrations of 40 pmol/l are seen (Guthrie et al 1996). However, during the postmenopausal years, irregular peaks of estradiol production can be observed.

Approximately 40–70% of postmenopausal women suffer from hot flushes and sweats, so-called “vasomotor symptoms” (Hammar et al 1984, Stadberg et al 1997a). Hot flushes, the most pronounced climacteric symptom, are most intense 3 months or more after menopause (Guthrie et al 1996). The frequency of hot flushes is associated with an increase in FSH, a decrease in estrogen, and a history of premenstrual complaints (Guthrie et al 1996). Other common climacteric problems are insomnia,

depressive mood swings, and vaginal dryness (Stadberg et al 1997a, Dennerstein et al 2000).



**Figure 1.** Overview of the hypothalamic-pituitary-gonadal axis.

The postmenopausal state in women lasts for about 30 years and has sometimes been regarded as a state of chronic estrogen deficiency. The effect of estradiol (E2) and conjugated equine estrogen (CEE) as a treatment for hot flushes and insomnia and to increase wellbeing in postmenopausal women has been widely documented (Campbell & Whitehead 1977, Sherwin 1991, Wiklund et al 1993).

In the 1960s and 1970s, the medical profession hesitated to offer women hormone replacement therapy (HRT) as estradiol was considered to potentially increase the risk of breast cancer, thrombosis, hypertension, stroke, and coronary heart disease. In Sweden in 1958, the popular health care book *Kvinnor se upp* addressed the estrogen-stimulating effect on breast cancer. “However,” the authors point out, “if the use [of hormones] is wise – and if the doctor prescribes them – there is no risk of developing cancer” (Ahltorp & Kuhnel 1958). Massive research in the 1980s and 1990s revolutionized opinion about HRT, and voices from the medical profession rose to encourage postmenopausal women to take estrogen. In case-control studies, women



treated with estrogen were reported to live longer than women without treatment (Henderson et al 1991, Ettinger et al 1996) and epidemiological studies indicated protective effects on bone mineral density and cardiovascular disease (Grodstein et al 1997, 1999). In 2 decades, estrogen use among Swedish postmenopausal women increased from 7% to 24% (Andersson et al 1996). The attitude towards estrogen treatment among gynecologists and general practitioners became more favorable, both in the medical practice and for treatment of their partners (Andersson et al 1996). In an article titled "The estrogen dilemma", published by the well-known *Time* magazine on estrogen therapy during menopause, menopause was pronounced to be "unnecessary". "Thanks to hormone therapy," it said, "women may look forward to prolonged well-being and extended youth." The possible drawbacks of therapy, such as an increased risk of breast cancer, were, however, also discussed (Wallis 1995).

Today it is known that estrogen therapy increases the risk of venous thrombosis (Daly et al 1996, Jick et al 1996, Grady et al 2000, Miller et al 2002). Moreover, long-term HRT appears to increase the risk of breast cancer (Magnusson et al 1999, Ross et al 2000, Olsson et al 2001, Santen et al 2001, Newcomb et al 2002). The latest discouraging study regarding HRT and chronic disease prevention had to be disrupted as treatment with estrogen and a progestin resulted in net harm in healthy women (Writing Group for the Women's Health Initiative Investigators 2002). Still, according to a recent study, 92% out of 435 gynecologists in North America and Israel routinely offer HRT to their menopausal patients (Kaplan et al 2002).

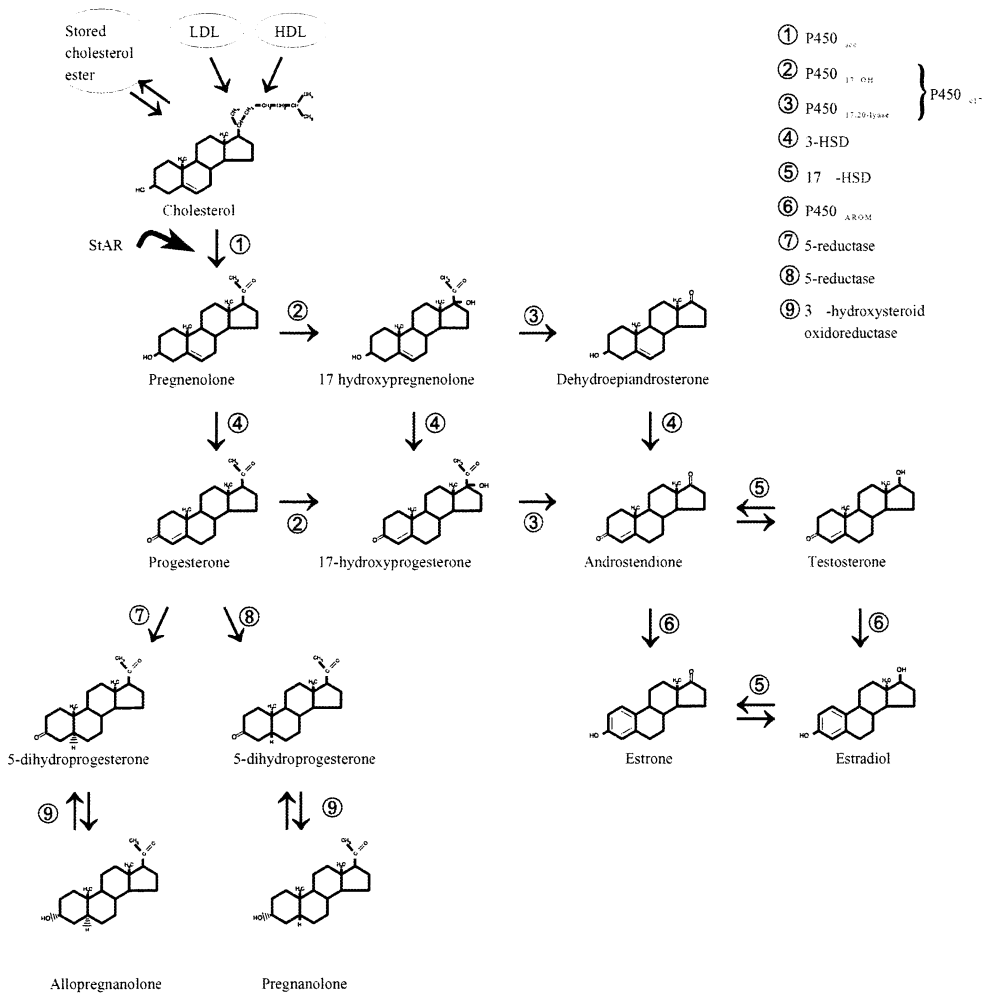
"Without a doubt, you are not really sane", as the Swedish comedian Tage Danielsson used to say.



The Thinker by A. Rodin

My personal reflection is that opinions and enthusiasm move in cyclic pathways, not unlike the ovarian cycles during the fertile period in women. In terms of HRT, we are back to where we started, somewhat wiser perhaps, having made numerous experiences along the way. There is no treatment which cures all conditions or gives eternal youth. We have to regard HRT with the same skepticism as we do other therapies, taking both the advantages and disadvantages into consideration. In managing HRT today, it is important not to frighten women currently on HRT, but to try to help patients in making choices beneficial to their wellbeing and quality of life, without increasing risk factors to unacceptable levels. Judgment of the individual risk is a very hazardous task and has not become easier with increasing knowledge, since many factors have to be weighed against each other and evaluated. There is, however, enough evidence to end routine use of HRT prescribed without proper indication for treatment. Estrogen is the drug of choice for treating climacteric complaints and treatment of osteoporosis still remains an indication. However, the benefit of treating older women with HRT to prevent osteoporosis is unclear and treatment effects on different age groups are under evaluation (SBU rapport 2002). Current data do not support giving HRT to healthy women for prevention of chronic disease, as the risks outnumber the benefits.

Nevertheless, research on the topic has for the past 50 years given us insight into the biological mechanisms of estrogen and progestins. Ovarian steroid effects on the brain have just recently begun to be elucidated and new techniques have opened possibilities to understand estradiol and progesterone influences on mood swings and dysphoric mood disorders. In this thesis, a clinical approach was used to study mental effects during postmenopausal HRT. The studies upon which this thesis is based started in 1996, and are to be seen from the perspective of hopes for and doubts about long-term beneficial health effects of estrogen treatment over the decades.



**Figure 2.** Diagram of the steroidogenic pathways. Enzymatic steps indicated by arrows and numbers. The synthesis of progesterone, estradiol and the progesterone metabolites allopregnanolone and pregnanolone.

## Estrogen and the endometrium

Crystalline estrone was first isolated from pregnancy urine in 1929, and was then named “theelin” (Furuhjelm 1977). In the same year, the first American report described treatment of menopausal symptoms with ovarian hormone. During the 1930s, several reports claimed that estrogen had an effect on climacteric symptoms, as well as on psychological disturbances during menopause. In the 1940s and 1950s, several studies on menopausal depression and psychosis reported estrogen-induced

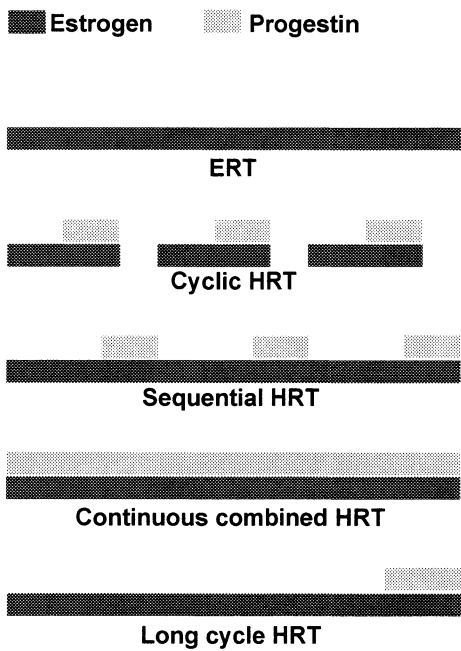
improvement of these disorders (Furuhjelm 1977). The estrogenic preparations were available both as injections and, introduced in 1941, in the oral form (Ettinger 1998). Back then, as well as today, the overall benefits and risks of estrogen treatment were discussed. Miriam Furuhjelm, one of the pro-estrogen debaters, wrote, "It is astonishing that estrogens are not given to all climacteric women who complain about symptoms nowadays" (Furuhjelm 1977).

However, there are two sides to every coin. The first report stating that the risk of endometrial cancer is increased when unopposed estrogen is used came out in 1975. The risk ratio (RR) given was 7.6 (Ziel & Finkle 1975). This finding was verified 20 years later in a meta-analysis including 30 studies (Grady et al 1995). The RR for endometrial cancer was associated with prolonged use of unopposed estrogen (RR 9.5 for 10 or more years). The incidence of abnormal vaginal bleeding, curettage, and hysterectomy was also reported to increase with long-term unopposed estrogen use (Ettinger et al 1988). Estrogen stimulates endometrial growth, which can induce hyperplasia, atypia, and malignancies. Hyperplasia of the endometrium, and in particular, complex hyperplasia, is considered to be a precursor of endometrial cancer (Gordon et al 1977). Since progesterone has an opposite effect on the endometrium, synthetic progestins are the drugs of choice to oppose estrogen in order to reduce the risk of endometrial cancer. The reason why synthetic alternatives are used is that natural progesterone given orally undergoes extensive first-pass liver metabolism and has a very short half-life in plasma. When a progestin is added to estrogen treatment, i.e. HRT, the increased risk of endometrial hyperplasia and carcinoma is reduced (Grady et al 1995, Woodruff & Pickar 1994, The Writing Group for the PEPI Trial 1996).

