TARJA VIHTAMÄKI

Towards Self-adjusted Postmenopausal Hormone Replacement Therapy

Biochemical and Clinical Parameters Associating with Percutaneous Treatment

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Tampere, for public discussion in the small auditorium of Building K, Medical School of the University of Tampere, Teiskontie 35, Tampere, on August 29th, 2003, at 12 o’clock.

Acta Universitatis Tamperensis 944
University of Tampere
Tampere 2003
ACADEMIC DISSERTATION
University of Tampere, Medical School
Tampere University Hospital, Department of Obstetrics and Gynecology
Ylöjärvi Health Center
Finland

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Distribution

University of Tampere
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 944
ISBN 951-44-5714-5
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 267
ISBN 951-44-5715-3
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2003
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4. Control of menopausal symptoms
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2. Compliance with HRT
3. Menopausal symptoms and achieved control of symptoms
4. Self-adjusted dose and serum estradiol, follicle-stimulating hormone, sex hormone-binding globulin
5. Effect of ascorbic acid on serum estradiol concentration
6. Influence of skin contamination on serum estradiol concentration

#### DISCUSSION

1. Prevalence and compliance with HRT
2. Self-adjustment of estrogen replacement therapy
3. Serum estradiol concentrations
4. Effect of skin contamination by estradiol gel on serum estradiol concentration
5. Effect of the time of blood sample taken on serum estradiol concentration
6. Effect of ascorbic acid on serum estradiol concentration
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to as I-V in the text. In addition, some previously unpublished data are also presented.


V Vihtamäki T, Luukkaala T, Tuimala R: Skin contamination by oestradiol gel – a remarkable source of error in plasma oestradiol measurements during percutaneous hormone replacement therapy. In press (Maturitas)
ABBREVIATIONS

AA Ascorbic acid
B-FSH Biological activity of follicle-stimulating hormone
BMI Body mass index, kg/cm²
CEE Conjugated equine estrogen
E Estrogen
E₁ Estrone
E₂ 17β-estradiol
ER Estrogen receptor
ERT Estrogen replacement therapy
FSH Follicle-stimulating hormone
HRT Hormone replacement therapy
I-FSH Immunological activity of follicle-stimulating hormone
KI Kupperman´s menopausal index
LH Luteinizing hormone
MPA Medroxyprogesterone acetate
RIA Radioimmunoassay
SHBG Sex hormone-binding globulin

CONVERSION FACTORS TO SI UNITS

E₁ : 1 pg/ml = 3.699 pmol/l
E₂ : 1 pg/ml = 3.671 pmol/l
INTRODUCTION

The average age for onset of menopause (51 years) has not changed over time, but life expectancy has. A woman can now anticipate living one-third of her life after menopause. Menopause results in estrogen (E) deficiency which in turn causes menopausal complaints for most women. The result is an impaired quality of life and accelerated loss of bone mass, increasing the risk of fractures. The benefits of postmenopausal hormone replacement therapy (HRT) are well recognized both for short term relief of menopausal symptoms, and long term protection against osteoporosis. However, compliance to treatment is generally poor, with few women taking HRT for more than one year.

Postmenopausal HRT also has disadvantages. Recently, a clinical trial, designed to clarify the risks and benefits of combination HRT, was terminated prematurely (The Writing group for the WHI trial 2002). The reason for stopping the trial after an average follow-up of 5.2 years was an increased risk of breast cancer in the hormone-therapy group and evidence that the greater overall risk outweighed the benefits of HRT. The results also showed an increased risk of heart disease, stroke and pulmonary embolism. The combination HRT reduced bone fractures and colorectal cancer. In reality, the absolute risks associated with combined HRT were small. Over one year, 10 000 women taking E plus progestin compared with placebo might experience seven more cardiovascular events, eight more strokes, eight more incidences of pulmonary embolism, and eight more invasive breast cancers (Skoyby 2002). Thus, the argument against using HRT for prevention of chronic diseases is not that the likelihood of adverse-effects is high, but rather that the potential harm may outweigh the potential benefits. The WHI study has been strongly criticized for its limitations. The major criticism is its chosen study population, which was not representative of the women typically considered for HRT (Genazzani et al. 2002). One considerable limitation is that the trial tested only one specific drug regimen, conjugated equine estrogen 0.625 mg/day, plus medroxyprogesterone acetate 2.5 mg/day, which is not available in most European countries. Thus, the findings of the WHI trial do not necessarily apply to other formulations of oral E and progestin, to other doses, or to other routes of administration.

Making decisions about HRT for a woman approaching menopause depends on her current symptomatology, health status and probable long-term health risks and benefits. Although the decision whether or not to use HRT is ultimately made by the woman, objective information and a physician’s recommendation are needed. Relief from menopausal symptoms and preventing
postmenopausal bone loss can be obtained from all forms and delivery systems of HRT. However, since the desired effects of E are achieved individually, with different doses being partly due to individual variations in E pharmacokinetics, individual HRT is recommended. Application of percutaneous gel allows for flexible dose adjustment. When compared with tablets, percutaneous therapy also results in relatively constant intra-individual serum estradiol (E$_2$) concentrations (Scott et al. 1991), a further advantage, considering estrogen replacement therapy (ERT) side-effects. Undesired side-effects associated with HRT are, according to several reports, the main reason for cessation of therapy.

One possibility for optimizing HRT, with no side-effects, but maximal benefits instead, may be self-adjusted dose titration based on the disappearance of menopausal symptoms. The present study was designed to investigate this novel method of HRT administration and related factors.
The female reproductive axis includes the hypothalamo-pituitary unit, the ovaries, and the uterus. In women of reproductive age, the ovaries are the major source of Estrogens (E). Estradiol (E₂) is the main, biologically active, circulating estrogen (E) in women and is a product of the ovarian granulosa cells. The pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), stimulate ovarian secretion of E₂, the inhibins from follicular granulosa cells, and androgens from the interstitial cells, including the theca.

During the reproductive years, the main sources of E in women are the dominant follicle, and the corpus luteum after ovulation. The principal E produced is 17β-E₂. Normal ovulatory cycles result in serum E₂ levels ranging individually during the follicular phase, at midcycle, and during the luteal phase (Table 1). This results in a mean integrated E₂ concentration of approximately 80 pg/ml (Kletzky et al. 1975, DeLignieres 1994). The dominant follicle and corpus luteum account for more than 95 % of the circulatory E₂ in premenopausal women (Lloyd et al. 1971, Baird et al. 1974). Peripheral conversion of estrone (E₁) to E₂ accounts for most of the remaining circulating E₂ (Baird et al. 1974). Estrone, the second major human E, is derived principally from the metabolism of E₂ and from the aromatization of androstenedione in adipose tissue (Baird et al. 1974). A small quantity is secreted directly by the ovary and adrenal. Serum E₁ levels during the menstrual cycle vary from 40 to 170 pg/ml, paralleling E₂ levels. Estradiol concentrations are identical in the forearm, uterine, and liver vessels, but are five to ten times higher in the efferent ovarian veins (DeLignieres 1994). The E₁/ E₂ ratio in fertile women is 1:2 (Kuhl 1990). In the circulation, approximately 38 % of E₂ is bound to SHBG, 60 % to albumin, while approximately 2 to 3 % is freely available for exchange with tissues (Kaufman 1997).

During ovulatory menstrual cycles, progesterone opposes the stimulatory effect of E and induces secretory transformation in the endometrium. During the luteal phase, the serum progesterone concentration rises up to 25ng/ml (Kuhl 1990). In the blood, circulating progesterone is bound to
corticosteroid-binding globulin and albumin. The metabolites of progesterone are conjugated to sulphates and glucuronides, then excreted in the urine (Kuhl 1990).

Inhibins are glycoprotein hormones consisting of two subunits: inhibin A and inhibin B. Serum concentrations of both inhibins fluctuate during the normal menstrual cycle. Inhibin A is dominant in the circulation during the luteal phase, while inhibin B dominates during the follicular phase of the cycle (Groome et al. 1996). Inhibin A, together with E₂, is derived from the dominant follicle in particular, and the ensuing corpus luteum (Roberts et al. 1993, Groome et al. 1996). Inhibin B is a secretory product of antral follicles (Roberts et al. 1993, Groome et al. 1996) and its circulating concentration reflects the number of follicles recruited from the primordial pool (Groome et al. 1996).

Table 1. Serum concentrations of E₂, E₁, progesterone (P) and gonadotropins in different phases during normal ovulatory cycles (adapted from DeLignieres 1993 and Hee et al. 1993).

<table>
<thead>
<tr>
<th></th>
<th>Follicular</th>
<th>Midcycle</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₂ (pg/ml)</td>
<td>40-80</td>
<td>80-300</td>
<td>40-150</td>
</tr>
<tr>
<td>E₁ (pg/ml)</td>
<td>40-60</td>
<td>170-200</td>
<td>40-80</td>
</tr>
<tr>
<td>P (nmol/l)</td>
<td>0.3-4.8</td>
<td></td>
<td>8-89.4</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>4-12</td>
<td>14-20</td>
<td>2-8</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>2-20</td>
<td>20-100</td>
<td>2-20</td>
</tr>
</tbody>
</table>

The menopausal transition, a period which averages four years in duration, begins with the first indications of the approach of menopause and ends with the final menses. Its morphological basis is the rapidly declining number of primordial follicles within the ovary (Richardson et al. 1990). A common initial marker of menopausal transition is alteration in menstrual regularity and/or bleeding, which may be accompanied by somatic or psychological symptoms. Due to simultaneous hormonal events, the frequency of the normal ovulatory cycles declines.
1.1.1. Gonadotropins

Serum FSH has been shown to increase in some women before the age of 40 particularly during the early mid-follicular and early luteal phases (Lee et al. 1988), but also throughout the cycle (Sherman et al. 1975), even though there may be no clinical indications of approaching menopause. The rise in FSH is significant from about five years before the menopause (Lenton et al. 1988, Rannevik et al. 1995), and correlates positively with age (Metcalf et al. 1985, Burger et al. 1999). Within the six month period surrounding menopause there is a further increase in serum FSH, which culminates 3-4 years after menopause. A slight decline in serum FSH begins around four years postmenopause but 10 years after menopause, FSH levels are still elevated, compared with FSH levels in fertile women (Rannevik et al. 1995).

Serum LH concentrations have been reported to rise slightly during the last 4 - 5 premenopausal years in regularly cycling women (Lee et al. 1988, Lenton et al. 1988). Within the six months surrounding menopause there is an increase in the serum LH level, which culminates during the first postmenopausal year. Thereafter, a continuous decrease in LH occurs for at least eight years. Even after this, LH is clearly elevated compared to LH levels during fertility (Rannevik et al. 1995).

1.1.2. Steroid hormones

During menopause, follicular function declines, with subsequent decreases in the concentrations of circulating Es. The ovaries fail to respond to gonadotropic stimulation, and E deficiency results. Postmenopausal women have serum E2 levels below 15 pg/ml and mean E1 levels of about 30 pg/ml resulting in an E1/E2 ratio of 2:1 (Judd 1976, Cauley et al. 1989, Kuhl 1990). The primary source of E1, which is the predominant E after menopause, is from peripheral aromatization of androstenedione in adipose tissue and liver (Longcope 1986, Siiteri 1987). Ninety-five percent of postmenopausal androstenedione production occurs in the adrenal gland, and 5 % in the ovaries. Aromatase enzyme transforms androstenedione into E1, and is present in nonendocrine tissues, including adipose tissue (Ackerman et al. 1981, Siiteri 1987). Increased conversion of androstenedione to E1 is proportional to increasing weight, and results in increased E levels (Cauley et al. 1989, Siiteri 1987). The primary source of E2 in postmenopausal women is from the peripheral conversion of E1.
As with E₂, the concentration of E₁ also decreases during and after menopause (Longcope et al. 1986a). The pattern of this fall is similar to that of E₂, and the concentrations of these two Es are strongly correlated.

Estrone sulfate, as a metabolite of both E₁ and E₂, is present in the circulation at higher levels than either E₁ or E₂, and with menopause, its concentration falls in a pattern similar to the other Es (Longcope et al. 1986). Although estrone sulfate has no biological activity of its own, it becomes an active E when the sulfate group is hydrolyzed.

During the premenopausal period an increasingly inadequate luteal function or anovulation occurs. This results in a lower level of serum progesterone during the luteal phase. Serum levels of progesterone decrease with age and in postmenopause are invariably low (Reyes et al. 1977, Rannevik et al. 1995).

Menopause is also associated with a decrease in ovarian androgen secretion (Judd et al. 1974, Aleen et al. 1985). After menopause, total androgen production decreases by about 50 %. The two androgens that demonstrate the greatest decrease after natural menopause are dehydroepiandrosterone and androstenedione, which decline by approximately 75 % and 50 %, respectively. In contrast, the ovaries continue to produce testosterone at nearly normal levels in the early phase of the menopausal transition. However, the fall in the secretion of androstenedione, a major source of testosterone, results in a decline in circulating testosterone in most postmenopausal women starting approximately five years after menopause (Longcope et al. 1986b, Bachmann 1999a).

