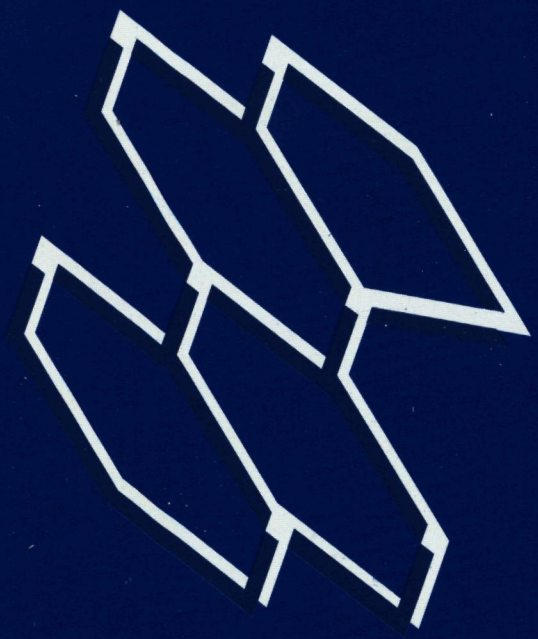
HORMONE REPLACEMENT THERAPY

Metabolic and Endometrial Changes, and the Role of Progestogens



M.J. van der Mooren

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Metabolic and Endometrial Changes, and the Role of Progestogens

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van de Medische Wetenschappen

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CONTENTS

CHAPTER 1

INTRODUCTION
AND
AIMS OF THE THESIS

Menopause is defined as the last menstrual bleeding. Menopause is the inevitable consequence of the ageing process of the ovarian function. Already 20 weeks after conception this process begins, and the initial amount of 5 to 7 million primordial follicles is reduced to about 10% at birth, and about 5% at the moment of menarche [1]. Of the remaining 300.000 follicles only about 400 fully mature and reach the moment of ovulation. The rest of the remaining follicles degenerates.

For a woman menopause means the cessation of the reproductive years. The average menopausal age is 51 years (*range 46 - 55 years*), and this has not changed much during the last decades [2,3]. The diagnosis of menopause can only be made in retrospect, and nowadays it is generally accepted that at this age a woman can be considered postmenopausal after one year of amenorrhea. After one year of amenorrhea the probability of having another menses is about 5% [4]. Average life expectancy for women is 81 years, so a woman can expect to live more than one third of her life in the postmenopausal state.

Menopause, and the problems accompanying the climacteric period, are phenomena that can be expected in an increasing number of women during the following decades. The 20th century has increased mean life expectancy amongst others by the introduction of modern medicine and improvement of hygiene and nourishment. Therefore, the number of postmenopausal women is now about four-fold higher than in the year 1900, and it is expected to double once more until the year 2030 [5-7].

The years around menopause, called the climacteric period, very often are accompanied with symptoms and complaints as hot flushes and sweats [8], due to the decreasing oestrogen concentrations with at the same time supraphysiological follicle stimulating hormone concentrations [9,10]. The oestradiol concentrations may fluctuate and this reportedly is the main cause of the hot flushes and sweats [11,12], although this mechanism has been questioned [13]. Anovulation and luteal insufficiency may be associated with dysfunctional bleeding. About 85% of climacteric women experience

climacteric problems to some degree; 25% of them seek medical care [14].

The climacteric signs and symptoms mentioned before vary with the cultural background [15]. They may often lead to secondary less typical problems, some of which may also be experienced independently, but they may all interfere with the feeling of well-being [16]. Complaints as sleeplesness, fatigue, mood changes, and sexuality problems, are often associated with the hormonal changes, but they may, however, very well be related to psychosocial problems that accompany the ageing woman, like the "empty nest syndrome" and furthermore the changing self-image of becoming an older women. These changes may alter the woman's life perspectives.

Little is known about the way menopause was experienced in the past. During the last decades the phenomena around the menopause gained more interest, and it became clear that climacteric symptoms and complaints are a result of the change in hormonal homeostasis. By some authors, the climacteric was defined as an oestrogen deficiency syndrome or even an endocrinopathy [17], an illness that needs hormonal treatment. Others, however, do emphasize the natural character of menopause, which does not need treatment, but needs understanding, acceptance and re-orientation on a new phase of life [18]. The choice for hormonal treatment of climacteric and postmenopausal complaints, also called hormone replacement therapy, is troubled by classical reports on increased risk for thrombo-embolic and cardiovascular complications like those reported for oral contraceptives [19,20], and the possibility of increased risk for breast cancer [21] or endometrial cancer [22].

In general, perimenopausal women are often experiencing many signs and symptoms. Some may also be related to the ageing process, and are not solely due to the oestrogen deficiency. However, ongoing research and experimental treatment with oestrogens gradually revealed that typical climacteric complaints, such as flushes and sweats, and urogenital problems, can be relieved very effectively with hormone supplementation [23-31].

Climacteric signs and symptoms introduce new complaints and may aggravate

already existing problems. Hormone replacement therapy obviously can not solve all the problems related to ageing, but it clearly may help to relieve serious symptoms, and by doing so it may also strengthen the climacteric woman in coping with the other ageing related problems.

Next to the relief of the symptoms and complaints mentioned before, there are long-term events that may impair women's health and well-being. Women after menopause are increasingly at risk for osteoporotic fractures and cardiovascular morbidity and mortality associated with the oestrogen deficiency. Administration of natural oestrogen reverses these risks [32-58] and forms another indication for hormone supplementation. This preventive treatment gains in popularity, but it is clear that the benefits of the preventive aspects should be balanced against possible disadvantages of hormone replacement therapy.

AIMS OF THIS THESIS

Our understanding of the events taking place at menopause has increased. Much more is known about the benefits and possible disadvantages of hormone replacement therapy. It has also become clear that hormone replacement therapy may have a profound impact on the chance of developing cardiovascular disease and osteoporosis. Consequently, the number of women seeking hormone replacement therapy has increased tremendously. Still, many aspects of hormone replacement therapy are greatly obscure, the hypotheses put forward on cause/relationship of several events are being disputed, and also our knowledge concerning negative aspects of hormone replacement therapy is still insufficient, thereby worsening to formulate the benefit/risk ratio of this type of treatment.

The aims of this thesis are therefore, (*1*) to investigate several metabolic aspects of various combined oestrogen/progestogen replacement therapies with regard to the risk of developing atherosclerosis, and with special reference to the additional influence of the progestogen, furthermore (*2*) to investigate the effects of combination hormone therapy on several histological aspects of the endometrium and (*3*) to gain insight into factors determining women's compliance for such a hormone treatment regimen.

As a base for these studies (*4*) we thoroughly reviewed the available literature concerning the relevant aspects of hormone replacement therapy as: indications and contra-indications, the cardiovascular impact, and some possible underlying pathogenetic mechanisms. Thereafter, benefits and risks will be balanced, and finally, the role of progestogens, and its consequences for patient compliance and acceptance will be addressed.

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CHAPTER 2

HORMONE REPLACEMENT THERAPY
A REVIEW

The purpose of hormone replacement therapy in postmenopausal women is to restore serum oestradiol concentrations to those seen in the early follicular phase of the menstrual cycle (*about 200 - 250 pmol/l*). Hormone replacement therapy can be administered as local and systemic treatment. Local applications, such as creams and vaginal suppositoria, are appropiate for the treatment of urogenital symptomatology, such as atrophic vaginitis, but normally the blood levels achieved by such treatment are too low to establish an effective relief of the other climacteric symptoms or to achieve the preventive purposes. Systemic hormone replacement therapy is possible by oral, transdermal (patch or cream), subcutaneaus and intra-muscular administration. As in local treatment, the effects are dose dependent. Regardless of its formulation or route of administration, most women can effectively be treated [1-18]. With oral dosages of 0.6 mg conjugated oestrogen, or 2 mg micronised 17ß-oestradiol or oestradiol-valerate the serum oestradiol concentrations specific for the early follicular phase of the menstraul cycle can be attained.

INDICATIONS

The main indications for systemic hormone replacement therapy [19-30] can be categorized in: climacteric complaints, typical and atypical, oestrogen deficiency complaints, and long term events. Table I describes each category in more detail.

The most typical climacteric complaints, as hot flushes and sweats, are obviously related to the hormonal imbalance and appear to respond well on oestrogen supplementation [1-15]. Treatment for one to two years, and if necesarry longer, may help the women to overcome these problems.

Table I: Indications for hormone replacement therapy

Climacteric complaints	Typical complaints:	Atypical complaints:	
	- hot flushes	- sleep disturbances	- irritability
	- (nightly) sweats	- low self-confidence	- agitation
		- palpitations	- fatigue
		- forgetfulness	- melancholia
		- concentration difficulties	- vertigo
		- paraesthesia hands & feet	- headaches
		- small-joint pain	- anxiety
Oestrogen deficiency complaints	Vaginal problems:	Urinary problems:	
	- dryness	- urge incontinence	
	- dyspareunia	- stress incontinence	
	- pruritis	- recurrent urinary infections	
	- senile kolpitis		
Long term events	Osteoporosis: risk profile:		
	- bone mass 1 SD or more below mean of reference population		
	Cardiovascular disease: risk profile:		
	- current cardiovascular disease		
	- risk factors: (familiary) hyperlipidaemia, diabetes mellitus, smoking habits, hypertension .		

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Atypical complaints are reported by many climacteric women, but are not necessarily related to the hormonal changes [31-33]. However, they may be the direct

consequence of hot flushes and sweats, and especially in the presence of the latter symptoms, they may respond well to oestrogen supplementation [10]. In the absence of hot flushes, atypical complaints may improve during oestrogen supplementation, but often to a lesser degree [10,34]. Some studies have demonstrated that oestrogens prolong R(*apid*) E(*ye*) M(*ovement*) sleep [35,36], and that sleep-disordered breathing periods are diminished [37]. Reportedly, women sleep longer during oestrogen supplementation [38]. In case of atypical complaints a probationary treatment of three months may help to find out or exclude the possible hormonal pathogenesis.

Complaints of oestrogen deficiency, such as vaginal dryness, itching and dyspareunia, often respond well on local as well as systemic therapy. Also symptoms of genital prolapsus may improve [14,15,39-41]. Even with low-dose therapy the atrophic changes may disappear completely, and a short term treatment may give long lasting improvement. Urinary problems due to mucosal atrophy, such as dysuria, pollakisuria and recurrent cystitides, often accompany the vaginal symptoms. These symptoms may improve during oestrogen treatment [42,43], and if so one may continue its administration. Besides creams and suppositoria, there are new developments in the field of local treatment by a vaginal oestrogen releasing ring [44], and intravesical oestriol instillation [45].

Women reach their peak bone mass around 20 - 30 years (Figure 1). In women the mean peak bone mass is about 15% lower than in men. After this age, the balance between bone formation and bone resorption becomes slightly negative, leading to on average 0.5% to 1% loss of skeletal bone every year. After menopause bone turnover increases, however in such a degree that bone resorption exceeds bone formation, and results in about 18% more loss of trabecular bone and 12% more loss of cortical bone than in men within a relatively short time. The trabecular bone mass decreases about 1 to 4% a year during the first 8 to 10 postmenopausal years [46-48].

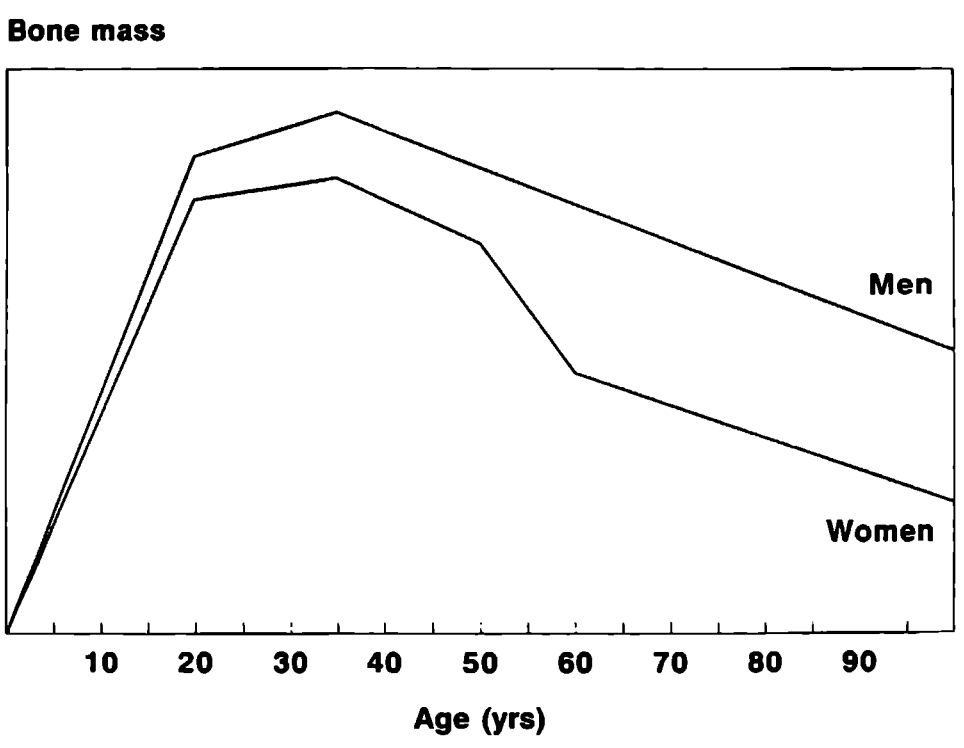

Figure 1: Simplified illustration of bone mass as function of age and gender.
(Adapted from: Barentsen R, 1989: Oestrogeen substitutie therapie. Waarom en hoe).

Bone loss, as a result of ageing and menopause, may lead to osteopenia and osteoporosis. Postmenopausal osteoporotic fractures commonly concern the distal radius, vertebra and in the older women also the hip. Osteoporotic fractures, and especially hip fractures, are associated with a high morbidity and mortality rate [49-51].

Oestrogen replacement therapy is associated with decreased bone resorption and fracture risk [52-60]. Oestrogen supplementation for 5 years or more reduces the

osteoporotic fracture risk in the wrist and hip on the average with 50% [61-65], and vertebral fracture risk even more [66]. Several large scale studies have demonstrated the preventive effect on the incidence of osteoporotic fractures of hormones alone, and even more when combined with calcium supplements [67-69]. Oestrogens combined with a progestogen may to some extent even promote new bone formation [70,71]. Several factors, summarized in table II, have been suggested to mediate the influence of sex steroids on the bone.

Table II: Actions of sex steroids on bone

Indirect effects	Systemic factors:	
	- Inhibition of insulin-like growth factor (IGF-I)	
	- Increase in growth hormone	
Direct effects	Increase of local growth factors:	Steroid receptors:
	- Insulin-like growth factor (IGF-II)	- Oestrogen receptors
	- Transforming growth factor-ß (TGF-ß)	- Progesterone receptors
	- Binding proteins of IGF-I/-II, TGF-ß	

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The last decades an increasing amount of reports has demonstrated that hormone replacement therapy reduces the risk of developing cardiovascular disease in postmenopausal women. It will probably only be a matter of time that hormone supplementation will be accepted as an important treatment modality for primary and secundary prevention at least in postmenopausal women at risk for cardiovascular disease. This issue will be discussed in more detail in the paragraph "Cardiovascular impact of hormone replacement therapy".

CONTRA-INDICATIONS

Although in most data-sheets on hormone supplementation many contra-indications are listed, it remains doubtful whether they are justified as they are not based on prospective scientific research. Many of the listed possible side-effects of low-dose natural oestrogen are the inheritance of the experiences with high-dose synthetic ethinyl-oestradiol containing oral contraceptives. Regrettably, it appears that natural oestrogen compounds as equine conjugated oestrogen and micronised 17ß-oestradiol, are often considered similar to synthetic oestrogen, and it will need more effort to dissociate the former of this undesirable and unjustifiable reputation.

Contra-indications [19-22] for administering hormone replacement are listed in table III. This section wil deal with the major objections for hormone replacement therapy.

Table III: Contra-indications for hormone replacement therapy

Contra-indications for oestrogen:	Contra-indications for progestogen:
- Breast cancer	- Meningeoma
- Endometrial cancer	
- Severe liver dysfunction	
- Porphyric disease	

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Breast cancer

Several conflicting reports have been published dealing with the effects of postmenopausal hormone treatment on the risk of developing breastcancer [78-94]. Although it still is a matter of debate, some of the latest reports have estimated a relative risk increasing with dose and duration of treatment. For long-term users (longer than 9 years) some have estimated a relative risk up to 1.7 [95]. In a meta-analysis, Dupont *et al.* [96], however, have questioned these data. These authors found overall relative risk estimates of 1.07 and 1.08 in women taking conjugated oestrogen dosages of 0.625 mg per day or less, and 1.25 mg per day or more, respectively, so no clear dosage related effect was found. Supplementation of oestrogen shorter than five years does not seem to increase the relative risk at all. Grady *et al.* [20] also reported no clear evidence for an increased risk with increasing oestrogen dosages and different treatment regimen. They estimated a slightly increased relative risk of 1.25 for women who used oestrogen for 8 years or more. Other possible variables concerning the risk of developing breast cancer are the type of oestrogen used, and the addition of a progestogen. Bergkvist *et al.* [95] reported a slightly increased risk for long-term users of oestradiol compounds, but not for users of conjugated oestrogen or other types of oestrogen. In addition, combined oestrogen-progestogen users showed no increased risk.

It still remains questionable whether the estimated increases in relative risk reported are in part due to surveillance bias, since the hormone treated group may consist of highly motivated women that get more regular medical examination including mammographic surveillance. Therefore, breast cancer is earlier diagnosed in this group. An underestimation of the relative risk as a result of investigating women with low treatment compliance can, however, also not be excluded. Although breast cancer disease may be diagnosed in a slightly higher frequency during hormone replacement therapy, breast cancer mortality has not been found to be increased due to hormone supplementation [97]. Still, every woman, independent of hormone treatment, is prone to a relatively high life-time risk of 10% for getting breast cancer. For every woman the experience of having a possible breast disease, especially in the case of breast cancer,

may introduce severe emotional distress. Therefore, any sign of a possible breast abnormality needs to be investigated thoroughly, by breast examination as well as by mammography [98,99]. In case of an increased risk for breast cancer, with respect to risk factors as: familial history of breast cancer [99], premalignant abnormalities [100,101], or history of prenatal diethylstilbestrol exposure [102], mammographic surveillance once a year is recommended. In all other cases mammographic surveillance once every two years is considered efficacious. Furthermore, it has been recommended to perform a mammography before commencement of postmenopausal hormone treatment [22]. Women using hormone replacement therapy should be properly informed by their physician on the possible effects of hormones on the breast, and regular self-investigation should be promoted to improve the early diagnosis of breast disease.

In women with a history of breast-cancer, hormone supplementation is considered contra-indicated. Oestrogens stimulate glandular tissue, especially in case of oestrogen and/or progestogen receptor presence. Therefore, growth of possible micro-metastases is considered a substantial risk. However, in some cases of extreme climacteric symptoms, hormone supplementation under good medical supervision may be justified in order to improve the quality of life [21,98,99].

Benign breast tumors as fibrocystic breast abnormalities are no risk factor for breast cancer, and therefore, are no contra-indication for hormone replacement therapy. About the non-carcinogenic effects of hormone treatment on fibrocystic abnormalities the literature is not consistent [103,104].

Endometrial cancer

Endometrial hyperplasia and carcinoma may develop as a result of prolonged exposure to oestrogen stimulation of the endometrial cells [105]. The risk of developing endometrial neoplasia increases with the dose and the duration of the oestrogen administration [106-113]. Grady *et al.* [20] estimated a relative risk for ever users of

oestrogen of 2.31, while women who used oestrogen for 8 years or more showed a relative risk of 8.22. Furthermore, oestradiol has much stronger hyperplastic and carcinogenic effects than oestriol, which is often used for local treatment of urogenital problems. Some authors have reported that endometrial carcinoma due to prolonged oestrogen exposure shows a low malignancy grade, and has a better prognosis [114]. After total hysterectomy for endometrial cancer, oestrogen treatment is still considered contra-indicated, to prevent de novo stimulation of possible micro-metastases. However, some small studies have reported that in stage I endometrial carcinoma the risk of relaps is very low [115-118]. Therefore, if firm indications for oestrogen supplementation are present, it has been proposed that in these cases oestrogens may be prescribed, but then continuous combination with a progestogen should be given. In cases with history of endometrial carcinoma with high-grade malignancy and five years free of relapse, oestrogen supplementation combined with continuous progestogen administration may be considered [22].

Liver disease

Liver adenoma is a very rare complication of oral contraceptive use [119], and is no contra-indication for hormone supplementation. Since oestrogens and progestogens are metabolised in the liver parenchyma, they must be considered contra-indicated in case of severely disturbed liver functions [22]. Little is known with respect to the effects of hormone supplementation in women with the rare Dubin-Johnson syndrome or the Roter syndrome. In such cases it is recommended to monitor the liver function during hormone supplementation [22].

Porphyric disease

Low-dose hormone treatment may disturb porphyrin metabolism [120,121]. Its implications for women with porphyria are still unknown, and therefore, cautiousness is

recommended.

Meningeoma

In meningeomas large amounts of progesterone receptors have been found [122-126], and meningeomas have been reported to shrink during treatment with anti-progestogens [127,128]. Therefore, until now meningeomas are considered as a relative contra-indication for progestogen administration, but there is no objection to unopposed oestrogens in this respect. However, recent studies [129-131] have questioned the significance of sex steroid influences on cerebral meningeomas. Further research may solve this controversy.

Table IV lists some points that need special attention and extra care in women using hormone replacement therapy. When present, these problems may aggravate during hormone supplementation and therefore thorough follow-up is needed. As aggravation occurs treatment must be stopped. Some of the risks listed will be discussed below.