The dose, intervals, and duration of progestin addition, as well as the dose of estrogen are of importance in prevention of endometrial hyperplasia and cancer. It has been suggested that in women who are given 0.625 mg CEE, sequential regimens should include 5 or 10 mg of medroxyprogesterone acetate (MPA) for 12 days or more (Gibbons & Thorneycroft 1999). When 2 mg estradiol (e.g., E2) or 0.625 mg CCE is combined with continuous progestin, the endometrium effect is opposed by 1 mg norethisterone acetate (NETA) or 2.5–5 mg MPA (The Writing Group for the PEPI Trial 1996, Wells et al 2002).

Long-cycle HRT models, such as those with 3 months of estrogen only, and 14 days of 20 mg MPA addition at the end of each 3-month cycle, appear to give better endometrial protection than 10 days of 1 mg NETA (Hirvonen et al 1995, Bjarnason et al 1999). If the estrogen dose is lowered

from the standard dose of 0.625 mg CCE to 0.3 mg in women aged >55 years, an interval of 6 months for the 14-day progestin addition of 10 mg MPA seems safe (Ettinger et al 2001). An intrauterine system containing either 10 ug or 20 ug levonorgestrel (LNG) suppresses the endometrium in women treated with either 2 mg oral estrogen or estrogen patch (50 ug/24 h) (Varila et al 2001, Raudaskoski et al 2002). Concern for the health of the endometrium has resulted in the different HRT models.



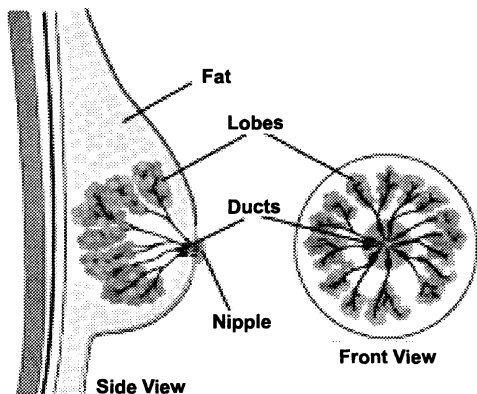
**Figure 3.** Modes of estrogen and hormone replacement therapy.

**Estrogen and the breast**

Breasts are target organs for ovarian hormones and contain estrogen receptors (ERs) and progesterone receptors (PRs). The structure and differentiation of breast lobuli develop throughout the woman’s life span. After menopause, lobuli change into the same type as they were during puberty and ordinary stroma is replaced by fat. The major influence on breast growth in puberty is estrogen. Estrogen stimulates proliferation by speeding up the cell cycle in the breast epithelium. Fluid secretion, mitotic activity, and deoxyribonucleic acid (DNA) production peak during the

luteal phase under the presence of both estrogen and progesterone (Speroff et al 1994).

It has long been debated whether progestins have a proliferative effect on breast cells or not. Experiments have shown conflicting results and in vitro studies have demonstrated both stimulating and inhibiting effects on breast cell proliferation (Santen et al 2001). In vivo studies indicate a possible additive stimulating effect on the breast by progestins in combination with estrogen (Söderqvist et al 1997, Cline et al 1998, Santen et al 2001). The type, dose, and duration of progestin influence may also be important.



**Figure 4.** The female breast.

Breast cancer is the most common female cancer, with an estimated 5,500 new cases in Sweden per year. Furthermore, the incidence is slowly increasing, by 1.4% per year (Swedish Cancer Register). Several studies have shown that long-term estrogen treatment increases the risk of breast cancer. The risk seems to increase with duration of use, and addition of continuous progestin further elevates the risk (Magnusson et al 1999, Schairer et al 2000, Ross et al 2000, Newcomb et al 2002). Hormone replacement therapy taken for less than 4 years does not appear to increase the risk of breast cancer, but after 4–10 years of treatment, the standard incidence ratio (SIR) increases to 1.92 compared with that for never users (Olsson et al 2001). In a review of four studies, the RR for breast cancer with unopposed estrogen treatment for more than 10 years has been reported to be 0.93–2.7. If a progestin is added continuously to the estrogen treatment, the RR increases to 1.79–2.95 (Santen et al 2001).

Mammographic density may be a risk factor for cancer development in the breast (Mandelson et al 2000). Estrogen in combination with progestin



treatment increases mammographic density (40%) in comparison with oral low-dose estrogen alone (6%) and transdermal estrogen (2%) (Lundström et al 2001). It has been suggested, however, that breast tumors detected in women receiving HRT have certain histological and biological characteristics that make these cancers less aggressive (Delgado & Lubian Lopez 2001) and that estrogen replacement therapy (ERT) may not increase the incidence of fatal breast cancer (Willis et al 1996).

## **Estrogen and the heart**

A number of observational studies have indicated that estrogen treatment decreases the risk of cardiovascular disease, and furthermore, that numerous intermediate markers of coronary disease are also positively influenced by HRT (Grady et al 1992, The Writing Group for the PEPI Trial 1995, Mendelsohn & Karas 1999). The frequently cited Nurses' Health Study indicates that current postmenopausal HRT users have a lower mortality (RR 0.63) than nonusers (Grodstein et al 1997). According to these epidemiological studies, it was recommended that women with coronary heart disease and women at high risk of coronary heart disease take hormone therapy.

Prospective randomized trials were, however, lacking until results of the Heart Estrogen/Progestin Replacement Study (HERS) were presented (Hulley et al 1998). Despite significantly beneficial changes of lipoprotein markers in the estrogen and progestin-treated group, the HERS results showed that events of myocardial infarction or coronary heart disease death did not decrease in HRT-treated women. The study group of 2,763 women with coronary disease, mean age 66.7 years, were randomized to receive either 0.625 mg CEE plus 2.5 mg of MPA, or placebo. More women in the HRT group than in the placebo group experienced venous thromboembolic events (RR 2.89), especially during the first years of treatment. After 4–5 years of treatment, coronary events decreased in the treated group. The conclusion from the HERS study was that estrogen treatment in combination with progestin could not be recommended for the purpose of secondary prevention of coronary heart disease, but that women already on treatment should be encouraged to continue (Hulley et al 1998).

Results of one of the largest prospective studies ever performed on postmenopausal HRT have recently been presented (Writing Group for the Women's Health Initiative Investigators 2002). Altogether 16,608 healthy women aged 50–79 with an intact uterus were randomly assigned to either 0.625 mg CEE plus 2.5 mg MPA daily, or placebo. The trial was

prematurely stopped after a mean follow-up time of 5.2 years, due to increased risk of breast cancer and increased net harm induced by HRT. Compared with the placebo group, the HRT group had higher rates of coronary artery disease (RR 1.29), stroke (RR 1.41), and breast cancer (RR 1.26), and more than twice the rate of venous thromboembolism although beneficial effects were seen in reductions in hip fractures and colorectal cancer. The authors concluded that HRT is more harmful than beneficial (Writing Group for the Women's Health Initiative Investigators 2002).

It appears from the research that the carbohydrate metabolism is deranged by progestins and beneficial lipid effects induced by estrogen are counteracted by progestins. These effects are dose-related and so far with doses used in HRT, no conclusive attenuating effects have been seen in observational studies. Progestins, especially the 19-nortestosterone-derived progestin, are not believed to be thrombogenic. However, an ongoing parallel trial of unopposed estrogen vs. placebo in postmenopausal women who have undergone hysterectomy will help answer questions about the influence of estrogen vs. progestin on event rates.

## **Estrogen and bone structure**

Bone loss and incidence of fracture accelerates faster among women than among men over 50 years of age, possibly due to estrogen deficiency. Observational studies indicate that use of estrogen treatment reduces the risk of fracture by half through inhibition of bone resorption (Christiansen et al 1982, Rozenberg et al 1995, Grodstein et al 1999). To achieve maximum benefit, HRT has been suggested to be started as soon as possible after ovarian failure and be continued in the long term (Lindsay 1993, Felson et al 1993, Cauley et al 1995). Progestins do not interfere with the effects of estrogen on the skeleton. When hormone treatment is withdrawn, bone loss accelerates within the first 2 years in a manner identical to that seen within the first 2 years after menopause (Tremollieres et al 2001). Few studies have been done thus far on the effects of HRT on fracture outcome. In a systematic review of 37 studies, only one small-scale preventive study was found (O'Connell et al 1998), which indicated a risk reduction with estrogen treatment on vertebral fracture (RR 0.63) (Lufkin et al 1992). Recently, two studies have supported the bone protection effects of estrogen. In a prospective randomized controlled trial in early postmenopausal women, it was shown that forearm fracture was reduced in women on HRT compared with women on no treatment (RR 0.24) (Mosekilde et al 2000). In addition, results of the Women's Health Initiative trial in 2002 showed a 33% reduction in the rate of hip fractures in women treated with estrogen plus progestin (Writing Group for the

Women's Health Initiative Investigators 2002). More information is, however, available on the effects of treatment on bone mass than on fracture outcome (O'Connell et al 1998).

## **Hormone replacement therapy and mood**

Quality of life, wellbeing, and depressed mood related to menopause improve when women with climacteric symptoms are treated with estrogen (Derman et al 1995, Rebar et al 2000, Schmidt et al 2000). Hot flushes, sleeping disturbances, and vaginal dryness are all decreased by estrogen (Wiklund et al 1992). Asymptomatic women without vasomotor symptoms, however, have not reported increased wellbeing with unopposed estrogen treatment (Girdler et al 1999, Skarsgard et al 2000). Ever since the report in 1975 on risk of endometrial cancer related to treatment with unopposed estrogen (Ziel & Finkle 1975), a progestin has been added to estrogen in treatment of women with a remaining uterus. The clinical impression, that progestin addition attenuates estrogen effects on mood, has been verified in some clinical trials. As early as 1985, it was reported that when cyclic lynestrenol is added to percutaneous estradiol treatment, negative mood and physical signs increase compared with estrogen only treatment (Hammarbäck et al 1985). In a study on women treated with estradiol and testosterone implants and a 7-day addition of 5 mg NETA, Magos and colleagues drew a parallel between symptoms during treatment and typical complaints of premenstrual syndrome (PMS) (Magos et al 1986). Women treated with 0.625 mg CEE plus 5 mg MPA given sequentially for 10 days responded with more negative mood and psychological symptoms than women continuously treated with 1.25 mg unopposed CEE (Sherwin 1991).

However, other studies contradict these findings. In a 3-year randomized placebo-controlled study, no influence on anxiety, cognition, or affect was seen when daily conjugated estrogens, CEE plus cyclic MPA, CEE plus daily MPA, CEE and cyclical micronized progesterone, and placebo were compared in parallel groups (Greendale et al 1998). Addition of MPA 10 mg for 14 days in cyclic transdermal estrogen therapy has been reported to produce no adverse physical or psychological effects on hysterectomized and oophorectomized women during treatment for one cycle, regardless of their PMS history (Kirkham et al 1991).

It appears that menopause as such does not increase the risk of clinical depression at menopausal age; however, a depressive mood and irritability are often reported among postmenopausal women (Hammar et al 1984, Stadberg 1997a). Depressed mood during menopause is associated with

vasomotor symptoms and sleeping disturbances, in particular if the perimenopausal period is long (Avis et al 1994, 2001). The issue of estrogen as treatment for clinical depression is currently under debate. A number of small and uncontrolled studies have indicated a possible beneficial effect (Soares et al 2001, Bukulmez et al 2001) but still there is a lack of well-designed clinical trials. In a study on 50 perimenopausal women with depressive disorder, treatment with transdermal estrogen for 12 weeks resulted in significantly higher rates of remission than seen with placebo (Soares et al 2001).