1.1.3. Inhibins

In the early perimenopausal phase the rise in serum FSH level is related to a fall in the circulating concentrations of inhibin B, while no significant change in the serum inhibin A level is found (Klein et al. 1996). Progression to late perimenopause is accompanied by a marked fall in inhibin A with no further change in inhibin B (Burger et al. 1998). After menopause, unlike inhibin B, small quantities of inhibin A is secreated into circulation (Ala-Fossi et al. 1998).
1.2. Symptoms and signs of estrogen deficiency

1.2.1. Menopausal symptoms

The most common menopausal symptoms are presented in Table 2. Vasomotor symptoms, like hot flashes, are the traditional sign of menopause, as well as the predominant complaint of perimenopausal and postmenopausal women in Western countries (Nachtingall 1998, Bachmann 1999b, Bardel et al. 2001). However, it was not until 1975 that serious scientific study of hot flashes was undertaken, proving their objective existence (Molnar 1975). A hot flash is a sudden transient episode of flashing, sweating and a sensation of heat, often accompanied by palpitations and a feeling of anxiety, sometimes followed by chills (Kronenberg et al. 1990). It may last from a few seconds to an hour and can range from mild to severe in intensity. Vasomotor complaints are present in 62 % - 91 % of Western perimenopausal women (Feldman et al. 1985, Bardel et al. 2002), and 30 % - 51 % are reported to have severe symptoms (Oldenhave et al. 1993). The frequency and intensity of vasomotor complaints gradually decreases within 0.5 - 5 years after natural menopause but may persist for more than 10 years in a small percentage of postmenopausal women (Oldenhave et al. 1994, Rodstrom et al. 2002). In women with surgical menopause vasomotor complaints have been reported to last on average longer, and be more severe than in those undergoing natural menopause (Kronenberg et al. 1990, Oldenhave et al. 1993, Nachtingall 1998, Berg et al. 1999). One reason for this may be that in women undergoing natural menopause, slower, continuous reduction in gonadal steroid levels results in a downward regulation of hormone receptors in the hypothalamus. By contrast, this downward regulation cannot occur gradually in surgically menopausal women (Nachtingall 1998).
Table 2. Symptoms associated with the climacteric and postmenopausal years in Western women. (Modified from Rutanen et al. 1998 and Dennerstein et al. 2000).

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Prevalence (%)</th>
<th>Symptom</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irritability</td>
<td>80 – 90</td>
<td>Backaches</td>
<td>30 - 40</td>
</tr>
<tr>
<td>Hot flashes and night</td>
<td>70 – 80</td>
<td>Vaginal dryness</td>
<td>30 - 40</td>
</tr>
<tr>
<td>Night sweats</td>
<td></td>
<td>Paresthesia</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Depression</td>
<td>70 – 80</td>
<td>Difficulty</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Headache</td>
<td>70 – 80</td>
<td>concentrating</td>
<td></td>
</tr>
<tr>
<td>Sleep disturbances</td>
<td>50 – 60</td>
<td>Nervousness</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Joint and muscle pain</td>
<td>50 – 60</td>
<td>Decreased libido</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Palpitations</td>
<td>40 – 50</td>
<td>Skin irritation</td>
<td>10 - 20</td>
</tr>
</tbody>
</table>

The exact pathophysiology of hot flashes has not been determined. Although hot flashes accompany E withdrawal during menopause, it alone is not responsible for them since there is no correlation between the presence of hot flashes and serum (Askel et al. 1976), urinary or vaginal (Stone et al. 1975) E levels. No differences in plasma E levels have been found in symptomatic versus asymptomatic women (Hutton et al. 1978). One hypothesis is that hot flashes are associated with thermoregulatory problems due to decreased E levels (Silva et al. 1986). Body temperature is regulated by the hypothalamus and involves blood flow through the skin so that heat production within the body is balanced by an outflow of heat (Savage et al. 1996). In a premenopausal state, it is thought that this thermoregulation zone is broad and adaptable while in postmenopausal women this zone would be somehow reset downward and would be narrowed (Kronenberg et al. 1990, Wyon et al. 2000). Thus, in menopause, a small increase in core body temperature might result in vasodilatation, increases in skin temperature, sweating, decreased skin resistance, and increased skin conductivity (Wyon et al. 2000). Kronenberg et al. (1990) suggest that there must be some other centrally mediated chemical trigger that is responsible in conjunction with the central and peripheral effects of E. Some possible triggers are a surge of LH and / or FSH (Tataryn et al. 1980), noradrenaline (Freedman 1998), calcitonin gene-related peptide (Wyon et al. 1998), and neuropeptide Y (Wyon et al. 2000). There is also evidence that hot flashes are preceded by a rise in
core body temperature (Freedman 1998). However, none of these hypotheses explains the absence of hot flashes in some peri- and postmenopausal women.

Insomnia, depression, anxiety, alterations of cognitive functions, and headaches in menopause are suggested to relate to alterations of the limbic system (Genazzani et al. 2002). In postmenopausal women changes in central serotoninergic and noradrenergic activities may cause mood disorders (Genazzani et al. 1996). Impairment of opioidergic peptide synthesis and secretion has also been reported to be involved in the modification of mood, behavior and pain perception in menopausal women (Linghtman et al. 1981, O'Donouhe et al. 1982).

Menopausal complaints are not universally experienced. The high prevalence of vasomotor symptoms in Western societies has not been reported in Chinese, Filipino, Thai, Mayan, Indonesian, Japanese, African, and Indian women, although their prevalence does vary between these countries (Table 3). For example, Japanese women report very few hot flashes, while Mayan women in Mexico do not report any symptoms during menopause at all, except for menstrual cycle irregularity (Beyene 1986, Martin et al. 1993, Lock M 1995). Reasons for these findings are not known but they may be due to differences in diet (Adlercreutz et al. 1992, Murkies et al. 1995, Nagata et al. 2001), exercise patterns, climatic conditions (Agoestina et al. 1984), or variations in cultural norms (Flint et al. 1990, Boulet et al. 1994, Zhao et al. 2000, Lock et al. 2001 ).
Table 3. Prevalence of vasomotor symptoms in postmenopausal women reported in different countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence of Vasomotor symptoms (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong</td>
<td>17</td>
<td>Leung et al. 2001</td>
</tr>
<tr>
<td>Indonesia</td>
<td>18</td>
<td>Boulet et al. 1994</td>
</tr>
<tr>
<td>Korea</td>
<td>49</td>
<td>Boulet et al. 1994</td>
</tr>
<tr>
<td>Malaysia</td>
<td>37</td>
<td>Boulet et al. 1994</td>
</tr>
<tr>
<td>Philippines</td>
<td>37</td>
<td>Boulet et al. 1994</td>
</tr>
<tr>
<td>Singapore</td>
<td>18</td>
<td>Chim et al. 2002</td>
</tr>
<tr>
<td>Taiwan</td>
<td>38</td>
<td>Pan et al. 2002</td>
</tr>
<tr>
<td>Thailand</td>
<td>26</td>
<td>Sueblinvong et al. 2001</td>
</tr>
<tr>
<td>Denmark</td>
<td>81</td>
<td>Oddens et al. 1997</td>
</tr>
<tr>
<td>Norway</td>
<td>67</td>
<td>Overlie et al. 2002</td>
</tr>
<tr>
<td>Sweden</td>
<td>91</td>
<td>Bardel et al. 2002</td>
</tr>
<tr>
<td>Australia</td>
<td>52</td>
<td>Dennerstein et al. 2000</td>
</tr>
<tr>
<td>Mexico</td>
<td>0</td>
<td>Beyene et al. 2001</td>
</tr>
<tr>
<td>Morocco</td>
<td>61</td>
<td>Obermeyer et al. 2002</td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>53</td>
<td>Rizk et al. 1998</td>
</tr>
</tbody>
</table>

1.2.2. Osteoporosis

The major consequence of E deficiency is osteoporosis which is defined as a reduction in bone mass or bone density accompanied by microarchitectural deterioration of the skeleton with an accompanying increase in the risk of fracture (Lindsay 1994). E deficiency, especially at the time of menopause, results in increased remodelling within the skeleton. An imbalance appears between bone resorption and bone formation, with resorption exceeding formation. The outcome of this is bone loss. All conditions of E deprivation result in bone loss. This includes natural or surgical
menopause and drugs that inhibit E production or its effects. Strenuous exercise or other conditions that provoke anovulation may also result in bone loss (Lindsay 1994).

Skeletal mass increases steadily throughout childhood and peaks between the ages of 20 and 30 (Recker et al. 1992, Slemenda et al. 1992). After a few years of relative stability, bone mass reduction starts when E levels begin to decrease (Slemenda et al. 1987, Ettinger et al. 1988). This reduction may begin before the onset of menopause (Drinkwater et al. 1984, Slemenda et al. 1987, Ettinger et al. 1988). Women lose bone at an accelerated rate, with approximately 10 % to 20 % of bone mineral density being lost within 10 years after the onset of menopause. By the age of 80, women may have lost 50 % of their skeletal mass (Riffée 1992). Bone loss increases the risk of fractures. The probability of a 50-year old woman fracturing her hip at some point during her remaining lifetime is estimated at 16 %, to fracture her wrist at 15 %, and to sustain a vertebral fracture at 32 % (Cummings et al. 1989).

The action of E on bone is mediated directly through estrogen receptors (ERs) in osteoblasts and osteoclasts. Estrogen also acts on ERs in fibroblasts of the bony extracellular matrix which produce collagen in bone connective tissue (Gray et al. 1987, Eriksen et al. 1988, Rickard et al. 1999). The exact cellular mechanism by which E withdrawal exerts its effect on osteoclast formation and function is not entirely known. It has been suggested that certain osteoclastogenic and resorptive cytokines are upregulated, leading to enhanced osteoclast formation and activity, followed by bone loss (Cenci et al. 2000).

Not all women will develop symptomatic osteoporosis (Christiansen 1994). They may be protected by the development of a high peak bone mass during their premenopausal years. A proportion of postmenopausal women may lose bone slowly during the first postmenopausal decade when bone loss is normally accelerated. These women will probably never lose enough bone to become osteoporotic (Christiansen 1994).

1.2.3. Skin and urogenital tract

The skin contains ERs and is therefore affected by E withdrawal after the menopause. Estrogen receptors have been identified on epidermal keratinocytes, and dermal fibroblasts and vessels (MacLean et al. 1990). With age, epidermal thickness reportedly decreases 5.7 % of its original value per decade in women while total dermal thickness decreases 6 % per decade (Branchet et al. 1990).
Also, after castration, the epidermis has been shown to get thinner, but this can be reversed with E therapy (Punnonen et al. 1971). In the dermis, the decline in skin collagen content after menopause occurs much more rapidly during the initial postmenopausal years. Some 30% of skin collagen is lost within the first five years after menopause, with an average decline of 2.1% per postmenopausal year over a 20-year period (Brincat et al. 1983, Brincat et al. 1987). As a result, the skin becomes thinner and dryer.

Estrogen receptors have been identified in the urogenital area including the vagina, vulva, urethra and urinary bladder (Iosif et al. 1981, MacLean 1990). After menopause, reduced levels of E cause atrophy of the vaginal epithelial cells and a decreased vaginal blood flow and secretion (Semmens et al. 1982). As glycogen diminishes, the vaginal pH increases, and lactobacilli disappear, allowing colonization of the vagina by Enterobacteriaceae (Molander et al. 1990, Milsom et al. 1993). The epithelial layers of the urethra and urinary bladder also atrophy (Greendale et al. 1993). Urogenital atrophy is the source of many postmenopausal complaints, such as irritation and burning, vaginal dryness, dyspareunia and urinary frequency, urgency, and infections.

2. Postmenopausal hormone replacement therapy (HRT)

2.1. Prevalence

During the last few years, the use of HRT has increased in many countries (MacLennan et al. 1998, Bakken et al. 2001, Bardel et al. 2002, Mueller et al. 2002). In Finland its use doubled between the years 1987 and 1997 (Martikainen 1998). The prevalence of HRT not only differs from country to country (Table 4), but also among regions within countries. For example, in Finland in 1995, 30% of women living in the Helsinki area used E preparations, compared to only 15% of those living in rural areas (Topo et al. 1995). HRT is more frequent among women aged 55 - 59 years, women with climacteric complaints, and hysterectomized women (Oddens et al. 1997, Mueller et al. 2002). HRT is also reported to be associated with healthy lifestyle such as the daily consumption of vegetables, use of vitamin supplements, and regular exercise. Previous use of oral contraceptives, higher education, age above 20 years at first pregnancy, less than four children, and not being overweight are also important determinants of current HRT use (Hammar et al. 1996)
Table 4. Prevalence of postmenopausal HRT in different countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence rate (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>&lt; 1</td>
<td>Haines et al. 1995</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>6</td>
<td>Haines et al. 1995</td>
</tr>
<tr>
<td>Japan</td>
<td>&lt;3</td>
<td>Nagata et al. 1996</td>
</tr>
<tr>
<td>Singapore</td>
<td>7</td>
<td>Chim et al. 2002</td>
</tr>
<tr>
<td>Taiwan</td>
<td>10</td>
<td>Pan et al. 2002</td>
</tr>
<tr>
<td>Estonia</td>
<td>&lt; 1</td>
<td>Fred Kirss (MD, personal message)</td>
</tr>
<tr>
<td>Germany</td>
<td>23</td>
<td>Mueller et al. 2002</td>
</tr>
<tr>
<td>Great Britain</td>
<td>20</td>
<td>Griffiths et al. 1995</td>
</tr>
<tr>
<td>Italy</td>
<td>3</td>
<td>Oddens et al. 1992</td>
</tr>
<tr>
<td>Netherlands</td>
<td>4</td>
<td>Oddens et al. 1994</td>
</tr>
<tr>
<td>Denmark</td>
<td>18</td>
<td>Oddens et al. 1997</td>
</tr>
<tr>
<td>Finland</td>
<td>21</td>
<td>Martikainen 1998</td>
</tr>
<tr>
<td>Norway</td>
<td>27</td>
<td>Bakken et al. 2001</td>
</tr>
<tr>
<td>Sweden</td>
<td>25</td>
<td>Bardel et al. 2002</td>
</tr>
<tr>
<td>Australia</td>
<td>26</td>
<td>MacLennan et al. 1998</td>
</tr>
<tr>
<td>Lebanon</td>
<td>15</td>
<td>Obermeyer et al. 1999</td>
</tr>
<tr>
<td>Morocco</td>
<td>5</td>
<td>Obermeyer et al. 2002</td>
</tr>
<tr>
<td>USA</td>
<td>38</td>
<td>Keating et al. 1999</td>
</tr>
</tbody>
</table>

2.2. Components of HRT

Estrogens used in HRT can be classified by chemical composition into natural or synthetic Es. Natural Es, chemically identical to Es biosynthesized in the human body, include E$_2$, E$_1$, and estriol and their conjugates such as E$_1$ sulphate and esters such as E$_2$ valerate. Synthetic Es, such as ethinylestradiol, have a stronger first-pass effect on the liver than natural Es and are therefore no
longer been for HRT (Kaufman 1997). As E₂ valerate is rapidly hydrolyzed after oral intake it is
dose-equivalent to micronized E₂ and the pharmacokinetics and effects of both are identical (Kuhl

Conjugated equine estrogen (CEE), derived from pregnant mare urine and mainly used in the US,
contains ten different Es including E₁ sulphate (50 %) and many non-human Es like equilin sulphate
(25 %) (Bhavnani 1998). As CEE is a mixture of different Es, the pharmacokinetics, with their long
half-lives, are complex. CEE derivatives have been measured in the serum several weeks after
termination of treatment (Ansbacher 2001). One milligram of micronized E₂ or E₂ valerate is
equivalent to 0.3 mg CEE, and two milligrams of E₂ are equivalent to 0.625 mg of CEE (Schneider
et al. 1999).