Table IV: Problems that need special attention

- Myoma uteri	- Familiairy hypertriglyceridaemia
- Endometriosis	- Venous thrombo-embolic disorders
- Migraine	- Risk profile breast cancer:
- Epilepsy	- Family history
- Gallstones	- Premalignant abnormalities
- Hypertension	- History of diethylstilbestrol-use

References 19-22

Gallstones

Oral contraceptive use and hormone replacement therapy do increase the risk of gallstone disease and the chance of having a cholecystectomy (*relative risk: 1.2 - 2.1*) [132-136]. This effect has so far not been found during transdermal hormone replacement therapy [137]. In the presence of gallstones, but also in case of history of cholestatic jaundice, it is recommended to administer hormones in dosages as low as possible, or to choose for non-oral treatment [22]. The recurrence risk of gallstones after cholecystectomy is very low.

Hypertension

Hormone replacement therapy has been associated with a small percentage of idiosyncratic hypertension [138], the reason why control of blood pressure after three months of treatment is recommended. This observation, however, must not be considered an argument for withholding hypertensive women postmenopausal hormone treatment. Most studies have reported an unchanged blood pressure or even small decreases in systolic, and sometimes also in diastolic, blood pressure during hormone replacement therapy [139-142].

Venous thrombo-embolic disorders

Although a history of venous thrombo-embolic disorders has been considered as contra-indication for hormone replacement, this statement has never been subscribed by clinical prospective studies. There is no clinical evidence that postmenopausal hormone replacement therapy increases the risk of thrombosis [143-145]. In women with history of spontaneous venous thrombosis, thrombosis during oral contraceptive use or during pregnancy, any deficiency of antithrombin III, protein-C or protein-S, must be excluded [22]. In women with one of these deficiencies, low-dose supplemention and good monitoring may be considered in case of severe climacteric complaints. Parenteral

administration may be more favourable than oral treatment, since it is reported to interfere less with liver metabolism, but no firm data are available to prove this. In case of thrombosis during hormone treatment it is recommended to stop the treatment, at least until adequate anti-thrombotic treatment is achieved [22].

CARDIOVASCULAR IMPACT OF HORMONE REPLACEMENT THERAPY

Until menopause, women are relatively protected against cardiovascular disease, as compared to men. In the latter, the incidence of cardiovascular morbidity increases gradually with age, while in women cardiovascular disease incidence increases exponentially after menopause until a percentage almost as high as in men [146,147]. Consequently, the increase in cardiovascular morbidity and mortality is delayed in women until after menopause [148] (Figure 2).

This phenomenon coincides with changes in the lipid profile, such as increases in the concentrations of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, while those of high-density lipoprotein cholesterol decrease [149]. Opposite to the suggested more or less abrupt changes in cardiovascular morbidity and mortality, and in the lipid profile, some authors state that these variables show gradual changes in time, with no significant relationship with the hormonal changes in the climacteric [150].

In the last decades an increasing amount of data has been gathered on the relationship between cardiovascular disease and oestrogen use in postmenopausal women [146,151-183]. Reports uniformly point in the same direction: oestrogen replacement therapy decreases the risk of developing cardiovascular disease as much as 50% [172]. Table V and VI summarize some of these studies, and all except one (The Framingham Study [184,185]) demonstrate relative risk estimates lower than one. However, after re-analysis of the latter data, the authors reversed their conclusion [169].

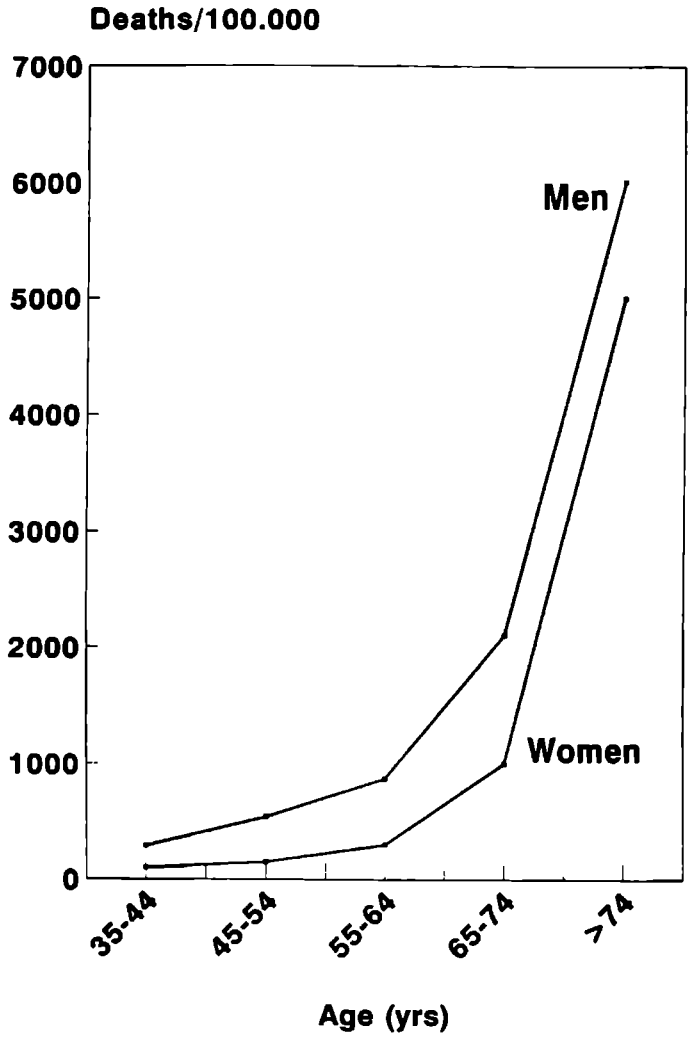

Figure 2: Cardiovascular death rates related to age and gender.

(Adapted from: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Center for Health Statistics, 1988: Vital statistics of the United States 1986. Vol II: Mortality, part A. Hyattsville, Maryland).

Table V: Effect of postmenopausal oestrogen replacement therapy on relative risk (RR) for cardiovascular disease in case-control studies

Authors		RR	Authors		RR
Rosenberg *et al.*[152]	1976	0.97	Ross *et al.*[155]	1981	0.40
Talbott *et al.*[174]	1977	0.34	Bain *et al.*[156]	1981	0.70
Pfeffer *et al.*[153]	1978	0.68	Adam *et al.*[157]	1981	0.65
Rosenberg *et al.*[175]	1980	1.00	Szklo *et al.*[158]	1984	0.61

Table VI: Effect of postmenopausal oestrogen replacement therapy on relative risk (RR) for cardiovascular disease in prospective studies

Authors		RR	Authors		RR
Burch *et al.*[159]	1974	0.40	Petitti *et al.*[163]	1986	0.50
Gordon *et al.*[184]	1978	1.00	Bush *et al.*[161]	1987	0.34
Hammond *et al.*[160]	1979	0.33	Hunt *et al.*[164]	1987	0.50
Nachtigall *et al.*[90]	1979	0.30	Bush *et al.*[180]	1987	0.40
Lafferty *et al.*[162]	1985	0.17	Criqui *et al.*[182]	1988	0.79
Wilson *et al.*[185]	1985	1.90	Henderson *et al.*[183]	1988	0.59
Stampfer *et al.*[170]	1985	0.30			

Investigation of the differential effects in subgroups with and without history of cardiovascular risk factors, such as: overweight, low socio-economic class, hypertension, smoking habits, hyperlipidaemia and history of coronary infarction [186], revealed very interesting results. Risk reduction appeared stronger in women having a risk factor.

Women with angiographically defined coronary heart disease and treated with oestrogens showed the same ten-year survival as women without coronary stenosis; the diffference between oestrogen users and non-users increased with increasing coronary stenosis [176,177,187,188]. These results stress the need for hormone replacement therapy, especially in women with an increased cardiovascular risk profile. However, because most other studies mentioned above have methodological impairments, these conclusions need confirmation [189].

Most studies investigated the changes in cardiovascular risk during unopposed oestrogen treatment. Since Ziel & Finkle [109] reported an increased risk for endometrial cancer during unopposed oestrogen treatment in non-hysterectomized women, more studies demonstrated the risk for developing endometrial hyperplasia and endometrial carcinoma [105-107,110-113]. Administration of a progestogen, continuously or cyclical for 12 days or more per 28 day cycle, protects the endometrium for these hazards [105]. Combined oestrogen/progestogen treatment reduces the incidence of endometrial hyperplasia and carcinoma to frequencies even below those seen in untreated women [108]. In Europe the monthly cyclical administration of a progestogen is common practise.

Until now many reports, not necessarily in postmenopausal women, have described possible unfavourable metabolic effects of progestogens, especially with respect to its influences on the lipid profile [190-193]. Since no direct data were available of progestogenic effects on cardiovascular disease, the results of these reports were extrapolated as that progestogens attenuate, or even reverse the cardiovascular protective effects exerted by oestrogen. Recently, some observational studies indicated that little difference exists between unopposed oestrogen and combined oestrogen/progestogen treatment with regard to the cardiovascular risk profile (Table VII). These results can be criticized because they lack randomised controlled groups. To clear this, large epidemiological randomised controlled trials are at present ongoing in the United States.

Table VII: Effect of combined oestrogen/progestogen therapy on relative risk (RR) for cardiovascular disease

Authors		RR	Authors		RR
Nachtigall *et al.*[90]	1979	0.3	Hunt *et al.*[165]	1990	0.3
Thompson *et al.*[194]	1989	0.9	Falkeborn *et al.*[167]	1992	0.5

MECHANISMS UNDERLYING THE HORMONE RELATED CHANGES IN CARDIOVASCULAR RISK

The explanation of the reported oestrogen related reduction in cardiovascular morbidity and mortality with up to 50% has been subject of many studies. These investigations can be divided in two categories: studies on direct (cardio)vascular effects and studies on non-direct effects, especially the metabolic studies.

Direct (cardio)vascular effects

Oestrogens exert acute and long-term vasodilatatory changes in coronary as well as periferal arterial vessels. They increase the blood flow through the brain and the heart [195]. The peripheral resistance is diminished [196,197], leading to lowered blood pressure, systolic as well as diastolic. Oestrogens have recently been described to have calcium-antagonistic effects on the vascular wall [198]. Furthermore, Rosano *et al.* [199] reported beneficial effects of sublingual oestrogens on the ECG during exercise-induced myocardial ischaemia in women with proven coronary artery disease.

Prostaglandins

Prostacyclins have a vasodilatatory effect, when released by the endothelial cells. 17ß-Oestradiol has been reported to stimulate the prostacyclin release [200,201], and by this mechanism it may exert its vasorelaxing effect.

Lipid profile

Most studies investigating the changes in lipids and lipoproteins during oestrogen supplementation in postmenopausal women have found a decrease in the atherogenic factors total cholesterol and low-density lipoprotein cholesterol, while the triglycerides and the anti-atherogenic high-density lipoprotein cholesterol increase [161,202-210]. Commonly, triglycerides decrease when high-density lipoproteins increase, but during oestrogen supplementation triglycerides rise, even when high-density lipoproteins increase. However, the rise in triglycerides reflects the rise in very low-density lipoprotein triglycerides, and these have a very low atherogenicity [211,212]. So, most changes during oestrogen administration can be considered favourable with respect to the risk of developing cardiovascular disease. Since unopposed oestrogen has been found to increase the risk of endometrial carcinoma combined oestrogen/progestogen treatment is recommended in non-hysterectomised women. This has prompted research to investigate the progestogen related effects on the lipid profile. The first reports indicated unfavourable androgenic effects, more so by the C-19 progestogens than the C-21 progestogens [190]. However, these effects also depend on the dosage, as well as the route and duration of administration of the progestogen [190-193,210]. In the recently developed low-dose C-19 progestogen containing preparations, the attenuation of the oestrogen induced beneficial changes in lipids and lipoproteins, if any, is comparable with those of the C-21 progestogens [213,214], especially during long-term treatment [215].

Until recently, changes in the conventional risk estimators as lipids and lipoproteins were considered the most important mechanism explaining the oestrogen

induced risk reduction in cardiovascular disease. However, recently, this mechanism has been stated only to explain up to 25% of the cardiovascular risk reduction [216]. In experimental animal studies, oestrogen and combined oestrogen/progestogen have been found to reduce atherosclerotic lesions in equal degree, even when serum lipoprotein concentrations were unchanged [217-222]. So, the reduction in cardiovascular disease seems to be the result of a conversion in the development of atherosclerosis, but this phenomenon can probably only in part be explained by the changes in the conventional risk estimators. This area is still open for further investigation to unravel other underlying mechanisms. However, the importance of lipids and lipoproteins as markers of metabolic changes must not be neglected.

Another factor that is involved in atherosclerosis lies in the qualitative properties of low-density lipoproteins. Low-density lipoproteins in its native (non-modified) form have a relatively low atherogenetic impact, but after modification by oxidation it becomes highly atherogenetic by binding to peripheral "scavenger receptors" in the vascular wall, after which it excessively accumulates in macrophages, the precursors of "foam cells". Accumulation of lipids leads to fatty streak formation, and these are the basis for atherosclerotic lesions [223].

Low-density lipoproteins are a heterogenetic group of particles with a diversity of size, density, molecular mass and chemical composition [224,225]. Small low-density lipoprotein particles have been associated with coronary heart disease [226-232], but this has been found to be accompanied with low high-density lipoprotein and with elevated triglyceride levels. Furthermore, small low-density lipoprotein particles are more susceptible to oxidative modification than large particles, and this may explain their higher atherogenetic potency [233]. Research on hormonal effects on low-density lipoprotein particle size and oxidizability may help to elucidate a part of the other 75% of cardiovascular risk reduction during postmenopausal hormone replacement therapy.

Carbohydrate metabolism

In postmenopausal women the pancreatic insulin secretion decreases, and the insulin fraction passing the liver increases. The insulin-resistance increases progressively after menopause. It has been reported that administration of oestradiol can attenuate this increased insulin-resistance, which phenomenon has not been demonstrated for other oestrogens [234]. These influences may possibly provide a mechanism contributing to the reduced cardiovascular risk during hormone replacement therapy. Moreover, it subscribes at least the opinion that women with diabetes mellitus, and therefore at risk for cardiovascular disease, rather benefit by oestrogens than the opposite.

Homocysteine

Homocysteine is the demethylated derivative of the essential amino acid methionine [235]. Its metabolic pathway is shown in Figure 3.

Elevated blood concentrations of homocysteine are an established risk factor for premature vascular disease. The autosomal recessive inherited metabolic disease "homocystinuria" is caused by a cystathionine ß-synthase deficiency, and is characterized by massive urinary excretion of homocysteine and severe elevation of blood methionine and homocysteine. Premature atherosclerosis and thrombo-embolism are life-threatening complications of homocystinuria. This is explained by homocysteine-induced endothelial cell injury, which may initiate the development of typical atherosclerotic lesions [236]. Several studies have demonstrated that even mildly elevated blood concentrations of homocysteine (hyperhomocysteinaemia) are associated with an increased risk of vascular disease [237-241]. Furthermore, hyperhomocysteinaemia has also been reported as risk factor for recurrent early pregnancy loss [242], neural-tube defects [243], and possibly placental abruption [244].

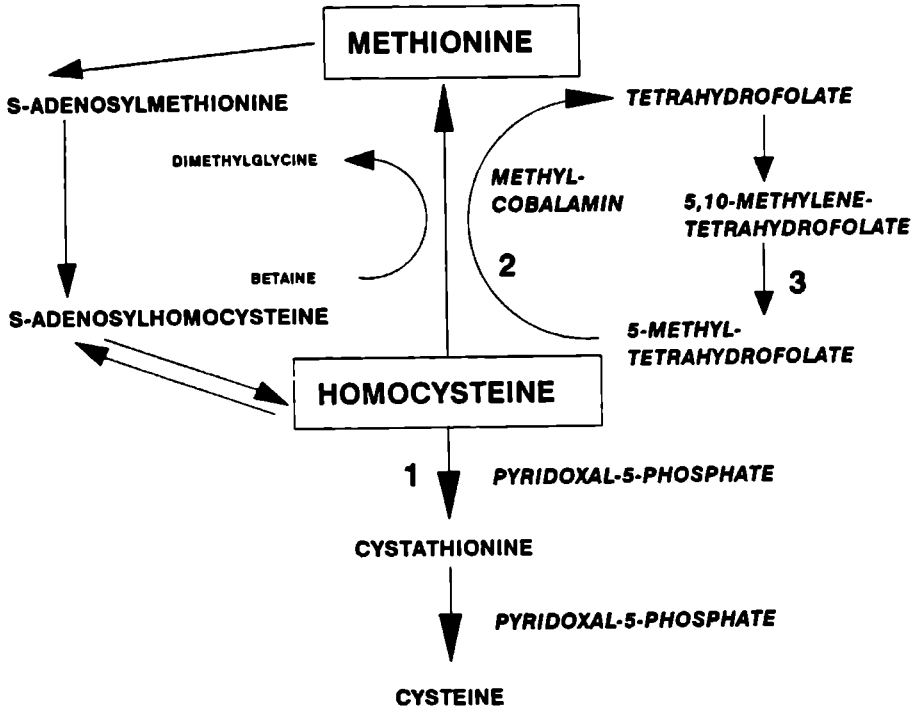

Figure 3: Simplified scheme of the methionine-homocysteine metabolism and enzymes and vitamines involved. (1) Cystathionine ß-synthase; (2) 5-methyltetrahydrofolate-homocysteine methyltransferase; (3) 5,10-methylene-tetrahydrofolate reductase.

(Adapted from: Wouters MGAJ, *et al.*, 1993: reference 242).

There are several reports that blood homocysteine concentrations, determined as free disulfides, as well as total homocysteine, are higher in men as compared to women. Furthermore, some studies, though inconsistently, have demonstrated higher homocysteine

levels in postmenopausal women as compared to those in premenopausal women [245-249]. Thus, homocysteine metabolism may indeed explain in part the difference in cardiovascular risk between pre- and postmenopausal women [246]. Until now, the role of homocysteine metabolism as a possible pathogenic mechanism mediating the hormone related changes in cardiovascular risk in the perimenopause as well as during hormone replacement therapy has only scarcely been investigated.

BENEFIT / RISK - RATIO OF HORMONE REPLACEMENT THERAPY

The increasing amount of epidemiological data on all aspects of hormone replacement therapy in postmenopausal women should help in the decision making by the general phycisian as well as the gynaecologist. For counseling the women in need for treatment of climacteric symptoms, knowledge of the latest epidemiological data is of course indispensable, although it may be hard to catch up with the growing amount of new data.

In general terms there is no doubt that hormone replacement is effective for the relief of climacteric symptoms and complaints, and for treatment of urogenital problems. Possible contra-indications as described before, although infrequently present, must be eliminated by proper (family) history taking and by physical, gynaecological and mammographic examination. The goal must be to achieve the most effective treatment of symptoms of complaints with as little as possible adverse reactions and, in non-hysterectomised women, as little as possible bleeds, by individualisation of the treatment.

Women without symptoms still may need hormone replacement therapy for the prevention of postmenopausal osteoporotic fractures. Bone densitometric measurement, a very accurate risk estimate for the occurrence of future bone fractures [250,251], may help to decide whether hormone replacement therapy must be advised. In such cases hormone treatment can at best be started soon after menopause. It should be realized that

prevention of osteoporosis by hormone replacement therapy requires long-term treatment.

At present many data indicate that hormone supplementation has a cardioprotective effect. Although tempting, at this moment it may be too premature to advocate oestrogen treatment as a new drug for the cardiologist. However, much would have been achieved when, in case of (suspected) cardiovascular disease or the presence of one or more risk factors, oestrogen treatment would no longer be abandoned. In the near future it can be expected that oestrogen administration will become a new treatment modality for postmenopausal women with (an increased risk of developing) cardiovascular disease.

In conclusion, hormone replacement therapy may benefit many women in their peri- and postmenopausal years, and when the few contra-indications are excluded it is unlikely that this type of treatment will introduce new hazards. Furthermore, cardiovascular morbidity, or at least existing risk factors for developing cardiovascular disease may become new indications for hormone treatment, instead of being considered as an objection for it [252-259].

ROLE OF PROGESTOGENS

As already mentioned before, the cyclic addition of a progestogen to the oestrogen is strongly recommended to prevent irregular bleeding episodes and the development of endometrial hyperplasia and ultimately endometrial cancer. However, cyclical oestrogen/progestogen regimen restore vaginal bleeding in 80% to 90% of women [260], and this may interfere with patient acceptance of the treatment [261-263] at an age when cessation of menses is anticipated. Progestogens are not believed to exert protective effects on all organ systems. For instance, progestogens may increase the mitotic index in breast tissue, although until now data on the progestogenic effects on the breast are inconsistent. The bone mass may benefit by combining oestrogen with a progestogen

[70,71], but also on this issue only few data are available. Progestogens may attenuate the oestrogen induced beneficial changes in the lipid profile. This depends on the type and the dosage of the progestogen, and on the route and the duration of its administration. Nevertheless, according to the latest reports, combining oestrogen with progestogens does not seem to alter the cardioprotective effects of oestrogen much.

Although the importance of progestogen administration seems obvious with respect to the prevention of endometrial disease, its impact on the other organ systems is yet incompletely known. More research is necessarry to elucidate the differential effects introduced by the different combined oestrogen/progestogen treatment regimen [21].

COMPLIANCE AND ACCEPTANCE

Most data on combined oestrogen/progestogen treatment concern a monthly sequential regimen. In most women sequential regimen, however, restore vaginal bleeds [260], and for many women this consequense of combined hormone treatment impairs the acceptance of the treatment [261-263]. Patient compliance after two years reportedly was less than 20% [264]. Considering the long-term preventive effects of hormone replacement therapy on osteoporosis and cardiovascular disease, these scores are disappointing, since most benefits can be expected when hormone treatment is started soon after menopause, and administered for a long period of time. Hormone supplementation for a shorter period, or starting the treatment later on after menopause, however, does not necessarily mean that the treatment is useless with respect to osteoporosis and cardiovascular disease prevention [64]. Consequently, to improve patient acceptance, more research is needed on different treatment regimen to determine their impact on the safety of the endometrium, the bleeding characteristics, patient acceptance and compliance.