## **Estrogen and cognition**

In epidemiological studies, estrogen is claimed to protect against Alzheimer's disease (Paganini-Hill & Henderson 1996) and in animal models, estrogen has been found to be associated with the maintenance and protection of brain structures (McEwen 2002). There is, however, little evidence regarding the effect of HRT or ERT on overall cognitive function in healthy postmenopausal women. In a meta-analysis of nine clinical trials located through the Cochrane database system, it was seen that most studies showed no evidence of an effect on verbal or visiospatial memory, mental rotations, speed, or accuracy measures. There was an effect on some verbal memory functions reported from a few small studies (Hogervorst et al 2002). Clinical data on the effects of estrogen in women with Alzheimer's disease are thus far conflicting (Miller et al 2001).

## **Premenstrual syndrome**

Cyclic mood swings seen in women treated with sequential hormonal therapy bear much resemblance with symptoms of PMS (Hammarbäck et al 1985, Magos et al 1986). In HRT, when a progestin is added to estrogen, negative mood symptoms, such as irritability, tension, and depressive mood increase a few days after the addition. Also, physical symptoms such as breast tenderness and bloatedness are more pronounced during the progestin addition.

Premenstrual syndrome, or put more correctly, premenstrual dysphoric disorder (PMDD), is a psychneuroendocrine disorder with psychiatric expression and association with other psychiatric conditions (Sundström, Bäckström et al 1999). According to the criteria given in the *Diagnostic and Statistical Manual of Mental Disorders* 4th edition (DSM-IV) published by the American Psychiatric Association, at least five distressing symptoms should be present during the premenstrual week, one of which must be depressed mood, anxiety, lability, or irritability, for the condition to be termed "PMDD". Other symptoms described in the DSM-IV are decreased

interest in daily activities, difficulty in concentrating, marked lack of energy, and hypersomnia or insomnia. These symptoms must be severe enough to interfere with essential activities (school, work performance, or interpersonal relationships). Furthermore, patients must be devoid of symptoms in the follicular phase to ensure that the premenstrual complaint is not merely an exacerbation of an underlying mood disorder. Finally, unlike other DSM-IV criteria, the PMDD diagnosis must be confirmed by daily ratings for at least 2 months to establish the temporal relationship between onset of symptoms and the premenstrual period, since none of the symptoms of PMDD are unique to the syndrome.

Between 2% and 6% of all fertile women have been said to suffer from severe PMDD, but in one female population studied, as many as 51% described their cyclic menstrual symptoms as “PMS” (Sveindottir & Bäckström 2000). Women with PMDD are reported to be more sensitive than controls to negative mood effects of oral contraceptives (OCs) (Cullberg 1972) and perimenopausal complaints and severity of vasomotor symptoms also seem to be correlated to a history of premenstrual symptoms (Skarsgard et al 1996, Morse et al 1998).

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Research on the mechanisms in PMDD is therefore useful in understanding the negative side effects in hormonal therapy. It is possible that there is an

association between sensitivity to ovarian steroid hormones, premenstrual discomfort, and negative side effects to HRT. While the temporal correlation between dysphoria and the menstrual cycle in PMDD is well established, the specific etiology of the disorder is more elusive. The only consistent endocrine finding in patients with PMDD is that symptom development in the luteal phase requires ovulation and the consequent formation of a corpus luteum. Premenstrual symptoms remit during spontaneously or GnRH agonist-induced anovulatory cycles (Hammarbäck & Bäckström 1988, Hammarbäck et al 1991). Although the temporal relationship of the affective state to progesterone levels in the luteal phase is well demonstrated, patients exhibiting symptoms of PMDD do not have significantly different absolute levels of progesterone, estradiol, cortisol, prolactin, or any other hormone tested from asymptomatic women (Bäckström et al 1983, Rubinow et al 1988). A promising avenue of research is the possible regulation of neurotransmitter receptors by steroid hormones. The progesterone metabolites allopregnanolone and pregnanolone seem to play a role in the disorder (Sundström, Bäckström et al 1999, Wang et al 2001) and evidence of abnormalities in the serotonergic system is abundant (Halbreich & Tworek 1993). Another finding which suggests a possible dysfunction in serotonergic neurotransmission is the fact that a number of well-designed studies have indicated that PMDD is successfully treated with selective serotonin reuptake inhibitors (SSRIs) (Steiner et al 1995, Perlstein 2002).

## Progestins

Progestins are widely used in different therapies for women. Oral contraceptives and intramuscular, subcutaneous, and intrauterine birth control systems all contain progestins. Progestins can be used to treat irregular bleeding and endometrial hyperplasia, to postpone menstruation, and to prevent relapse of endometrial cancer. In this thesis, the focus will be on progestins as an addition to estrogen in HRT.

Currently, progestins are derived from either 19-nortestosterone, with more androgenic effects, or from 17-alpha-hydroxyprogesterone, with more progesterone-like effects, table 1.

The progestins used in our studies are those used most frequently today in Europe and North America, NETA and MPA. Progestins can bind to receptors other than the PR and have more or less androgenic and glucocorticoid effects. The newer progestins, the so-called "goanes" (gestodene, norgestimate, and desogestrel), have less androgenic effect than do the older ones. Equipotency between different progestins is based



upon effects on the endometrium, which does not necessarily mean that effects on the brain, the cardiovascular system, and the breast are equivalent.

**Table 1.** Synthetic progestins

19-nortestosterone	17-alpha-hydroxyprogesterone
norethisterone (NETA)	medroxyprogesterone acetate (MPA)
lynestrenol	cyproterone acetate
levonorgestrel (LNG)	megestrol acetate
norgestimate	dydrogesterone
gestodene	
desogestrel	

The following are considered to be equipotent doses of progestins, table 2.

**Table 2.** Equipotent doses of synthetic progestins.

MPA	10 mg
NETA	0.7–1 mg
LNG	0.075 mg
Micronized progesterone	200–300 mg
Dydrogesterone	20 mg

Among the two progestins studied, NETA and MPA, bioavailability and absorption differ. Medroxyprogesterone acetate has low and variable absorption, and maximum plasma levels are generally reached within 1–4 hours, although this may vary from 8 to 10 hours, depending on the individual. Norethisterone acetate, on the other hand, has a bioavailability of 60–65% and maximum plasma levels occur within 1–2 hours of oral administration. The half-life of MPA is relatively long, 40–60 hours, while NETA has a shorter half-life, of 8–11 hours. The 19-nor progestins are bound to albumin and sex hormone-binding globulin (SHBG) for plasma transportation. Progestins are fairly lipophilic and are therefore metabolized before being excreted renally. The major metabolic pathway by all delta4-3-keto steroids is to be 5-alpha or beta-reduced and 3alpha gamma aminobutyric acid<sub>A</sub> (GABA<sub>A</sub>)-hydroxylated. The 21-steroids are further hydroxylated at the 20 position to a dehydroxylated steroid. All steroids with hormonal activity except estrogens are metabolized via the

same pathway and some end up as steroids with a chemical structure which is active on the GABA<sub>A</sub> receptor (Meyerson 1967; Picazo et al 1998; Stoffel-Wagner 2001; Ganong 2001). Natural progesterone is absorbed orally only if ingested in a micronized form (Grow 2002). Orally ingested progesterone is largely metabolized to 3alpha-hydroxy-5alpha/beta-pregnanolone (de Lignieres et al 1995).

## **Estrogen and progesterone and the central nervous system**

Due to their high lipid solubility, estradiol and progesterone easily cross the blood-brain barrier and the brain is an important target organ of steroid hormones. Moreover, an extensive steroid metabolism occurs in the brain and several brain regions are well equipped with enzymes necessary for steroid hormone biosynthesis (Stoffel-Wagner 2001). Steroid hormones play an important role in the development, growth, maturation, and differentiation of the brain.

Estradiol and progesterone were previously thought to interact with the central nervous system (CNS) mainly through their respective intracellular receptors. In doing so they regulate gene expression and protein synthesis in the brain (McEwen & Woolley 1994). We know that both ERs and PRs are found in the brain (McEwen 1994). A second ER, ER- $\beta$ , was recently discovered (Enmark et al 1997) and both ER- $\alpha$  and ER- $\beta$  have properties in the brain. Estrogen receptor  $\alpha$  messenger ribonucleic acid (mRNA) expression is mainly found in the amygdala and hypothalamus (Österlund, Keller et al 2000), whereas ER- $\beta$  is found in hippocampus and cerebral cortex (Österlund, Gustafsson et al 2000). The hippocampus is a brain region related to emotions and memory function (McEwen 2002), as is the cerebral cortex. Along with the hypothalamus, the limbic system, which includes the amygdala and hippocampus, is concerned with feeding, sexual behavior, fear, emotions, and motivation.

A number of important neurotransmitter systems in the brain, such as the GABA, serotonin (5-hydroxytryptamine (5-HT)), and N-methyl-D-aspartate (NMDA) systems, are influenced by sex steroids. Serotonin is involved in behavioral and emotional processes, such as mood, affect, sex, learning, memory, and aggression (Bethea et al 1998). There is accumulating evidence that estrogen and progesterone affect numerous functional properties of the serotonin neural system. The actions through which this effect occurs are complex, involving pre- and postsynaptic receptors, the serotonin reuptake transporter, and the gene expression of tryptophan hydroxylase (TPH), the enzyme which synthesizes serotonin (Bethea et al 1998). Estrogens regulate PR expression in both 5-HT and

non-5-HT neurons, and in mice, estrogen induction of progesterin receptors has been reported (McEwen 2002.) However, as the interaction between estradiol and progesterone and the serotonin system differs from brain region to brain region and depending on the time course of treatment (short-term vs. long-term effects), general conclusions about the effect of ovarian steroids and serotonergic function are sometimes difficult to draw. Through NMDA receptors, estrogen is involved in formation of new excitatory synapses and may regulate events at the sites of synaptic contact (McEwen 2002).

The physiological actions induced by estradiol and progesterone binding to their respective receptors generally occur within hours or days. However, a number of estradiol and progesterone-induced effects in the brain have such a rapid onset that gene transcription is unlikely to explain these events. Increasing evidence points to the possibility of more direct effects on CNS neurotransmission (McEwen 2002). Nongenomic actions of estradiol can be mediated through the plasma membrane ER (Levin 1999) but also, through interaction with second messenger systems (Fugger et al 2000).

The most obvious nongenomic effect of ovarian steroids is the interaction between progesterone metabolites and the GABA<sub>A</sub> receptor. The GABA system is the major inhibitory system of the mammalian CNS, with an abundance of GABA in approximately 30% of the brain synapses. Benzodiazepines, barbiturates, and, to some extent, alcohol exert their actions through binding to the GABA<sub>A</sub> receptor and consequently, drugs that bind to the GABA<sub>A</sub> receptor have anxiolytic, sedative, and anesthetic properties. Progesterone has potent anesthetic properties in the brain and a dampening effect on brain excitability (Landgren et al 1978, Bäckström et al 1984, Norberg et al 1987, Bixo & Bäckström 1990). The anesthetic effect of progesterone is mediated by its metabolism to GABA-active progesterone metabolites, such as allopregnanolone and pregnanolone (Paul & Purdy 1992). As the enzymes required for this reduction, 5 $\alpha$ -reductase and 3 $\alpha$ -hydroxysteroid oxidoreductase, are present in the brain, they are called "neurosteroids", a term coined in the early 1980s (Baulieu 1981). Neurosteroids bind to the GABA<sub>A</sub> receptor and potentiate the action of GABA, which in turn leads to increased hyperpolarization of the postsynaptic neuron, and consequently, inhibition of synaptic transmission (Lambert et al 1995). The neurosteroids have potent anxiolytic (Wieland et al 1991), anesthetic (Norberg et al 1987), and antiepileptic (Bäckström et al 1984, Landgren et al 1987) effects on the CNS. Although there is evidence for de novo synthesis of neurosteroids also in the human brain (Stoffel-Wagner 2001), it is conceivable that progesterone, produced by

the corpus luteum, is a major source for neurosteroids in fertile women (Bäckström et al 1986, Bixo et al 1997, Genazzani et al 1997).