During oral administration, E₂ undergoes bio-transformation in the intestinal mucosa and in the
liver, on the first passage, with 80-90 % of the dose being metabolized to E₁ and E₁ sulphate (Kuhl
1990). The oral route therefore leads to E₁ serum concentrations which are 3-6 times higher than
those of E₂. The E₁/E₂ ratio, which is 1:2 in fertile women and 2:1 in postmenopausal women,
increases to 4:1 after oral treatment with E₂ or E₂ valerate. Since the half-life of the sulphate is
prolonged, the level of E₁ sulphate is considerably higher and serves as a hormonally inactive E
reservoir (Kaufman 1997). There is a reversible equilibrium between E₂, E₁ and E₁ sulphate.
Maximum serum E₂ and E₁ concentrations are achieved 2 - 6 hours after oral ingestion (Kaufman
1997). Daily administration of 2 mg micronized E₂ results in serum E₂ concentrations between 80
and 150 pg/ml after several hours, and between 40 and 60 pg/ml after 24 hours (Powers et al. 1985,
Kaufman 1997). Absorption of orally administered Es results in wide fluctuations between peak
and nadir serum concentrations, which in turn result in serum levels outside the therapeutical range.
This could increase the incidence of side-effects.

Estrone as E₁ sulphate can also be used in oral HRT but has no advantage over E₂ (Stumpf 1990).

Estriol is a weak E which cannot be converted to E₂. After oral intake, estriol is conjugated in the
intestine to glucuronides and sulphates, and only 1-2 % of estriol reaches the circulation. The
metabolism of estriol is more limited after vaginal application, and high systemic concentrations
have been attained, this way. Comparable serum estriol concentrations are obtained after vaginal
application of 0.5 mg estriol and oral administration of 8mg (Kuhl 1990). In clinical practise, estriol is mostly used for the treatment of urogenital symptoms (Willhite et al. 2001).

Progestin in HRT is added to counteract the proliferative effect of E on the endometrium, and thus prevent development of endometrial hyperplasia and its consequences. In cyclic therapy, the recommended duration of progestin phase is 10 to 14 days, but the frequency of administration can be reduced without increased risk of hyperplasia (Hirvonen 1996). The antiproliferative effect of progestin on the endometrium is based on several different mechanisms such as decrease DNA synthesis and mitotic activity, and down-regulation of ERs (Hirvonen 1996). Moreover, progestins exert a broad spectrum of various agonistic, antagonistic, and synergistic effects through interaction with progesterone, androgen, estrogen, glucocorticoid and mineralocorticoid receptors (Kuhl 1990). Therefore, progestin may also have different actions on other target organs such as the liver, breasts, bone, brain, and on the immunological and cardiovascular systems, depending on progestin dose and androgenic potency (Hirvonen 1996). Progestin may also cause subjective side-effects and thus the minimum effective antiproliferative dose is recommended.

Progestins can be classified into natural progesterone and synthetic progestins which can be further divided into 17α-hydroxyprogestins, retrosteroids and 19-norprogestins according to their chemical structure and distinctive biological effects. The biological activity of progestins and current antiproliferative doses given per os in conventional HRT are presented in Table 5.
Table 5. Biological activity of different progestins and their transformation doses. (Modified from Hirvonen 1996 and Rozenbaum 1996)

<table>
<thead>
<tr>
<th>Progestin</th>
<th>Progestogenic effect</th>
<th>Androgenic effect</th>
<th>Antiestrogenic effect</th>
<th>Dose (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>17α-hydroxyprogestins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medroxyprogesterone acetate</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>5-10</td>
</tr>
<tr>
<td>Megestrole acetate</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>Cyproterone acetate *</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td><strong>Retrosteroids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dydrogesterone</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>10-20</td>
</tr>
<tr>
<td>Progesterone (micronized)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>200-300</td>
</tr>
<tr>
<td><strong>19-norprogestins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norethisterone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0.3-1</td>
</tr>
<tr>
<td>Norethisterone acetate</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0.7</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>0.150</td>
</tr>
<tr>
<td>Desogestrel</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0.150</td>
</tr>
<tr>
<td>Gestodene</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>0.075</td>
</tr>
<tr>
<td>Norgestimate</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>0.250</td>
</tr>
</tbody>
</table>

* also exhibits antiandrogenic effect
After oral administration, progestins are absorbed efficiently and metabolized in the gut and liver, but the degree of first-pass metabolism varies greatly between progestins and individuals (Kuhl 1990, Rozenbaum 1996). Besides oral administration, progestins can be administered transdermally, percutaneously, vaginally and intrauterinely.

2.3. Benefits of HRT

2.3.1. Documented benefits

Placebo-controlled studies have documented the effectiveness of E therapy for climacteric symptoms such as hot flashes. Estrogen administered by a variety of routes reduces both perceived and objectively measured hot flashes (Coope et al. 1975, Steingold et al. 1985, Haas et al. 1988). The reduction in vasomotor symptoms is dependent on both dose and time (Jensen et al. 1983, Haas et al. 1988, Archer et al. 1992). Notelovitz et al. compared the efficacy of different oral doses of E2 for the relief of moderate to severe hot flashes and found a significant inverse correlation between dosage and number of hot flashes per week (Notelovitz et al. 2000). At week 4, half of the women on placebo had reduced hot flashes of at least 52 %, the corresponding figures were 56 %, 69 %, 86 % and 91 % for 0.25 mg, 0.5 mg, 1 mg and 2 mg, respectively, and at week 12, 77 %, 97 %, 100 % and 100 %, respectively. The most rapid reduction in hot flashes was achieved with 1 mg and 2 mg doses (Notelovitz et al. 2000). It is suggested that more than two weeks of E therapy should elapse before adjustments in E dose are implemented for the control of hot flushes (Haas et al. 1988).

A number of observational studies have shown HRT to prevent postmenopausal bone loss and reduce the risk of vertebral and hip fractures (Torgerson et al. 2001, Marcus et al. 2002). Although a prospective clinical trial showed a reduction in vertebral fractures with transdermal E2 (Lufkin et al. 1996), no prospective data for hip fractures were available until the results from the Women’s Health Initiative study are published (The Writing group for the WHI trial 2002). This randomized, placebo-controlled, 5.2-year trial showed a 34 % reduction in hip fractures and a 24 % reduction in total fractures in postmenopausal women treated with an orally administered combination of CEE and MPA.

The WHI trial also corroborated epidemiological studies showing that E reduces the risk of colorectal cancer (The Writing group for the WHI trial 2002). An analysis of these studies showed
a 20% reduction in colon cancer among women who had previously undergone HRT, and a 34% reduction among current HRT recipients, compared to women who had not received HRT (Grodstein et al. 1999). Duration of HRT use did not influence risk estimates.

In randomized placebo-controlled trials E therapy has been documented to be efficacious in the treatment of symptoms caused by urogenital atrophy (Willhite 2001). Low-dose vaginal E preparations are as effective as systemic HRT (Willhite 2001). Estrogen thickens the vaginal mucosa and helps restore the vaginal pH and normalize the bacterial flora.

### 2.3.2. Uncertain documentation

Both clinical and basic scientific data suggest that E benefits the cardiovascular system in menopausal women. Estrogen receptor-mediated mechanisms have been shown to improve serum lipid levels, enhance endothelial function, dilate coronary arteries, and inhibit the progression of atherosclerosis (The Writing group for the PEPI trial 1995, Adams et al. 1999, Mendelsohn et al. 1999, Mendelsohn et al. 2001, Mosca et al. 2001). In addition, epidemiological evidence suggests that E protects against cardiovascular morbidity. Several observational studies have concluded that HRT reduces overall mortality by 25-50%, largely attributable to a reduction in cardiovascular deaths (Henderson et al. 1991, Etttinget et al. 1996, Grodstein et al. 1997).

The first prospective, placebo-controlled, randomized trial of HRT assessed the efficacy of HRT for secondary prevention of cardiovascular disease, demonstrating that a fixed combination of 0.625 mg/day of CEE and 2.5 mg/day of MPA had no effect on the incidence of fatal or nonfatal cardiac events compared to placebo subjects (Hulley et al. 1998). This result was supported by the findings of two randomized trials that assessed the effect of HRT on the progression of atherosclerosis (Herrington et al. 2000, Angerer et al. 2001). These three prospective trials concluded that HRT has no benefits with respect to secondary prevention of cardiovascular disease.

Recently, the first randomized, placebo-controlled trial assessing the efficacy of HRT for primary prevention was prematurely terminated because of an increased risk of breast cancer (The Writing group for the WHI trial 2002). Unexpectedly there was a 29% increase in the risk of myocardial infarction, which translates to four additional coronary events for every 1000 women over a 5.2 year period (Solomon et al. 2003).
This study has met with enormous criticism for its limitations concerning the health status and age of the participants (Genazzani et al. 2002). Out of all participants 35% were being treated for hypertension, 35% were overweight (BMI 25-29), 34% were obese (BMI > 30), 4% had diabetes, 12.5% had elevated cholesterol levels requiring medication, 6.9% used statins, and 20% used acetyl salicylic acid. The women enrolled in the study were 50-79 years old at the time of recruitment (mean age: 63.3 years) of whom 67% were 60-79 years of age. Only 33% of the participants were 50-59 years old, reflecting HRT users in Europe. Moreover, the participants in the WHI trial were not typical HRT users, being asymptomatic. They had diverse ethnic backgrounds, implying different genetic backgrounds. In spite of this heterogenous group, the results were published uniformly across different age groups and different races. Also, many other questions have arisen such as whether these HRT results can be extrapolated to other HRT regimens, the role of different Es and progestins, and other routes of administration (Genazzani et al. 2002, Skoyby 2002).

Randomized, controlled studies, using formal testing to measure the effects of E on cognition, showed that women symptomatic from menopause improved in verbal memory, vigilance, reasoning and motor speeds, but not in other areas. In asymptomatic women generally, no benefits were observed (Ditkoff et al. 1991, Polo-Kantola et al. 1998, Shaywitz et al. 1999). In a recent, randomized, prospective study, in which the therapeutic efficacy of HRT and the Alzheimer medication, tacrine, were compared, both treatment methods improved cognition and mood, with HRT being better for “activities in daily living” as measured by neuropsychological tests (Yoon et al. 2003).

A main indicator of skin aging is skin thickness, which reflects the status of the collagen and elastin tissue. In controlled studies HRT has been shown to cause a thickening in both the epidermis and dermis when investigated histologically, or by ultrasound (Punnonen 1971, Punnonen et al. 1974, Bricant et al. 1985, Maheux et al. 1994, Sator et al. 2001). The measurements of skin layers, however, have mostly been carried out after a short HRT period, and the results of long-term treatment are missing. The effect of HRT on epidermal hydration and elasticity has also been investigated but with conflicting results (Jemec et al. 1989, Callens et al. 1996, Sator et al. 2001).

In placebo-controlled trials, E therapy and E plus androgen therapy have been shown to improve quality of life and sexual well-being in postmenopausal women when measured with psychological tests (Wiklund et al. 1993, Sherwin 2002).
2.4. Harms of HRT

2.4.1. Side-effects

In HRT both progestin and E may cause undesirable and even similar side-effects (Table 6). Moreover, different progestins derivatives may cause different side-effects. Acne, oily skin and greasy hair are reported more frequently with the testosterone derivatives, norethisterone and norgestrel (Whitehead et al. 1992). The occurrence of progestin related side-effects may be dose-dependent. When compared to a placebo, the addition of 5 mg/day norethisterone for 7 days each month in women with E₂ implants was associated with a statistically significant increase in the occurrence of adverse effects such as headache, fluid retention, weight gain, breast tenderness, concentration difficulties, anxiety, emotional lability and depression. However, in a similar, placebo-controlled study, the addition of 2.5 mg/day norethisterone, did not cause statistically significant changes in symptomatology or behaviour. Besides its subjective side-effects, progestin may cause metabolic side-effects such as increased LDL, decreased HDL, hyper-insulinemia, and deterioration of glucose tolerance (Hirvonen 1996).