Recently, new developments in research are concentrating on the effects of

alternative treatment regimen, such as combined continuous oestrogen/progestogen administration [265-270] and prolonged treatment cycles [271,272]. Until now, the first data indicate that combined continuous treatments induce an atrofic endometrium and that withdrawal bleeds disappear. However, this condition is often established only after several months, during which period breakthrough bleeds may occur. Breakthrough bleeding may be associated with a hyperplastic endometrium, as well as with an atrophic endometrium. Therefore, these unexpected bleeds may introduce diagnostic difficulties. Until recently unexpected genital bleeds were investigated by dilation and curettage procedure. New developments as ultrasound [273,274] and endometrial biopsy [275] may in the near future simplify the diagnostic procedure. To avoid these diagnostic problems, it is at present advised to delay the administration of combined continuous treatment regimen until the women are postmenopausal for some time [21]. In this respect, however, six months as well as two years are recommended [21,276]. Before that moment, sequential regimen are prefered. Although little data are available on other regimen than the monthly sequential treatment, it appears that in general practise many physicians choose to prolong the treatment cycle to two or even three months of continuous oestrogen supplementation, combined with 10 - 14 days administration of a progestogen at the end of the treatment cycle. Reducing the incidence of withdrawal bleeds seems an interesting strategy to improve patient acceptance and compliance, but further research still has to support this hypothesis.

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CHAPTER 3 * Part I

BENEFICIAL EFFECTS ON SERUM LIPOPROTEINS BY 17ß-OESTRADIOL - DYDROGESTERONE THERAPY IN POSTMENOPAUSAL WOMEN; A PROSPECTIVE STUDY

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ABSTRACT

Objective: To study the possible changes in reproductive hormones, sex hormone binding globulin, serum lipids and lipoproteins, lipoprotein(a) included, coagulation and plasma glucose in postmenopausal women treated with continuous 17ß-oestradiol and cyclic dydrogesterone for 14 days per 28 days treatment cycle.

Design: Open longitudinal prospective study.

Duration: Twelve 28 days treatment cycles.

Setting: Gynaecological department of a university hospital.

Subjects: Twenty-seven healthy non-hysterectomised postmenopausal women.

Results: After treatment for six cycles serum concentrations of FSH and LH decreased significantly with 43.0% and 24.4%, respectively. Serum concentrations of oestradiol and oestrone increased significantly with 302% and 792%, respectively, and SHBG increased as well with 111% ($P<0.01$). Serum total cholesterol decreased with 9.0% ($P<0.01$). Serum VLDL-cholesterol did not change significantly. Serum LDL-cholesterol decreased with 16.3% ($P<0.01$) and HDL-cholesterol increased with 8.0% ($P<0.01$). This was accompanied with similar significant changes in the apolipoproteins: apolipoprotein A-I rose with 14.4% and apolipoprotein B decreased with 6.0%. Serum triglycerides and VLDL-triglycerides increased significantly with 14.4% and 17.9%, respectively. Lipoprotein(a) decreased with 17.5% ($P<0.01$). These results more or less sustained at cycle twelve of treatment. Serum concentrations of antithrombin III and plasma glucose did not change. Fibrinogen decreased slightly but significantly below the initial value.

Conclusions: This combination replacement therapy gives beneficial changes in lipid metabolism indicating a reduced risk of developing coronary heart disease without unfavourably changing coagulation and glucose metabolism. The expected beneficial changes with oestradiol alone are not counteracted by the intermittent addition of dydrogesterone. Therefore, this oestrogen/progestogen scheme can indeed be recommended for use in HRT.

INTRODUCTION

Hormonal replacement therapy (HRT) for women with postmenopausal complaints has become of increasing interest during the last decades. Hot flushes, perspiration and vaginal dryness, and also many subjective complaints, diminish or disappear in a majority of women soon after starting HRT [1].

Slowing down the early postmenopausal osteoclastic activity by oestrogen supplementation is of great importance in the prevention of osteoporotic fractures. In women with low bone mass and approaching menopause HRT is recommended [2].

Several large studies show that oestrogen replacement therapy (ERT) also reduces cardiovascular morbidity and mortality risk by approximately 50 percent [3,4]. Postmenopausal women with manifest coronary heart disease (CHD) appear to have the largest benefit of HRT [5].

Next to vasodilatatory effects oestrogens and progestogens also influence the serum concentrations of lipids and lipoproteins. Oestrogens have beneficial effects on plasma lipids by decreasing serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and increasing high-density lipoprotein cholesterol (HDL-C). Progestogens may have oppositional effects [6]. These effects of HRT appear to depend on the type and the dosage of oestrogen and progestogen, and the route and the duration of their administration [7].

The usual prescribed dosages of HRT restore serum oestradiol (E_2) concentrations to values as found during the early follicular phase of the menstrual cycle. At these concentrations oestrogen use is not associated with thrombogenesis and glucose-intolerance [8,9].

In the present report we describe the effects of treatment with continuous administration of micronised 17ß-E_2 (Zumenon®) in combination with dydrogesterone (Duphaston®) [10] cyclic for 14 days per 28 days treatment cycle in healthy postmenopausal women. Dydrogesterone is an unique progestogen, exerting little if any

influence on serum lipids which is in marked contrast with many other progestogens. The effects of this treatment on reproductive hormones and sex hormone binding globulin (SHBG), serum lipids and lipoproteins, including lipoprotein(a) (Lp(a)), coagulation factors and glucose metabolism was followed for up to one year.

SUBJECTS AND METHODS

The study was approved by the ethical committee of our hospital on beforehand. Included were generally healthy non-hysterectomised postmenopausal women, aged 49 to 59 years, who were recruted by advertisement in a local newspaper during the months January until May 1990. The women were amenorrhoeic for at least six months and were screened to have follicle-stimulating hormone (FSH) serum concentrations within the range, characteristic for the postmenopausal phase. Excluded were women using drugs affecting lipid metabolism or who used hormonal therapy for the previous two weeks. Before entering the study the purpose of the protocol had been explained and written informed consent was obtained.

All women were treated with micronised 17ß-E_2 (Zumenon®), 2 mg daily, and with dydrogesterone (Duphaston®), 10 mg daily for the first half of each 28 days treatment cycle, both orally administered (Solvay-Duphar, Weesp, The Netherlands).

Clinical examination, endometrial biopsy performed by micro-curettage (Vabra® endocurette, Farina Lanfranco, Venezia, Italia), and fasting venous blood sampling took place before study entry and on day 12, 13 or 14 of the combined 17ß-E_2 and dydrogesterone intake of cycles 6 (± 1 cycle) and 12 (± 1 cycle) for the assessment of reproductive hormones and SHBG (cycle 12 excluded), serum lipids and lipoproteins, antithrombin III, fibrinogen and glucose.

Previous to study entry assessment of liver enzymes (ALAT, ASAT and gamma GT) and mammography was performed. At cycle 4, additional assessment of antithrombin III and fibrinogen was performed in order to diagnose a possible early deterioration in the coagulation system.

Serum concentrations of LH and FSH were measured with specific immunoradiometric assays (Medgenix, Fleurus, Belgium) and have been described elsewhere [11]. Serum concentrations of E_2 were measured with an in-house RIA [12] and serum concentrations of oestrone (E_1) were measured by oestrogen-specific dextran-coated charcoal (DCC) radioimmunoassay described by Heineman [13]. Serum concentrations of SHBG were measured by a commercially available non-competitive liquid-phase immunoradiometric assay (Farmos Diagnostica, Turku, Finland) described by Hammond *et al.* [14].

Very low-density lipoproteins (VLDL) were isolated within 5 days after blood sampling by ultracentrifugation at d = 1.006 g/ml using a Kontron TFT 45.6 rotor for sixteen hrs at 168000 x g at 14°C in a Beckman L7-55 ultracentrifuge [15]. HDL-C in these sera was determined by the polyethylene glycol 6000 method [16]. For HDL-C the inter-assay coefficient of variation amounted to 2.3% (n = 20). Serum TC and triglycerides (TG) were measured by enzymatic methods using commercially available reagents (CHOD-PAP cholesterol reagent, cat. no. 237574, Boehringer Mannheim, Mannheim, FRG, and SERA-PAK TG cat. no. 6684, Miles, Italia). Both measurements were performed with a centrifugal analyser (Multistat III). Inter-assay coefficients of variation were 1.7% and 1.5%, respectively (n = 20). The accuracy checked for cholesterol against an Abell-Kendall method approved by the Centers for Disease Control (Atlanta, USA) was within 3% of target values. LDL-C was calculated by subtracting the cholesterol content in the $d<1.006$ g/ml fraction and in the HDL-fraction from total serum cholesterol. Sera to be analysed for apolipoproteins were stored at -80°C until the end of the study. To minimize the imprecision all samples from the same subject were analysed in the same run in duplicate. Apolipoprotein (Apo) A-I and B were measured by immunonephelometry [17,18]. Lp(a) was measured with a radioimmunoassay procedure

(Pharmacia, Uppsala, Sweden). In this method plasminogen up to a concentration of 5 g/l gives no measurable crossreactivity. The inter-assay coefficients of variation for single measurements of Apo A-I and Apo B were 5.8% and 6.2%, respectively (n = 12), and for Lp(a): 6.6% (n = 20).

Antithrombin III was measured with the chromogenic substrate S2238 (Kabi Diagnostica AB, Mölndal, Sweden) using a microtiter technique. The fibrinogen concentration was determined with a clotting test using a KC 10 coagulometer (Amelung) as described by Clauss [19]. The intra-assay and inter-assay variabilities for means of duplicate measurements were calculated from several pools of plasma and were, respectively, 3.0% and 5.2% in the case of antithrombin III, and were 3.9% and 4.5% in the case of fibrinogen (n=10).

After screening, 27 healthy postmenopausal women were included in the study. Characteristics are given in Table I. One woman was incorrectly admitted to the study as the initial endometrial biopsy showed focal adenomatous hyperplasia. Therefore she was excluded. There were three drop outs due to several reasons.

In six women the dydrogesterone dosage was increased from 10 to 20 mg because of repeated spotting or withdrawal bleeding before day ten of the combined 17ß-E_2 and dydrogesterone intake [20]. In the case of one woman venous blood sampling took place by mistake on day nineteen instead of day twelve to fourteen of the combined 17ß-E_2 and dydrogesterone intake. Another woman missed dydrogesterone seven days in two cycles and fourteen days in one cycle, due to repeated hospitalisation because of total hip surgery, without further violation of the protocol. Excluding the obtained data from these eight women did not change our results significantly, the reason why analyses on data of 27 women will be discussed.

Statistical analysis on the data of 27 women was performed on an "intention-to-treat" basis. The last visit carried forwards (LVCF) analysis was used because of one exclusion due to screening failure and three drop outs. Apolipoprotein analyses were performed on data of 23 women (data on one screening failure and three drop outs were not available). In case of normal distribution (descriptive statistics) the Student's paired *t* test

and the repeated measures analysis of variance (ANOVA) was used, in the other cases we used the Wilcoxon's signed-rank test and the Friedman's two-way ANOVA. *P*-values in tables are given for comparing data of cycle 6 and cycle 12 versus cycle 0. A *P*-value of 0.05 or less is considered to be statistically significant. Statistical analyses were performed with the Dyna-stat computer program (Dynamic Microsystems, Inc., Washington, D.C., Pittsburgh, PA, USA).

Table I: Descriptive statistics[a] before treatment (cycle 0) and at cycle 12

		cycle 0	cycle 12	P^b
Age	(years)	54.0 ± 3.6		
Amenorrhea	(months)	52.8 ± 38.9		
Body-mass index	(kg/m^2)	24.5 ± 2.8	24.5 ± 3.0	NS
Blood pressure: systolic	(mmHg)	126 ± 15	134 ± 15	<0.01
diastolic	(mmHg)	82 ± 7	84 ± 6	NS

[a]: mean ± SD (N=27); [b]: Student paired *t* test, verified with repeated measures ANOVA

RESULTS

Mean age was 54.0 years and the mean period of amenorrhoea was 52.8 months (Table I). Thirteen women had used HRT before with a mean wash out period of one year (*range: 14 days - 4 years*). Six women (=22%) had a wash out period ranging between 14 days and 3 months. Mean body-mass index was 24.5 kg/m^2. The mean systolic blood pressure increased from 126 to 134 mmHg ($P<0.01$). Seven women

(=26%) were cigarette-smokers, only three of them (=11%) smoked five cigarettes or more per day.

During the first six cycles mean serum concentrations of FSH and LH both decreased, respectively from 83 to 48 IU/l and from 31 to 23 IU/l ($P<0.01$) (Table II). The mean serum concentrations of E_2 and E_1 both increased, respectively from 145 to 583 pmol/l and from 261 to 2329 pmol/l ($P<0.01$). Mean serum concentration of SHBG increased as well from 53 to 111 nmol/l ($P<0.01$).

Table II: Reproductive hormones and SHBG before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycle 6[a]

		cycle 0	cycle 6	P[b]
FSH	(IU/l)	83 ± 27	48 ± 26	<0.01
LH	(IU/l)	31 ± 10	23 ± 13	<0.01
E_2	(pmol/l)	145 ± 150	583 ± 339	<0.01
E_1	(pmol/l)	261 ± 131	2329 ± 1594	<0.01
SHBG	(nmol/l)	53 ± 25	111 ± 45	<0.01

[a]: mean ± SD (N=27); [b]: Wilcoxon signed-rank test; FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2: oestradiol; E_1: oestrone; SHBG: sex hormone binding globulin.

During the first six cycles mean serum TC decreased from 6.32 to 5.75 mmol/l ($P<0.01$) (Table III and Figure 1). Thereafter it slightly increased to 5.96 mmol/l which is still below the initial value ($P<0.01$).

Table III: Lipids and (apo)lipoproteins before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 6 and 12[a]

		cycle 0	cycle 6	P^b	cycle 12	P^b	ANOVA
Total cholesterol	(mmol/l)	6.32 ± 1.36	5.75 ± 0.94	<0.01	5.96 ± 0.96	<0.01	*
HDL-cholesterol	(mmol/l)	1.50 ± 0.29	1.62 ± 0.33	<0.01	1.66 ± 0.35	<0.01	*
LDL-cholesterol	(mmol/l)	4.47 ± 1.40	3.74 ± 0.96	<0.01	3.92 ± 0.97	<0.01	*
VLDL-cholesterol	(mmol/l)	0.35 ± 0.28	0.40 ± 0.22	NS	0.38 ± 0.28	NS	NS
Triglycerides	(mmol/l)	1.25 ± 0.65	1.45 ± 0.56	<0.05	1.42 ± 0.68	<0.05	*
VLDL-triglycerides	(mmol/l)	0.67 ± 0.57	0.79 ± 0.42	<0.05	0.73 ± 0.54	NS	*
Apolipoprotein A-I	(mg/l)	1417 ± 191	1621 ± 161	<0.01	1672 ± 195	<0.01	*
Apolipoprotein B	(mg/l)	1589 ± 434	1494 ± 381	<0.01	1554 ± 398	NS	NS
Lipoprotein(a)	(mg/l)	303 ± 467	250 ± 375	<0.01	239 ± 382	<0.01	*

[a]: in mean ± SD ; N=27 (N=23 for apolipoprotein A-I & B and lipoprotein(a)); [b]: *P*-values versus cycle 0; Wilcoxon signed-rank test; *: statistical significance verified with Friedman's two-way ANOVA.

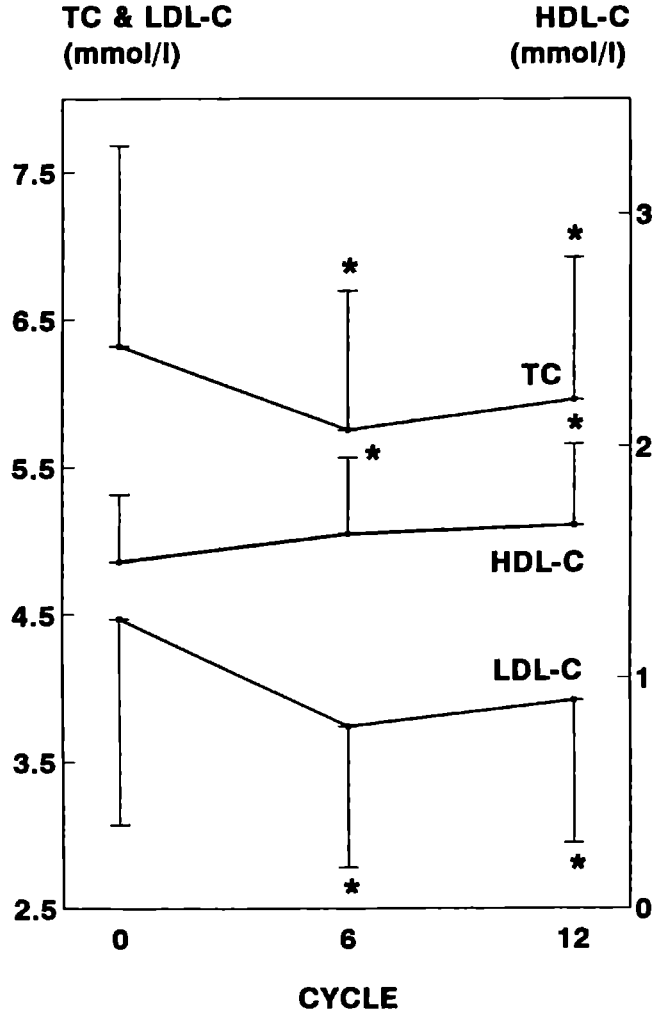

Figure 1: Mean serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in mmol/l before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 6 and 12 (N=27); *: $P<0.01$.

The changes in mean serum TC were reflected in the mean serum LDL-C concentration which decreased from 4.47 via 3.74 to 3.92 mmol/l ($P<0.01$ versus the initial value). During the study period mean serum HDL-C increased from 1.50 to 1.66 mmol/l ($P<0.01$). Mean serum VLDL-C did not change significantly. During the study period mean serum TG increased from 1.25 to 1.42 mmol/l ($P<0.05$), which is reflected in a rise in VLDL-TG from 0.67 via 0.79 to 0.73 mmol/l (statistically significant with ANOVA). Mean serum Apo A-I rose from 1417 to 1672 mg/l ($P<0.01$) (Table III and Figure 2). Mean serum Apo B decreased from 1589 via 1494 to 1554 mg/l ($P>0.05$ versus the initial value). In this study population the serum concentration of Lp(a) was highly skewed (skewness 2.48). During the study period mean serum Lp(a) decreased from 303 to 239 mg/l ($P<0.01$). The median serum concentration decreased from 76 via 67 to 71 mg/l. Re-analysis of the logarithmic of serum Lp(a) (skewness 0.22) showed a decrease from 2.01 ($SD=0.69$) to 1.90 ($SD=0.68$) ($P<0.001$; Student's paired t test). There was no correlation between serum Lp(a) and serum concentrations of TC, HDL-C, LDL-C, VLDL-C, TG, Apo A-I or B. The [TC]/[HDL-C] ratio decreased from 4.41 ($SD=1.41$) via 3.74 ($SD=1.07$) to 3.78 ($SD=1.12$) ($P<0.01$ versus the initial value). The recently introduced atherogenic index ATH-INDEX [21] (=[TC - HDL-C]x[Apo B]/[Apo A]x[HDL-C]) decreased from 4.48 ($SD=3.37$) via 2.78 ($SD=1.99$) to 2.88 ($SD=2.09$) ($P<0.01$ versus the initial value).

Thrombolysis was estimated by means of serum antithrombin III, thrombogenesis by means of serum fibrinogen. During the study period there was no significant change in mean serum antithrombin III (Table IV). Mean serum fibrinogen slightly increased in the first four cycles from 2364 to 2436 mg/l. Thereafter it decreased to 2245 mg/l ($P<0.05$ versus the initial value).

Mean plasma glucose concentration decreased during the study period from 5.3 via 5.0 to 5.1 mmol/l ($P>0.05$ versus the initial value).

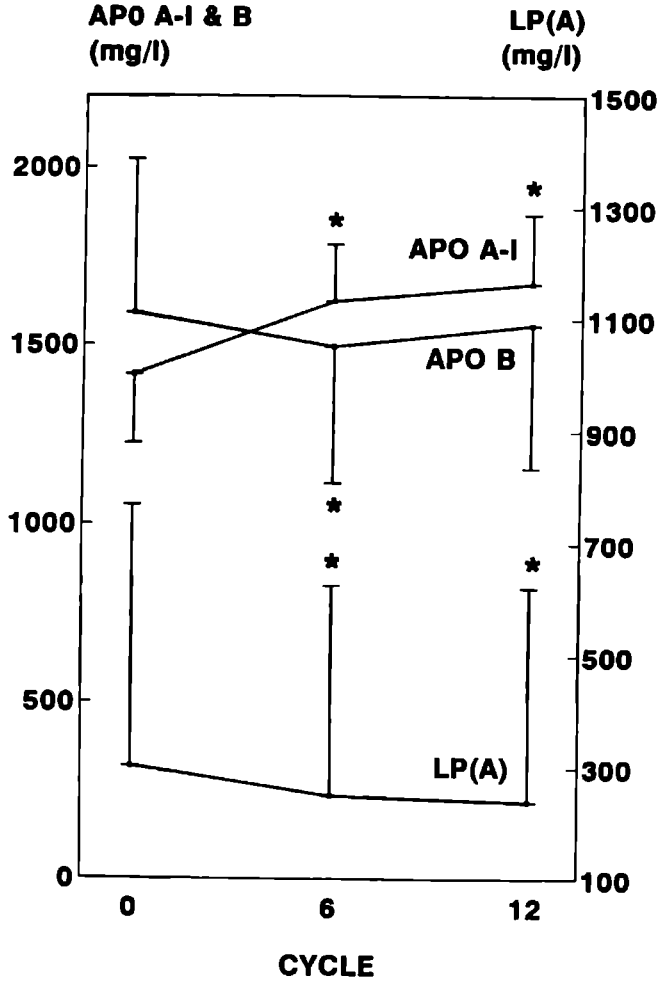

Figure 2: Mean serum concentrations of apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B) and lipoprotein(a) (Lp(a)) in mg/l before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 6 and 12 (N=23); *: $P<0.01$.