Micronized progesterone given orally has sedative effects, but when given vaginally, only low concentrations of sedating metabolites can be found in plasma (de Lignieres et al 1995). The 19-nortestosterone-derived progestins are different from progesterone, in that they have no sedative effects (Meyerson 1967). Medroxyprogesterone acetate is sedative, but not as potent as progesterone (Meyerson 1967).

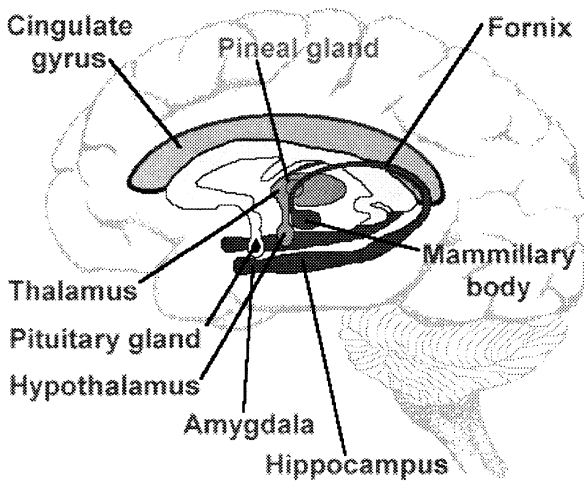


Figure 5. The limbic system.

**Hormone replacement therapy and compliance**

Following this introduction to HRT, we need to take a brief look at how well people have been reported to comply with medicinal therapy in general, and with HRT in particular. Poor compliance or adherence is a common problem in most medical therapies. Phenomenographic studies on the subject describe people as preferring to be self-dependent rather than compliant (Fallsberg 1991). Drug dependency is known to cause loss of self-control.

Several reports describe low compliance with HRT (Cano 1994, Kotzan et al 1999). One out of three women have been reported to discontinue HRT within a year mainly due to unwanted bleeding or spotting and fear of cancer (Wren & Brown 1991). Never filling the prescription or taking hormones intermittently is also common. In studies on osteoporosis and

treatment with hormones, 40–50% of subjects were not taking the prescribed drug 8–12 months after initiation of treatment (Ryan et al 1992, Torgerson et al 1995). Older women, women who use a progestin, women who experience side effects, and women who see a male gynecologist or physician have been reported to be more likely to be noncompliant with estrogen therapy (Berman et al 1997). In one study, white women were found to be more willing to continue treatment than black women, and women treated with bleed-free continuous combined therapy have been said to be more likely to continue the treatment than women treated with sequential therapy (Dören et al 1995, Hill et al 2000, Eiken & Kolthoff 2002). After 8 years, fewer than half of the women in one study population were still on treatment (Eiken & Kolthoff 1995). In women treated with 0.625 CEE plus 2.5 mg MPA, adherence declined to 81% after 1 year and after 6 years, only 45% continued therapy (Hulley et al 2002).

It seems controversial on the one hand that HRT improves wellbeing and was during the 1980s and 1990s believed to have beneficial effects on health, but on the other hand, that its discontinuation rates have been high. There is reason to assume that women are not fully satisfied with the treatment and that explanations may be found in unwanted side effects due to modes of treatment. Discontinuation is partly due to irregular bleeding and fear of cancer (Dören et al 1995, Stadberg 1997b), but other factors have to be considered. Studies on effects on mood caused by progestin addition to estrogen have been inconclusive and many questions remain unanswered. In this thesis, we aim to elucidate some of the elements deteriorating mood during HRT.

# AIMS

The aims of this work were to –

- explore the frequency of discontinuation and describe the reasons why peri- and postmenopausal women discontinue HRT
- compare the effects of two different progestins, MPA and NETA, on mood and physical symptoms during sequential HRT
- establish whether women with a history of premenstrual syndrome are more sensitive than controls to the adverse mood effects of the progestin addition during HRT
- evaluate whether there is a dose-dependent effect of MPA on mood during the progestin phase of HRT
- evaluate whether there is a dose-dependent effect of estradiol on mood during the progestin phase of HRT.

# SUBJECTS AND METHODS

## Subjects

### Paper I

All women (n=453) who consulted a gynecological practice in Piteå, northern Sweden, for climacteric symptoms between September 1991 and December 1992 were considered for the study. Patients were excluded if there were contraindications to estrogen treatment. Women already on estrogen treatment were likewise excluded. At the end of 1996, 356 women received a questionnaire about their hormone treatment. The socioeconomic status of the study population was compared with that in a similar population of women aged 45–54 years in the county of Norrbotten (n=14,181) and in the community of Piteå (n=3,872).

### Papers II–IV

Fifty-one women were recruited to study II, 36 to study III, and 38 to study IV. In total, 125 women were recruited to three clinical trials at three gynecological centers in northern Sweden. The Department of Obstetrics and Gynecology at Umeå University Hospital, Umeå, and Läkarhuset Björnen in Piteå participated in all trials and the Department of Women's and Children's Health at Östersund Hospital, Östersund, participated in studies II and III. The Department of Obstetrics and Gynecology at Lycksele Hospital, Lycksele, took part in study IV. The subjects either consulted the outpatient clinics with climacteric complaints or were recruited through advertisements in the local newspapers. None of the women were included in more than one of the clinical trials. All women were more than 6 months postmenopausal, had two or more climacteric symptoms, had no contraindications to HRT, and had serum FSH levels of >18 IU/l. All women had a remaining uterus, had no history of psychiatric disease, and were not being treated with any psychopharmacological drugs. Both women with and without a history of PMS were included.

Table 3 displays demographic data on all participants who completed the clinical trials.

**Table 3.** Demographic data of three study groups, n = 107

	Paper II	Paper III	Paper IV
N	46	33	28
Mean age, Ys, (range)	52 (46-60)	53 (45-61)	50,5 (39-55)
Working, n (%)	44 (96%)	31 (94%)	28 (100%)
Previous pregnancy, n (%)	43 (94%)	31 (94%)	26 (93%)
Former users of OC's, n (%)	35 (76%)	28 (85%)	25 (89%)
Hot flushes, n (%)	43 (94%)	31 (94%)	26 (93%)
Sleeping disturbances, n (%)	34 (74%)	24 (73%)	27 (96%)
Depressed mood, n (%)	25 (54%)	19 (58%)	15 (54%)
Vaginal dryness, n (%)	21 (46%)	14 (42%)	13 (46%)

## Design

### Paper I

This study was carried out as a retrospective longitudinal study. At the end of 1996, 356 women treated with estrogen or estrogen plus progestin received a questionnaire containing 14 questions. The questionnaire was evaluated by the Department of Epidemiology at the University in Umeå. The questions were as follows: Have you started/Did you ever start your HRT? If not, why? For how long have you been treated? If you have discontinued the HRT, why? What side effects do, or did, you have? During what period of treatment do/did they occur? Have you had unexplained depressive symptoms during treatment? During your fertile life, did you have premenstrual symptoms, such as headaches, bloatedness, irritability, tension, a depressed mood, and breast tenderness? The respondents were asked to give their height and also, their weight before and after the treatment. Respondents were asked to indicate the mode of HRT, including changes, and other medication. The questionnaire also asked about negative side effects, such as premenstrual symptoms, weight gain, abdominal pain, and irregular bleeding. Reasons for never starting or for discontinuing HRT were asked for, with side effects, fear of cancer or thrombosis, weariness of bleeding, aim to deal with symptoms “naturally”, a desire to find out whether the symptoms had meanwhile disappeared, or “other reasons” given as options. The subjects responded by marking one or more



alternatives listed for each question. Personal comments were allowed. Additional data, such as time from menopause to start of treatment, duration and mode of treatment, disruption of treatment, and complaints of negative side effects brought to the health care professional's notice at the first follow-up, were taken from the medical records. Data from the questionnaires were compared with complaints noted in the records to validate the retrospective data.

The women starting HRT were given oral and written information about the treatment at the first visit and a follow-up consultation was arranged after 3–6 months. In the meantime, patients had access to telephone consultations with the doctor or a nurse. After the follow-up visit at 3–6 months, yearly visits were made.

Papers II–IV

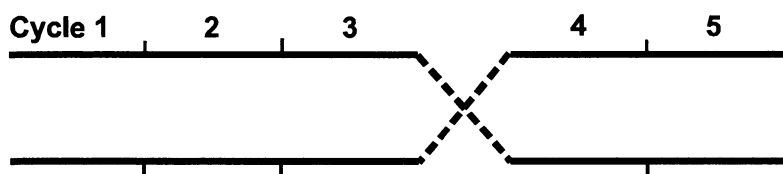
Three clinical trials were carried out in a prospective, randomized, double-blind cross-over study. The study design of the three trials was identical except for Paper IV, which included a treatment-free cycle at the beginning of the study. Each treatment cycle was 28 days and the study period continued for five cycles.

The participants were randomly assigned to one out of two treatment groups in each study. All patients were treated with estradiol continuously for five 28-day cycles. A progestin was added during the last 12 days of each cycle (days 17–28). In study II, two different progestins were used, 10 mg MPA or 1 mg NETA, and the estradiol dose was kept at 2 mg. In study III, two different doses of MPA were used, 10 mg or 20 mg, while the estradiol dose was 2 mg. In the last study, the same progestin addition and dose, 10 mg of MPA for 12 days, was used, while the two groups received either 2 or 3 mg estradiol continuously.

The subjects in each study received the one treatment for three cycles (including the run-in cycle); thereafter, a cross-over to the other treatment was made for another two cycles. The study design is shown in Figures 6 and 7.



Figure 6. One HRT cycle.



**Figure 7.** Cross-over design during five 28-day cycles.

The medicines were prepared by Apoteksbolaget, Production and Laboratories, Umeå Sweden. The capsules were made to look identical and were packed in 28-day blister cards by the pharmacy at Umeå University Hospital. Randomization was carried out by the pharmacy and codes were kept secret until termination of the studies. During the study period, the women were seen three times by a gynecologist, at inclusion in the study, after 8–10 weeks of treatment, and at termination. A gynecological examination and breast examination were performed at inclusion and at termination of the studies. Serum concentrations of FSH, weight, and blood pressure were monitored throughout the study period.

## The cyclicity diagnoser

The main outcome measure in Papers II–IV was the daily rating scales kept by the women throughout the studies. We used a modified form of the cyclicity diagnoser (CD), which is a Lickert scale designed for cyclic symptoms. The CD has been validated as an instrument for diagnosing cyclic conditions (Sundström, Bixo et al 1999). Our modified scale contained four physical symptoms: abdominal bloating, breast tenderness, hot flushes, and withdrawal bleeding, and seven psychological symptoms: tension, irritability, fatigue, depressed mood, friendliness, cheerfulness, and libido. The effects on daily life caused by the symptoms were graded. The scale in study II is a 9-point scale, where 9 represents maximum severity of a symptom and 1 means absence of the symptom. In studies III and IV, we changed the grading to 0–8, with endpoints of the scale being similar to the previously used 9-point scale. This change was due to the input from the participants of the first clinical trial who thought it seemed more logical. The women kept rating scales from the first day of treatment until termination of the studies, except in study IV, where they also rated mood during a 1-month baseline period.

The Lickert scale is similar to the visual analog scale (VAS), which is frequently used to evaluate intensity of pain. The difference between the two scales is the lack of scale steps in the VAS. Scale steps visually facilitate the reading of scales. Both scales are mainly used for intraindividual analysis and not for comparisons between individuals, which is why the studies required a cross-over design. Continuous daily ratings on the scales provide the opportunity to analyze onset of symptoms and gradual symptom changes over time, and are superior to intermittent ratings. The Lickert scale has been validated with a high internal consistency and time reproducibility in occupational studies on perceived stress (Consoli et al 1997). In a follow-up study, the scale was used also for interindividual analyses showing significant differences with regard to gender and occupation. Consideration should, however, be given to possible individual differences in baseline scores, as with all instruments of self-assessment in the interpretation of interindividual analysis. The scale is powerful enough to detect a difference in mood with each scale step (Seippel & Bäckström 1998).