In a study by Nachtigall (1990), the most frequently reported side-effects given as reasons for discontinuing therapy were withdrawal bleeding, irregular bleeding, breast tenderness and edema. Different bleeding disorders are also commonly reported in other studies (Wren et al. 1991, Stumpf et al. 1994, Leung et al. 2001). Hyperestrogenic symptoms during HRT are significant breast tenderness, nipple sensitivity, leg cramps, nausea, and fluid retention (Whitehead et al. 1992). The occurrence of these symptoms and their continuation for more than 6 - 8 weeks after initiation of HRT may necessitate an E₂ dose reduction. Skin irritation caused by E₂ patches has been reported more frequently than with topical gel.
Table 6. Some possible side-effects related to HRT (Whitehead et al. 1992)

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>Progestogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding disorders</td>
<td>Acne and seborrhea</td>
</tr>
<tr>
<td>Bloating</td>
<td>Anxiety and irritability</td>
</tr>
<tr>
<td>Breast tenderness</td>
<td>Bleeding disorders</td>
</tr>
<tr>
<td>Headaches and migraine</td>
<td>Breast tenderness</td>
</tr>
<tr>
<td>Leg cramps</td>
<td>Depression</td>
</tr>
<tr>
<td>Limb pains</td>
<td>Headaches</td>
</tr>
<tr>
<td>Nausea</td>
<td>Hot flashes</td>
</tr>
<tr>
<td>Weight gain and fluid retention</td>
<td>Weight gain and fluid retention</td>
</tr>
</tbody>
</table>

2.4.2. Other consequences

Current use of HRT is reported to be associated with an increased risk of breast cancer in observational studies (Sillero-Arenas et al. 1992, Colditz et al. 1993). The results indicate an increased risk with longer duration of use. The findings are consistent with the controlled WHI trial where a 26% increase in the risk of invasive breast cancer was associated with combined HRT (The Writing group for the WHI trial 2002). This increased risk means four additional breast cancers for every 1000 women over 5.2 years. The role of progestin in HRT is currently being discussed, and some studies suggest that the concurrent use of progestin confers a significantly greater risk of breast cancer than the use of E alone (Colditz et al. 1995, Ross et al. 2000, Schairer et al. 2000). However, contrasting data regarding the role of HRT in breast cancer risk also exist (De Lignieres 2002, Hulley et al. 2002). The influence of various types and routes of ERT and E doses on the risk of breast cancer has not been studied. The effects of achieved serum E levels and different progestins on the incidence of breast cancer also needs further investigation.

Both epidemiological and randomized, controlled studies report an increase in risk for deep vein thrombosis and pulmonary embolism among E users, with the highest risks occurring during the first year of use (Hulley et al. 2002, Miller et al. 2002, The Writing group for the WHI trial 2002). The absolute increase is not high, being 1.5 venous thromboembolic events per 10 000 women in one year. Some studies report a higher risk with increased E dose (> 0.625 mg of CEE), as well as for
the use of E combined with progestin versus the use of E alone (Daly et al. 1996, Perez-Gutthann et al. 1997). However, mortality from these events is not increased in HRT users.

Contrasting data exist regarding the role of HRT in an increased risk of stroke. In observational studies, stroke incidence, but not mortality, has been reported to increase among ever users (Pedersen et al. 1997, Sourander et al. 1998, Grodstein et al. 2000). This risk was elevated for thromboembolic stroke (Pedersen et al. 1997) but not subarachnoid (Grodstein et al. 2000) or intracerebral stroke (Pedersen et al. 1997). In general, no differences were shown between current or previous HRT users. The WHI trial indicated an increased risk of nonfatal stroke among HRT users (RR 1.41), while another prospective trial reported no increase in strokes (Grady et al. 2002, The Writing group for the WHI trial 2002).

In the Nurses’ Health Study the risk for cholecystitis among HRT users increased after 5 years, and remained elevated for women after 10 or more years of use (Grodstein et al. 1994). This finding is supported by Hulley et al. (2002) who report an increase in biliary tract surgery among HRT users compared with placebo during 6.8 years of follow-up. However, this outcome has not yet been reported by the WHI trial (The Writing group for the WHI trial 2002).

Unopposed E therapy is associated with an increased risk of endometrial cancer and the risk increases with duration of use (Grady et al. 1995). When progestin is added to E sequentially, the risk of endometrial hyperplasia and cancer is considered to be eliminated (The Writing group for the PEPI trial 1996). Observational studies show conflicting results regarding the risk of endometrial cancer with combined regimens. However, prospective, controlled studies have not reported an increase in endometrial cancer (Hulley et al. 2002, The Writing group for the WHI trial 2002).

3. Alternatives to HRT

Tibolone is a tissue-specific compound with estrogenic and weak progestogenic and androgenic properties that has been used to control climacteric symptoms (Albertazzi et al. 1998). Using a dosage of 2.5 mg/day, a double-blind, randomized trial demonstrated tibolone to be effective in controlling hot flashes (Hammar et al. 1998). In addition, due to its tissue-specific activity and
androgenic properties, other beneficial effects of tibolone include prevention of bone loss (Lindsay et al. 1980), lack of endometrial proliferation, and increased libido (Moore 1999).

Progestins, with variable success, have been used to control menopausal symptoms. A study conducted in women with breast cancer established the efficacy of megestrol acetate for the treatment of hot flashes (Loprinzi et al. 1994). The initial dose of 40 mg/day was used until hot flashes subsided and was then titrated to 20 mg/day or every other day (Quella et al. 1998). Treatment with propranolol 120 mg/day was tested in 25 perimenopausal women but was found to be no more effective than placebo (Coope et al. 1978). Bellergal®, a combination product of belladonna alkaloids and phenobarbital, has been used for the treatment of hot flashes. The published data on the efficacy of this treatment is, however, conflicting (Bergman et al. 1987). Bellergal® is no longer available in Finland.

Antidepressants have been used for the treatment of climacteric complaints. One novel antidepressant, used for treatment of hot flashes, is venlafaxine, also available in Finland. Venlafaxine is classified as a serotonin noradrenergic reuptake inhibitor (Loprinzi et al. 1998). In a randomized, placebo-controlled trial hot flashes were reduced by 40% and 60% in women treated with 37.5 mg and 75 mg/day of venlafaxine, respectively, compared with a reduction of 27% in the placebo group. The adverse effects related to venlafaxine were mouth dryness, decreased appetite, constipation and nausea (Loprinzi et al. 2000). Alternative therapies, such as herbal medications and soy protein, appear to be borderline effective at best (Davis 2001).

Despite adequate calcium and vitamin D intake, there are other ways to prevent or treat postmenopausal osteoporosis. Bisphosphonates alendronate and risedronate maintain bone density similar to HRT and reduce the risks of both hip and vertebral fractures. Raloxifene, a selective ER modulator, prevents osteoporosis but not hip fractures (Ettinger et al. 1999). Raloxifene may also exacerbate hot flashes in some women.

4. Parenteral estrogen replacement therapy

Parenteral routes of administration include transdermal patches, subcutaneous implants, intramuscular injection, and intravaginal, nasal, sublingual, and percutaneous gel administration. All of these forms of administration avoid the first-pass metabolism of E₂ to E₁ in the liver and
more physiological E\textsubscript{2}/E\textsubscript{1} ratio (1:1) in serum is achieved (Holst et al. 1983a, Laufer et al. 1983, Chetkowski et al. 1986, Scott et al. 1991).

Transdermal patches are either reservoir-type patches with a membrane controlling the release of E\textsubscript{2}, or matrix patches (Kaufman 1997). In matrix-type patches, E\textsubscript{2} is dissolved or dispensed into the adhesive matrix of the batch and the rate of release is controlled by the specific formulation of the matrix. Patches releasing 25 - 100 ug/24h of E\textsubscript{2}, administered once or twice a week, are available. With patches, the total delivery of E\textsubscript{2} depends on the content and area of the patch (Kaufman 1997). After the application, peak concentrations of E\textsubscript{2} are attained within 2 to 8 hours, after which the levels tend to decrease (Kuhl 1990). The average serum E\textsubscript{2} concentration of a 50 µg/24h patch is about 40 pg/ml with continuous application and a change of plaster twice a week, but low E\textsubscript{2} concentrations have been reported in some users (Reginster et al. 1997). Inter-individual variations in serum E\textsubscript{2} levels are larger than with gel (Järvinen et al. 1999, Paoletti et al. 2001).

Subcutaneous implants have been reported to produce fairly constant E\textsubscript{2} concentrations (Notelovitz et al. 1987, Kaufman 1997). There are, nevertheless, significant variations in the total time during which adequate levels are maintained. Accumulation can occur if a new pellet is implanted before the E concentrations have decreased to their initial values (Kuhl 1990). Injectable Es have been used in HRT, but they have shown variable plasma concentrations, with a high E\textsubscript{2} peak 2 days after administration (Kuhl 1990). Vaginal application of micronized E\textsubscript{2} rapidly produces a higher peak E\textsubscript{2} level than with peroral administration, but it also decreases rapidly. A vaginal ring with continuous release of E\textsubscript{2} results in relatively constant E\textsubscript{2} levels over a period of weeks or months (Kaufman 1997).

4.1. Percutaneous therapy

4.1.1. Pharmacokinetics

Estradiol-containing hydro-alcoholic gel, when applied to the skin, penetrates the stratum corneum and diffuses through the epidermis to the capillary plexus in the dermis and connective tissue into the systemic circulation over a period of several hours (Wendker et al. 1976). The skin, especially the stratum corneum, is metabolically active, and functions as a drug reservoir and an E diffusion
regulating membrane (Sitruk-Ware 1989, Simon et al. 1990). Approximately 10% of the applied dose reaches the circulation in a steady-state condition (Sitruk-Ware 1989). Daily application of 1.5 and 3 mg E$_2$ (0.6 mg E$_2$/gram gel) causes serum E$_2$ levels to rise during the first 3 to 5 days, after which a plateau is reached with an average concentration of about 70 - 80 pg/ml and 120 - 150 pg/ml, respectively (Basdevant et al. 1983, De Lignieres et al. 1986, Scott et al. 1991). Intra-individual serum E$_2$ levels have been shown to be relatively stable (Scott et al. 1991).

Large variations inter-individually have been suggested to be a consequence of individual differences in percutaneous absorption due to variations in moisture of the skin, thickness of the epidermis, retention time within the skin, and vascularity of the adipose tissue (Guy et al. 1987, Ranade 1991, Karjalainen et al. 1997). Furthermore, the size of the application area has a tremendous impact on absorption (Chanez et al. 1989, Scott et al. 1991). Also, individual variations in E metabolism may result in fluctuations in serum E$_2$. The pharmaceutical formulation and drug content of different percutaneous preparations also affect the pharmacokinetics and bioavailability of a drug (Järvinen et al. 1999).

With gel containing 0.6 mg E$_2$/gram, the biological availability of E$_2$ improves, and the variability of serum concentrations decreases with respect to the surface area of application. A reduction in the surface area from 750 to 400 cm$^2$ may result in a 50% reduction of the achieved steady state levels (Chanez et al. 1989, Scott et al. 1991). Spreading the gel over a larger area of skin is estimated to enhance the reservoir effect of the skin and thus minimize hourly and daily variations in serum E$_2$ concentrations (Chanez et al. 1989, Scott et al. 1991).

The site of application is not considered to be an important factor contributing to absorption. Studies where the gel was applied to the abdomen alone, or to the shoulders and arms showed similar serum E$_2$ concentrations. In a study by Holst et al. (1987), application to the inner thigh was as effective as application to the lower abdomen.

Three commercial percutaneous E$_2$ gel formulations are available in Finland, but their composition and application instructions differ. The recommended dose of Oestrogel © (0.6 mg E$_2$/gram) is 2.5 g gel over a 750 cm$^2$ skin area. Divigel © (1.0 mg E$_2$/gram), 0.5 - 1.5 g gel is required to be spread over a 200 - 400 cm$^2$ skin area. The permeation of E$_2$ through the skin is similar in these two gel formulations at the recommended doses (Waltres et al. 1998). The initial 0.5 g dose of
the third E2 gel, Estrena ® (1.0mg E2 / gram), is recommended to be titrated individually according to response. It should be applied to an area of skin equivalent to the area of the palms of two hands.

4.1.2. Efficacy

In placebo controlled trials percutaneous E has been effective in alleviating vasomotor symptoms in postmenopausal women (Jensen et al. 1987, Dupont et al. 1991). In one study of treatment efficacy, after 12 weeks of percutaneous treatment with 1.5 mg E2, 95 % of the E2 gel group showed improvement in vasomotor symptom severity compared with 39 % of the placebo group. The E2 gel group also showed a significant decrease (85 %) in frequency of hot flashes than did the women in the placebo group (30 %) (Kornafeld et al. 1992). Compared with oral therapy, percutaneous E2 has been equally effective in relieving hot flashes, insomnia, night sweats, and vaginal atrophy (Dupont et al. 1991). Percutaneous E also prevents postmenopausal bone loss. In placebo-controlled, double-blind studies, percutaneous E was effective at a daily dose of 1.5 g E2 (Riis et al. 1987a, Riis et al. 1987b). When treated with 3 mg E2, there was an additional 4.5 - 6.4 % reduction in bone mineral content. In all women the values either remained constant or showed a slight increase in all bone compartments over a two-year period (Riis et al.1987a).

4.1.3. Adverse effects

The reported incidence of adverse skin reactions to E2 gel is low. In a 5.7 year study by Moyer et al. (1993) none of the 157 women reported skin irritation. This is noteworthy, considering reports of skin reactions with transdermal E2 patches (Stanczyk et al. 1988, Sentrakul et al. 1991). Influence on skin histology of repetitive application of E2 gel has been investigated and no changes were found in the stratum corneum and stratum granulosum. The number of cell layers and their thicknesses within the epidermis did not change before and after treatment (Holst et al. 1982).

4.1.4. Percutaneous versus transdermal administration

Both E2 patches and gel have been shown to relieve menopausal symptoms and provide protection against osteoporosis. Depending on which gel product is being used, different absorption figures have been obtained. For example, some, give a peak E2 concentration four hours after dosing
reflecting that of tablets, but with a lower magnitude (Järvinen et al. 2001). Another gel product offers relatively constant E₂ levels throughout the 24-hour period after application. In general, when comparing gels with patches, more E₂ fluctuation has been reported with patches than with gel. However, variations in bioavailability between patches also exists (Reginster et al. 1997).