Table IV: Antithrombin III and fibrinogen before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 4, 6 and 12[a]

	cycle 0	cycle 4	cycle 6	cycle 12	P^b	A^c
AT III (%)	97 ± 4	98 ± 4	97 ± 5	96 ± 5	NS	NS
Fibr (mg/l)	2364 ± 446	2436 ± 537	2304 ± 482	2245 ± 514	<0.05	*

[a]: in mean ± SD (N=27); [b]: *P*-values versus cycle 0; Wilcoxon signed-rank test;
*: statistical significance verified with Friedman's two-way ANOVA (A^c).
AT III: antithrombin III; Fibr: fibrinogen.

DISCUSSION

Our study population was selected by advertisement in stead of using an outpatients department population for the purpose of shortening the inclusion period. Although this may have caused some selection bias, it has the advantage of a more homogeneous group as far as seasonal influences are concerned.

All women in this study experienced a satisfactory relief of symptoms and complaints on the given combination therapy, with preponderantly few side-effects well within acceptable limits. In six women (=22%) the dydrogesterone dosage had to be increased from 10 to 20 mg because of repeated spotting or withdrawal bleeding before day ten of the combined 17ß-E_2 and dydrogesterone intake. This may indicate that the optimal dose of this drug is in between 10 and 20 mg daily. This increase did not change the obtained results significantly.

Although serum FSH and LH concentrations did not reach premenopausal values after six cycles of HRT, serum E_2 was increased to normal premenopausal values as seen

during the follicular phase of the menstrual cycle. A remarkable increase is observed in the E_1 serum concentration due to conversion from E_2 to E_1. We speculate that the good and stable results as far as relief of climacteric symptoms are concerned depend on this interconversion where E_1 acts as substrate (buffer) for E_2.

The observed metabolic changes are not likely to be exclusively due to seasonal changes, because the start and the end of the study were in the same season. The lipid and (apo)lipoprotein analyses show marked beneficial changes: during the first six cycles we observed a rise in HDL-C with 0.12 mmol/l (8.0%, $P<0.01$) that after twelve cycles even was 0.16 mmol/l. An increase of HDL-C with 10 mg/dl (equivalent to 0.26 mmol/l) has been associated with a decrease in risk of CHD of as much as 50 percent [3], although recently the anti-atherogenic role of an increase in HDL-C has been questioned [22]. LDL-C decreased at the same time with 16.3% ($P<0.01$). These changes were accompanied with similar changes in the apolipoproteins: Apo A-I rose with 14.4% ($P<0.01$) and Apo B decreased with 6.0% ($P<0.01$). TC decreased with 9.0% ($P<0.01$). TG were elevated without clinical relevance. Hence the atherogenic indexes [TC]/[HDL-C] and the recently introduced ATH-INDEX [21] decreased significantly with 15% and 38%, respectively. These results more or less sustained at cycle twelve of treatment. The ATH-INDEX shows the greatest decrease compared with the other atherogenic indexes, so we speculate that the ATH-INDEX might be clinically useful in describing changes in lipid metabolism.

The main purpose of this study was to investigate whether or not the expected beneficial changes in lipid metabolism with E_2 alone sustain during intermittent intake of dydrogesterone. Studies have questioned the use of some progestogens in HRT for the reason of reversal of beneficial changes in lipid metabolism [7]. With intermittent dydrogesterone administration the expected beneficial effects on lipid metabolism do sustain. This confirms the similar observation reported by Siddle *et al.* [23]. Therefore this oestrogen/progestogen scheme can indeed be recommended for use in HRT.

The predictive value of changes in Lp(a) metabolism is not all clear [24,25]. An association between elevated Lp(a) concentrations and CHD seems to be established, but

the basic pathophysiological mechanisms behind this are still poorly understood. Little is known about possible changes in Lp(a) during HRT. Only recently a significant decrease in Lp(a) was found during norethisterone treatment, 5 mg twice daily, in postmenopausal women [26]. As dydrogesterone has no androgenic properties, our findings suggest that the decrease in Lp(a) during norethisterone treatment must be a progestational effect, more than an androgenic effect of norethisterone. A possible role in thrombogenesis and atherogenesis seems established and, although until now unproven, a fall in Lp(a) is probably beneficial, but this still needs further investigation by clinical trials. As in other studies Lp(a) was highly skewed in our study population but despite this we observed a significant decrease of 17.5% after six cycles and of 21.1% after twelve cycles in the mean serum concentration, which was not correlated with any other change in lipids and (apo)lipoproteins. In the light of our knowledge concerning the role of Lp(a) in predicting atherosclerosis and thrombosis, the significant decrease during 17ß-E_2 - dydrogesterone therapy may be of great importance.

Our analyses of antithrombin III and fibrinogen not only confirm the assumption that low dosage HRT does not increase thrombosis risk, but it even slightly points in the opposite direction by a small but significant reduction in fibrinogen after twelve cycles.

CONCLUSIONS

It is concluded that HRT with continuous micronised 17ß-E_2, 2 mg daily, in combination with dydrogesterone, 10 mg or more daily and cyclic for fourteen days during each 28 days treatment cycle gives beneficial changes in lipid metabolism, including Lp(a), indicating a reduced risk of developing CHD without unfavourably changing coagulation or glucose metabolism. The expected beneficial changes with E_2 alone are not counteracted by the intermittent addition of dydrogesterone. Therefore this oestrogen/progestogen scheme can indeed be recommended for use in HRT.

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CHAPTER 3 * Part II

A 2-YEAR STUDY ON THE BENEFICIAL EFFECTS OF 17ß-OESTRADIOL - DYDROGESTERONE THERAPY ON SERUM LIPOPROTEINS AND LP(a) IN POSTMENOPAUSAL WOMEN: NO ADDITIONAL UNFAVOURABLE EFFECTS OF DYDROGESTERONE

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ABSTRACT

Introduction: Postmenopausal hormone replacement therapy (HRT) has been described to reduce the risk of developing cardiovascular disease (CVD), which can be attributed at least in part to beneficial effects of oestrogens on serum lipoproteins. Little is known about a possible counteracting effect by the progestogen integrated in modern HRT regimen.

Objective: To study the possible changes in serum lipids, lipoproteins and apolipoproteins during HRT with special emphasis on the possible progestational effect.

Study Design: In an open-label longitudinal non-comparative study 23 healthy non-hysterectomised postmenopausal women were treated with continuous micronized 17ß-oestradiol, 2 mg daily, in combination with cyclic dydrogesterone, 10 mg daily, the first 14 days of each 28-days treatment cycle. The women were followed for up to two years.

Results: After two years serum total cholesterol and low-density lipoprotein cholesterol had decreased with 9.0% and 18%, respectively ($P<0.01$), while high-density lipoprotein cholesterol had increased with 13% ($P<0.01$). The latter change was accompanied with similar increases in apolipoprotein A-I (+16%; $P<0.01$) and A-II (+13%; $P<0.01$), while apolipoprotein B remained unchanged. Serum very low-density lipoprotein (VLDL) cholesterol and VLDL-triglycerides increased with 28% and 21%, respectively, the latter reflecting the slight increase in serum triglycerides with 21%. These values, however, remained within the normal range. Serum lipoprotein(a) decreased with 16% ($P<0.01$). All calculated atherogenic indices decreased ($P<0.01$) during the study period. Serum lipids and (apo)lipoproteins did not change after withdrawal of dydrogesterone for 14 days during the combination therapy in the last cycle studied. Serum fibrinogen decreased with 8.4% ($P<0.01$) in the first 12 cycles, after which it increased to 13% above baseline value ($P<0.01$ vs. baseline). Antithrombin III did not change and plasma glucose decreased with 5.7%.

Conclusions: This HRT regimen induces, also when given for a longer period, beneficial changes in the lipid profile, without affecting important indicators of thrombosis. Also the glucose metabolism does not seem to be interfered with. Cyclic administration of dydrogesterone does not unfavourably affect serum lipids and (apo)lipoproteins when combined with 17ß-oestradiol supplementation. Therefore, this combination hormone regimen can be recommended for use in HRT.

INTRODUCTION

Oestrogen replacement therapy (ERT) repeatedly has been described to reduce the risk of developing cardiovascular disease (CVD). In literature the estimates of this important preventive effect of ERT on CVD have been reported up to 50% [1-4]. Changes in the lipid profile during oestrogen supplementation have been considered to partly explain this phenomenon [1,5,6]. In the treatment of climacteric complaints in non-hysterectomised women the addition of a progestogen is generally accepted to prevent the development of endometrial hyperplasia and carcinoma [7]. Progestogens, however, may counteract the ERT induced beneficial changes in the lipid profile [8,9]. This may depend on the structure and the dosage of the progestogen, and the route and duration of its administration.

Dydrogesterone has been described as a metabolic inert progestogen to have little or no effect on lipid metabolism [10-12]. Recently, we reported on the beneficial changes on serum lipids and (apo)lipoproteins during one year of supplementation with micronised 17ß-oestradiol and cyclic administration of dydrogesterone [13]. Since studies investigating the changes in lipid metabolism within six to twelve months have often been criticized for their short duration, we here report the 2-year results of this hormone replacement therapy (HRT) regimen. In addition we studied the effects of withdrawal of dydrogesterone for 14 days on the course of serum lipids and (apo)lipoproteins. Further-

more, some blood clotting parameters and fasting plasma glucose were evaluated to determine a possible increase in the risk of developing thombosis or a deterioration in glucose metabolism.

SUBJECTS AND METHODS

The study was approved by the ethical committee of our hospital on beforehand. Included were healthy non-hysterectomised postmenopausal women, aged 49 to 59 years, who were recruited by advertisement in a local newspaper during the months January to May 1990. All women suffered climacteric symptoms and complaints and were amenorrhoeic for at least six months. They were screened to have follicle-stimulating hormone concentrations within the range, characteristic for the postmenopausal phase. Excluded were women using drugs affecting lipid metabolism or who used hormonal therapy for the previous two weeks. Before entering the study the purpose of the protocol had been explained and written informed consent was obtained. The results presented here concern the data of 23 women. Characteristics of the study population are given in Table I.

All women were treated with micronised 17ß-oestradiol (Zumenon®), 2 mg daily, and with dydrogesterone (Duphaston®), 10 mg daily for the first half of each 28-days treatment cycle, both orally administered (Solvay-Duphar, Weesp, The Netherlands).

Fasting venous blood sampling was performed before study entry and on day 12, 13 or 14 of the combined 17ß-oestradiol - dydrogesterone intake of cycles 6 (± 1), 12 (± 1) and 24 (± 1). Blood sampling took place after a 12-hours fast. To evaluate the possible differences in serum lipids and (apo)lipoproteins between the combined oestradiol-dydrogesterone phase and the oestradiol-only phase extra blood was sampled on cycle day 26, 27 or 28 of cycle 23 or 24.

Table I: Characteristics[a] of the study population before treatment (cycle 0) and at cycle 24

		cycle 0	cycle 24	P^b
Age	(years)	54.3 ± 3.5		
Amenorrhea	(months)	56 ± 40		
Body-mass index	(kg/m^2)	24.6 ± 2.5	24.7 ± 2.9	NS
Blood pressure: systolic	(mmHg)	126 ± 15	137 ± 16	<0.01
diastolic	(mmHg)	82 ± 7	84 ± 6	NS

[a]: mean ± SD (N=23); [b]: Wilcoxon signed-rank test.

The assay procedures for quantitation of reproductive hormones and sex hormone binding globulin (SHBG), lipids and (apo)lipoproteins, antithrombin III and fibrinogen have been described previously [13]. To determine a possible change in the ratio of apolipoprotein (Apo) A-I/A-II we measured Apo A-II at the end of the study. It was determined in the serum samples, stored at -80°C, by radial immunodiffusion, using a specific antiserum raised in rabbit against purified Apo A-II. The between-day coefficient of variation for control sera was 7.4%. All samples were measured in duplicate and were repeated if the duplicates differed more than 10%.

Statistical analysis was performed on data of the 23 women who completed the 2-year study period. Baseline values of the 4 women who did not complete the full 2-year study, for reasons reported earlier [13], were not different from the whole study population. All variables were analysed with the Wilcoxon signed-rank test. In order to adjust for multiple testing a *P*-value less than 0.01 was considered statistically significant.

RESULTS

Clinical aspects of the treatment

All women experienced a satisfactory subjective relief of symptoms and complaints during the given HRT regimen. This was the main reason that 19 of the 23 women (83%) who completed the study desired to continue the treatment after the 2-year study period. During the study period the mean systolic blood pressure increased slightly but remained within the normal range (Table I).

The mean fasting plasma glucose concentration decreased ($P<0.05$) from 5.3 (*SD*: 0.6) to 5.0 (*SD*: 0.4) mmol/l.

Reproductive hormones and SHBG

After six treatment cycles the mean serum concentrations of follicle stimulating hormone (FSH) and luteinizing hormone decreased significantly to one-half and two-third of the initial values, respectively (Table II). The mean 17ß-oestradiol concentration increased significantly almost five-fold to values seen in the follicular phase of the menstrual cycle. Furthermore, mean serum oestrone and SHBG increased significantly ten-fold and two-fold, respectively.

Table II: Reproductive hormones and SHBG before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycle 6[a]

		cycle 0	cycle 24	P^b
FSH	(IU/l)	84 ± 26	42 ± 19	<0.01
LH	(IU/l)	31 ± 10	21 ± 12	<0.01
E_2	(pmol/l)	143 ± 155	658 ± 307	<0.01
E_1	(pmol/l)	263 ± 128	2689 ± 1443	<0.01
SHBG	(nmol/l)	54 ± 26	122 ± 38	<0.01

[a]: mean ± SD (N=23); [b]: Wilcoxon signed-rank test; FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2: oestradiol; E_1: oestrone; SHBG: sex hormone binding globulin.

Lipids and (apo)lipoproteins

After six cycles the mean serum concentration of low-density lipoprotein (LDL) cholesterol decreased significantly from 4.47 to 3.63 mmol/l, whereas mean high-density lipoprotein (HDL) cholesterol increased significantly from 1.49 to 1.63 mmol/l (Table III). The decrease in LDL cholesterol was reflected in the significant decrease in total cholesterol from 6.32 to 5.66 mmol/l. Similarly, these changes were accompanied by a decrease in mean Apo B (1589 to 1494 mg/l; $P<0.01$) and an increase in Apo A-I (1417 to 1621 mg/l; $P<0.01$). Furthermore, Apo A-II rose from 395 to 423 mg/l ($P<0.05$), resulting in no significant changes in the Apo A-I/A-II ratio. Very low-density lipoprotein (VLDL) cholesterol did not change significantly. Serum triglycerides (TG) increased from 1.27 to 1.48 mmol/l ($P<0.05$), which could mostly be attributed to the rise in VLDL-TG ($P<0.05$).

Table III: Concentrations of serum lipids, (apo)lipoproteins and atherogenic indices before treatment (cycle 0), on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 6, 12 and 24, and on day 26, 27 or 28 (17ß-oestradiol-only phase) of cycle 23 or 24[a]

	cycle 0	cycle 6[e+p]	P^b	cycle 12[e+p]	P^b	cycle 24[e+p]	P^b	cycle 24[e]	P^c
Lipids & lipoproteins[d]									
Total cholesterol	6.32 ± 1.46	5.66 ± 0.96	<0.01	5.91 ± 1.00	<0 01	5.75 ± 0 95	<0.01	5.82 ± 0 91	NS
LDL cholesterol	4.47 ± 1.52	3 63 ± 0.99	<0 01	3.84 ± 1 02	<0.01	3.65 ± 0.94	<0 01	3 68 ± 0 92	NS
VLDL cholesterol	0 35 ± 0 29	0.41 ± 0.23	NS	0.38 ± 0 30	NS	0.45 ± 0 23	NS	0.46 ± 0 24	NS
HDL cholesterol	1.49 ± 0 28	1 63 ± 0.33	<0 01	1 68 ± 0 35	<0.01	1.68 ± 0.25	<0 01	1 69 ± 0 29	NS
Triglycerides	1.27 ± 0 69	1.48 ± 0.59	NS	1.45 ± 0.73	NS	1.54 ± 0.57	NS	1 53 ± 0 63	NS
VLDL triglycerides	0.67 ± 0.61	0.81 ± 0.44	NS	0.73 ± 0.57	NS	0.81 ± 0.44	NS	0 87 ± 0 53	NS
Apolipoproteins[f]									
Apolipoprotein A-I	1417 ± 191	1621 ± 161	<0.01	1672 ± 195	<0.01	1642 ± 211	<0.01	1607 ± 214[g]	NS
Apolipoprotein A-II	395 ± 48	423 ± 59	NS	443 ± 58	<0.01	445 ± 67	<0.01		
Apolipoprotein B	1589 ± 434	1494 ± 381	<0 01	1554 ± 398	NS	1608 ± 407	NS	1622 ± 416[g]	NS
Lipoprotein(a)	303 ± 467	250 ± 375	<0 01	239 ± 382	<0.01	254 ± 390	<0.01	250 ± 390[g]	NS
Atherogenic indices									
TC/HDL-C	4.45 ± 1 51	3 66 ± 1.13	<0.01	3 71 ± 1.19	<0.01	3 52 ± 0 80	<0.01	3.56 ± 0.90	NS
LDL-C/HDL-C	3.18 ± 1.38	2 38 ± 0.97	<0.01	2 44 ± 0.99	<0.01	2 25 ± 0.72	<0.01	2 27 ± 0.78	NS
Apo B/Apo A-I	1 15 ± 0 37	0 94 ± 0.29	<0.01	0.95 ± 0 29	<0.01	1.00 ± 0 31	<0.01	1 03 ± 0 33[g]	NS
ATH-IND	3.47 ± 2.57	2 20 ± 1.57	<0 01	2 26 ± 1.62	<0.01	2.15 ± 1.28	<0.01		

[a]. mean ± SD (N=23); *P*-values versus [b]: cycle 0, and versus [c]. cycle 24[e+p]; Wilcoxon signed-rank test; [e]: 17ß-oestradiol-only phase; [e+p]: combined 17ß-oestradiol-dydrogesterone phase; [d]: in mmol/l; [f]: in mg/l, [g]. N=22.

Serum lipoprotein(a) (Lp(a)) was positively skewed (skewness: 2.48; kurtosis: 6.34; range: 15 - 1890 mg/l; see figure) and the mean decreased significantly from 303 to 250 mg/l, whereas the median decreased from 76 to 67 mg/l. Furthermore, all atherogenic indices, including the recently introduced ATH-IND [14], decreased significantly. Comparing the serum lipid and (apo)lipoprotein concentrations of cycle 24 with cycle 12, no significant differences were found. However, after two years of treatment mean serum Apo B had returned to values not significantly different from the initial value, and mean Apo A-II had increased to 445 mg/l, which was significantly higher than baseline.

Effects of withdrawal of dydrogesterone

No significant differences were found comparing the serum lipid and (apo)lipoprotein concentrations of the oestradiol-only phase (cycle day 26, 27 or 28) with the combined oestradiol - dydrogesterone phase (cycle day 12, 13 or 14) of cycle 23 or 24.

Blood clotting parameters

During the 2-year study period the mean serum concentration of antithrombin III remained unchanged, whereas mean serum fibrinogen initially decreased significantly from 2367 to 2169 mg/l during the first year, after which it increased slightly though significantly to 2666 mg/l (Table IV).

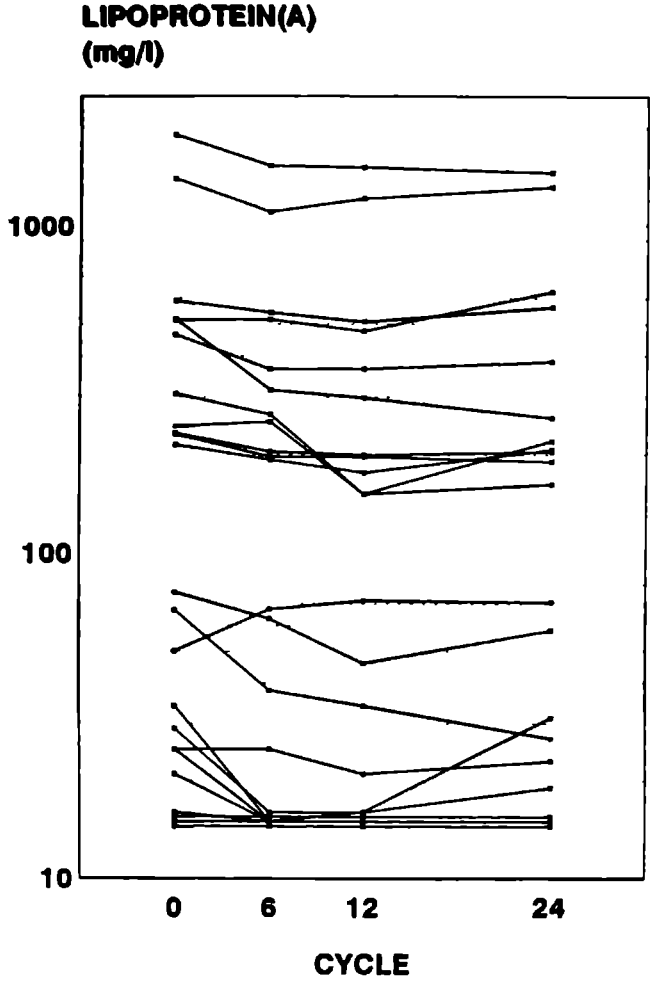

Figure: Serum concentrations of lipoprotein(a) in mg/l (*semi-logarithmic scale*) of all 23 women before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 6, 12 and 24.