### **Premenstrual syndrome diagnosis**

At the inclusion interview, the women were asked about a history of premenstrual symptoms that had affected their daily life, partner, family, and work. Premenstrual symptoms were defined as mood deterioration before menstruation, which decreased or disappeared at the onset of menstrual bleeding. The diagnoses of PMS history in our studies were retrospective, since the women were already postmenopausal at inclusion. Therefore, the diagnoses do not define women as having PMDD or not, but give premenstrual symptoms as described by the women themselves. Before entering the study, subjects were also asked about previous psychiatric conditions and medication.

### **Primary care evaluation of mental disorders**

In study IV, presence of psychiatric disorders was identified using a complementary structured psychiatric interview, the primary care evaluation of mental disorders (Prime-MD), which has been validated for use in primary care settings (Spitzer et al 1994). It conforms to the diagnostic criteria of the DSM-IV and is used to diagnose depressive, anxiety, and eating disorders. Experience from other studies using the Prime-MD encouraged us to make efforts to reveal especially concealed depression and anxiety conditions.

## Hormone assays

The only hormone analyzed in our studies was serum FSH, to confirm the menopausal state along with the medical history. The serum concentrations were measured by microparticle enzyme immunoassay (MEIA) (AxSYM, Abbot Laboratories, IL, USA). The assay sensitivity was 0.37 IU/l, with intra- and interassay coefficients of variation being 3.5% and 2.3%, respectively.

## Statistics

### Paper I

Data were analyzed with the statistical computer program Epi Info of the World Health Organization (WHO). Demographic data and data from the questionnaire were analyzed in frequency tables and crosstables. Fisher's exact test was used to compare negative side effects among subjects who had continued or discontinued HRT. A p-value of  $<0.05$  was considered significant. The socioeconomic index of the Statistical Bureau of Sweden was used to classify socioeconomic status of the participants and compare subjects with women of the same age group in the community and the county. The subjects were divided into rural or urban residents. Weight and body mass index (BMI) were estimated before treatment and at the time of the study, and differences between group medians were calculated using the Mann-Whitney U test.

### Papers II-IV

The first treatment cycle was not included in the analysis, since it was used as a run-in cycle. As estrogen is known to increase wellbeing, the first treatment cycle was excluded to avoid interference with mood effects due to reduction of vasomotor symptoms (Holst et al 1989). Hence, in Papers II and III, cycles two to five were included in the analyses, whereas Paper IV also includes baseline mood scores. Symptoms were analyzed both separately and in clusters of symptoms. Related symptoms were grouped together as mean scores of summarized symptoms, so that "negative mood symptoms" included tension, irritability, fatigue and a depressed mood, and "positive mood symptoms" referred to friendliness and cheerfulness, and "physical symptoms" to abdominal bloating and tenderness of the breasts. Cyclicity of symptoms in Papers II and III was investigated using two-way analysis of variance (ANOVA), with the cycle phase (best phase vs. worst phase) as the independent variable. The effects of the different progestins, different doses of MPA, and different doses of estradiol on each symptom and on summarized symptoms were analyzed by two-way ANOVA with repeated measures. Independent factors in these analyses were drugs used and cycle day. In study II, drugs used were

NETA vs. MPA, in study III, MPA 10 mg vs. 20 mg, and in study IV, estradiol 2 mg vs. 3 mg. In all studies, the term “cycle days” was used to refer to the progestin phase, days 17–28. In studies II and III, a history of PMS vs. no PMS history, progestin phase, and type of progestin or dose of MPA were analyzed by three-way ANOVA. Possible groups in terms of drug interactions were followed up by two-way ANOVA.

# RESULTS

## Compliance (Paper I)

Altogether 92% of the women responded to the questionnaire. The study group matched the control population with regard to work and years of tertiary education, the majority (58%) being workers and employees with <3 years of tertiary education. Forty-six per cent of the study group came from a rural area and 54% from the town of Piteå. Mean age was 50.1 years (range 36–69 years) and 75% were aged between 45 and 54 years. Ninety-eight per cent had started HRT and 2% had not.

A total of 75% were continuing their HRT at the time of the study, with the minimum length of treatment being 3 years. Seventy-seven per cent of the women starting HRT had experienced negative side effects, mainly weight gain, breast tenderness, bloatedness, and a negative mood. Among the women who had discontinued treatment, unacceptable side effects including a negative mood was the most common reason for stopping treatment (35%). Another 26% of patients had discontinued treatment to find out whether symptoms had disappeared, and 25% had stopped for fear of cancer or thrombosis. The results are shown in Table 4.

**Table 4.** Reasons for discontinuing HRT (more than one reason possible).

Unacceptable side-effect including negative mood symptoms	35%
Wish to find out if symptoms have disappeared	26%
Fear of cancer or thrombosis	25%
Weariness of bleeding	19%
Aim to deal with the symptoms “naturally”	15%
Other reasons (increase in weight, no effect of therapy, itch, growing fibroids, nausea, fibromyalgia or SLE changing for the worse, forgetting to take treatment)	32%

Only four patients were advised by somebody else to quit.

Among the symptoms reported as negative side effects, depression, irritability, and tension were reported significantly more frequently in subjects who had discontinued than in subjects who had continued their treatment (Table 5).

**Table 5.** Symptoms reported as negative side-effects of HRT (more than one alternative possible).

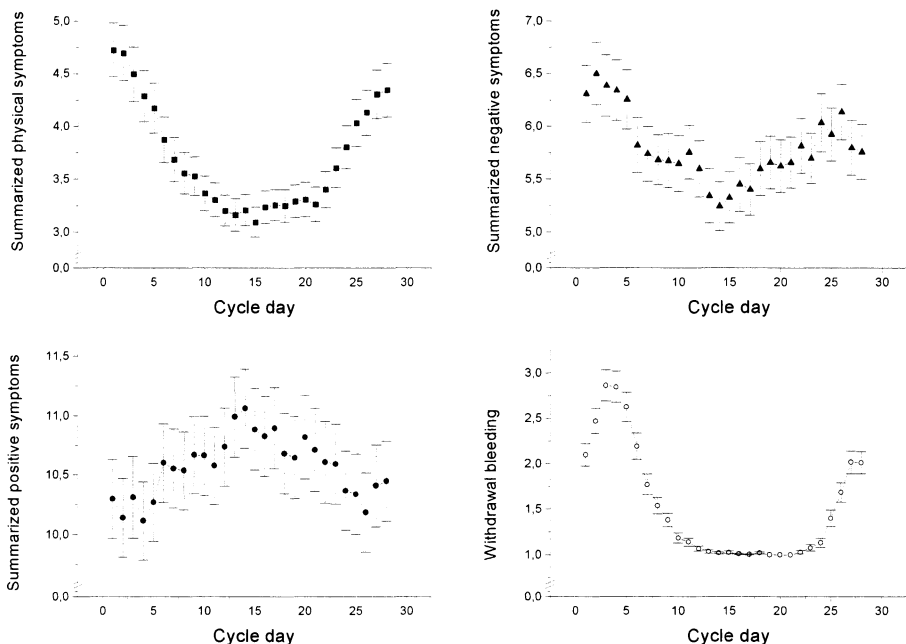
	Total group (n = 321)	Discontinued group (n = 75)	Continued group (n = 246)	p
Weight gain (%)	38	39	38	ns
Breast tension (%)	25	28	24	ns
Depression (%)	13	22	11	0.016
Bloating (%)	12	15	10	ns
Headache (%)	10	13	9	ns
Irritability (%)	10	19	7	0.004
Tension (%)	6	11	4	0.024
Pelvic pain (%)	3	4	2	ns
Irregular bleeding (%)	3	4	2	ns
Other reasons (%)	8			

**Follicle-stimulating hormone, weight, and blood pressure (Papers II–IV)**

Levels of serum FSH decreased significantly from pretreatment levels to termination in all three studies ( $p<0.001$ ). Weight and blood pressure were unaltered.

**Symptom cyclicity (Papers II and III)**

All symptoms on the CD scale displayed a significant difference between the worst and the best phase of a 28-day cycle. We found that the best period, when mood was at its best and physical symptoms at their lowest levels, occurred during mid-cycle (days 10–16). The worst period had its peak in the late progestin phase (days 25–28) and lasted a few days into the next cycle (days 1–3). Scores of breast tenderness, bloatedness, hot flushes, tension, irritability, depressed mood, fatigue, and effect on daily life were all significantly increased during the worst phase of treatment ( $p<0.001$ – $0.005$ ). Symptoms started to increase 1–3 days after the progestin addition. Likewise, scores of cheerfulness, friendliness, and libido all significantly decreased during the worst phase of treatment ( $p<0.001$ – $0.005$ ). Withdrawal bleeding culminated on days 4 and 5. There was no breakthrough bleeding. The intensity of symptoms, in particular negative mood symptoms, was significantly reduced with time (although cyclicity in symptoms did remain), as seen when cycles 2 and 5 were compared ( $p<0.001$ – $0.05$ ). Cyclicity results are shown in Figure 8.



**Figure 8.** Cyclicity during HRT. Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of summarized physical, negative and positive mood symptoms, and withdrawal bleeding during sequential estrogen/progestin treatment. Each point represents the mean  $\pm$  SEM of treatment cycles 2-5 among all women (n=46). All related symptom groups displayed significant cyclicity between best and worst phase of the treatment cycle.

## Differences between pretreatment and treatment symptoms (Paper IV)

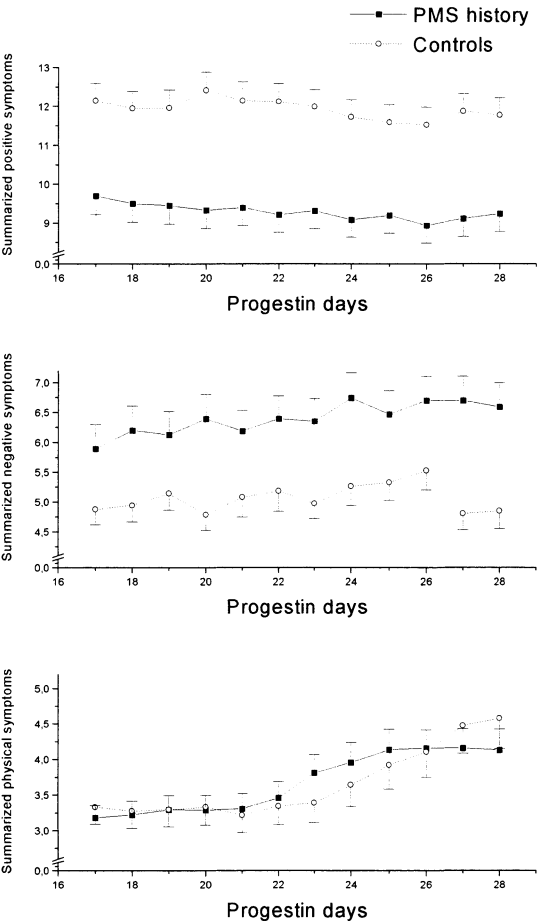
In study IV, the women scored presence of all symptoms during a 1-month baseline period. There was, however, no cyclicity in symptoms during this pretreatment period. Compared with pretreatment ratings, hot flushes and irritability decreased significantly during sequential HRT ( $p < 0.001$  and  $p < 0.05$ , respectively). Breast tenderness, on the other hand, increased during treatment ( $p < 0.05$ ).