4.2. Parenteral versus oral administration

In ERT, parenteral dosage has many advantages over oral dosing. In oral administration, a large part of E₂ is transformed into biologically less active E₁ and conjugates via presystemic metabolism (Lyrenäs et al. 1981, Chetkowski et al. 1986) whereas in parenteral administration, the presystemic metabolism of E₂ is prevented and more physiological E₂/E₁ ratio is achieved (Holst et al. 1983b, Laufer et al. 1983). This is because the bioavailability of parenterally administered E₂ is tenfold compared with oral therapy in which higher doses of E₂ are needed. Moreover, parenterally administered E does not lead to induction of hepatic protein synthesis, which after oral therapy is estimated to be associated with adverse effects such as hypertension, hypercoagulability and cholelithiasis (Geola et al. 1980, Selby et al. 1989, Crook 1997). On the other hand, women with an unfavourable blood lipid profile in menopause may benefit more from oral therapy over parenteral therapy since greater positive changes in lipid metabolism are observed during oral ERT. (Tikkanen et al. 1982, Basdevant et al. 1991, Pang et al. 1993). The effect of different administration routes of E on glucose metabolism and insulin sensitivity has been studied. Average doses of oral E do not seem to have a significant negative effect on carbohydrate metabolism (Godsland et al. 1993). However, parenteral E₂ administration appears to be more beneficial with regards to effects on glucose metabolism (Godsland et al. 1993). When compared with tablets and patches, percutaneous gel allows more flexible dose adjustment.

5. Compliance with HRT

Compliance with HRT has been poor despite the recognized advantages of HRT. It has been suggested that physicians advise and prescribe HRT more often than women actually use it (Ravnikar 1987, Topo et al. 1993). In studies of compliance with HRT 6 - 30 % of women who had been prescribed HRT never started the therapy (Nachtigall 1990, Oddens et al. 1997), and 10 % took the medication sporadically (Nachtigall 1990). In other trials, 31 % ceased the therapy within 6 months and 27 - 61 % within 1 - 2 years of initiation (Koster et al. 1990, Wren et al. 1991).
A considerable number of users cease HRT because of its E- and / or progestin-related side-effects. The most frequently cited side-effect is bleeding episodes (Wren et al. 1991, Buist et al. 1999, Doren et al. 1999). At an American clinic specializing in informing patients before prescribing HRT, the main reasons for discontinuing the therapy after one year of treatment were bleeding disorders, breast tenderness, and fluid retention (Nachtingall 1992). At the same clinic, in a study of 1330 women on transdermal HRT, causes for cessation were also bleeding problems, followed by skin irritation, and edema. Compliance in both studies was high. Only 7 % and 5 % discontinued HRT, mainly because of bleeding disorders. Progestin in HRT may cause headaches, edema, breast tenderness, and irritability (Nachtingall 1992). These symptoms can almost always be controlled with a change in the type, dose, or duration of the progestin.

Other reasons for early cessation of HRT are various concerns by a woman or a physician and lack of knowledge. In a study of 1015 Danish women, 35 % of previous users had ceased HRT because of a fear of possible health risks, and 16 % were advised to cease HRT by a physician (Oddens et al. 1997). The corresponding figures in another study were 37 % and 13 %, respectively (Leung et al. 2001).

Compliance with HRT is, however, increasing in many countries along with the prevalence rate (Bakken et al. 2001, Mueller et al. 2002 ). In Australia, the median compliance rate rose from 24 to 60 months between the years 1991 and1995 (MacLennan et al. 1998). In studies where long-term compliance was investigated, 5 and 10 year compliance figures were 71 % and 87 %, respectively (Eiken et al. 2002, Leung et al. 2001). Absence of withdrawal bleeding and the encouragement of a physician enhance long-term compliance with HRT (Eiken et al. 2002, Leung et al. 2001).
AIMS OF THE STUDY

The aims of this study were:

1. To investigate the factors affecting the use and compliance of HRT and the applicability of self-adjusted percutaneous ERT (Study I, II).

2. To obtain information about self-adjusted percutaneous estrogen doses in relation to disappearance of climacteric symptoms, serum E₂, FSH and SHBG concentrations (Study II, III).

3. To study the possible interaction between ascorbic acid (AA) and E₂ in women on percutaneous ERT with stable serum E₂ concentrations (Study IV).

4. To study the influence of skin contamination by E₂ gel on serum E₂ concentration during percutaneous HRT (Study V).
MATERIALS AND METHODS

1. Subjects

A summary of the materials and methods used in the original publications is presented in Table 7. The sample of women for our study (I) was selected from the age-sex register of the suburban health care region of Ylöjärvi and included all women (n=1056) who were born between 1935 and 1945 and were therefore aged 50-60 at the time of the survey. The Ylöjärvi area, with 22 500 inhabitants in 1995, comprises the population from the local councils of Ylöjärvi and Kuru and is situated in southern Finland, 15 kilometers north-west of Tampere. A postal questionnaire, an explanatory letter, and a pre-paid reply envelope were sent in December 1995. A reminder and a second copy of the questionnaire were sent to non-responders in January 1996. After the reminder, 884 (84 %) completed questionnaires were obtained (study I). In the questionnaire the women were also asked if they were interested in participating in studies related to postmenopausal HRT. Two-hundred and eighty-five women who volunteered for further investigation were interviewed and all underwent clinical, gynecological and breast examinations (T.V.). Of the 285 women who volunteered and were examined, 55 were only interested in consulting a physician and being examined free of charge. They had no intention of participating in further studies. Eight women had contraindications with respect to ERT such as a history of malignancy and tromboembolism, and 51 had illnesses or gynaecological abnormalities, for example, fibroids. They were excluded from the study. Furthermore, women whose duration of amenorrhea was less than 12 months were excluded. The remaining volunteers participated in our study (Studies II-V). Participants who were currently undergoing HRT had a wash-out period of two weeks before the start of the study (Studies II, III, V). Twenty-five women from study II also participated in study IV, and six women from study II participated in study V.
Table 7. The study material and the design of studies I-V.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
<th>Study V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>884</td>
<td>32</td>
<td>32</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Age of women</td>
<td>50-60</td>
<td>51-59</td>
<td>51-59</td>
<td>51-59</td>
<td>52-58</td>
</tr>
<tr>
<td>Objectives</td>
<td>To study the prevalence, use and compliance of HRT in Finland</td>
<td>To study the relationship between climacteric symptoms, E2 dose and serum E2 in self-adjusted model</td>
<td>To evaluate the hormonal profile during ERT with reference to B-FSH in self-adjusted model</td>
<td>To study the possible interaction between AA and E2</td>
<td>To study if skin contamination by E2 gel has an influence on serum E2 levels</td>
</tr>
<tr>
<td>Design</td>
<td>Questionnaire study</td>
<td>Prospective follow-up study</td>
<td>Prospective follow-up study</td>
<td>Prospective follow-up study</td>
<td>Prospective follow-up study</td>
</tr>
<tr>
<td>Main outcome measures</td>
<td>Prevalence of HRT, menopausal symptoms, compliance, side-effects of HRT</td>
<td>E2 dose, S-E2, Kupperman Index</td>
<td>E2 dose, S-E2, S-I-FSH, S- B-FSH, S-SHBG Kupperman Index</td>
<td>S-E2, S-Ascorbic acid</td>
<td>S-E2 from both cubital veins after every two weeks when gel spreading protocol was changed</td>
</tr>
</tbody>
</table>
2. Study medications

All women (Studies II-V) received percutaneous 17β-estradiol gel (Estrogel ®, Leiras Oy, Turku, Finland) which contains 1.5 mg of E₂ per 2.5 g gel releasing 150 µg E₂ into the circulation. This hydro-alcoholic gel was applied to a large surface area (750 cm²) of the skin. The accuracy of E₂ dosage was emphasized to participants before the study and during control visits since the administration was performed with a dosing plastic ruler. The E₂ gel was in a tube from which it was pressed first in the measuring groove of the dosing plastic ruler and applied to the skin. Before the study started the participants were taught how to press the correct dose by the author, including how to press equally thick gel braids. Women with intact uteri also received oral MPA (Megestin ®, Leiras Oy, Turku, Finland) 10 mg per day for 10 days a month (studies II, III, IV) in order to initiate withdrawal bleeding. All subjects in study IV received 500 mg of AA (Ascorbin-C-vitamin ®, Leiras Oy, Turku, Finland) twice a day.

3. Study designs

Study I.
A postal questionnaire study as described earlier.

Studies II and III.
Thirty-two women applied percutaneous E₂ gel to the skin at an initial dose of 1 mg E₂, administered once a day in the evening. The subjects observed their symptoms and increased the daily treatment dose by 0.5 mg every two weeks until they felt comfortable with it. Each woman continued at that treatment dose for 3 months. Twelve hysterectomized women were treated with E₂ gel only, while 20 women with intact uteri also received oral MPA 10 mg per day for 10 days per month.

Study IV
Twenty-five women had been on percutaneous E₂ for 10 - 12 months and had self-adjusted their estrogen doses on the basis of disappearance of climacteric symptoms. Stabilized doses varied individually from 0.5 to 2 mg of E₂ per day (mean 1.5 mg/day). The gel was applied to the skin of the abdomen using the right hand, in the evenings. With HRT continuing at the same doses, 500
mg of AA was added twice a day for 3 months. Thirteen women with intact uteri also received oral MPA, 10 mg per day for 10 days per month.

Study V
Ten hysterectomized women applied 1.5 mg percutaneous E$_2$ gel once a day in the evening to an arm or thigh with either a bare or gloved hand in two week periods according to the schedule: E$_2$ was applied to a thigh with a disposable glove on the right hand, to the left arm with a disposable glove on the right hand, to a thigh with a bare right hand, and to the left arm with a bare right hand.

4. Control of menopausal symptoms

Menopausal symptoms were recorded before the study (Studies II, III), and during the treatment, at two weeks intervals: each time the E$_2$ dose was increased, and when a suitable, self-adjusted treatment dose was established (Studies II, III). Symptom relief was evaluated using the sum of the numerical conversion factor from the Kupperman Index (KI) (Kupperman et al. 1953) of the eleven most common menopausal complaints: hot flashes, sweating, insomnia, nervousness, depression, vertigo, fatigue, pain in the joints and muscles, headache, palpitation, and formications (paresthesia and itching). The severity of each symptom was graded by women using a scale of 0 - 3 where 0 means no symptoms, and 1, 2, and 3 indicate mild, moderate, and severe symptoms, respectively. Scores for hot flashes are multiplied by four and those for sweating and insomnia by two.

5. Laboratory assays

Blood samples for assays were collected twelve hours after the gel (Studies II-V) and AA tablet (Study V) administration. Blood samples were not taken from the same side cubital vein with which hand the gel had been spread. The specimens were stored at –20°C and all samples from each subject in each study (II-V) were assayed together.
1. Serum E₂
Concentrations of E₂ were measured by RIA (Sorin biomedica, S.p.A., Saluggia, Italy) (Studies II-V).
Studies II and III. Blood samples for the determination of serum E₂ concentrations were obtained at baseline, each time before the self-adjusted E₂ dose was increased independently by the subjects, and at month 3.
Study IV. Blood samples for E₂ assay were collected from the left cubital vein at 0, 1 and 3 months. Blood samples were not taken during the progestin phase.
Study V. Blood samples for serum E₂ measurements were collected at the same time, but separately from both cubital veins, after a two-week wash-out period and after each two-week treatment period.

2. Serum FSH
Study III. The immunological activity of FSH (I-FSH) was monitored by Delfia®, immunofluorometric technique (Wallac Oy Pharmacia, Turku, Finland). Biological activity of serum FSH (B-FSH) was monitored as described earlier (Gudemann et al. 1994) using a cell system transfected permanently with FSH receptor cDNA. Blood samples for the I-FSH and B-FSH determinations were drawn at baseline, each time the dose was increased, and at the end of the study.

3. Serum SHBG
Study III. Serum sex-hormone binding globulin levels were measured by time-resolved fluororimmunoassays (Wallac Oy Pharmacia, Turku, Finland). Blood samples for the SHBG determinations were collected according to the same schedule as FSH described above.

4. Serum AA
Study IV. Serum concentration of AA was measured by high performance liquid chromatography, with an electrochemical detector, according to the method described by Frei et al. (1989).
The sensitivity, intra-assay and interassay coefficient of variation of the methods and normal ranges of the values are shown in table 8.
Table 8. Characteristics of the hormonal assays

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Intra-assay CV</th>
<th>Range of measurements</th>
<th>Normal values after menopause</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₂</td>
<td>RIA</td>
<td>0.01 nmol/l</td>
<td>4.2%</td>
<td>0.02-3.7 nmol/l</td>
</tr>
<tr>
<td>I-FSH</td>
<td>IRMA</td>
<td>0.8 IU/l</td>
<td>4.8%</td>
<td>1.48-50.2 IU/l</td>
</tr>
<tr>
<td>SHBG</td>
<td>IRMA</td>
<td>0.05 nmol/l</td>
<td>1.3-1.8%</td>
<td>18.6-117 nmol/l</td>
</tr>
</tbody>
</table>

6. Statistical analyses

Continuous normalized data were described by their means and standard error or standard deviation. In skewed distribution data, or in data with a reduced number of subjects, nonparametric methods with median and range values were reported. The significance of differences between the groups with normally distributed data was determined using the t-test or chi-square test. When repeated measures were obtained, the two-way analysis of variance was used to detect differences within and between the groups. The significances of observed changes in different hormonal parameters were evaluated by Wilcoxon’s test (Studies III, IV, V). In order to estimate the changes in AA concentrations between the three different time points, Friedman’s test was applied (Study IV). The same test was used in study V to assess the differences between E₂ gel-contaminated samples and between uncontaminated samples. For association correlation between different parameters, Spearman’s correlation coefficient was used (Studies III, IV). A p-value <0.05 was considered statistically significant.