Table IV: Serum concentrations of antithrombin III and fibrinogen before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 4, 6, 12 and 24[a]

	Antithrombin III (%)	P^b	Fibrinogen (mg/l)	P^b
cycle 0	96.4 ± 4.4		2367 ± 463	
cycle 4	97.2 ± 3.7	NS	2382 ± 443	NS
cycle 6	96.2 ± 5.6	NS	2239 ± 340	NS
cycle 12	95.6 ± 5.5	NS	2169 ± 378	<0.01
cycle 24	95.0 ± 6.3	NS	2666 ± 533	<0.01

[a]: mean ± SD (N=23); [b]: *P*-values versus cycle 0; Wilcoxon signed-rank test.

DISCUSSION

Only in two studies [11,12] the effects of a HRT regimen with dydrogesterone on the serum lipid and lipoprotein concentrations have been described. However, none reported on a study interval of two years. During HRT in healthy postmenopausal women with 17ß-oestradiol and cyclic dydrogesterone we found favourable changes in all serum lipids and (apo)lipoproteins measured. This favourable effect was reached within six months of treatment already, indicating that within six months a new and consistent "metabolic equilibrium" has developed. Studies on changes in lipid metabolism of six to twelve months have often been criticized for their short duration, but from our observation this seems unjustified.

In the present study the changes in the Apo A-I/A-II ratio were used as an indicator for those in the HDL_2/HDL_3 ratio. Since no changes occurred we assume that

the used HRT regimen did not influence the HDL subfraction distribution.

This HRT regimen appeared effective in decreasing the Lp(a) concentration, which can be considered as an independent risk factor for CVD [15-18]. Until now only nicotinic acid reportedly has an Lp(a) decreasing effect [19]. Earlier, postmenopausal ERT for six months showed a substantial, though insignificant, decrease in the mean Lp(a) concentration with 32% [18]. The insignificance in this decrease, however, may be attributable to the small population studied and to the large inter-individual variations of this parameter measured. In our study there was, irrespective of the pretreatment Lp(a) concentration, a gradual decreasing trend in the Lp(a) values during 24 cycles of treatment. This is in contrast to the observation of Lobo *et al.* [18]. In our study HRT was also found to be effective in women with high Lp(a) concentrations being the most at risk for developing CVD. Seven (30%) women had Lp(a) concentrations higher than 300 mg/l, which increased their CVD risk about two-fold. Consistent with a 47% decrease of Lp(a) observed during postmenopausal administration of norethisterone, 10 mg daily [20], we found a 16% decrease of Lp(a) in our study population. Our study provides additional evidence that the decrease in CVD risk during postmenopausal HRT may result in part from the significant decrease in the serum Lp(a) concentrations, and that especially women with increased CVD risk with respect to their Lp(a) concentration may benefit the most of HRT.

Furthermore, no significant differences were observed between the oestradiol-only phase and the combined oestradiol-dydrogesterone phase of cycle 23 or 24, which confirms our earlier suggestion [13] that dydrogesterone does not attenuate the beneficial changes during oestradiol-only therapy.

During the first year of treatment serum fibrinogen decreased with 8.4% to values significantly lower than baseline ($P<0.01$). Thereafter, it increased with 13% to values significantly higher than baseline during the second year ($P<0.01$ vs baseline). All values, however, remained low normal (*normal range: 2 to 4 g/l*). As these values were determined in the daily routine haematology laboratory, we consider these fluctuations as the extremes in the analytical variation which amounted to 8%. Since high levels of Lp(a)

have been demonstrated to have antifibrinolytic effects [15,21] the observed Lp(a) decrease adds to the conclusion based on the other thrombosis parameters studied, that the specific HRT regimen used has not increased thrombosis risk.

The systolic blood pressure was found to be slightly increased after two years of HRT, but stayed well within the normal range. No good explanation can be given for this change. However, since the diastolic blood pressure did not change, we may conclude that this slight change does not have any clinical relevance.

It is concluded that HRT with continuous 17ß-oestradiol, 2 mg daily, and cyclic dydrogesterone, 10 mg daily, given the first half of each 28-days treatment cycle results in substantial changes in the serum lipids and (apo)lipoproteins, Lp(a) included. All these changes suggest a decreased risk for the development of CVD. The alterations can already be observed after a treatment of six months and are maintained for at least two years. In addition, dydrogesterone did not counteract the beneficial changes in serum lipids and (apo)lipoproteins observed during 17ß-oestradiol alone.

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CHAPTER 4

EFFECT OF CONJUGATED OESTROGEN WITH AND WITHOUT MEDROGESTONE: A PROSPECTIVE STUDY

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ABSTRACT

Objective: To study the possible changes in serum lipids and (apo)lipoproteins during hormone replacement therapy with special emphasis on the possible additional effects brought about by the progestogen.

Design: Open-label randomised prospective comparative study.

Setting: Gynaecological outpatient department of a university hospital.

Patients: Thirty-three healthy hysterectomised postmenopausal women.

Interventions: Continuous oral supplementation with conjugated oestrogens, 0.625 mg daily, was administered either alone (group I; N = 18) or in combination with cyclic medrogestone, 5 mg daily during the last 12 days of each 28 days treatment cycle (group II; N = 15).

Main Outcome Measure: Changes in serum lipids, lipoproteins and apolipoproteins after three, six and thirteen treatment cycles.

Results: After one year of treatment significant increases were observed in mean concentrations of high-density lipoprotein (HDL) cholesterol, its subfractions, and apolipoprotein A-I in group I and II: HDL cholesterol: +25.2% and +12.1%, respectively; HDL_2 cholesterol: +47.4% and +23.5%, respectively; HDL_3 cholesterol: +18.1% and +11.2%, respectively; apolipoprotein A-I: +23.0 and +14.8%, respectively. Comparing the two study groups no significant differences were found in lipid changes during the study period, except for HDL_2 cholesterol.

Conclusion: Supplementation with conjugated oestrogens, with and without medrogestone, and given for a longer period, demonstrated a beneficial influence on serum lipoproteins with almost no differences between the two treatment regimen.

INTRODUCTION

Oestrogen replacement therapy reduces the risk of developing coronary heart disease in postmenopausal women [1-3]. This can be partly attributed to the beneficial changes in lipid metabolism. Especially a rise in high-density lipoprotein (HDL) cholesterol is considered as a beneficial change, whereas an increase in low-density lipoprotein (LDL) cholesterol indicates an increased risk of developing coronary heart disease [4,5]. In non-hysterectomised women it is recommended to combine oestrogen replacement therapy with a progestogen for prevention of hyperplasia and carcinoma of the endometrium. However, some progestogens may offset the effects of oestrogens, depending on the type and the dosage of the progestogen and the route and duration of its administration. Medrogestone is a derivative of 17α-hydroxy-progesterone of which only few studies exist concerning its effects on lipid metabolism [6-8].

The purpose of this randomised prospective study was to investigate the effects on lipid metabolism of continuous oral supplementation with conjugated oestrogens in combination with cyclic oral administration of medrogestone. Changes in the concentrations of serum lipids, lipoproteins and apolipoproteins were followed up to one year and were compared with a control group, receiving conjugated oestrogens only.

SUBJECTS AND METHODS

Sample size, necessary to detect a 20% difference in HDL cholesterol concentration between both study groups, with 80% power using a two-sided test at the 5% significance level, was estimated at 16 women per study group. Volunteers were recruited by advertisement in a local newspaper during the months June to November 1990. Thirty-five healthy hysterectomised postmenopausal women were selected with a serum FSH concentration greater than 40 IU/l and a serum oestradiol (E_2) concentration

lower than 150 pmol/l. Excluded were women with history or active presence of thrombo-embolic disorders, cerebrovascular accident or ischaemic heart disease, chronic renal or hepatic disease, and known or suspected oestrogen-dependent neoplasia. Also excluded were women with gallbladder disease, neuro-ocular disorders, known hypersensitivity to oestrogens and/or progestogens and use of oral oestrogen or progestogen containing medication within three months prior to the prestudy screening. Also not included were women with blood pressure higher than 160/90 mmHg, liver disease, or with endocrine diseases, malabsorbtion disorders, any malignancy and cervical Papanicolaou smear of Class III or greater (in case of supra-vaginal hysterectomy). Women smoking more than 15 cigarettes a day, with obesity, known alcohol or drug abuse and with any evidence of premalignant or malignant changes in the prestudy mammogram were also not included. During the study period chronic steroid medication, oestrogens or progestogens other than the study medication, and lipid lowering agents were not permitted. Baseline serum total cholesterol and triglyceride levels had to be respectively 6.7 mmol/l and 2.8 mmol/l or lower.

The study was approved by the ethical committee of our hospital on beforehand. Before entering the study the purpose of the protocol had been explained and written informed consent was obtained.

Physical examination and fasting venous blood sampling were performed at prestudy screening and between day 22-28 of the 28 days cyclic treatment regimen of cycles three, six and thirteen. Mammography was carried out at prestudy screening and cycle thirteen. Fasting venous blood sampling for lipid baseline values was repeated at least one week after prestudy screening, but prior to starting the treatment regimen. Venous blood sampling was performed after a minimum 12 hours fast. Laboratory safety screening (hematology, blood chemistry and routine urinalysis) was done at prestudy screening and at cycles six and thirteen.

Serum concentrations of FSH were measured with a specific immunoradiometric assay (Medgenix, Fleurus, Belgium) which has been described elsewhere [9]. The intra-assay and inter-assay variablities for means of duplicate measurements were calculated

from several pools of serum and ranged between 1.8-3.6% and 6.8-8.2%, respectively. Serum concentrations of E_2 were measured with an in-house radioimmunoassay as described elsewhere [10]. The intra-assay and inter-assay variabilities for means of duplicate measurements were respectively 4.3% and 7.9%.

Serum lipid and lipoprotein levels were determined by standard laboratory procedures. Serum total cholesterol and triglycerides were measured by enzymatic methods using commercially available reagents (CHOD-PAP cholesterol reagent, cat. no. 236691, and GPO-PAP triglycerides reagent, cat. no. 701904, Boehringer Mannheim, Mannheim, FRG). HDL cholesterol in serum was determined by using the precipitation method with sodium phosphotungstate-Mg^{2+} [11] and LDL cholesterol was calculated with the Friedewald-formula [12]. Serum lipoproteins were isolated by density gradient ultracentrifugation [13] using a SW-40 Ti rotor for 18 hours 40,000 r.p.m. at 4°C. Afterwards the top fraction (about 1.5 ml), containing very low-density lipoprotein was collected with a Pasteur pipette. The gradient was then fractionated into 40 fractions of 250 microliter each using a capillary placed on the bottom of the tube attached to a micropump and a fractioncollector. Cholesterol was measured in each fraction using an enzymatic method (CHOD-PAP, Boehringer Mannheim, Mannheim, FRG). To find the precise position of the density gradient along the lipoprotein pattern (1.210 - 1.063 kg/l) and the cut-off point around and between HDL_2 cholesterol and HDL_3 cholesterol (1.125 kg/l) the density of ten fractions was measured with a DMA 602 M densitometer (Mettler Paar, Austria) in each gradient. The volume of each fraction was calculated from its weight and density. Small differences between total HDL cholesterol and the sum of HDL_2 cholesterol and HDL_3 cholesterol could occur because any cholesterol in the very high-density lipoprotein fraction with a density higher than 1.210 kg/l was not measured. Apolipoprotein A-I and apolipoprotein B were determined by rate-nephelometry using polyvalent monospecific anti-apolipoprotein A-I and anti-apolipoprotein B antiserum in a Beckman Array Protein System [14]. The intra- and inter-assay coefficients of variation were 5% and 8%, respectively.

We selected 35 women who were in chronological order and at random allocated

to one of the two study groups by using a computerized randomisation list. Group I, containing 18 women, was treated with conjugated oestrogens, 0.625 mg (Premarin®) daily continuously. Group II, containing 17 women, was treated with conjugated oestrogens, 0.625 mg daily continuously, in combination with cyclic medrogestone, 5 mg (Colpro®) daily, given the last 12 days of each 28 days treatment cycle (Wyeth Laboratories, Hoofddorp, The Netherlands).

There were five drop outs, two in group I and three in group II. In group I one woman stopped the medication in treatment cycle four because of progressive nausea. The second one had to stop the medication in treatment cycle eight because a haemangioma of the liver was diagnosed by sonography that was performed in order to explain severe atypical epigastric pain. In group II one woman suffered from painful varicosis of the left calf and stopped the medication in treatment cycle two. The second one discontinued the medication in treatment cycle three because of vertigo during the medrogestone intake and finally the last woman had to stop the medication in treatment cycle ten because of symptomatic cholelithiasis.

Statistical analysis: Lipid baseline values were calculated by averaging the screening value and the value obtained at least one week later. End point analyses were performed on the data of 33 women after excluding two women (group II) who dropped out before lipid assays in cycle three could have been performed. The last visit carried forward (LVCF) procedure was used in the case of the other three drop outs (two women in group I and one woman in group II). Since almost all variables were normally distributed (tested by skewness and kurtosis), statistical significance of within-group changes and between-group differences was tested with the repeated measures analysis of variance (ANOVA, two-factor analysis). For analysis of separate within-group changes we used the Student paired *t* test and for between-group differences (in descriptive statistics) the Student unpaired *t* test was applied. Variables are given as means ± standard deviation (SD). A *P*-value lower than 0.05 was considered to be statistically significant. Statistical analyses were performed with the Dyna-stat computer program

(Dynamic Microsystems, Inc., Washington, D.C., Pittsburgh, PA, USA).

RESULTS

Characteristics of the two study groups are given in Table I. There were no statistically significant differences between the groups with regard to age at study entry, age at hysterectomy, wash out period, serum concentrations of FSH and E_2, body-mass index (BMI) and blood pressure.

Table I: Characteristics of the study groups[a]

		Group I		Group II		P^b
Age	(years)	53.1 ± 2.4	(50-59)	54.6 ± 3.3	(50-59)	NS
HE-age[c]	(years)	41.8 ± 6.0	(30-51)	43.3 ± 3.3	(36-47)	NS
Wash-out	(months)	15.5 ± 2.1[d]	(3.5-60)	33.0 ± 48.9[e]	(5-120)	NS
FSH	(IU/l)	87 ± 17	(50-120)	87 ± 30	(43-140)	NS
Oestradiol	(pmol/l)	100 ± 20	(75-140)	92 ± 19	(75-120)	NS
BMI	(kg/m^2)	25.1 ± 2.4	(20.4-28.3)	25.3 ± 1.2	(23.5-27.9)	NS
Blood pressure:						
- systolic	(mmHg)	128 ± 18	(100-155)	135 ± 13	(110-160)	NS
- diastolic	(mmHg)	83 ± 8	(60-90)	83 ± 6	(70-90)	NS

Group I (N=18): conjugated oestrogens 0.625 mg only; Group II (N=15): conjugated oestrogens 0.625 mg and cyclic medrogestone 5 mg on days 17-28 of the 28 days treatment regimen.

[a]: values are means ± SD (range); [b]: Student unpaired *t* test for between-group differences; [c]: age at hysterectomy; [d]: N=10; [e]: N=5.

In group II we found a temporary decrease in the mean systolic blood pressure to 127 ± 8 mmHg ($P<0.05$ vs baseline) during cycle six that returned to the baseline value at cycle thirteen.

During the hormone therapy all women experienced a satisfactory relief of climacteric and postmenopausal complaints and symptoms. Twenty-three of the 30 women (77%) who completed the study desired to continue the oestrogen replacement therapy. Of the five drop outs the nausea and vertigo in two patients may obviously be a result of the given medication, whereas we can not exclude that the other diagnosed reasons for drop out were present already at study entry.

Comparing the two study groups no significant differences were found in lipid changes during the study period, except for HDL_2 cholesterol ($P<0.05$; Table II). After three cycles serum total cholesterol initially decreased in both groups (group I: 2.2%, $P>0.05$; group II: 6.7%, $P<0.05$) after which there was a rise resulting in concentrations not significantly different from baseline values. Furthermore, the serum concentrations of very low-density lipoprotein cholesterol tended to increase, but this was not found statistically significant. During the first three cycles serum concentrations of LDL cholesterol decreased significantly ($P<0.01$), with 11.2% and 13.7% in group I and II, respectively. In group I this was followed by a rise during the course of the study to values that were not significantly different from baseline values whereas in group II the decrease sustained. This difference between the treatment groups did not reach statistical significance. HDL cholesterol serum concentrations rose significantly in both groups ($P<0.01$) , but the most (25.2%) in group I (Figure 1). The cholesterol values along the density-gradient between 1.210 and 1.063 kg/l (about 30 fractions) revealed a bimodal distribution in most patients reflecting HDL_2 cholesterol (the lesser dense subclass) and HDL_3 cholesterol. The determination of the two subclasses was based on calculation of the area under the curve. The recovery was 99.7 ± 5%.

Table II: Serum concentrations of lipids and (apo)lipoproteins in group I and II[a]

		baseline	cycle 3	cycle 6	cycle 13	AN^W	AN^B
TC[c]	I	5.86 ± 0.75	5.73 ± 0.73	5.83 ± 0.75	6.08 ± 0.89^{*,S}	#	*
	II	6.29 ± 0.56	5.87 ± 0.49$^{\#}$	5.87 ± 0.75$^{\#}$	5.90 ± 0.80*		
VLDL-C[c]	I	0.57 ± 0.22	0.66 ± 0.33	0.63 ± 0.29	0.70 ± 0.36	NS	NS
	II	0.74 ± 0.21	0.74 ± 0.29	0.77 ± 0.37	0.71 ± 0.36		
LDL-C[c]	I	3.84 ± 0.66	3.41 ± 0.76$^{\$}$	3.51 ± 0.77$^{\#}$	3.65 ± 0.81	$	NS
	II	4.10 ± 0.50	3.54 ± 0.46$^{\$}$	3.56 ± 0.68$^{\$}$	3.60 ± 0.64$^{\$}$		
HDL-C[c]	I	1.39 ± 0.28	1.67 ± 0.36$^{\$}$	1.69 ± 0.41$^{\$}$	1.74 ± 0.42$^{\$}$	$	*
	II	1.40 ± 0.28	1.58 ± 0.31$^{\$}$	1.55 ± 0.31$^{\$}$	1.57 ± 0.35$^{\$}$		
HDL_2-C[c]	I	0.38 ± 0.15	0.59 ± 0.24$^{\$}$	0.60 ± 0.29$^{\$}$	0.56 ± 0.26$^{\$}$	$	#
	II	0.34 ± 0.13	0.51 ± 0.21$^{\$}$	0.42 ± 0.16$^{\$}$	0.42 ± 0.19$^{\$}$		
HDL_3-C[c]	I	0.94 ± 0.16	1.01 ± 0.17$^{\#}$	1.03 ± 0.17$^{\$}$	1.11 ± 0.23$^{\$}$	$	NS
	II	0.98 ± 0.19	1.01 ± 0.17	1.06 ± 0.21*	1.09 ± 0.23$^{\#}$		
TG[c]	I	1.26 ± 0.42	1.52 ± 0.78$^{\#}$	1.59 ± 0.77$^{\#}$	1.54 ± 0.73$^{\#}$	#	NS
	II	1.58 ± 0.46	1.71 ± 0.68	1.79 ± 0.75	1.77 ± 0.86		
VLDL-TG[c]	I	0.57 ± 0.28	0.73 ± 0.54	0.71 ± 0.46	0.75 ± 0.49*	NS	NS
	II	0.81 ± 0.35	0.83 ± 0.45	0.80 ± 0.48	0.89 ± 0.59		
Apo A-I[d]	I	152 ± 20	179 ± 22$^{\$}$	179 ± 24$^{\$}$	187 ± 32$^{\$}$	$	NS
	II	155 ± 24	179 ± 23$^{\$}$	176 ± 25$^{\$}$	178 ± 27$^{\$}$		
Apo B[d]	I	134 ± 24	129 ± 25	130 ± 27	136 ± 27	NS	NS
	II	148 ± 18	138 ± 23*	141 ± 30	140 ± 30		

Group I (N=18): conjugated oestrogens 0.625 mg only; Group II (N=15): conjugated oestrogens 0.625 mg and cyclic medrogestone 5 mg on days 17-28 of the 28 days treatment regimen.

[a]: values are means ± SD; concentrations in [c]: mmol/l and [d]: mg/dl; statistics by [S]: Student paired *t* test (differences versus baseline) and ANOVA (AN^W: within-group changes; AN^B: between-group differences); *: $0.05 \leq P < 0.1$; #: $P < 0.05$; $: $P < 0.01$; TC: total cholesterol; VLDL-C: very low-density lipoprotein chol; LDL-C: low-density lipoprotein chol; HDL-C: high-density lipoprotein chol; HDL_2-C: high-density $lipoprotein_2$ chol; HDL_3-C: high-density $lipoprotein_3$ chol; TG: triglycerides; VLDL-TG: very low-density lipoprotein TG; Apo A-I: apolipoprotein A-I; Apo B: apolipoprotein B.