**Difference in symptoms between women with and women without a history of premenstrual syndrome (Papers II and III)**

During their fertile life, 52% of subjects had had premenstrual symptoms affecting their daily life. In study II, subjects with a history of PMS reported significantly lower scores of positive symptoms during progestin addition than did women without a history of PMS,  $F(1,44) = 5.22$ ;  $p < 0.05$ , figure 9. Subjects with a history of PMS also displayed increased negative mood symptoms in comparison with controls, although this difference did not reach significance,  $F(1,44) = 3.35$ ;  $p = 0.074$ .

In study III, there was no significant difference in mood scores during the progestin phase between women with a history of PMS and women with no such history.



**Figure 9.** Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of summarized physical, negative, and positive symptoms during the progestin-phase of sequential estrogen/progestin treatment in women with a history of PMS (n=24) and women without (n=22). Each point represents the mean  $\pm$  SEM of treatment cycles 2-5. PMS patients displayed significantly lower scores for positive mood symptoms during the progestin-phase  $F(1,44) = 5.22$ ;  $p < 0.05$ .

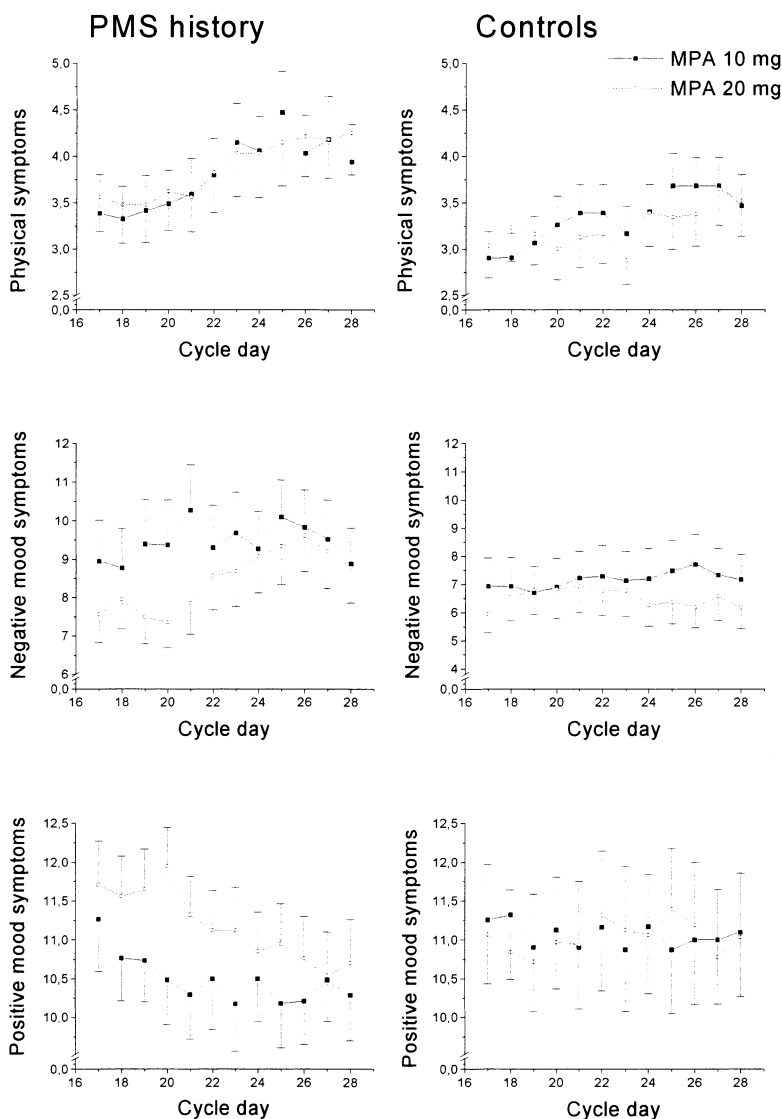
## **Difference between norethisterone acetate and medroxyprogesterone acetate (Paper II)**

Women with no history of premenstrual symptoms reported more negative mood symptoms,  $F(1,21) = 16.99$ ;  $p < 0.001$  and fewer positive symptoms,  $F(1,21) = 29.75$ ;  $p < 0.001$  during treatment with NETA compared with MPA. Medroxyprogesterone, on the other hand, induced more physical symptoms than NETA,  $F(1,44) = 9.80$ ;  $p < 0.01$ . Women with a history of PMS reported inconsistent scores when the two progestin compounds were compared. Medroxyprogesterone induced more negative mood symptoms,  $F(1,23) = 10.45$ ;  $p < 0.001$ , whereas NETA induced more negative effects on daily life,  $F(1,21) = 2.43$ ;  $p < 0.01$  in women with a previous history of PMS.

## **Difference between 10 mg and 20 mg medroxyprogesterone acetate (Paper III)**

Both women with and women without a history of PMS reported fewer negative mood symptoms during treatment with 20 mg MPA than during treatment with 10 mg,  $F(1,16) = 20.24$ ;  $p < 0.001$ , and  $F(1,15) = 7.82$ ;  $p < 0.01$ , respectively. Women with previous PMS reported more positive mood symptoms during treatment with 20 mg MPA compared with 10 mg,  $F(1,16) = 22.27$ ;  $p < 0.001$ ). Women without a history of PMS displayed no significant differences in positive mood scores when the two MPA doses were compared, figure 10.

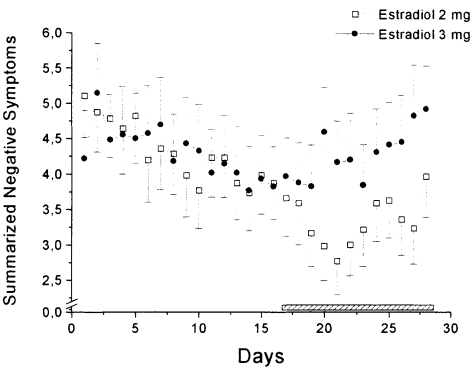
With regard to physical symptoms, there was no difference between the two MPA doses; also, there was no difference in physical symptoms between women with and women without a PMS history.



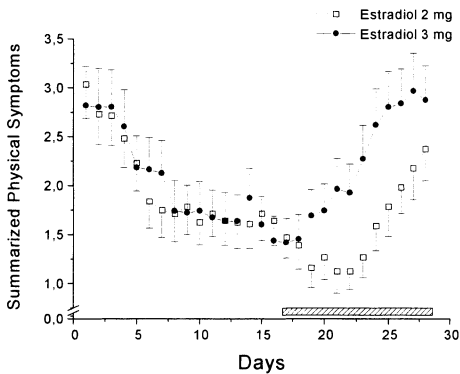
**Figure 10.** Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of summarised physical, negative, and positive symptoms during the progestin-phase (day 17–28) of sequential estrogen/progestin treatment in women with a history of PMS ( $n=17$ ) and women without ( $n=16$ ). Each point represents the mean  $\pm$  SEM of treatment cycles 2-5. Women with a PMS history, as well as women without, scored less negative mood symptoms when treated with 20 mg MPA compared to 10 mg,  $F(1,16) = 20.24$ ;  $p < 0.001$  and  $F(1,15) = 7.82$ ;  $p < 0.005$ , respectively. MPA also induced more positive mood symptoms than 10 mg MPA in women with a PMS history,  $F(1,16) = 22.27$ ;  $p < 0.001$ .

**Difference between 2 mg and 3 mg estradiol during the progestin phase (Paper IV)**

During the progestin phase of the treatment cycle, the higher estrogen dose significantly increased scores of negative mood symptoms compared with the lower dose,  $F(1,27) = 19.35$ ;  $p < 0.001$ ). Tension, irritability, and depressed mood, but not fatigue, were all significantly augmented during cycles with 3 mg estradiol, figure 11. Physical symptoms, in particular breast tenderness and hot flushes, increased during the progestin phase when the higher estrogen dose was used,  $F(1,27) = 84.32$ ;  $p < 0.001$ , and  $F(1,27) = 5.92$ ;  $p < 0.05$ , respectively, figure 12. Negative effects on daily life increased during the progestin phase of 3 mg estradiol cycles compared with 2 mg cycles,  $F(1,27) = 11.40$ ;  $p < 0.001$ ). However, during cycles with 2 mg, but not 3 mg of estradiol, progestin addition resulted in lower negative mood scores than seen during the previous 12 days of estrogen only treatment,  $F(1,27) = 6.21$ ;  $p < 0.05$ .



**Figure 11.** Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of summarized negative symptoms during sequential estrogen/progestin treatment. Each point represents the mean  $\pm$  SEM of treatment cycles 2-5 among all women ( $n=28$ ). During progestin phase (day 17-28), the higher dose of estrogen caused significantly more negative mood symptoms than the lower dose.  $F(1,27) = 19.35$   $p < 0.001$ .



**Figure 12.** Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of summarized physical symptoms during sequential estrogen/progestin treatment. Each point represent the mean  $\pm$  SEM of treatment cycles 2-5 among all women ( $n=28$ ). Physical symptoms increased during the progestin phase (day 17-28), of 3 mg estradiol cycles compared to 2 mg cycles  $F(1,27) = 51.41$   $p < 0.001$ .

# DISCUSSION

Our results indicate that addition of a progestin to estrogen leads to unfavorable effects in mood and physical symptoms. These negative side effects, which bear resemblance to premenstrual symptoms in fertile women, are an important reason for women to discontinue HRT. Women with a history of PMS are more vulnerable to adverse mood effects induced by HRT than women without a history of PMS. The addition of medroxyprogesterone to estrogen is preferable to that of norethisterone with respect to mood symptoms in women with no history of PMS. Furthermore, with respect to mood symptoms, results have shown that the overall aim to lower MPA doses in HRT is uncalled for. We suggest that estrogen and progestin together are responsible for mood effects during HRT. Moreover, if the estrogen dose is increased during the progestin phase of HRT, negative mood and physical symptoms are augmented.

There are, however, some methodological considerations which may bias the strength of our conclusions and they are discussed below.

## **Methodological considerations**

The diagnosis of PMS in women taking part in our studies is a weakness. Since the women were already postmenopausal at inclusion in the studies, the PMS diagnosis was a retrospective one. A proper PMS/PMDD diagnosis should be based on prospective symptom scores during two menstrual cycles (Hammarbäck, Bäckström et al 1989, American Psychiatric Association 1994). To meet the criteria for PMDD, women are required to report at least five distressing symptoms during the luteal phase, which must interfere with social or occupational functioning. Also, they should not have any ongoing mood or anxiety disorder. The PMS diagnosis of women participating in our studies was made based on reported cyclic negative mood symptoms, which decreased or disappeared at the onset of bleeding. The diagnosis therefore did not include PMDD, according to the DSM-IV definition. Fifty-two per cent of the women in our clinical trials had experienced premenstrual symptoms, whereas only 2–6% of an

average female fertile population are said to suffer from severe PMDD (Sveindottir & Bäckström 2000). The number of women with self-reported, retrospective PMS symptoms in our studies was similar to the expected number of such cases in population-based studies (Sveindottir & Bäckström 2000). Naturally, when counseling women with climacteric problems, the only PMS diagnosis that can be obtained at that point is retrospective and based on self-report.

Serum FSH was measured before inclusion in our studies and levels above 18 U/l were considered postmenopausal. In other studies on HRT, the postmenopausal FSH level is often set at 35 U/l. We chose the level used in clinical practice; however, serum FSH was not used as our sole criterion of the postmenopausal state. At least two climacteric symptoms had to be present and if there was any reason to suspect another cause for amenorrhea, an endocrinological investigation was performed.

The Prime-MD should have been used in all three clinical trials but was used only in study IV. It has been shown to be an accurate instrument for evaluation of depression and anxiety disorders (Spitzer et al 1994). When mood effects are studied it is important to rule out masked psychiatric conditions, which otherwise may influence mood experiences. Furthermore, approximately 26% of women with climacteric complaints have been found to have depression and anxiety disorders (Bixo et al 2001). Unfortunately, we did not have any personal experience with this diagnostic instrument until 2000, when study II and III had already been completed. However, a psychiatric history was taken before inclusion and women with previous psychiatric disorders were excluded from the studies.