7. Ethical considerations

The study protocol was approved by the Ethics Committee of Tampere University Hospital and Health Center of Ylöjärvi. Written informed consent was obtained from each subject.
RESULTS

1. Prevalence of HRT and characteristics of users

Of the 884 responders, 773 had reached menopause, of whom 302 (39 %) were current HRT users, 126 (16 %) were previous users, and 345 (45 %) were non-users (Study I). There were no significant differences in medication or illnesses between current HRT users, previous users and non-users, excluding malignant diseases. Fourteen women (2 %) had been treated for breast cancer and none of them were using HRT at the time of the survey. Five women, however, had used oestrogens previously. Two women out of 14 being treated for another malignant disease were current HRT users. The number of pregnancies did not appear to have an influence on the use of HRT, whereas gynaecological operations were more common among current users. Of the 302 current users of HRT, 74 % had peroral preparations, and 26 % used a transdermal form of HRT.

Reasons for estrogen therapy were climacteric symptoms (79 %), prevention of osteoporosis (7 %), and other reasons (11 %). Three percent of the women on HRT were unsure of the reason for starting treatment. The majority of the current users (88 %) had received their prescriptions from a gynaecologist, whereas general practitioners and other specialists had prescribed 8 % and 4 % of the prescriptions, respectively. General practitioners had not prescribed the transdermal form of HRT to anyone, nor had they prescribed estrogens for the prevention of osteoporosis. Of the women on HRT, 85 % had experienced the advantages of estrogen treatment, while 15 % were unsure of the advantages. Of the 302 current users, 11 % had been advised to discontinue the treatment by a friend, 2 % by a physician, and 2 % by another person.

2. Compliance with HRT

Twenty-eight percent of the current users had used HRT for more than 5 years, and 8 % for more than ten years (Table 9). Of the 126 previous users, 27 % had used HRT for less than 6 months, and almost half of the previous users (46 %) had ceased treatment within one year (Table 9). The reasons for discontinuation of HRT are presented in Table 10 and 11. Sixty women (20 %) of the current users reported having continuous side-effects from HRT (Table 11). There were no significant differences in side-effects between women with intact uteri and hysterectomized women except for weight gain and fluid retention, which was more frequent among women with intact uteri (p<0.005). Treatment regimens did not correlate with side-effects.
Table 9. Duration of HRT among current and previous users.

<table>
<thead>
<tr>
<th>Time</th>
<th>Current users (n=302) (%)</th>
<th>Previous users (n=126) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 months</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>17</td>
<td>46</td>
</tr>
<tr>
<td>&gt; 1-3 years</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>&gt; 3-5 years</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>&gt; 5-10 years</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>&gt; 10 years</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Unsure about</td>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 10. Reasons for discontinuation of HRT among previous users.

<table>
<thead>
<tr>
<th>Reason</th>
<th>Previous users (n=126) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side-effects</td>
<td>41</td>
</tr>
<tr>
<td>Fear of cancer</td>
<td>16</td>
</tr>
<tr>
<td>Recommendation of a physician</td>
<td>12</td>
</tr>
<tr>
<td>Inefficiency</td>
<td>4</td>
</tr>
<tr>
<td>Advice of a friend</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
</tr>
</tbody>
</table>
Table 11. Side-effects of HRT among current users 1) and previous users (as a reason for discontinuation)

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>Current users (n=60) (%)</th>
<th>Previous users (n=52) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain and fluid retention</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Headache</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Breast tenderness</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Increase of blood pressure</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Hot flashes and sweating</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Irregular bleeding</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Hot flashes and sweating</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

1) more than one side-effect possible

3. Menopausal symptoms and achieved control of symptoms

Different menopausal symptoms experienced by current HRT users, previous users, and non-users are presented in Table 12 (Study I). Menopausal symptoms were experienced more often by current HRT users than non-users and there was a significant difference in the incidence of sweating, trouble sleeping, and depression (p<0.005) between the two groups.
Table 12. Menopausal symptoms among current HRT users, previous users and non-users. (More than one symptom possible).

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Current users (n=302) (%)</th>
<th>Previous users (n=126) (%)</th>
<th>Non-users (n=350) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweating</td>
<td>64</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>Hot flashes</td>
<td>31</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Trouble sleeping</td>
<td>26</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>Depression</td>
<td>16</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Irritability</td>
<td>11</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Palpitations</td>
<td>10</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Headache</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Anxiety</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vaginal dryness</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Joint pains</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

When the women self-adjusted E doses based on reduction of menopausal symptoms, during three months of treatment, climacteric symptoms, especially vasomotor episodes, decreased in all women when evaluated by Kupperman’s menopausal index (KI) (p<0.001) (Study II). The dose of 1 mg percutaneous E2 per day was sufficient to relieve symptoms in 29 % (n=9) of the women, 52 % (n=16) required 1.5 mg, and 19 % (n=6) 2 mg or more. The initial KI had no correlation with the final self-adjusted treatment doses but a weak correlation was observed between KI and B-FSH (Figure 1) and I-FSH (Figure 2) (Study III). In addition, no correlation was found between decreased KI and serum E2 after three months of treatment.
Twenty-nine out of 32 women who self-adjusted their percutaneous E doses according to the disappearance of climacteric symptoms followed the study protocol strictly and completed the study symptom-free having given themselves suitable treatment doses (Study II). Two participants digressed from the study protocol: after increasing the dose to 1.5 mg, they decreased it back to the initial dose because of breast tenderness. They completed the study compliant with the original E$_2$ dose. One woman ceased participating in the study after 5 weeks of treatment because of side-effects. Twenty-five women who participated in study IV, continued with their self-adjusted E doses and were still compliant after 10-12 months. Six women from study II who participated in study V were compliant with their E$_2$ doses at the start of Study V.
4. Self-adjusted dose and serum estradiol, follicle-stimulating hormone, sex hormone-binding globulin

Initial serum E₂ concentrations were at postmenopausal levels in all women (Studies II, III, V). After 2 weeks of treatment with 1 mg percutaneous estradiol, serum E₂ concentrations showed a wide range, fluctuating between 17 and 195 pg/ml (Figure 3) (Study II).

Serum E₂ concentrations, after three months of treatment with self-adjusted E₂ doses, were at postmenopausal levels (<50 pg/ml) in seven women (22 %) (Figure 4) (Study II). In all, 14 participants (45 %) showed serum E₂ concentrations under 60 pg/ml, eight of whom applied gel at a dose of 1mg/day. Nine subjects (29 %) showed better therapeutic levels of 60 - 100 pg/ml, their doses varying from 1.5 mg to 6 mg estradiol daily. Eight participants (26 %) had serum E₂ concentrations greater than 100 pg/ml, their daily doses being from 1 to 3 mg. There were no significant differences either in gel doses or E₂ levels between women with intact uteri and hysterectomized women, nor was there any correlation between serum E₂ concentration and body mass index (BMI) (Study II).

Figure 3. Serum E₂ concentrations after 2 weeks of treatment with 1 mg percutaneous E₂ gel in 32 postmenopausal women.

Figure 4. Self-adjusted estrogen doses and the respective serum E₂ concentrations after three months of treatment with percutaneous E₂.
The correlation between serum B-FSH and I-FSH in postmenopausal women on self-adjusted percutaneous ERT is shown in Figure 5 and the correlations of serum I-FSH and B-FSH with serum E₂ are presented in Figures 6 and 7 (Study III). Serum E₂ levels showed a weak positive correlation with serum SHBG levels (Figure 8) (Study III).
There were two participants who, after the initial dose, increased it to 1.5 mg but due to breast tenderness, decreased the dose back to 1 mg (Study II). Their E\textsubscript{2} concentrations during the period of mastalgia were 95 and 147 pg/ml. One woman ceased participating in the study after 5 weeks of treatment because of cardiac ectopic beats and increased blood pressure. Her estradiol dose was 1 mg/day and her serum E\textsubscript{2} was at a postmenopausal level (19 pg/ml). Nevertheless, she felt herself to be symptom-free (KI from 33 to 0) (Study II).

5. Effect of ascorbic acid on serum estradiol concentration

The initial median serum AA level was 78.9 µmol/l (range 51 – 138 umol/l), increasing to 112.9 µmol/l (range 80 - 181 µmol/l) at month 1, and to 133.6 µmol/l (range 91 - 254 µmol/l) at month 3 (p<0.01). In the group as a whole, the serum E\textsubscript{2} levels, having become stabilized individually during the preceding 10 months, were raised by 20.8 % from a baseline level of 0.24 nmol/l (range 0.08 - 0.71 nmol/l) to 0.29 nmol/l (range 0.08 - 0.92 nmol/l) after 1 month of daily AA supplementation, a non-significant difference.
As the effect of AA was expected to be higher in women with low initial AA levels, the group was split into two at the initial level of 70 µmol/l. In the initially low AA group (n=9), E$_2$ levels increased by 55% (from 0.20 – 0.31 nmol) after 1 months’ daily AA supplementation, which approached significance (p=0.063). In the initially high AA group no change in E$_2$ levels were noticed (p=0.959; from 0.32 - 0.30 nmol/l)(Figure 9). Two months’ further treatment resulted in no additional E$_2$ increase in either group.

When the group was split into two, according to baseline E$_2$ concentrations (at 0.20 nmol/l), in subjects with low initial E$_2$ levels (n=9), E$_2$ levels doubled from 0.13 nmol/l (range 0.08 - 0.19 nmol/l) to 0.26 nmol/l (range 0.08 - 0.58 nmol/l; p=0.028)(Figure 10). In subjects with initial E$_2$ concentrations above 0.20 nmol/l there was no change in E$_2$ levels (from 0.37 to 0.36 nmol/l)(Figure 10). Two additional months of treatment did not cause any further change in E$_2$ levels.

There were positive correlations between AA and E$_2$ after 1 month (r= 0.717) and 3 months (r=0.791) of AA supplementation in the initially low E$_2$ subgroup (n=9). In the subgroup of initially high E$_2$ (n=16) no correlation between AA and E$_2$ was found either after one month (r=-0.211), or after three months( r=0.028).
Figure 9. Serum E₂ concentrations before and after daily AA supplementation in two groups: initial serum AA concentration below 70 µmol/l (n=9) and above 70 µmol/l (n=16). In the initially low AA group, serum E₂ levels were increased by 55% (p=0.063).

Figure 10. Serum E₂ concentrations before and after daily AA supplementation in two groups: initial E₂ below 0.20 nmol/l (n=9) and above 0.20 nmol/l (n=16). The increase is significant in the initially low E₂ group (p=0.028).
6. Influence of skin contamination on serum estradiol concentration

Baseline samples from the right and left cubital veins showed no significant differences in E\textsubscript{2} concentrations, either intra-individually or inter-individually (Study V). When the gel was applied with a disposable glove on the right hand to either thigh there were no differences in E\textsubscript{2} concentrations between the right (median 0.21 nmol/l) and the left (median 0.17 nmol/l) cubital vein (p=0.34). After applying the E\textsubscript{2} gel to the left arm with a disposable glove on the right hand, markedly higher E\textsubscript{2} concentrations were measured in the left cubital vein (median 0.26 nmol/l) in all subjects (p=0.03). In addition, higher E\textsubscript{2} concentrations were found in right forearm vein samples (median 0.21 nmol/l) after applying the gel to a thigh with a bare right hand (p=0.07). When the gel was applied to the left arm with a bare right hand there was no significant difference between E\textsubscript{2} concentrations in the right (median 0.32 nmol/l) and left (median 0.34 nmol/l) cubital veins (p=0.72). When assaying E\textsubscript{2} gel-contaminated samples together there were no significant differences (p=0.52). Neither were any differences found when uncontaminated samples were assayed together (p=0.52). However, when comparing data from all contaminated samples with data from uncontaminated samples, there was a significant difference (p=0.01) (Study V). Serum E\textsubscript{2} concentrations in each woman are shown in Figure 11 (contaminated samples) and Figure 12 (uncontaminated samples).
Figure 11. Contaminated samples. Serum E2 concentrations in 10 women treated with percutaneous E2 1.5 mg/day. E2 gel was spread (1) to a thigh with a bare right hand (the sample for E2 was taken from the right cubital vein), (2) to the left arm with a bare right hand (the sample for E2 was taken from the left cubital vein), (3) to the left arm with a bare right hand (the sample for E2 was taken from the right cubital vein), and (4) to the left arm with a disposable glove on the right hand (the sample was taken from the left cubital vein).

Figure 12. Uncontaminated samples. Serum E2 concentrations in 10 women treated with E2 gel 1.5 mg/day. E2 gel was spread (1) to a thigh with a disposable glove on the right hand (the sample for E2 was taken from the right cubital vein), (2) to a thigh with a disposable glove on the right hand (the sample for E2 was taken from the right cubital vein), (3) to the left arm with a disposable glove on the right hand (the sample for E2 was taken from the left cubital vein), (4) to a thigh with a bare right hand (the sample for E2 was taken from the right cubital vein).
DISCUSSION

1. Prevalence and compliance with HRT

In the last 15 years there has been an increasing tendency to use HRT in Finland. In 1991 12% of postmenopausal women used HRT but by 1998, the prevalence rate was 22%. This increase has been even greater in large cities, and in southern Finland, where physicians and treatment facilities are more accessible. The 39% prevalence rate observed in the present study (I), confirms this, and may be due to increased knowledge and changes in attitudes of physicians towards hormone substitution. The increasing use of HRT has also been reported in other Scandinavian and European countries (Hammar et al. 1996, Bardel et al. 2002, Mueller et al. 2002). When sales of HRT drugs in Finland are compared to sales in neighbouring countries, there are big differences. In Sweden and Norway the use of HRT has been equal to that of Finland, while in Estonia the prevalence rate of HRT is less than 1% (personal communication, Fred Kirss, MD, University Hospital of Tartu, Estonia). According to 1997 sales figures for the Nordic countries, HRT use was most frequent in Iceland, and lowest in Denmark (Sogaard et al. 2000).