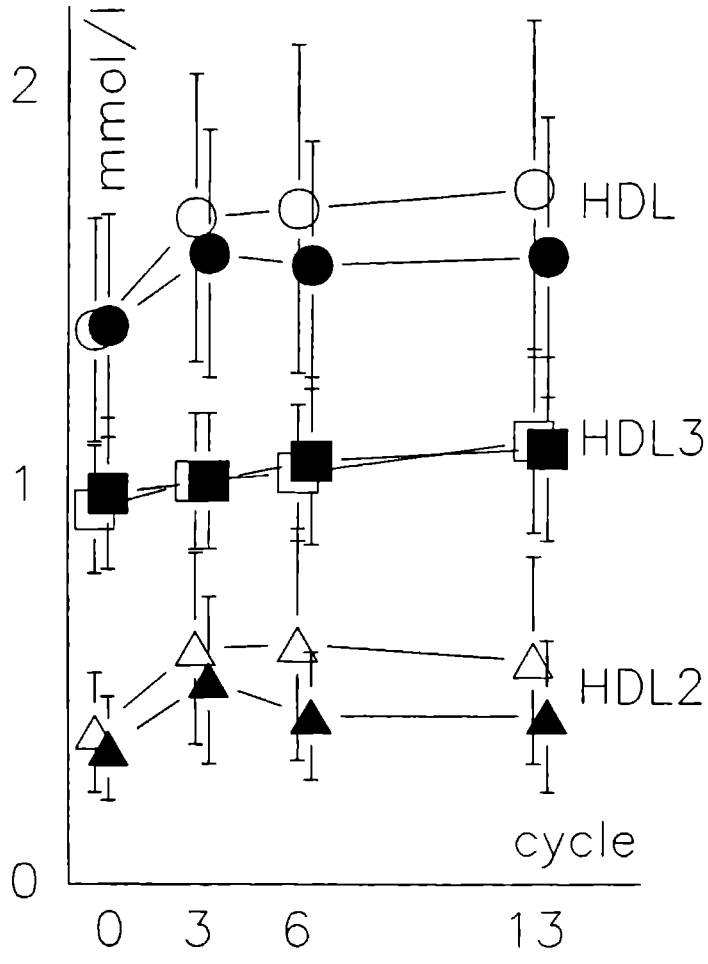

Figure 1: Serum concentrations of total HDL cholesterol (HDL) and its subfractions HDL_2 (HDL2) and HDL_3 (HDL3) cholesterol in mean ± SD at baseline (cycle 0) and on day 22-28 of treatment cycle 3, 6 and 13. Group I (N=18; *open symbols*): conjugated oestrogens 0.625 mg only; Group II (N=15; *filled symbols*): conjugated oestrogens 0.625 mg and cyclic medrogestone 5 mg on days 17-28 of the 28 days treatment regimen.

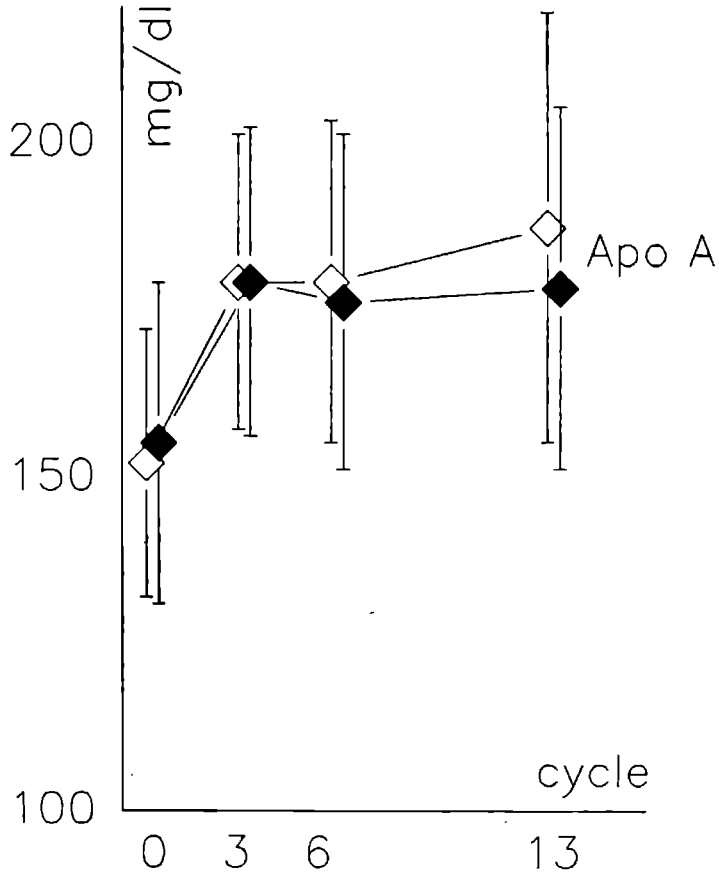

Figure 2: Serum concentrations of apolipoprotein A-I (Apo A) in mean ± SD at baseline (cycle 0) and on day 22-28 of treatment cycle 3, 6 and 13. Group I (N=18; *open symbols*): conjugated oestrogens 0.625 mg only; Group II (N=15; *filled symbols*): conjugated oestrogens 0.625 mg and cyclic medrogestone 5 mg on days 17-28 of the 28 days treatment regimen.

During the first three cycles serum concentrations of HDL_2 cholesterol rose significantly ($P<0.01$) in both groups (55.3% in group I and 50.0% in group II) and in the course of the study HDL_2 cholesterol levels slightly decreased in group II but remained significantly higher than baseline values ($P<0.05$) whereas in group I the rise sustained. Furthermore, serum concentrations of HDL_3 cholesterol rose significantly in both groups (group I: 18.1%, $P<0.01$; group II: 11.2%, $P<0.05$). Serum triglycerides rose with 22.2% ($P<0.05$) in group I and with 12.0% ($P>0.05$) in group II and similar increases, though statistically not significant, were seen in very low-density lipoprotein triglycerides levels. During the study period apolipoprotein A-I rose significantly ($P<0.01$) in both groups (group I: 23.0%, group II: 14.8%; Figure 2) whereas the apolipoprotein B concentrations remained similar.

The atherogenic indices (Table III) apolipoprotein B/apolipoprotein A-I, LDL cholesterol/HDL cholesterol and total cholesterol/HDL cholesterol all decreased significantly in both groups ($P<0.01$).

Table III: Atherogenic indices in group I and II[a]

		baseline	cycle 3	cycle 6	cycle 13	AN^W	AN^B
Apo B/A	I	0.90 ± 0.21	0.74 ± 0.21$^{\$,S}$	0.75 ± 0.22$^{\$}$	0.75 ± 0.22$^{\$}$	\$	NS
	II	0.98 ± 0.23	0.79 ± 0.19$^{\$}$	0.82 ± 0.22$^{\$}$	0.80 ± 0.22$^{\$}$		
LDL/HDL	I	2.88 ± 0.85	2.16 ± 0.73$^{\$}$	2.21 ± 0.74$^{\$}$	2.20 ± 0.69$^{\$}$	\$	NS
	II	3.08 ± 0.87	2.35 ± 0.66$^{\$}$	2.46 ± 0.75$^{\$}$	2.44 ± 0.80$^{\$}$		
TC/HDL	I	4.36 ± 1.03	3.61 ± 0.98$^{\$}$	3.64 ± 0.99$^{\$}$	3.67 ± 0.96$^{\$}$	\$	NS
	II	4.66 ± 0.99	3.86 ± 0.89$^{\$}$	3.94 ± 0.92$^{\$}$	3.92 ± 0.98$^{\$}$		

Group I (N=18): conjugated oestrogens 0.625 mg only; Group II (N=15): conjugated oestrogens 0.625 mg and cyclic medrogestone 5 mg on days 17-28 of the 28 days treatment regimen.
[a]: values are means ± SD; statistics by ^{S}: Student paired *t* test (differences versus baseline) and ANOVA (AN^W: within-group changes; AN^B: between-group differences); \$: $P<0.01$; Atherogenic indices: apolipoprotein B/apolipoprotein A-I (Apo B/A), LDL cholesterol/HDL cholesterol (LDL/HDL), and total cholesterol/HDL cholesterol (TC/HDL).

DISCUSSION

The hormone replacement regimen with continuous conjugated oestrogens and cyclic medrogestone as used in this study has been described as effective and satisfactory concerning relief of climacteric complaints and symptoms [15,16]. It has also been described as being effective in inducing secretory changes in a proliferative endometrium [17].

The purpose of this study was to investigate possible changes in serum lipids, lipoproteins and apolipoproteins during the given combination therapy and to determine any additional effect attributable to the cyclic administration of medrogestone. As to the latter, no significant differences were found between the lipid parameters measured during the treatment with conjugated oestrogens alone and combination therapy with conjugated oestrogens and cyclic medrogestone, except for serum HDL_2 cholesterol concentrations. Since the difference in HDL cholesterol between the study groups was 10%, no statistical significance can be expected considering the sample size estimation. In order to detect small differences as observed in this study, and reach statistical significance, one should include at least 80 women in each study group. Although our findings are in agreement with earlier reports [6-8] there were clear differences in the design of the study. In our study we supplemented conjugated oestrogens 0.625 mg instead of 1.25 mg [6,7], with continuously administered conjugated oestrogens instead of using a pill-free interval [7,8]. Teichmann *et al.* [6] described a cyclic treatment regimen of "22 + 6 days" of which the nature can not be clearly understood. They found small but significant increases in HDL cholesterol and apolipoprotein A-I whereas Sonnendecker *et al.* [8] found significantly increased levels of HDL cholesterol and HDL_2 cholesterol and significantly decreased levels of total cholesterol and LDL cholesterol. After thirteen cycles of hormone treatment we found in both groups a significant increase in serum levels of HDL cholesterol, HDL_2 and HDL_3 cholesterol, where in group I the rise in HDL_2 cholesterol was significantly stronger ($P<0.05$) then in group II (Figure 1). Concerning the value of HDL subfraction analysis, Gordon *et al.* [18] found a consistent inverse relation between

HDL cholesterol levels and coronary heart disease event rates. According to Demacker *et al.* [19] the tedious subfractionation of HDL fails to add more information to that of total HDL cholesterol, and any rise can be regarded as beneficial. On the other hand, HDL_2 cholesterol is the most variable part of HDL cholesterol, and its determination could enhance the discriminatory power to find statistical differences [20]. Indeed, HDL_2 cholesterol showed the largest percentual changes, as compared to total HDL cholesterol and HDL_3 cholesterol, and it was the only variable in which significant differences were found between the changes in both groups. This difference may indicate a less beneficial effect on HDL_2 cholesterol due to the addition of medrogestone. However, despite of this difference, HDL_2 cholesterol rose in both groups, which can be considered as beneficial with regard to the risk of developing coronary heart disease. This also applies for the changes in HDL cholesterol and HDL_3 cholesterol as found in this study, whereas HDL_2 cholesterol demonstrated the most potential to detect differences between the two groups. Serum LDL cholesterol decreased in both groups, even though the decrease in group I was not statistically significant after thirteen cycles. Serum triglyceride levels rose but remained below 2 mmol/l. In this study apolipoprotein A-I, which is the main carrier protein of HDL, rose significantly and in parallel with HDL cholesterol in both groups (Figure 2) as was described by Teichmann *et al.* [6], but was not found by Sonnendecker *et al.* [8]. Teichmann *et al.* also found a decrease in apolipoprotein B in contrast to our findings. The changes in the calculated atherogenic indices indicate in another way the beneficial effect on the lipoproteins of both treatment regimen.

Therefore, in both treatment groups changes in lipoprotein levels occurred which can be considered as beneficial with regard to the development of cardiovascular disease: a rise in HDL cholesterol and its major apolipoprotein, apolipoprotein A-I, while apolipoprotein B remained unchanged. Except for HDL_2 cholesterol no other significant differences were observed between the two study groups, but this may, however, be the result of the small sample size. To draw a firm conclusion on this issue it is recommended to extend the study with larger study groups.

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CHAPTER 5

CHANGES IN THE LOW-DENSITY LIPOPROTEIN PROFILE DURING 17ß-OESTRADIOL - DYDROGESTERONE THERAPY IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Introduction: Postmenopausal women are at increased risk for developing coronary heart disease (CHD) most likely due to alterations in plasma lipoproteins modulated by hormonal changes. Oestrogen supplementation may exert a protective role on CHD partly by the beneficial effects of oestrogens on lipid metabolism. Especially a rise in high-density lipoprotein (HDL) is considered as a beneficial change whereas an increase in low-density lipoprotein (LDL) indicates an increased risk for developing CHD. Also LDL subfractions have been suggested to be of importance in the development of atherosclerosis.

Objective: To study the changes in the LDL subfraction profile during postmenopausal hormone replacement therapy.

Design: An open-label, non-comparative, prospective study.

Patients: Twenty-three healthy non-hysterectomised postmenopausal women.

Intervention: Continuous oral supplementation with 17ß-oestradiol, 2 mg daily, and cyclic administration of dydrogesterone, 10 mg daily for the first 14 days of each 28 days treatment cycle.

Duration: One year.

Measurements: LDL subfractions, as found by gradient gel electrophoresis. Changes were studied by visual observation of the gels and by analysis of the densitometric scans of the gels.

Results: During treatment we observed significant changes in the LDL subfraction profile towards a smaller particle size ($P<0.001$). These changes could almost completely be attributed to the hormonal treatment regimen ($P<0.01$).

Conclusions: The observed changes in LDL particle size may indicate an effect, partially opposite to the other reported changes in the lipid profile. However, it appears that a shift in LDL particle distribution to a smaller size may not per se be positively correlated with cardiovascular disease. There is a need for more prospective research

towards the effects of hormone replacement therapy in the protection against cardiovascular disease.

INTRODUCTION

Hormone replacement therapy (HRT) reportedly has been described to reduce the risk of developing coronary heart disease (CHD) in postmenopausal women, which may be partly due to the beneficial changes in the lipid profile [1,2].

Small low-density lipoprotein (LDL) particles are associated both with the male sex and increased age [3], and have also been identified as risk factor for CHD [4-6].

We studied possible changes in the LDL subfraction profile, as found by gradient gel electrophoresis (GGE), in healthy postmenopausal women during one year of HRT. The changes were related to those in serum lipids, lipoproteins and apolipoproteins.

SUBJECTS AND METHODS

The study was approved by the ethical committee of our hospital and before study entry the participants gave their written informed consent. Recruited were 23 healthy non-hysterectomised postmenopausal women (*mean age* 54.3 yrs; *SD* 3.5 yrs), who were amenorrhoeic for at least six months (*mean* 56 mths; *SD* 40 mths) and who were screened to have serum follicle-stimulating hormone (FSH) concentrations within the range, characteristic for the postmenopausal phase (*mean* 84 IU/l; *SD* 26 IU/l). Selection criteria and assays have been described elsewhere [7].

All women were treated with micronised 17ß-oestradiol (Zumenon®), 2 mg daily,

and with dydrogesterone (Duphaston®), 10 mg daily for the first half of each 28 days treatment cycle, both orally administered (Solvay Duphar, Weesp, The Netherlands).

Determination of the LDL subfraction profile was performed in venous blood samples (evacuated collection tubes; Corvac®, Becton Dickinson, 38241, Cedex, France), taken after a twelve hours fast, before and after one year of HRT in all 23 women who completed the one year study period. Sera were stored at -80°C until the end of the study, and then assayed. Storage under these conditions has been shown not to influence the LDL subfraction profile [3,8].

LDL subfractions were separated by GGE as described previously [8], using 2-16% polyacrylamide gradient gels (PAA 2/16 gels; Pharmacia, Uppsala, Sweden). Serum aliquots were mixed with glycerol and bromphenol blue. Gels were pre-electrophoresed for 20 min at 70 V, afterwards we added 10 μl of the prepared samples to each lane of the gel, and electrophoresis was continued for another 30 min at 70 V, followed for 5.5 h at 400 V and 8°C. After electrophoresis the gels were fixated in ethanol and the lipoprotein bands were stained with Sudan Black B (Paragon Lipostain; Beckman Instruments) followed by destaining in ethanol. Samples of the same subject were run on the same gel and in two nearest lanes. A serum standard was prepared by pooling sera to cover the complete spectrum of subfractions. In each gel this serum was applied in two lanes to serve as reference for the LDL bands in the samples. The pooled serum consisted of sera of six persons with a predominant LDL-1 (*d*: 1.030 to 1.033 kg/l), LDL-2 (*d*: 1.033 to 1.040 kg/l), or LDL-3 (*d*: 1.040 to 1.045 kg/l) subfraction [8].

Changes in the LDL banding pattern with respect to change in the migration distance, reflecting LDL particle size, and the relative intensity, reflecting relative concentration, of each band were judged qualitatively by unblinded, but independent, visual inspection of the gels by three of the authors.

Gels were also analysed quantitatively after densitometric scanning in triplicate with the LKB 2202 Ultrascan XL enhanced laser densitometer. The scans were analysed by calculating the change in relative peak height after therapy to determine changes in the

relative contribution of each LDL subfraction to total LDL. Furthermore, we evaluated the change in the migration distance and relative peak height of the predominant LDL band.

Statistical analysis

All changes were tested by means of the Wilcoxon signed-rank test. Qualitative changes in the LDL banding pattern, with respect to change in the migration distance and the relative intensity of each band, determined by visual inspection were tested with the sign-test. Inter-observer agreement on qualitative changes was measured by calculating the *kappa*-coefficient [9]. Stepwise multiple regression analysis was used to examine significant contributions of serum lipids and (apo)lipoproteins to the variation in the LDL subfraction profile. By using the test on the intercept we examined whether HRT had a significant additional influence on the LDL subfraction profile. Statistical analyses were performed with the Statistical Analysis System software package (SAS Institute Inc., Cary, NC).

RESULTS

Lipids and lipoproteins

During the hormone therapy mean LDL cholesterol decreased from 4.47 (*SD* 1.52) to 3.84 (*SD* 1.02) mmol/l ($P<0.001$), parallel with the decrease in total cholesterol from 6.32 (*SD* 1.46) to 5.91 (*SD* 1.00) mmol/l ($P<0.01$). High-density lipoprotein (HDL) cholesterol increased from 1.49 (*SD* 0.28) to 1.68 (*SD* 0.35) mmol/l ($P<0.001$), while serum triglycerides (TG) increased slightly, but significantly ($P<0.05$) from 1.27 (*SD* 0.69) to 1.45 (*SD* 0.73) mmol/l. Apolipoprotein (Apo) A-I increased from 1417 (*SD* 191) to 1672 (*SD* 195) mg/l ($P<0.001$), while Apo B showed a slight non-significant decrease

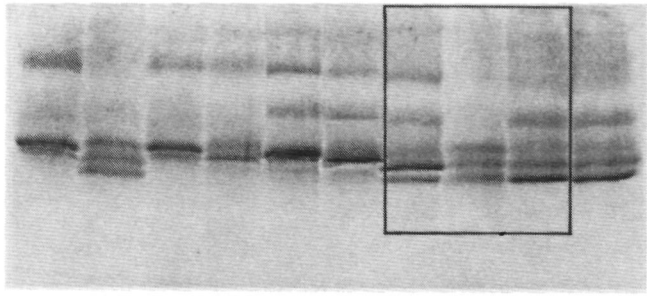
from 1589 (*SD* 434) to 1554 (*SD* 398) mg/l. Lipoprotein(a) decreased from 303 (*SD* 467) to 239 (*SD* 382) mg/l ($P<0.001$).

LDL subfraction profile

GGE of the individual sera revealed discrete LDL subfraction bands, that were identified as LDL-1, LDL-2 and LDL-3, with particle sizes of on average 267 Å, 257 Å and 251 Å, respectively (data originating from work by Austin *et al.* [5]) (see Figure). This predominance of three LDL subfractions was confirmed by densitometric scanning of the gels. Each lane was scanned in triplicate. Within all triplicates, the number of peaks and the migration distance of each peak agreed well, and the shapes of the densitometric curves were similar.

Visual inspection of the gels revealed that during the therapy in 48% ($P<0.05$) of the gels a shift in the total LDL profile towards a smaller LDL particle size was observed by all three observers (Table). In 74% similar observations were made by all the three authors (*kappa* = 0.69; SE = 0.10) with respect to change in the migration distance and relative intensity of each band. According to the calculated *kappa*-coefficient there was a substantial interobserver agreement [9].

Before treatment the mean percentual contributions of each LDL band to total LDL, calculated as mean relative peak heights, were: LDL-1: 36% (*SD* 22%; *median* 34%, Q_1 19%, Q_3 55%), LDL-2: 44% (*SD* 18%; *median* 41%, Q_1 28%, Q_3 55%), LDL-3: 20% (*SD* 15%; *median* 15%, Q_1 11%, Q_3 23%), and the predominant peak: 59% (*SD* 9%; *median* 58%, Q_1 51%, Q_3 63%). In 21 individuals (91%) the predominant peak was the LDL-1 or LDL-2 peak.



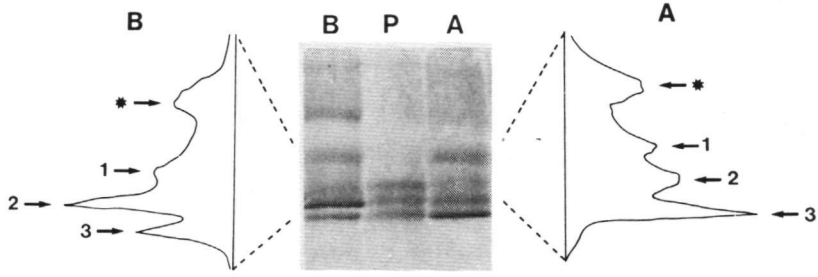


Figure: Effect of HRT on the LDL subfraction profile of one subject as observed by gradient gel electrophoresis and densitometric scanning of the gel.
During HRT the size of the LDL shifts towards a predominance of smaller LDL particles:
Top: Upper part (40%) of one gel showing the VLDL and LDL range, covering the molecular size between 180 Å and 800 Å.
Bottom: Middle: lanes of one subject before (B) and after (A) HRT, and of the pool serum (P), as shown in the frame (*top*). *Left*: densitometric scan of the gel before HRT. *Right*: densitometric scan of the gel after HRT.
1: LDL-1 (267 Å); *2*: LDL-2 (257 Å); *3*: LDL-3 (251 Å); *: Lp(a)-band; The values of molecular size distribution of the main subfractions originate from Austin *et al. (ref. 5)*.

Table: Qualitative observations[a] of changes in the LDL banding profile[b] related to particle size

	Author			Three Similar Observations	
	I	II	III		
Particle size[c]					
- Increase	2	2	4	2	(9%)
- No change	8	8	6	4	(17%)
- Decrease	13 [e]	13 [e]	13 [d]	11	(48%)
Total	23	23	23	17 [f]	(74%)

[a]: independent visual observations by three of the authors; [b]: with respect to changes in the migration distance (reflecting LDL particle size), and the relative intensity (reflecting relative concentration), of each band; [c]: changes are described as change in LDL particle size; changes tested with the sign-test: [d]: $P<0.05$; [e]: $P<0.01$ (decrease versus increase in particle size); [f]: inter-observer agreement measured by *kappa*-coefficient: *kappa* = 0.69 (*SE* = 0.10); N = 23.