In study IV, but not in studies II and III, baseline mood scores were kept for 28 days. After completing the first two clinical trials we realized the value of describing pretreatment mood and physical symptoms for a more stringent evaluation of cyclicity and intensity of treatment effects. This could naturally not be accomplished in retrospect; however, the experience taught us how to improve the study protocol.

There were no washout phases included between each treatment cycle or at the cross-over. Since we only investigated progestin-induced effects this was considered acceptable. The estradiol only phase of each treatment cycle can be regarded as a washout phase as it is unlikely that any progestin effects from the previous treatment cycle would still be present at day 17 of the next cycle. However, due to the design of the studies, we have refrained from drawing any conclusions in terms of estradiol only effects, in particular in study IV, where two different doses of estradiol were used.

Serum concentrations of estradiol and progestins were not measured in our trials. The women in our studies were more than 6 months postmenopausal, at which time, endogenous estradiol levels have not yet reached their nadir. As estradiol dose has an impact on mood symptoms (Paper IV), fluctuating low levels of endogenous estradiol could have interfered with the mood effects seen during the progestin phases of all three clinical trials. However, the doses used in our studies were similar to those used in clinical practice, and these doses are also standard doses in most clinical trials on HRT. In addition, the treatment was given in a randomized order and should therefore not have been influenced by a gradual decrease in the endogenous production.

The bioavailability of NETA is 60–65%, but metabolization can differ from individual to individual. The bowel uptake of MPA is even more varied and this drug's half-life is longer than that of NETA. These pharmacological differences suggest that MPA and NETA are not equivalent and this can bias mood responses. Dose equivalence has previously been based upon endometrial effects, but dose equivalence for CNS effects is unknown.

The results of study II indicated a significant difference in mood response between women with a history of PMS and women without a history of PMS. This could not be replicated in the following trial (study III). In study III, scores for negative mood symptoms indicated a possible difference between women with and women without previous PMS during the progestin phase, but the statistical analyses did not reveal any significant difference. The reason for this may have been the size of the study group, which was considerably smaller in study III (n=33) than in study II (n=46). Also, one subject in the control group (i.e., the group with no previous history of PMS) of study III most probably was already depressed at inclusion and reported consistently high scores for negative mood throughout the study period. Since no diagnostic tool for evaluation of mood disorders was used at inclusion, however, the fact of this probable ongoing depression was missed. Removal of this particular subject from the statistical analysis revealed significant differences between the two study groups. As we had no reason to exclude her, however, the study findings remained. This finding further emphasizes the importance of using psychiatric tools to evaluate depression and anxiety before a patient is included.

## Estrogen and mood

Our studies on sequential HRT indicate that negative mood symptoms, i.e., fatigue, depressed mood, tension, and irritability, are at their lowest and that positive mood symptoms, i.e., cheerfulness and friendliness, are at their highest during the estrogen only phase. Hot flushes and irritability significantly decrease during HRT compared with before treatment. Estrogen replacement therapy has been reported to have effects in relieving vasomotor symptoms, enhancing wellbeing, and improving mood in women with climacteric symptoms (Wiklund et al 1993, Sherwin 1999). Estrogen is known to influence a number of neurotransmitter systems, which in turn are important for depressive mood and anxiety. Most importantly, estradiol has a link to the serotonin and glutamate systems. This was first discovered in animals when it was found that estrogen treatment decreases serotonin-catalyzing monoamine oxidase (MAO) in the limbic system of the rat (Luine 1975). Many subsequent studies have shown that estrogen can regulate the serotonin system and influence mood (Bethesda et al 1998). In a prospective, double-blind, cross-over study on 31 hysterectomized and oophorectomized postmenopausal women, depression scores decreased during treatment with 10 mg of intramuscular estradiol injection compared with placebo (Sherwin & Suranyi-Cadotte 1990). The mood enhancement was associated with an increase in the density of the imipramine binding site on platelets, which are involved in the active transport of serotonin. When meta-chlorophenylpiperazine, a serotonin agonist, is given orally to postmenopausal women, the cortisol and prolactin responses have been reported to be increased during estrogen treatment compared with placebo, indicating an estrogen-induced augmentation of serotonergic activity (Halbreich et al 1995). In a small study on five postmenopausal women, estrogen and micronized progesterone increased the 5-HT receptor-binding potential in the cerebral cortex, as shown by positron emission tomography (Moses et al 2000). In this regard, it must be pointed out, however, that our studies did not aim to investigate unopposed estradiol effects on mood. Although some preliminary conclusions can be drawn from the estradiol only phase of each treatment cycle, spillover effects from the previous progestin phase cannot be ruled out. There were no washout phases included between cycles or at the cross-over, hence it is difficult to establish with certainty what effects are due to estradiol on its own.

## Progestin and mood

We know of only one placebo-controlled, double-blind cross-over study in postmenopausal women treated with cyclical MPA alone without the combination of estradiol (Prior et al 1994). Eleven women received 10 mg



MPA for 10 days per month during 2 consecutive months, keeping daily diary scales on mood and physiologic experiences. No adverse symptoms were seen during progesterone only treatment compared with placebo (Prior et al 1994). From this study, it appears plausible that either estrogen or MPA given alone do not readily explain adverse mood symptoms during HRT.

## **Hormones and premenstrual syndrome**

Onset of premenstrual symptoms in fertile women requires both estrogen and progesterone. Symptoms of PMS disappear during anovulatory cycles when progesterone levels, in particular, are low (Hammarbäck & Bäckström 1988, Hammarbäck et al 1991). In women with moderate to severe PMS treated with estrogen during the luteal phase, mental and physical symptoms of PMS are aggravated (Dhar & Murphy 1990). It has also been shown in interindividual as well as intraindividual studies that elevated plasma estradiol concentration in the luteal phase is related to increased severity of symptoms. More pronounced premenstrual symptoms are found in cycles with high luteal phase plasma estradiol and progesterone concentrations, compared with cycles with lower levels of these hormones (Hammarbäck, Damber et al 1989). Also, in a study of 30 PMS patients, the symptom severity was correlated to cycles with a high concentration of luteal phase estradiol and luteinizing hormone (LH) (Seippel & Bäckström 1998). Finally, women with PMS are more susceptible than women without PMS to mood provocation during treatment with OCs (Cullberg 1972). The results of our first clinical trial indicate that women with a history of PMS are more vulnerable to adverse mood effects of HRT than women without prior PMS. Women with previous PMS reported fewer positive symptoms during progestin addition than women without.

A number of issues remain to be addressed. Firstly, it is still not known whether women with a history of PMS are simply a group of women with more mood symptoms at baseline than seen in women without a history of PMS. Secondly, it also remains to be determined whether it is only the progestin addition that provokes the negative mood changes, or whether unopposed estradiol has a similar effect in vulnerable women. To elucidate the effect of estradiol only in women with a history of PMS, we would have to perform a randomized clinical trial addressing this specific issue.

From the studies we have performed so far, a number of conclusions regarding women with prior PMS and progestin type and dose can be

drawn. Type of progestin seems to be of less importance to women with previous PMS. Whereas controls responded with increased negative and decreased positive mood scores when treated with NETA as compared with MPA, the findings in women with a PMS history were more inconsistent. On the one hand, the addition of MPA to estrogen provoked more negative mood symptoms, but on the other hand, NETA increased negative effects on daily life in women with previous PMS.

### **Progestin dose and mood**

The dose of a progestin added to estrogen appears to have a greater impact on mood response than the type of progestin. Surprisingly, the addition of 20 mg MPA enhanced positive mood in women with a PMS history, compared with the addition of 10 mg MPA. Negative mood scores decreased during the higher MPA dose in both women with previous PMS and women with no PMS history. The mood-enhancing effect of the higher dose of MPA is interesting and may be explained by responses in the GABA system. The anesthetic effect of progesterone is mediated via its metabolite allopregnanolone, through modulation of the GABA<sub>A</sub> receptor (Mok & Krieger 1990). Medroxyprogesterone acetate is also metabolized into GABA-active metabolites, although the effect of MPA as an anesthetic is less potent than that of progesterone (Meyerson 1967). Administration of high doses of allopregnanolone has shown acute anxiolytic effects in laboratory animals (Wieland et al 1991, Bitran et al 1995). It has, however, been shown that allopregnanolone has a biphasic effect on the GABA<sub>A</sub> receptor (Mehta & Ticku 1998, Srinivasan et al 1999). At low concentrations, allopregnanolone inhibits the effects of GABA, whereas higher concentrations enhance its effect (Srinivasan et al 1999). Inhibitors of GABA<sub>A</sub> receptor are known to be anxiogenic (Ninan et al 1982, Prado de Carvalho et al 1983).

If a dose-response effect exists between progestins and negative mood, the subjects should have felt worse on a higher MPA dose than on a lower dose. Indeed, the dose dependence that was observed in this work (Papers II–IV), between no treatment with progestin and progestin treatment, has been reported in a number of studies (Hammarbäck et al 1985, Magos et al 1986, Sherwin 1991). However, when 10 mg MPA was compared with 20 mg MPA, the group treated with the higher MPA dose displayed no further mood deterioration (Paper III). This may be explained by the fact that several GABA<sub>A</sub> receptor agonists, including the progesterone metabolite allopregnanolone, have biphasic dose response curves, in both humans and animals. Since it is possible to induce anesthesia with MPA (Meyerson 1967), it is likely that metabolites of MPA act in a similar way

to allopregnanolone. At low doses, GABA<sub>A</sub> receptor agonists induce negative reactions, such as loss of impulse control, negative mood, anxiety, and aggression/irritability, both in humans and in animals (Miczek et al 1993, 1997, Ferrari et al 1997, Masia et al 2000, Beauchamp et al 2000, Fish et al 2001). On the other hand, high doses of GABA<sub>A</sub> agonists have anxiolytic, sedative, and antiepileptic effects. Some of these effects have been reported in both humans and animals, whereas others have only been seen in animals (Bäckström et al 1984, Landgren et al 1987, Carl et al 1990, Mok & Krieger 1990, Wieland et al 1991, Sundström et al 1998). Hence, an inverse relation between the dose and effect can be expected when the progestagen dose used has passed the maximum negative effect on the dose response curve.

A number of studies have confirmed this hypothesis. A low dose of allopregnanolone given into the cerebral ventricular of rats has been observed to produce a conditioned place aversion, while a higher dosage has no effect (Beauchamp et al 2000). Allopregnanolone therefore increases aggression in rats up to a certain level, similarly to alcohol in alcohol-heightened aggression. After this level has been reached, a further increase in allopregnanolone, as in alcohol dose, decreases aggressive behavior (Fish et al 2001). Moreover, low-dose diazepam given to men has been reported to elicit more aggression than placebo, even under experimental conditions (Ben-Porath & Taylor 2002). Barbiturates have been seen to induce severe negative mood reactions in 2.5–6% of patients undergoing amobarbital provocation as a workup for epilepsy surgery (Lee et al 1988, Kurthen et al 1991, Masia et al 2000). Milder emotional reactions seem common following amobarbital injections, while severe emotional outbursts have been reported to be rare and to seem to be related to previous negative psychological experiences, such as traumatic events (Masia et al 2000). The prevalence of severe reactions (2.5–6%) to barbiturate treatment is interesting, as it is similar to the reported prevalence of PMDD (2–6%) (Sveindottir & Bäckström 2000).

Whether the mood-improving effect of the higher dose of MPA is an effect mediated through the metabolites of MPA or whether it is an effect mediated through MPA binding to the PR is of course a speculation beyond the findings of the present study.