Compliance with HRT has traditionally been poor despite recognition of the consequences of E deficiency and advantages of HRT. In the last few years, however, compliance rates for HRT have increased in many countries (MacLennan et al. 1998, Bakken et al. 2001, Bardel et al. 2002, Mueller et al. 2002). This may be partly because the new postmenopausal generation is less preoccupied with HRT than those who were engaged in the ideological debate during the mid 1970s. With E therapy, not only are woman and physician acceptance involved but, in addition, family and friends may impose their opinions on its use (Groeneveld et al. 1998, Andersson et al. 1998). This was also revealed in our study (I) where 15% of previous users had ceased E therapy on someone’s advice. The media has also played an important role in forming public opinion and acceptance of HRT. The way new study results are reported to the public cannot be without consequence. Objective information concerning the risks and benefits of HRT is essential, since media reports constitute the main source of information for many postmenopausal women (60% in the present study I). Excessive hope and other false beliefs invested in HRT are impairing compliance. The recent WHI trial report of the risks of HRT stopped us from rethinking the contra-indications of E therapy (The Writing group for the WHI trial 2002). While receiving enormous criticism concerning, mainly, the characteristics of the chosen study group (Genazzani et al. 2002,
Skouby 2002), the results of the trial cannot be without consequence for HRT compliance in the near future.

Most women seek help for unfavourable menopausal complaints, and if HRT does not reduce symptoms and thus improve quality of life, the therapy is ceased. In our study (Study I), 20% of current users had continuous side-effects related to HRT, and almost as many (15%) were unsure about the advantages achieved with the therapy. It is obvious that these women are the most likely to cease treatment. Women who have no side-effects and who understand why the therapy is prescribed are the most compliant (Nachtigall 1990). Side-effects appeared to be the main reason among previous users to discontinue E therapy, but fear of cancer was also remarkable. Different fears related to the therapy are also reported from other countries (Wren et al. 1991). In a study by Oddens et al. (1997), even 35% of women had stopped HRT due to fear of possible health risks.

Contrary to other studies, both current and previous users seem to have suffered most from weight gain and fluid retention, although various bleeding disorders have often been reported to be more important reasons for cessation of HRT (Nachtigall 1990, Wren 1991). When comparing the duration of HRT, few differences are found between our results and others’. Approximately half of HRT users cease treatment within less than one year, and approximately 27% stop the treatment within 6 months. This suggests that long-term compliance with HRT, which is important, taking into consideration the prevention of osteoporosis and fractures, is poor. According to epidemiologic data estrogen therapy given for at least 5 years immediately after menopause reduces fracture incidence by at least 50%. In our study, only 9% of previous users had been on HRT for more than 5 years, the corresponding rate being 28% among current users.

Compliance with percutaneous ERT in the present study (II-V) was good. Only one woman ceased participating in the study (II), due to side-effects consisting of increased blood pressure, and cardiac ectopic beats. Neither irregular bleeding episodes nor any other side-effects were reported. The women were motivated to follow up the study protocol in spite of its related obligations. This may be due to the fact that before study II, the participants were suffering from menopausal symptoms, and many had previously been on unsuccessful HRT. Further evidence of good compliance, was demonstrated by two participants who after the initial dose increased it, but then, experiencing breast tenderness, decreased the dose back to the initial one. Thereafter they continued satisfied with that dose. This occurred independently, of any advice the women had received. Serum E$_2$ concentrations of the women during the period of mastalgia were 95 and 147 pg/ml while,
in another study, the incidence of breast tenderness increased not until serum E₂ levels elevated above 150 pg/ml (DeLignieres 1981).

2. Self-adjustment of estrogen replacement therapy

The present study (II) with percutaneous E₂ gel revealed that E therapy can be carried out by the self-adjusted method in symptomatic menopausal women. This is the first study in which postmenopausal women independently titrated suitable E doses for themselves using their symptoms as markers. Percutaneous E₂ gel was efficient in alleviating menopausal symptoms in all women. The dose, however, in which symptom control was achieved varied from 1mg to 6 mg E₂/day. Twenty-nine percent of participants managed with a lower dose compared with the dose recommended by the pharmaceutical manufacturer of the product, 52 % were symptom free with the recommended dose, and 19 % required greater doses. There was a tendency for women with low symptom scores to increase the dose more cautiously than those with higher scores. This is in accordance with data demonstrating the dose-response relationship of ERT in alleviation of vasomotor complaints (Steingold et al. 1985, Notelovitz et al. 2000). The woman with the dose of 6 mg E₂ daily was compliant, did not have any hyper-estrogenic side-effects, and her steady state serum E₂ concentration was not supraphysiological (92 pg/ml).

Compared with E₂, placebo has also demonstrated considerable effectiveness in the treatment of menopausal complaints at the outset of the study. Coope et al. (1975) showed that during the first 3 months, hot flashes were reduced by about 90 % in women on peroral E and by about 62 % in women on placebo. In a double-blind, placebo-controlled, twelve-week study with E₂ gel carried out by Kornafeld et al. (1992), severity of hot flashes was significantly reduced in both E₂ and placebo groups during the first treatment week, after which the reduction in hot flashes was only significant in the E₂ gel group. The decrease in symptom scores among our participants was the greatest during the first two weeks of treatment which also may partly be due to a placebo-effect at the beginning of the study. Thereafter, however, the symptom scores continued to decrease and after 12 weeks with the self-adjusted dose, all participants had good symptom control. The subjects who later participated in other studies (IV, V) were still compliant with their self-adjusted doses.

The woman who ceased therapy because of cardiac ectopic beats and increase in blood pressure utilized an E₂ dose of 1 mg/day, and her serum E₂ concentration was at a postmenopausal level (19
In spite of this she felt herself to be symptom free and her KI had decreased from a baseline value of 33 to 0. Cardiac ectopic beats and increase in blood pressure, have been reported during E therapy, but they have mainly been associated with peroral treatment rather than parenterally administered HRT (Selby et al. 1989, Crook et al. 1997).

3. Serum estradiol concentrations

Serum E₂ concentrations, after two weeks of treatment, fluctuated between 17 and 195 pg/ml, with a daily dose of 1 mg E₂. This large E₂ variation cannot be due to the short treatment period, since steady state in the circulation with the used pharmaceutical product is reported to be achieved in 3 - 5 days (Sitruk-Ware et al. 1980, Lyrenas et al. 1981). Individual variations in serum E₂ were also observed after three months among women who had self-administered equal doses of E₂ (1 mg, 1.5 mg, 2 mg) (Study II). The variation was highest among those with the 1.5 mg dose, including three women whose serum E₂ concentrations were between 214 and 328 pg/ml.

This large, inter-individual E₂ fluctuation found in our participants was unexpected. However, considering the values as a whole, the great majority of them correspond to E₂ values of midluteal phase during fertile age. Large variations inter-individually have been suggested to be a consequence of individual differences in percutaneous absorption due to variation in humidity of the skin, epidermal thickness, retention time within the skin, and vascularity of the adipose tissue (Guy et al. 1987, Ranade 1991, Karjalainen et al. 1997). Absorption is slower through dehydrated skin and through skin with a thick epidermis. However, absorption through palmar areas, despite their considerably thick epidermis, is rapid and comparable to forearm absorption (Turakka 1997). Individual differences in E metabolism may partly explain variations in E₂ concentrations (Kaufman 1997). In the present study (II), the need for accuracy of the E dosage was emphasized to participants before the study and during control visits, since self-administration was performed with a dosing plastic ruler. Using the ruler required carefulness on the part of the women, not only in pressing the right gel dose to the measuring groove of the ruler, but also in emptying it from the gel to the skin completely. Although the women received instruction on how to administer the right dose, failures are possible.

Fluctuations in serum E₂ concentrations during percutaneous treatment, reported in some studies, may partly be explained by unstandardized conditions at the site of gel application or at the time
point of collection of the blood samples. For example, in the study by Järvinen et al. (2001) the accuracy of the results is questionable because the site of the gel application was not specified. Although blood samples were drawn from the indwelling cannula of one of the forearm veins, abnormally high E₂ concentrations were obtained if the gel was spread using the cannula-containing hand, or if the gel was spread to the cannula forearm. Furthermore, in another study where the pharmacokinetic profiles of 17β-E₂ were investigated, the gel was spread on the subjects’ arms, shoulders and thighs, and blood samples were collected via venopuncture of an unspecified antecubital arm vein (Paoletti et al. 2001). In both studies, skin contamination by the gel has obviously contributed to the variations seen in serum E₂.

No general consensus exists about the need to monitor serum E₂ concentrations systematically during HRT. However, many clinicians agree, that it is necessary in cases with therapy-related problems, such as poor symptom control. Neither is there a consensus on which serum E₂ level should be reached in order to obtain the benefits of HRT. Steingold et al. (1985) found a significant negative correlation between serum E₂ levels and frequency of hot flushes in women suffering from them. The number of hot flushes decreased by half when serum E₂ was approximately 60 pg/ml. In another study, where serum E₂ was above 60 pg/ml, 84.4 % of the women treated with percutaneous E₂ were compliant and did not complain of any symptoms (DeLignieres 1994). Differing opinions also exist regarding serum E₂ values adequate for the prevention of osteoporosis, ranging from 40 to 100 pg/ml (Wimalawansa 1990, DeLignieres 1994). Some authors agree that monitoring is unnecessary. However, in a recent, prospective, 5-year randomized study, 11 % of the women on conventional per oral HRT lost bone from the spine, and 26 % from the hip. The women with no bone response to HRT had significantly lower serum E₂ concentrations compared with E responders (Komulainen et al. 2000). This is a concern of the women in the present study (II) whose serum E₂ concentration remained at a postmenopausal level for they may not have adequate, long-term osteoporosis protection. Moreover, a serum concentration above 40 pg/ml, reached in 81 % of the participants, can be estimated to be sufficient for osteoporosis prevention. However, genetic factors and many other as yet unknown factors may be associated with bone mass preservation.
4. *Effect of skin contamination by estradiol gel on serum estradiol concentration*

In percutaneous E therapy the material is applied to the skin using a bare hand. Besides being absorbed from the gel-application skin area, E₂ is also absorbed from the skin of the gel-spreading hand. Depending on from which forearm samples for E₂ assays are collected, falsely high E₂ concentrations may occur (Hirvonen et al. 1997). In the present study (V) we compared serum E₂ concentrations collected at the same time from both cubital veins, thereby taking into consideration the absorption of E₂ gel through the hands. Skin contamination by the gel significantly affected E₂ concentrations. As expected, intra-individual fluctuation in serum E₂ in all participants was greater in contaminated samples compared with non-contaminated ones. Inter-individual E₂ levels also varied significantly in contaminated samples and considerably high serum E₂ levels were detected in some of the women. Intra-individually, serum E₂ concentrations were constant when non-contaminated samples were evaluated. Only one participant showed slight fluctuation in E₂. These results suggest that at least some of the high E₂ concentrations in our study (II) may be due to skin contamination, although this possibility was attempted to eliminate carefully in the study protocol.

5. *Effect of the time of blood sample taken on serum estradiol concentration*

The point of time when blood samples are taken has an influence on reported serum E₂ levels. In published studies most of the samples were either collected 12 or 24 hours after the gel administration, or the collection time is not specified (Holst et al. 1983, Scott et al. 1991). These differences in collection times have resulted in variations in serum E₂ suggesting that E₂ values from different studies are not always comparable. Also, analytical assay specificity and sensitivity varies, and differing results in serum E₂ may be achieved depending on whether extraction is used during the procedure (Diver 1992). In the present study (II-V) blood samples for E₂ assays were collected twelve hours after the gel application, since twelve hours is the collection time normally used for determining of circulating drug concentrations. Furthermore, the E₂ gel used in the present study has been shown to provide relatively constant E₂ concentrations for 24 hours (Scott et al. 1991). Practical considerations also supported 12-hour sample collection time.
6. Effect of ascorbic acid on serum estradiol concentration

In addition to E formulation and route of administration, other factors, such as cigarette smoking (Byrjalsen et al. 1993) and co-administered pharmaceutical preparations may affect E availability (Notelovitz et al. 1981). Cigarette smoking can increase the hepatic metabolism of oral Es. In two studies, Jensen et al. (1985, 1988) discovered an up to 50 % reduction in E₁ and E₂ levels in postmenopausal women who smoked, compared with those who did not. However, with percutaneous ERT, E₁ and E₂ concentrations were unaltered by smoking (Jensen et al. 1988).

The effect of ascorbic acid (AA) on serum E₂ concentration was studied in women with stable serum E₂ (Study IV). That AA ingestion might affect the bioavailability of Es was supported by subjects with the lowest blood concentrations of either AA or E₂. This finding agrees with results from studies showing that AA may increase tissue ethinylestradiol levels in women taking oral contraceptives, although the changes we observed, (21 % in the group as whole) were less than the 40 - 60 % increases reported (Kalesh et al. 1971, Back et al. 1981). This may be due to the relatively high initial serum levels of AA in our subjects (mean 79 umol/l), which were much greater than those of previous studies (Kalesh et al. 1971).

We had expected to find more participants with low initial AA levels. In many subjects the tissues were probably already saturated with AA, leaving little room for observable increases in AA levels. However, an increase in E₂ concentration was seen in participants with the lowest initial AA levels. Ascorbic acid also increased serum E₂ concentrations in women whose initial serum E₂ had been low (below 0.20 nmol/l) by as much as 100 % (from 0.13 to 0.26 nmol/l). It can be surmised that the lowest levels of E₂ during E therapy may represent either poor bioavailability or increased utilization or elimination.

One possible interaction between AA and Es could be antioxidation. Ascorbic acid is an effective and important water-soluble antioxidant present in serum (Frei 1991), whereas Es are highly effective, lipid-soluble phenolic antioxidants of cell structures (Moosmann et al. 1999). The antioxidative potential of E₂ has been demonstrated clinically, for example in perimenopausal women (Sack et al. 1994, Tranquilli et al. 1995)). The tissue levels of Es are extremely low and are
only a minute fraction of the concentrations of the other antioxidants. Any support from the more quantitatively important antioxidants would spare low E\textsubscript{2} reserves.