Further, analysis of data obtained by densitometric scanning revealed that HRT was associated with on average a small decrease in the mean relative heights of the LDL-1 (*mean* -4%, *SD* 16%; *median* -4%, Q_1 -14%, Q_3 +1%; P=0.154) and LDL-2 (*mean* -3%, *SD* 18%; *median* +1%, Q_1 -22%, Q_3 +10%; P=0.930) peaks in combination with a considerable increase in the relative height of the LDL-3 peak (*mean* +7%, *SD* 12%; *median* +5%, Q_1 +1%, Q_3 +7%; P=0.0005). So, the relative contribution of LDL-3 to total LDL increased, indicating a more dense subfraction profile. The mean relative

height of the predominant peak decreased significantly (*mean* -5%, *SD* 14%; *median* -5%, Q_1 -14%, Q_3 0%; P=0.025) and its relative migration distance increased (*mean* +0.21 units, *SD* 0.58 units; *median* +0.13 units, Q_1 -0.20 units, Q_3 +0.70 units; P=0.131), reflecting a change towards a smaller LDL particle size. Thus, during HRT the average size of the LDL particles decreased.

Stepwise regression analysis on all lipids and (apo)lipoproteins described in the results section, revealed that only total serum Apo B levels (as determined by nephelometry) correlated significantly to the relative height of the LDL-3 peak ($P<0.05$). An estimate of the additional effect of the given HRT on the relative height of the LDL-3 peak, as found by the test on the intercept, is 0.08 (SE=0.02) ($P<0.01$). So, HRT had a significant additional influence on the relative height of the LDL-3 peak.

DISCUSSION

Earlier, we reported on the changes in serum lipids and (apo)lipoproteins during sequential 17ß-oestradiol - dydrogesterone therapy in postmenopausal women. With regard to the risk for CHD, these changes can be considered to be favourable with the exception of the small rise in the serum TG [7]. The concentration of LDL cholesterol decreased while that of total serum Apo B, which in these normo-triglyceridemic women is largely present in LDL, remained almost similar. The observed changes in the LDL cholesterol/Apo B ratio indicate a shift towards smaller LDL particles [3]. This could indeed be confirmed by GGE, a method which has proved to be valid for detecting LDL heterogeneity [8]. Recently, Campos *et al.*, who already demonstrated smaller LDL particles in postmenopausal women [10], also reported decreased LDL cholesterol, and increased HDL cholesterol, Apo A-I and TG levels in postmenopausal women treated with conjugated oestrogen monotherapy. In women with predominantly large LDL particles (> 272 Å) they found a significant decrease in the LDL peak area and in the

particle diameter, while in women with predominantly small LDL particles (261-272 Å) these parameters did not change [11].

A possible explanation for decreasing LDL particle size during HRT is given by Deckelbaum *et al.* [12] who hypothesized that size and density of LDL are at least partly determined by exchange of TG from very low-density lipoprotein (VLDL) for cholesteryl esters from LDL. As TG levels rise during oestrogen supplementation, due to increased VLDL synthesis, the hypothesized mechanism explains how LDL particles become enriched by an excess amount of TG at the expense of cholesteryl esters, and then allow continued particle size reduction through lipase action, which may finally result in lipid poor, and thus protein enriched LDL particles of relatively high density.

A predominance of small LDL particles has earlier been identified in a lipoprotein pattern with increased serum TG and decreased HDL cholesterol levels [3]. In previous studies comparing LDL particle size of patients with CHD and controls [4-6] the LDL particle size difference between these two groups was reduced to non-significant after adjusting for TG and HDL cholesterol levels. This indicates that small LDL particles may not independently be associated with CHD. The reported association with low HDL cholesterol and elevated TG suggests that the LDL size reflects a series of alterations, that is deviating in CHD. Indeed, LDL of different size, induced by lipid exchange reactions, differ in their susceptibility to oxidative modification in vitro [13], a phenomenon considered to underlie the excessive accumulation of cholesterol in atherosclerotic lesions [14].

HRT, including the hormone treatment used in the present study, is associated with beneficial changes in the lipid and lipoprotein parameters predicting the risk for CHD [7]. The favourable effect of HRT concerning the risk for atherosclerosis is supported by animal experiments in which it was shown that LDL cholesterol accumulation in the arterial wall is attenuated by oestrogen monotherapy and combined oestrogen-progestogen therapy, even when plasma lipids, lipoproteins and apoproteins remain unchanged [15]. As a matter of fact, also in these animal studies a decrease in LDL molecular weight was observed [15].

In summary, as a result of the diversity of influences on women's metabolism exerted by HRT, it remains difficult to make a proper CHD risk estimation, based on the common lipid parameters. From earlier reports on changes in LDL density and LDL particle size during HRT in experimental and clinical studies, that can be confirmed by our own observation, it appears that a shift in LDL particle distribution to a smaller size may not per se be positively correlated with cardiovascular disease. Clearly, there is a need for more prospective research towards the effects of HRT in the protection against cardiovascular disease.

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CHAPTER 6

HORMONE REPLACEMENT THERAPY MAY REDUCE HIGH SERUM HOMOCYSTEINE IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Introduction: Postmenopausal women are at increased risk for developing cardiovascular disease as compared to premenopausal women. During hormone replacement therapy the risk of developing cardiovascular disease decreases up to 50%. Homocysteine is an established independent risk factor for cardiovascular disease. Conflicting reports have indicated possible elevated homocysteine concentrations in postmenopausal women when compared to premenopausal women.

Objective: To investigate the possible changes in fasting serum total homocysteine concentrations during hormone replacement therapy.

Study Design: In a prospective study 21 healthy non-hysterectomised postmenopausal women were given continuous micronised 17ß-oestradiol, 2 mg daily, in combination with cyclic dydrogesterone, 10 mg daily during the first 14 days of each 28 days cycle. Changes were followed for up to two years.

Results: During the first six cycles mean serum homocysteine decreased by 10.9% (P=0.013), after which no further significant changes were found during the two years of treatment. A 16.9% decrease (P=0.017; N=8) was found in women with high homocysteine concentrations, while in women with low homocysteine concentrations (N=13) no significant changes were observed.

Conclusion: The observed decrease in high homocysteine concentrations in postmenopausal women may in part contribute to the decreased risk of developing cardiovascular disease during hormone replacement therapy.

INTRODUCTION

Cardiovascular morbidity and mortality are important health problems in developed countries. In men the risk of cardiovascular disease (CVD) increases proportionally with age, while women are relatively protected until menopause. After menopause the risk of CVD increases rapidly. Menopause is hormonally characterised by decreasing serum oestrogen concentrations. Furthermore, serum concentrations of total cholesterol and low-density lipoprotein cholesterol increase, while high-density lipoprotein cholesterol decreases after menopause [1]. During postmenopausal hormone replacement therapy (HRT) the risk of developing CVD decreases up to 50% [1,2]. This has been partly explained by the beneficial changes in the lipid profile [3].

Elevated blood concentrations of homocysteine are an established independent risk factor for premature vascular disease [4-6]. Several investigators have reported increased homocysteine concentrations in postmenopausal women when compared to premenopausal women [7-9]. This observation, however, could not be confirmed by others [10-13]. Elevated homocysteine concentrations may in part contribute to the increased risk of developing CVD after menopause [8].

To investigate a possible effect of HRT on homocysteine concentrations, we studied the changes in fasting serum total (free and protein-bound) homocysteine concentrations during two years of supplementation with a sequential 17β-oestradiol - dydrogesterone regimen in postmenopausal women.

PATIENTS AND METHODS

The study was approved by the ethical committee of our hospital on beforehand. Included were healthy non-hysterectomised postmenopausal women, who were amenorrhoeic for at least six months. Excluded from study were women using drugs

affecting lipid metabolism or women who used hormonal therapy for the previous two weeks. Previous to study entry, assessment of serum follicle-stimulating hormone (FSH) concentrations, liver enzymes (alanine aminetransferase, aspartate aminetransferase and gamma-glutamyltranspeptidase) and mammography were performed, and were found to be normal. Before entering the study the purpose of the protocol had been explained to the participants and written informed consent was obtained. None of the women reported intake of vitamin B_6, B_{12}, or folate supplements at study entry, or during the course of the study.

After screening, 27 healthy postmenopausal women, aged 49 to 59 years, were included in the study. All women were given micronised 17ß-oestradiol (Zumenon®), 2 mg daily, and cyclic dydrogesterone (Duphaston®), 10 mg daily for the first half of each 28 days cycle, both orally administered (Solvay Duphar, Weesp, The Netherlands). Twenty-three women completed the two-year study period. The reasons for exclusion of four women have been reported elsewhere [14]. Another two women had to be excluded from statistical analyses because their serum FSH concentrations (18 and 26 IU/l, respectively) appeared too low for the range, characteristic for the postmenopausal phase (FSH $\geq$ 40 IU/l). Therefore, statistical analyses were performed on data of 21 women, with a mean age of 54.5 years (*range: 49 - 59 years*), who were amenorrhoeic for 55.1 months (*range: 9 - 120 months*), and whose mean body-mass index was 24.4 kg/m^2 (*range: 20.8 - 29.3 kg/m^2*).

Physical examination and fasting venous blood sampling were performed before study entry and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 6 ($\pm$ 1) , 12 ($\pm$ 1) and 24 ($\pm$ 1). Blood sampling was performed after a 12 hours fast. Furthermore, assessment of lipids and lipoproteins (total cholesterol, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, very low-density lipoprotein triglycerides, apolipoprotein A-I, A-II, and B, and lipoprotein(a)), coagulation factors (antithrombin III and fibrinogen) and glucose was performed to determine possible statistical correlations

with serum concentrations of total homocysteine.

Serum concentrations of FSH were measured with a specific immunoradiometric assay (Medgenix, Fleurus, Belgium) as described elsewhere [15]. Serum concentrations of 17ß-oestradiol were measured with an in-house radioimmunoassay [16] and serum concentrations of oestrone were measured by oestrogen-specific dextran-coated charcoal radioimmunoassay described by Heineman [17].

For serum total homocysteine measurement blood was drawn into evacuated collection tubes (Corvac®, Becton Dickinson, 38241, Cedex, France). After clotting for one to two hours, serum was isolated after centrifugation for 15 min at 3500 x *g*. Sera were stored at -80°C until analysis. To minimize the imprecision of assay all samples from the same subject were analysed in the same run. Serum total homocysteine was measured by High Performance Liquid Chromatography (HPLC) and fluorimetric detection according to Fiskerstrand *et al.* [18]. The detection limit was 0.5 µmol/l and the intra- and interassay coefficients of variation amounted both to $< 5\%$.

Assays on lipids, lipoproteins and coagulation factors have been described elsewhere [14].

Statistical analysis was performed on the data of 21 women. Hormonal parameters were tested by the Student paired *t* test. Homocysteine was tested by Friedman's two-way analysis of variance and Wilcoxon's signed-rank test. The *P*-values were two-tailed, and a *P*-value lower than 0.05 was considered to be statistically significant. Statistical analyses were performed with the Dyna-stat computer program (Dynamic Microsystems, Inc., Washington D.C., Pittsburgh, PA, USA).

RESULTS

Hormonal parameters

During the first six cycles of HRT the mean serum concentration of FSH decreased significantly with 52% and the mean serum concentrations of oestrone and oestradiol increased significantly 10-fold and 5-fold, respectively (Table 1).

Table I: Serum concentrations of reproductive hormones before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol - dydrogesterone therapy of cycle 6[a]

		Cycle 0	Cycle 6	P^b
FSH	(IU/l)	90 ± 18	43 ± 19	<0.0001
E_1	(pmol/l)	232 ± 75	2612 ± 1486	<0.0001
E_2	(pmol/l)	104 ± 30	635 ± 310	<0.0001

[a]: values as mean ± SD (N = 21); [b]: Student paired *t* test; E_1: oestrone; E_2: oestradiol.

Serum homocysteine

After the first six treatment cycles the mean concentration of serum homocysteine was significantly lower as compared to baseline (*Total* group: -10.9%; P=0.013) (Table 2), after which no further statistically significant changes were observed during the two years of treatment. When dividing the total group in subgroups with homocysteine concentrations above the mean (*High-level* group: homocysteine > 13.8 µmol/l; N = 8) and below the mean (*Low-level* group: homocysteine < 13.8 µmol/l; N = 13), the *High-level* group showed a 16.9% decrease (P=0.017), while in the *Low-level* group no significant changes were observed.

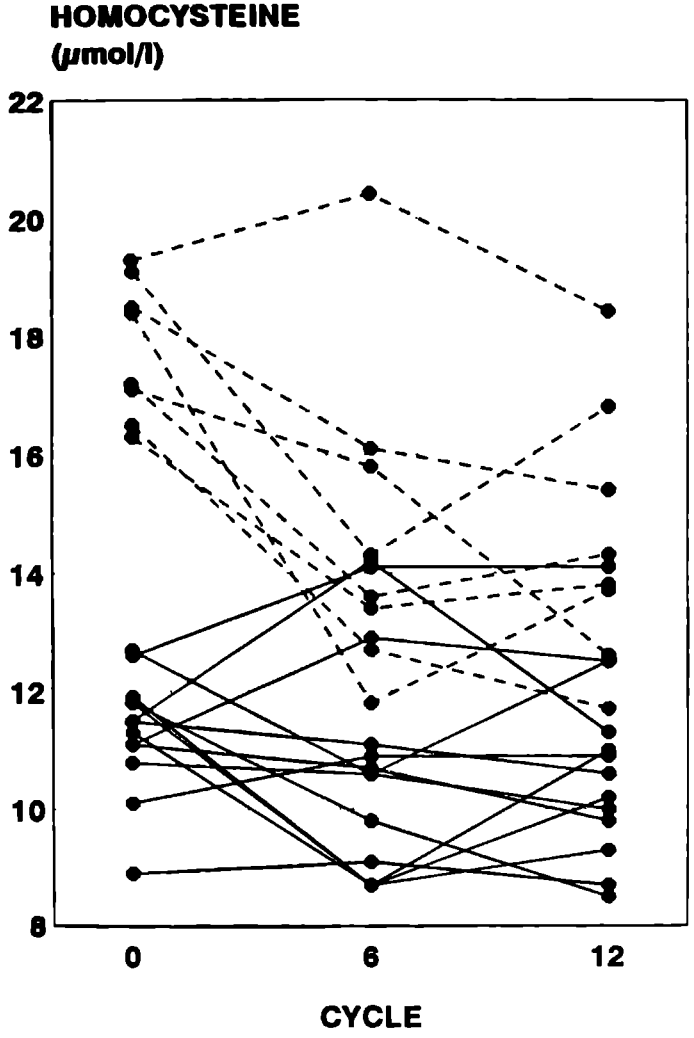

Figure: Individual changes in fasting serum total homocysteine concentrations during the first 12 treatment cycles with a sequential 17ß-oestradiol - dydrogesterone regimen (N = 21), ●– – –● *High-level* group (homocysteine > 13 8 µmol/l), ●——● *Low-level* group (homocysteine < 13 8 µmol/l)

Table II: Serum concentrations of homocysteine[a] before treatment (cycle 0) and on day 12, 13 or 14 of the combined 17ß-oestradiol - dydrogesterone therapy of cycles 6, 12 and 24[b]

	Cycle 0	Cycle 6	Cycle 12	Cycle 24	ANOVA[c]
High-level group	17.8 ± 1.2	14.8 ± 2.7	14.6 ± 2.2	15.6 ± 2.0	
	17.8 (16.3-19.3)	14.0 (11.8-20.4)	14.1 (11.7-18.4)	16.0 (11.2-17.4)	
P^d		0.017	0.012	0.018	<0.01
Low-level group	11.3 ± 1.0	10.8 ± 1.9	10.7 ± 1.6	11.0 ± 1.6	
	11.5 (8.9-12.7)	10.6 (8.7-14.2)	10.6 (8.5-14.1)	10.7 (8.0-13.1)	
P^d		NS	NS	NS	NS
Total group	13.8 ± 3.4	12.3 ± 2.9	12.2 ± 2.6	12.7 ± 2.8	
	11.9 (8.9-19.3)	11.8 (8.7-20.4)	11.7 (8.5-18.4)	12.4 (8-17.4)	
P^d		0.013	0.0017	0.006	<0.01

[a]: fasting serum total homocysteine (µmol/l); *High-level* group (N = 8): homocysteine > 13.8 µmol/l; *Low-level* group (N = 13): homocysteine < 13.8 µmol/l; *Total* group (N = 21): combined *High-* and *Low-level* group; [b]: values as mean ± SD and median (range); [c]: Friedman's two-way ANOVA; [d]: *P*-value versus cycle 0 (Wilcoxon's signed-rank test).

No significant correlations were found between serum homocysteine and serum oestrogens, lipids and lipoproteins or coagulation factors.

DISCUSSION

We found significantly decreased serum homocysteine concentrations during HRT. To the best of our knowledge, this observation has not been reported before. The authors realize that their study design lacks a control group. However, a mean decrease of 11% in six months that sustained thereafter obviously excludes the possibility of a mere drift in time or variation due to storage. Earlier, it was demonstrated that homocysteine concentrations show little changes in time, when re-investigated after 6 - 12 months [10].

Differences in homocysteine concentrations have been demonstrated during pregnancy and during intake of oral contraceptives (OC). In pregnant women, having high oestradiol concentrations, homocysteine concentrations were found to be lower as compared to non-pregnant women [19,20]. Surprisingly, high concentrations of homocysteine have been found during the use of OC's [21], but this observation was not confirmed by others [22,23]. It must be emphasized that synthetic oestrogens like ethinyl-oestradiol, which is the most common oestrogen in OC's, may have different metabolic interactions when compared with natural oestrogens, as used in the present postmenopausal HRT study.

Until now the relationship between homocysteine metabolism and the hormonal changes in climacteric women has not been clearly established. Boers *et al.* [7] described the efficiency of the methionine metabolism in premenopausal women leading to lower homocysteine concentrations as compared to postmenopausal women. This has also been demonstrated by others [8,9]. However, some investigators reported non-significant differences [10,11], or no difference at all [12,13] between pre- and postmenopausal women. This may be due to differences in, or imprecision of the assay-procedure. Some

authors determined free homocysteine (homocysteine-cysteine mixed disulfide and homocystine) [7,8,13], while others determined total (free and protein-bound) homocysteine [9,11,12], or both [10]. Recently, we observed clearly elevated total plasma homocysteine concentrations, fasting as well as after methionine loading, in postmenopausal women when compared to premenopausal women [24]. Increased homocysteine concentrations may well in part contribute to the increased risk of developing CVD after menopause [8].

After six months of treatment, seven of eight women in the *High-level* group, and after 12 months all eight women showed a substantial decrease (*average -18%; P*=0.012) in serum homocysteine. In the *Low-level* group no significant changes were observed. This indicates that especially those women, having a higher homocysteine-related risk for CVD, may favour the homocysteine-lowering influence of HRT, while HRT does not seem to influence low-normal homocysteine concentrations.

Elevated homocysteine concentrations have been related to an increased risk of developing CVD [4-6], but cardioprotective influences of homocysteine lowering therapy have not been published yet. Therefore, one can only speculate that the decrease in homocysteine concentrations in postmenopausal women due to HRT will have a cardioprotective influence.

In conclusion, high serum homocysteine concentrations in postmenopausal women decreased during HRT. The observed decrease may in part contribute to the decreased risk of developing CVD during HRT. Still, there are many obscurities and controversial observations concerning the influence of sex hormones on the homocysteine concentration in postmenopausal women that warrant further investigation.

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CHAPTER 7

CHANGES IN THE WITHDRAWAL BLEEDING PATTERN AND ENDOMETRIAL HISTOLOGY DURING 17ß-OESTRADIOL - DYDROGESTERONE THERAPY IN POSTMENOPAUSAL WOMEN; A 2 YEAR PROSPECTIVE STUDY

M.J. van der Mooren, A.G.J.M. Hanselaar, G.F. Born, R. Rolland

Adapted from:
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ABSTRACT

Objective: To describe changes in the withdrawal bleeding pattern and endometrial histology during a sequential 17ß-oestradiol - dydrogesterone regimen in postmenopausal women.

Design: Open-label, non-comparative, prospective study.

Setting: Gynaecological outpatient department of a university hospital.

Patients: Twenty-seven healthy non-hysterectomised postmenopausal women.

Interventions: Continuous micronised 17ß-oestradiol supplementation, 2 mg daily, and cyclic administration of dydrogesterone, 10 mg daily for the first half of each 28 day treatment cycle.

Main Outcome Measures: Changes in the characteristics of the withdrawal bleeding pattern and the endometrial biopsy histology during two years of treatment.

Results: The initial withdrawal bleeds were comparable to normal menstruation with respect to amount and duration. During the two years of treatment the bleeding showed a significant tendency to become shorter with less blood loss. This was mainly the result of the decrease ($P<0.001$) in the number of days per cycle with bleeding grade II (normal menstruation). None of the women developed endometrial hyperplasia, and in almost all women the given hormone replacement therapy regimen induced secretory or atrophic changes of the endometrium.

Conclusions: This sequential 17ß-oestradiol - dydrogesterone regimen can be regarded as safe with respect to the prevention of endometrial disease. Furthermore, it appears to foster patient compliance.

INTRODUCTION

Postmenopausal hormone replacement therapy (HRT) has become of increasing interest in general and gynaecologic practices, not only for its effective relief of climacteric and postmenopausal symptoms and complaints, but also for its important preventive effects on bone mineral loss and cardiovascular disease (CVD) [1-5].