Our results, in summary, indicate that previous PMS may be a risk factor for adverse mood effects during sequential HRT.

## Estrogen and progestins and mood

Our results furthermore suggest that progestins deteriorate mood during HRT. In our studies, we found that NETA is slightly more prone to provoke negative mood symptoms, at least in controls with no history of PMS, whereas MPA induces more physical symptoms. Several review papers describe negative side effects and therapeutic problems with the progestin addition during HRT (von Schoultz 1986, Panay & Studd 1997, Sherwin 1999) and there are a number of clinical trials to support these findings. For instance, in a double-blind cross-over study, nondepressed menopausal women treated with cyclic estrogen and estrogen plus progestin, or with placebo, responded with adverse mood effects to the progestin addition (Klaiber et al 1996). Furthermore, Magos and colleagues found a significant increase in pain, difficulties in concentrating, behavioral changes, water retention, and negative affect during 7 days of addition of 5 mg norethisterone to subcutaneous estradiol and testosterone treatment. The addition of 2.5 mg norethisterone or placebo to subcutaneous estradiol and testosterone did not induce any of these negative side effects (Magos et al 1986). The authors concluded that the symptoms expressed could be used as a model for the PMS. In coherence with the study by Magos, Hammarbäck and colleagues described cyclic mood swings as in PMS during transdermal estrogen therapy, where a negative mood started to appear 1–3 days after addition of lynestrenol (Hammarbäck et al 1985). Finally, in a study by Holst and colleagues, postmenopausal women in two groups were treated with either cyclic transdermal estrogen alone or cyclic transdermal estrogen in sequential combination with lynestrenol. The women kept daily ratings for 6 months of therapy. All negative mood symptoms were more pronounced in the group treated with a combination of estrogen and progestin (Holst et al 1989). Our results are in agreement with these findings. Smith and coworkers, however, proposed that cyclic norethisterone during continuous estrogen treatment is more likely to cause pain, but less likely to cause negative affect symptoms, than either medroxyprogesterone or dydrogesterone (Smith et al 1994).

At odds with these findings, two studies, besides the 1-month study of Kirkham et al in 1991, report no adverse effects with addition of MPA to continuous estrogen treatment. In the first, a 3-year prospective, double-blind, placebo-controlled study, participants were assigned to either placebo, daily CEE, CEE plus cyclic MPA, CEE plus daily MPA, or CEE plus cyclic micronized progesterone. At 1 and 3 years, participants reported absence or presence of 52 symptoms on a checklist. Progestin-containing regimens were significantly associated with higher levels of breast discomfort, but neither anxiety nor cognitive and affective symptoms differed with treatment assignment (Greendale et al 1998). Symptoms were,

however, not reported daily and the study was designed as a parallel study, instead of a cross-over study.

In the second named study, 23 nondepressed, early postmenopausal women participated in a single-blind pilot study with the following sequence of treatments: 1 week of no substance, 2 weeks of placebo, 2 weeks of progestogen only, 1 week of placebo, and 2 months of sequential standard HRT consisting of continuous 0.625 mg CEE and an addition of progestin 2 weeks per month. Half of the group received 5 mg MPA as a progestin addition and the other half was given 200 mg micronized progesterone. Daily records of mood and somatic symptoms were kept. None of the hormone treatments had a detectable effect on mood. Medroxyprogesterone acetate users reported more breast tenderness and vaginal bleeding than the progesterone users. The authors concluded, "In contrast with the widely held belief among psychiatrists that progesterone depresses mood, neither of the progestogens we used in normal, non-depressed and non-anxious women showed this effect" (Cummings & Brizendine 2002). Again, mood symptoms were assessed between treatment groups rather than within individuals.

Finally, to emphasize the importance of intraindividual comparisons, our results are in line with a 6-month placebo-controlled study on 54 postmenopausal women. The participants, who at the time of the study were asymptomatic and without psychiatric history, were randomly assigned to either estrogen treatment alone or estrogen in combination with cyclic progesterone. Mood ratings were obtained every day for 30 days prior to treatment and during the last 30 days of treatment. The women randomized to estrogen plus progesterone exhibited a significant increase in daily depression, cramping, and breast tenderness as well as a marginally significant increase in daily anxiety. The increases were mild and did not interfere with normal functioning (Girdler et al 1999). In our studies, where ratings were kept daily for 5 months and a cross-over design was used, mood and physical symptoms significantly changed when a progestin was added. The changes were small also in our studies, but detectable with the primary outcome measure, the CD. The mood changes affected daily life, according to the records kept by the women, but not daily functioning with the partner, family, or work. Among women who dropped out of the three studies referred to above, several reported severe premenstrual side effects with an extensive influence on daily functioning. The discrepancies between our studies and the above cited studies with contradictory results are most likely due to the design of the studies. The changes noted in our studies were small, although detectable, and it is clear that intraindividual studies are superior to parallel studies when

mood symptoms are addressed. Although VAS ratings and other mood scores are often used to compare different groups of subjects, a comparison within individuals is clearly a superior way of evaluating mood scores. Furthermore, we have shown that the effect of HRT is greater in women with a prior history of PMS than in women with no prior PMS, which may also explain the mood-provoking effects of progestin addition in our studies.

In contrast to our previous studies, in Paper IV we found that addition of 10 mg MPA to 2 mg estradiol had a beneficial effect on mood. The most likely reason for discrepancies between this study and studies II and III is the use of MPA instead of nortestosterone-derived progestins. In study II, we showed that NETA provokes more negative mood symptoms in women with a history of PMS than in controls with no such history. Furthermore, the mood effects during the progestin phase remained for up to 3 days in the estrogen only phase, which affected mood scores during estrogen only treatment since there were no washout periods between treatment cycles. This phenomenon was, however, present in all three studies.

The combination of estradiol and progestin appears to be essential to mood provocation. The mechanism behind this is not fully understood, but some data suggest that estradiol upregulates PRs in certain brain areas. Recently, Alves and colleagues found that estrogen treatment significantly increases PR cells in the midbrain raphe, where serotonin neurons originate (Alves et al 2000). The urinary metabolite of serotonin, 5-hydroxyindole acetic acid (5-HIAA), increases significantly during estrogen only treatment, whereas estradiol in combination with NETA has been reported to abolish this effect (Mueck et al 1997). Blood platelet serotonin content and imipramine binding are peripheral markers of serotonin activity. Continuous treatment with CEE and MPA has been suggested to increase platelet [<sup>3</sup>H]-imipramine binding and improve mood in postmenopausal women (Bukulmez et al 2001). However, other studies have not been able to show any effects of unopposed estradiol or HRT in terms of peripheral markers of serotonin activity on healthy postmenopausal women (Wihlbäck, Sundström-Poromaa, Allard et al 2001).

The GABA system is also most likely to be involved in mood responses to HRT. The neuroactive progesterone metabolite pregnanolone exerts a sedative effect through the GABA<sub>A</sub> receptor. Wihlbäck and colleagues have shown that pregnanolone sensitivity increases with deterioration in mood symptoms during addition of progestins during HRT (Wihlbäck, Sundström-Poromaa, Nyberg et al 2001). Both MPA and NETA are metabolized into GABA-active metabolites, although their potency is less

than that of the progesterone metabolites (Meyerson 1967). Furthermore, our results suggest that the estrogen dose is of vital importance to mood deterioration during progestin addition. We found that treatment with 3 mg continuous estradiol resulted in significant increase of tension, irritability, depressed mood, bloatedness, breast tenderness, and hot flushes, as well as effect on daily life during the progestin addition of 10 mg MPA, compared with treatment with 2 mg estradiol. A study on estradiol treatment levels and mood during HRT supports these findings. Women with a long duration of menopause and higher treatment serum estradiol levels had significantly more dysphoria when given a combination of estrogen plus progestin than did the women with a short duration of menopause and lower treatment serum estradiol levels (Klaiber et al 1997). The dose relationship of serum estradiol plus progesterone and mood was investigated in a study with 56 postmenopausal women treated with transdermal estrogen for 3 months while plasma estrogen levels were measured (de Lignieres & Vincens 1982). Natural progesterone was administered during the last 10 days of treatment. When plasma levels were low, moderate depressive symptoms were seen. Only when a moderate increase in estradiol levels was induced by the treatment did the estradiol treatment itself lead to a pleasant feeling of wellbeing. When excessive increase in estradiol levels was induced by the treatment, irritability and aggressiveness were reported. Progesterone had very few psychological effects when estradiol levels were low or only slightly increased. When plasma estradiol was high, a moderate elevation of plasma progesterone induced a pleasant tranquilizing effect. A massive elevation of plasma progesterone levels immediately induced an inadequate hypnotic effect, sometimes with dizziness (de Lignieres & Vincens 1982). However, we studied dose-dependent effects on mood and consequently, comparisons involving peripheral steroid hormone levels should be drawn with caution. In a 1-year randomized study, women treated with 0.625 mg CEE for 25 days and 5 mg MPA during days 15–25 experienced more negative moods and more psychological symptoms compared with women treated with 1.25 mg CEE and placebo (Sherwin 1991).

We know that estrogen also exerts its CNS effects by enhancing the excitatory effect of glutamate (McEwen 2002). The glutamate system is the largest excitatory system in the brain and is the excitatory counterpart to the inhibitory GABA system. The glutamate system is known to be involved in induction of depression and glutamate antagonists are used as antidepressants (Krystal et al 2002). A situation in which the recall of unpleasant depressant/anxiogenic memories is enhanced by estrogen-induced stimulation of the glutamate system, combined with disinhibition

of the GABA system, may allow negative emotional memories to surface. In such a concept, the effects of progestins and estrogen, as shown in the results of the present studies, are understandable.

In summary, there are women with an increased vulnerability to mental side effects of HRT, but there are also women who do not seem to be particularly sensitive to these effects at all. Our study on compliance with HRT showed that both women who were compliant and women who were noncompliant experienced negative side effects, but not to such an extent that the disadvantages outweighed the advantages of treatment.



# GENERAL CONCLUSIONS

- Negative side effects and mood deterioration are important reasons for noncompliance with HRT.
- Sequential HRT provokes cyclic mood swings and cyclic physical symptoms during treatment cycles of 28 days. The best phase, when mood is at its best and physical symptoms are at their lowest, occurs during the last part of the estrogen only phase.  
A negative mood and physical symptoms start to appear 1–3 days after the progestin addition and remain until 1–3 days into the next cycle.
- Estradiol in combination with a progestin provokes negative mood symptoms and physical symptoms during HRT.
- Women with a history of PMS have a different vulnerability to adverse mood effects induced by HRT than women with no history of PMS. Women with previous PMS respond with lower positive mood scores than seen in women without previous PMS during progestin addition. None of the tested progestins seem to be favorable for women with a history of PMS. On the other hand, in our study when the MPA addition was increased from the standard dose of 10 mg to 20 mg during continuous estrogen treatment, women with a history of PMS surprisingly responded with higher positive mood scores.
- Medroxyprogesterone acetate appeared to have some favorable mood effects compared with NETA during sequential HRT in women with no history of PMS. On the other hand, MPA appeared to induce more physical symptoms than did NETA.
- A higher dose of MPA during sequential HRT did not impair mood compared with the standard dose of MPA. On the contrary, 20 mg as progestin addition during HRT may have mood-relieving properties both in women with a history of PMS and in women with no such history.
- An increase of the dose of estradiol from 2 mg to 3 mg during sequential HRT was found to enhance a negative mood and breast tenderness during progestin addition. Hot flushes also increased with the higher estradiol dose during progestin addition.
- Physically and mentally healthy women without a history of PMS had little influence on mood when treated with 2 mg estradiol and cyclic addition of 10 mg MPA

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On the seventh hour, and on the seventh day  
On the seventh month, the seventh doctor say...

*Willie Dixon*