7. Serum follicle-stimulating hormone concentration

Serum FSH levels have been shown to increase already early in menopause (Sherman et al. 1975, Lenton et al. 1988, Rannevik et al. 1995), and are generally considered to be reliable laboratory parameters of the onset of perimenopause (Metcalf et al. 1985, Burger et al. 1999). The concentrations of serum FSH are traditionally measured using immunochemical assays, which measure the protein levels of the hormone but do not evaluate the biological activity of the gonadotropin. In different clinical settings, differences in the biological activity may be found, although immunometric assays indicate no alterations in hormone levels, suggesting that the molecular modification of the hormone protein is an important step in regulation of gonadotropic action (Jaakkola et al. 1990, Matikainen et al. 1992). These findings have led to the development of cell-based assays where cells are permanently transfected with FSH receptors and the bioactivity of FSH is estimated by the altered production of second messengers such as cAMP and cDNA (Guderman et al. 1994).

In the present study (III), in addition to conventional immunological measurements (I-FSH), we measured the bio-potency of FSH (B-FSH) using a cell-based assay during percutaneous E\textsubscript{2} treatment. In general, both serum FSH levels correlated negatively to serum E\textsubscript{2} levels demonstrating that increases in serum E\textsubscript{2} during percutaneous treatment result in a negative feedback effect on the pituitary similar to that occurring in women of reproductive age. Suppression of gonadotropins by both oral and transdermal administration of Es have been reported. Some authors have concluded that this is a sign that levels of circulating E\textsubscript{2} are necessary for efficacy (Powers et al.1985). Furthermore, serum B-FSH correlated highly to I-FSH suggesting that the negative feedback effect observed is primarily due to decreased synthesis or secretion of FSH by the pituitary. However, in individual women the B-FSH/I-FSH ratio was increased up to threefold suggesting that in some women, the B-FSH is increased while the gonadotropin protein level is increased more modestly. Thus in some women, the molecular nature of secreted FSH is altered. It is known that FSH is secreted from the pituitary in the form of various iso-hormones which may differ in their glycosylation patterns resulting in multiple proteins in isoelectric focusing (Chappel et al. 1995). Both B-FSH and I-FSH correlated positively to climacteric symptoms. We had
expected that B-FSH rather than I-FSH might serve as a marker of effective E therapy, but there were no significant differences from that point of view.

8. Serum sex hormone-binding globulin concentration

In circulation Es are freely available for exchange with the tissues if they are not protein-bound. Serum binding affects the availability of E to diffuse across cell membranes and express biological activity (Kaufman 1997). Alterations in SHBG levels change the concentration of unbound E_2, also affecting the bioavailability of E_2. Orally-given Es cause induction of liver enzymes and also increase SHBG levels whereas parenterally administered HRT has been shown to by-pass the first-pass liver metabolism resulting in smaller changes to liver metabolism than peroral HRT (Holst et al. 1983). In the present study with percutaneous ERT (Study III), serum E_2 concentration had a positive correlation with serum SHBG levels suggesting that even with percutaneous therapy, SHBG production may be induced. Similar results were reported in a study carried out by Kraemer et al. (2003) where treatment with transdermal patch (0.1 mg) increased serum SHBG levels significantly. The increase in SHBG levels was associated with increased serum E_2 levels (above 60 pg/ml in all participants). Compared with other treatment groups with significantly lower serum E_2 levels, no increase in serum SHBG was noticed (Kraemer et al. 2003).

9. Aspects of ideal HRT

Postmenopausal E therapy is used to manage the climacteric symptoms that impair the quality of life of a substantial number of women. The difficulty is achieving the desired effects with minimal side-effects and no adverse health risks. Improvement of quality of life may also include longer-term benefits of HRT such as prevention of bone loss, fractures, and urogenital atrophy. Individual variation in E pharmacokinetics and thus bioavailability results in the need for individual E dose administration.

The results of the WHI trial indicate that more data on prospective, controlled trials of the risks associated with the use of HRT are required. More documentation is also needed about the dose-related risks of HRT, and the consequence of serum E_2 concentration during HRT. Since the data on these aspects are limited, the lowest effective dose to control menopausal symptoms may be the
safest. Percutaneous self-adjustment is feasible and provides flexible individual dosage resulting in good control of menopausal symptoms with the lowest possible dose.

However, within the self-adjusted ERT administration procedure, some factors need further investigations such as determining the appropriate progestogen doses in women with intact uteri. In order to avoid bleeding disorders, obviously progestin doses and phases should also be adjusted individually according to the self-adjusted E₂ dose. Although the compliance rate in the present study was good, we do not know whether the long-term compliance with the used method would be better than with the conventional method for carrying out the therapy. Finally, as with self-adjusted doses, serum E₂ concentrations may remain at a postmenopausal level in some women, it would be worthwhile investigating whether the achieved E₂ concentrations also ensure longer-term benefits of HRT.
This thesis was undertaken to evaluate the factors affecting the use and compliance of HRT in Finland and to examine the applicability and effectiveness of percutaneous ERT carried out by a woman with a novel self-adjusted method. In addition, the different factors, producing variations in serum E2 concentrations during percutaneous ERT were investigated.

The population of the present study was selected from the age-sex register of the health care region of Ylöjärvi and included all women (n=1056) who were born between the years 1935 and 1945. A postal questionnaire was sent and after a reminder, 884 completed questionnaires were obtained (Study I). Some of these women volunteered, and, after interviews and examinations, postmenopausal, healthy women with no contraindications to HRT were selected as subjects for this study (Studies II-V).

Of the 773 postmenopausal women, 39 % were current users of HRT, 16 % were previous users, and 45 % had never undergone E therapy. Menopausal symptoms had been more frequently experienced by current users compared with non-users. This was also the reason for initiating ERT in 79 % of the current users. Seven percent had HRT for osteoporosis prevention. Of the previous users, 27 % had used HRT for less than 6 months, and almost half (46 %) had ceased treatment within one year. With respect to osteoporosis prevention, only 9 % had utilized HRT for more than 5 years. The main reason for cessation of HRT was side-effects which were weight gain and fluid retention (35 %), bleeding disorders (25 %), headache (11 %), and breast tenderness (11 %). Other reasons for discontinuing HRT were fear of cancer (16 %), recommendation by a physician (12 %), and inefficacy (4 %). Twenty percent of current users reported having continuous side-effects from the treatment.

Thirty-two postmenopausal women self-adjusted their percutaneous E doses on the basis of disappearance of climacteric symptoms. The initial dose of 1 mg percutaneous E2 per day was sufficient to relieve symptoms in 29 % of the women, 52 % required 1.5 mg, and 19 % 2 mg or more. The initial Kupperman Index (KI) had no correlation with the final self-adjusted treatment doses, but there was a correlation between KI and biologically active follicle stimulating hormone (B-FSH) and immunologically active follicle stimulating hormone (I-FSH). With a 1 mg dose, serum E2 concentrations fluctuated inter-individually between 17 and 195 pg/ml. After three months, with self-adjusted doses, serum E2 was at a postmenopausal level (<50 pg/ml) in 22 % of the women. In all, 45 % showed serum E2 remaining under 60 pg/ml, and 8 out of 14 applied the gel at a dose of 1 mg/day. Twenty-nine percent had serum E2 levels of 60 - 100 pg/ml, their doses
varying from 1.5 mg to 6 mg E₂ daily. Twenty-six percent showed serum E₂ of more than 100 pg/ml and their daily doses varied from 1 to 3 mg. A negative correlation was observed between serum E₂ concentration and both B-FSH and I-FSH, the latter producing a slightly higher correlation. Serum E₂ concentration also correlated with serum sex hormone-binding globulin (SHBG), indicating a minor induction in SHBG production.

The effects of ascorbic acid (AA) supplementation on serum E₂ concentrations were studied in 25 women on self-adjusted percutaneous HRT with stable serum E₂ concentrations. One month of treatment with 1000 mg AA daily increased serum E₂ levels by 21%. Greater responses were seen in two subgroups. In women who initially had the lowest serum concentrations of AA (<70 µmol/l) there was an increase of 55% in serum E₂ levels. In the subgroup with the lowest initial E₂ levels (<55 pg/ml) there was a significant increase from 35 to 71 pg/ml in serum E₂ concentration.

The effect of skin contamination by E₂ gel on circulating serum E₂ concentration was studied in ten hysterectomized women. Skin contamination by the gel significantly affected serum E₂ concentrations by producing false, highly fluctuating values. Intra-individually, serum E₂ concentrations were constant when non-contaminated samples were evaluated. Only one participant showed minor fluctuation.
CONCLUSIONS

Prevalence of HRT has increased in Finland during the last 15 years but compliance with HRT is poor. Approximately 30% of users discontinue the therapy within six months and almost half (46%) within one year, mostly because of side-effects (41%) related to the therapy. Also current HRT users (20%) are reporting continuous side-effects from the therapy.

Symptomatic, compliant, co-operating menopausal women may benefit from the self-adjusted method of percutaneous ERT: women are tailoring individual E doses for themselves based on the disappearance of symptoms. Since the method provides good control of menopausal symptoms, compliance with HRT may improve.

The E2 dose in which symptom control is achieved varies largely inter-individually. Compared with the dose recommended by the pharmaceutical manufacturer of the product, some women can manage with lower doses (29%), others become symptom-free with the recommended dose (52%), and a moderate proportion need greater doses (19%).

Intra-individual fluctuation in serum E2 concentrations during percutaneous ERT is minimal when the source of error, caused by skin contamination by E2 gel, is eliminated. Estradiol measurements can be utilized in the adjustment of individual HRT.

In certain cases interaction between estrogens and ascorbic acid may influence estrogen pharmacokinetics, and ascorbic acid supplementation during percutaneous ERT may improve estrogen bioavailability.
ACKNOWLEDGEMENT

The present study was carried out in Ylöjärvi Health Center and at the Department of Obstetrics and Gynecology of Tampere University Hospital.

My warmest thanks belong to my supervisor, Docent Risto Tuimala, MD., Ph.D., who suggested the inspiring topic of the research. His wide experience and knowledge of menopause and HRT, his professional help in organizing the studies, friendly support and encouragement have been motivating me through these studies.

I wish to extend my deep gratitude to Professor Pertti Kirkinen, MD., Ph.D. for his interest, strong support and supervision during the final preparation of the thesis. Due to his skilful guidance and great resolution this thesis has now reached this point.

I express my sincere gratitude to Risto Savilahti MD., former Head Doctor of Ylöjärvi Health Center for arranging excellent facilities to carry out the clinical trials in Ylöjärvi Health Center. I also thank him for providing me assistance in the use of personal computer and data management. I wish to thank all nurses and laboratory staff in Ylöjärvi Health Center for taking care of the participants of my studies.

I am most grateful to my co-workers Jouko Parantainen, M.Sc. and Docent Kimmo Vihko, MD., Ph.D. for their valuable collaboration and indispensable help. I also acknowledge Docent Timo Metsä-Ketelä (deceased), as well as to Professor Eberhard Nieschlag, MD, Ph.D and Manuela Simoni, MD, Ph.D., from the Institute of Reproductive Medicine of the University of Münster, for their friendly co-operation.

I wish to express my gratitude to Anna-Maija Koivisto, B.Sc., Tiina Luukkaala and Professor Pekka Laippala, D.Sc.(deceased), for providing me invaluable assistance in statistical analyses.

I would like to extend my warm thanks to the former Head of the Department of Obstetrics and Gynecology, Professor Emeritus Reijo Punnonen, MD., Ph.D. and to Professor Pentti Heinonen MD., Ph.D. as well as to Docent Erkki Kujansuu, MD., Ph.D. for their supporting attitude and intrest in my study.
I sincerely thank Professor Pentti Tuohimaa, MD., Ph.D. and Professor Timo Ylikomi, MD., Ph.D. for providing me superior circumstances to continue my studies when I worked as an assistant at the Department of Anatomy.

I owe my sincere thanks to the official reviewers of the thesis, Professor Olavi Pelkonen MD., Ph.D. and Docent Lars Rönnberg, MD., Ph.D. for their valuable suggestions for improvement of the manuscript.

I wish to thank all my colleagues at the Department of Obstetrics and Gynecology, for their interest and support during these years. Special thanks belong to my Ahma family Outi Palomäki (Ahma mother), Kati Tihtonen (Pentu Ahma), Leena Vasara (my Ahma twin sister B) and Maarit Vuento (my Ahma twin sister C). I also want to thank current and previous research-workers in our clinic like Beata Stach-Lempinen, Sirkka-Liisa Ala-Fossi, Merja Vainio and Kari Nieminen for their support. Warmest thanks are due to all members of Team Ahma , for enjoyable and exciting moments in Ahma trips which have pleasantly balanced my research work.

I sincerely thank to all my friends, but especially to Tuula Liiti and Flora sisters: Kersti Liber, Eija Hirvonen, Suvi Karhu and Eija Pokkinen, for their friendship and ongoing support. Very special thanks are sent to Velho of Lapland who helped me to focus my concentration on the thesis. I also want to thank Hannu Elo M.Sc. for his help and support during the first research years.

Finally, I wish to express my warmest and most heartfelt thanks to my mother and father for their support during these years. To both my twin sister Paula and brother Jukka I want to express my deep gratitude for their valuable help in several occasions. In spite of this, I warmly thank Paula for working as a study nurse in our study V and Jukka for advising me with computers hundreds of times.

My warm thanks are due to Mrs. Jean MacKenzy, for revising the English of this thesis. In addition, I wish to thank Nicholas Bolton, Ph.D., for reviewing the language of the original publications.

I will inclue a special acknowledgement to all women who participated in this investigation and made this work possible.
This research was supported by grants from the Medical Research Fund of Tampere University Hospital, the Research Fund of the Finnish Gynecological Association, the Finnish Menopause Society and Leksmedi Oy, which are all gratefully acknowledged.

I thank Elsevier Scientific Publishers B.V., Elsevier Science and the Society of the European Journal of Endocrinology for permission to reproduce the original contributions in this thesis.
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