Unopposed oestrogen therapy in non-hysterectomised women reportedly increases the incidence of irregular bleeding and the risk of developing endometrial hyperplasia and carcinoma. Nowadays, it is generally accepted practise to add a progestogen, either continuously or cyclical to the oestrogen therapy [6]. In Europe, the latter is the most commonly prescribed HRT regimen. The progestogen transforms the oestrogen-induced proliferative and hyperplastic endometrium into a secretory or even atrophic endometrium. Sequential HRT regimen, however, restore vaginal bleeding, which may reduce patient compliance. Furthermore, progestogens may attenuate the beneficial changes in lipid metabolism brought about by unopposed oestrogen therapy. The degree of attenuation depends on the type and the dosage of the progestogen, as well as the route and duration of its administration [7,8]. Therefore, the optimal oestrogen-progestogen combination prescibed for postmenopausal non-hysterectomised women must firstly garantee the safety of the treatment with regard to endometrial disease and limit vaginal bleeding, and secondly must induce beneficial changes in the lipid metabolism which favour CVD risk.

Dydrogesterone has been described as a metabolic inert progestogen to have little or no influence on the lipid metabolism [9-12]. Recently, we reported on the changes in serum lipids and (apo)lipoproteins during two years of continuous supplementation of micronised 17ß-oestradiol and cyclic administration of dydrogesterone [13]. These changes were in favour of lowering the risk of developing CVD. In order to document the compliance and safety of this HRT regimen, we investigated the changes in characteristics of the withdrawal bleeding pattern, and studied the endometrial histology.

SUBJECTS AND METHODS

The study received prior approval by the ethical committee of our hospital. Healthy non-hysterectomised postmenopausal women were recruited by advertisement in a local newspaper during the months January until May 1990. The women were amenorrhoeic for at least six months and were screened to have follicle-stimulating hormone (FSH) serum concentrations within the range, characteristic for the postmenopausal phase. Excluded were women using drugs affecting lipid metabolism or who used hormonal therapy for the previous two weeks. Before entering the study the purpose of the protocol had been explained and written informed consent was obtained.

After screening 27 women, aged 49 - 59 years, were included in the study. Characteristics of the study population are given in Table I. In two cases the FSH and oestradiol concentrations exceeded the postmenopausal range (*normal range*: FSH: $\geq$ 36 IU/l; oestradiol: $<$ 150 pmol/l) as a result of a short wash-out period (*i.e.* 2 weeks; FSH: 18 IU/l; oestradiol: 290 pmol/l) or a relatively short amenorrhea (*i.e.* 7 months; FSH: 26 IU/l; oestradiol: 820 pmol/l).

Table I: Characteristics of the study population[a]

Age	(years)	54.0	(49 - 59)
Amenorrhea	(months)	53	(7 - 120)
Body-mass index	(kg/m^2)	24.5	(20.1 - 30.9)
FSH[b]	(IU/l)	83	(18 - 140)
Oestradiol	(pmol/l)	145	(75 - 820)

[a]: values given as mean (range); N = 27; [b]: follicle-stimulating hormone.

All women were treated with micronised 17ß-oestradiol (Zumenon®), 2 mg daily, and with dydrogesterone (Duphaston®), 10 mg daily for the first half of each 28 day treatment cycle, both orally administered (Solvay Duphar, Weesp, The Netherlands). Before starting the study medication the women were at first given a progesterone challenge test (PCT), by administration of dydrogesterone 10 mg daily for 12 days.

During the study the women recorded any vaginal bleeding on a diary card. Bleeding for which no sanitary protection was needed (spotting) was coded as bleeding grade I. Bleeding identified as being like normal menstruation was coded as grade II, and heavy vaginal bleeding as grade III.

Physical examination and endometrial biopsy took place before study entry and on day 12, 13 or 14 of the combined 17ß-oestradiol and dydrogesterone intake of cycles 12 (± 1 cycle) and 24 (± 1 cycle). Endometrial biopsy was performed by micro-curettage (Vabra® endocurette, Farina Lanfranco, Venezia, Italia). Endometrial samples were collected in neutrally buffered formaldehyde solution (4%), and embedded in one paraffin block each. Six micrometer thin sections were cut and stained with haematoxylin and eosin for routine pathological examination. The slides were analysed and histological diagnosis was established by a consultant pathologist at the Institute of Pathology, University Hospital Nijmegen, The Netherlands, using strict histologic criteria.

Twenty-three women completed the two year study period. One woman was incorrectly admitted to the study as the initial endometrial biopsy showed focal adenomatous hyperplasia. Therefore she was excluded. There were three drop outs due to various reasons [13].

In six women the dydrogesterone dosage was increased from 10 to 20 mg, according to instructions in the protocol, since repeated spotting or withdrawal bleeding occured before day ten of the combined 17ß-oestradiol and dydrogesterone intake [14]. Another woman missed dydrogesterone seven days in two cycles and fourteen days in one cycle, due to repeated hospitalisation because of total hip surgery, without further violation of the protocol.

Statistical analysis was performed on the data of all 27 women included on an "intention-to-treat" basis. The last visit carried forwards (LVCF) procedure was used because of one exclusion and three drop outs. To evaluate the withdrawal bleeding pattern the following characteristics were analysed: number of days per cycle with respectively bleeding grade I, grade II, grade III, and total bleeding days per cycle, the mean bleeding grade per cycle and the first cycle day of the withdrawal bleeding. The raw data of these bleeding characteristics showed the biggest changes during the first six months, while the second study year only showed a modest continuation of the trend seen in the second half of the first year. Therefore, for analysis of the bleeding characteristics it was decided to divide the two year study period into five intervals: cycle 1, cycles 2-3, cycles 4-6, cycles 7-12 and cycles 13-24. All bleeding characteristics were analysed by Friedman's two way Analysis of Variance.

Endometrial biopsy histology was categorized as: no passage to the uterine cavity achieved, insufficient tissue (for appropiate diagnosis), atrophic endometrium, secretory endometrium, proliferative endometrium and hyperplasia. Data on changes in endometrial histology are merely descriptive since the population size was too small to justify any statistical analysis.

RESULTS

Withdrawal bleeding pattern

Of the 27 women five (19%) had a positive PCT after 12 days of dydrogesterone challenge, previous to treatment. During the study period the total number of bleeding days per cycle decreased, after an initial increase during the first three cycles, from 6.6 (*mean*; *SD* 2.0) to 5.3 (*SD* 1.5) days per cycle (P=0.0001; cycle interval 2-3 versus 13-24; see Figure).

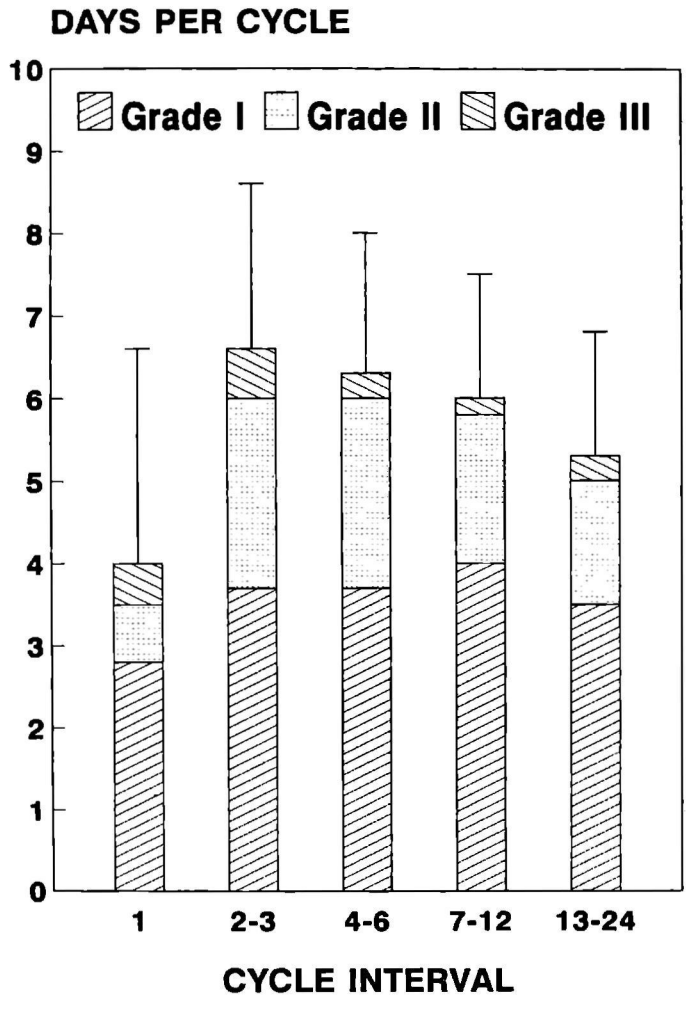

Figure: Withdrawal bleeding pattern during 17ß-oestradiol - dydrogesterone therapy in 27 women. *Bleeding characteristics:* number of days per treatment cycle with respectively bleeding grade I (spotting; no protection needed), grade II (normal menstruation), grade III (heavy vaginal bleeding), and standard deviation (error bars) of total bleeding days.

This fall could mostly be attributed to the reduction, also after an initial increase, in bleeding grade II, from 2.3 (*SD* 1.8) to 1.5 (*SD* 1.1) days per cycle ($P<0.001$; cycle interval 2-3 versus 13-24). No significant changes occurred in withdrawal bleeding grade I and III. The mean bleeding grade per cycle decreased, after an initial increase, from 1.44 (*SD* 0.35) to 1.36 (*SD* 0.25) ($P<0.05$; cycle interval 2-3 versus 13-24) and the mean first bleeding day was delayed with 1.4 days from cycle day 12.3 (*SD* 2.5) to cycle day 13.7 (*SD* 1.8) ($P<0.05$; cycle interval 2-3 versus 13-24).

Endometrial histology

The findings are summarized in Table II. Before treatment the initial endometrial biopsy of two women (7.4%) showed a proliferative endometrium, and they also had a positive PCT. Both women bled between five and eight days per cycle, one stopped the medication because of progressive varicosis, the other kept moderately heavy withdrawal bleeds (grade II and III) until the end of the study period. Another woman's initial biopsy, also following a positive PCT, showed focal adenomatous hyperplasia, and she was therefore excluded from the study. Until the moment of exclusion she had regular withdrawal bleeds, and follow up biopsies showed no signs of hyperplasia. In the other two women with a positive PCT, and in whom the 10 mg dydrogesterone dosage had to be increased to 20 mg because of commencement of withdrawal bleeding before cycle day ten [14], the endometrium showed secretory changes after 24 treatment cycles. The other four women in whom the dydrogesterone dosage was increased showed secretory changes (N = 3; 11%) or an atrophic endometrium (N = 1). In 21 women the initial endometrial biopsies showed atrophic endometrial tissue (N = 1) or insufficient tissue for appropiate diagnosis (N = 20; 74%), which can in most cases be regarded as a symptom of the atrophic status of the postmenopausal endometrium. In three women (11%) no passage to the uterine cavity was achieved.

After 12 treatment cycles the endometrium of 17 women (63%) showed secretory changes. After 24 treatment cycles the endometrial biopsies showed in most cases (N =

18; 67%) secretory changes, some in combination with slight proliferative changes. Further, atrophic tissue (N = 1) and insufficient tissue (N = 2) were observed, or no passage to the uterine cavity was achieved (N = 2). In none of the women hyperplastic changes of the endometrium were observed during the treatment.

Table II: Endometrial histology before (cycle 0) and after 12 and 24 cycles of 17ß-oestradiol - dydrogesterone therapy

Category	Cycle 0	Cycle 12	Cycle 24
No passage[a]	3	3	2
Insufficient tissue	20	2	2
Atrophy	1	1	1
Secretory	-	17	18
Proliferative	2	-	-
Hyperplasia	1[b]	-	-
Drop out/exclusion	-	4	4

N = 27; [a]: no passage to the uterine cavity; [b]: excluded from study.

DISCUSSION

Since Ziel & Finkle in 1975 [15] reported an increased risk of endometrial cancer during unopposed oestrogens, this finding has been confirmed by others [16-18], including the increased risk of endometrial hyperplasia. In modern HRT regimen, the addition of a progestogen, cyclical or continuously, has become generally accepted. The addition of a progestogen during at least 12 days per treatment cycle prevents the increase

in risk of hyperplasia or carcinoma due to oestrogen treatment [19-23]. Although both the cyclic and the continuous administration seem equally effective with regard to the latter, in Europe the cyclical regimen are more commonly prescribed.

Dydrogesterone, a retroprogesterone, lacks the androgenic properties [24] as seen in many other progestogens, which is probably the reason why it does not affect the lipid profile unfavourably [10,12,13]. To establish secretory changes in the oestrogen-primed endometrium a daily dose of 10 to 20 mg is required [25-27]. Recently, it has been demonstrated that 14 days administration of 10 to 20 mg of dydrogesterone opposes the proliferative effects of a 50 mg oestradiol implant, and that 20 mg is sufficient to counteract the effects of a 100 mg oestradiol implant [28,29].

The present study demonstrates that the given sequential oestradiol-dydrogesterone regimen introduced a withdrawal bleeding that was initially comparable to normal menstruation with respect to amount and duration, and that during two years of treatment the bleeding showed a tendency to become shorter with less blood loss. The first can be concluded from the decrease in the total bleeding days per cycle, and the latter from the decrease in the number of days with bleeding grade II, and the decrease in the mean bleeding grade per cycle. These changes can be regarded as favourable with respect to patient compliance. Nineteen of the 23 women (83%) who completed the study desired to continue the treatment after the two year study period, which underlines the degree of patient acceptability.

In six women (22%) with irregular blood loss, the dydrogesterone dosage had to be increased to 20 mg daily according to the protocol. This resulted in an acceptable bleeding pattern indicating that individualisation of HRT regimen is preferred to fixed-dose HRT combinations.

After two years of HRT with dydrogesterone, 10 or 20 mg for 14 days each 28 day cycle, 19 of the 23 women who completed the study period (83%) showed a secretory or atrophic endometrium. Even in the one case that the initial biopsy showed focal adenomatous hyperplasia, a regular withdrawal bleeding pattern and absence of hyperplastic or proliferative changes was established during cyclic administration of 10

mg dydrogesterone in combination with 17ß-oestradiol.

In view of earlier demonstrated beneficial changes in serum lipids and (apo)lipoproteins during this sequential 17ß-oestradiol - dydrogesterone regimen, and the data presented here on bleeding pattern and endometrium histology, we conclude that this HRT regimen appears safe, promotes good compliance and is therefore well suited for the purpose of HRT.

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CHAPTER 8

GENERAL DISCUSSION

For the purpose of treatment of climacteric complaints hormone replacement therapy is practisized for a long period of time. From the literature it is clear that hormone replacement therapy benefits the quality of a climacteric or postmenopausal woman's life. Especially the typical complaints as flushes and sweats, and the urogenital complaints due to the oestrogen deficiency, respond well to the supplementation of natural oestrogen. Also less typical complaints, for example sleep disturbances, forgetfulness, fatigue and melancholia, frequently related to the severity of flushes and sweats, often improve as well.

In addition to the direct benefits of hormone replacement therapy on typical and atypical climacteric complaints there is also growing evidence on other favourable effects of hormone supplementation. Oestrogens appear to be a key hormone in bone metabolism and have proven effectivity in the prevention of osteoporosis. Addition of progestogens even may strengthen this effect on bone metabolism. Hormone replacement therapy has reduced the incidence of osteoporotic fractures in postmenopausal women. Large scale hormone prescription to postmenopausal women at risk for osteoporosis can be expected to have a high impact on the socio-economic consequences of oseoporosis.

The literature contains an extensive amount of data indicating that oestrogen also is a key hormone in the regulation of cardiovascular function, and that oestrogen is of importance in secondary and probably also in primary prevention of cardiovascular disease. Hormone replacement therapy reduces cardiovascular disease risk with up to 50%. Women at risk to develop cardiovascular disease due to obesity, smoking habits, hyperlipidaemia or history of coronary heart disease may benefit even more.

These benefits have made hormone replacement therapy increasingly popular. However, the reported increased incidence of endometrial cancer in the past made oestrogen treatment less popular. Prolonged oestrogen exposure was associated with hyperplastic and carcinogenic changes in the endometrium. Better understanding of the

endometrial physiology led to the addition of cyclic progestogen administration in non-hysterectomised women, which reduced the incidence of endometrial hyperplasia and carcinoma to frequencies even below those seen in untreated women [1]. Therefore, it is nowadays generally accepted to reserve unopposed oestrogen treatment only for hysterectomised women. Non-hysterectomised women should always be treated with a progestogen combined with the oestrogen therapy for at least 10 days.

As stated before, the importance of progestogens for the safety of the endometrium is generally accepted, and the studied oestradiol-dydrogesterone regimen in this thesis was confirmative with other reports on the safety of dydrogesterone in this respect. However, the effects of progestogens on the other organ systems is yet unclear. Progestogens apparently do not protect the breasts against cancer, they even may stimulate the proliferation of breast glandular tissue [2]. Until recently it was believed that progestogens attenuate or even reverse the beneficial effects of oestrogen on lipids and lipoproteins. Therefore an unfavourable influence concerning the cardiovascular system seemed evident.

In this thesis two combined oestrogen/progestogen treatments were investigated concerning their influences on lipids and lipoproteins: an oestradiol-dydrogesterone regimen and a conjugated oestrogen-medrogestone regimen. Both hormone treatments induced beneficial changes in the lipid profile. In both studies the additional effects of progestogens when combined with oestrogen were investigated. The progestogen administration did not induce unfavourable additional changes in lipids and lipoproteins. Until now this item was often neglected in the literature. Both studies dealt with rather small groups, which may have impaired the statistical power. The observed changes in the oestradiol-dydrogesterone study were very clear and a larger population to prove the effectiveness of the treatment is not needed. The chapter on the conjugated oestrogen-medrogestone treatment reported preliminary data from a larger study [3], that demonstrated almost comparable changes as described in this thesis. So, our studies on lipids and lipoproteins did not support the suggested unfavourable progestogenic influence on the cardiovascular system, in agreement with other recent reports [4-8].

The reported effects of hormone treatment on the conventional lipid and lipoprotein risk estimators can only be held responsible for about 25% of the cardiovascular risk reduction, and more research on other risk factors is needed to elucidate this intriguing matter. In this thesis three new risk factors in the field of cardiovascular research in postmenopausal women were studied: lipoprotein(a), the low-density lipoprotein profile and the homocysteine metabolism. The decrease in lipoprotein(a) very well adds to the other beneficial changes in the lipid profile. Still, evidence is lacking that reducing lipoprotein(a) decreases cardiovascular risk. The observed changes in the low-density lipoprotein profile during hormone treatment are confirmative with some earlier reports, but the demonstrated shift towards smaller low-density lipoprotein particles is still difficult to integrate with the changes in the other lipids and lipoproteins. Apparently, in our understanding so far the observed shift indicates an unfavourable influence of the hormone treatment, but the significance of this effect when compared with the substantial increase in high-density lipoprotein cholesterol, and decreases in total low-density lipoprotein cholesterol and lipoprotein(a) is questionable. Further research on hormone induced changes in the low-density lipoprotein profile, and also changes in its oxidizability, is yet ongoing. The first data indicate that hormone supplementation does reduce low-density lipoprotein oxidizability [9], which can be regarded as favourable in respect to this lipoprotein's atherogenicity. In contrast to the low-density lipoprotein profile, the study of hormonal effects on serum homocysteine is still unique. Until now the literature on hormone related changes related to menopause in homocysteine concentrations are scarce and inconsistent. Very recently, our research group reported substantial differences in total plasma homocysteine, fasting and after methionine loading, between healthy pre- and postmenopausal women [10]. At least in part this observation may help to explain the difference in cardiovascular risk between pre- and postmenopausal women. This thesis demonstrates that above normal homocysteine concentrations in postmenopausal women decrease with 17% during hormone replacement therapy, while low normal homocysteine concentrations do not change. Therefore, it may be important to identify women that are at risk for developing

cardiovascular disease related to the high homocysteine concentration, since these women may benefit the homocysteine reducing influence of hormone supplementation. Unpublished data of evidence in a new trial confirm these findings [11]. Here, a 10% decrease in mean homocysteine concentration was measured during a sequential conjugated oestrogen and medrogestone treatment. Therefore, it is likely that a decrease in homocysteine may explain part of the reduction in cardiovascular disease in hormonally treated postmenopausal women. Our results on the observed reduction in homocysteine concentrations during hormone replacement therapy pleads for recognition of homocysteine metabolism in further postmenopausal research.

For the prevention of osteoporosis hormone treatment for at least five years is advised, while only a small percentage of women is taking the treatment longer than two years [12]. With respect to cardiovascular disease, the relation between treatment duration and preventive effects is yet unclear, but it seems justified to think of a comparable necessarry minimal period of treatment. Especially with respect to the aspects of secundary prevention life-long treatment needs consideration. Regarding the apparent problem of patient compliance, at least in non-hysterectomised women, patient compliance reportedly was inversely correlated with the inconvenience of the withdrawal bleeds. To improve compliance, the bleeding aspects of existing treatments need to be evaluated, and new research must help to find ways to reduce the bleeds in duration, frequency and severity. In this thesis we demonstrate that during monthly sequential oestradiol-dydrogesterone treatment the withdrawal bleeding is acceptable and shows a tendency to become shorter with less blood loss, which favours the acceptance of the treatment. This was also confirmed by the high percentage of women that asked to continue the treatment after two years of hormone replacement.

Alternative treatment modalities have been introduced, such as the continuous combined oestrogen-progestogen treatment. Although promising for women that are postmenopausal for one or more years, this treatment still needs thorough evaluation, primarily concerning its effects on the endometrium and the bleeding aspects, but also

with respect to its consequences for osteoporosis, cardiovascular disease and the breast disease. Recent investigations on endometrial receptor kinetics may be promising with respect to the development of alternative treatment regimen with combined oestrogen and interrupted progestogen administration [13]. A prolonged treatment cycle, inducing withdrawal bleeds every two or three months instead of every month, may offer a reasonable alternative. Although prolonged treatment cycles seem common practise for general practitioners and gynaecologists, literature data on this issue are very scarce. Presently, new studies investigating three-monthly treatment regimen, with respect to their effects on the endometrium and the bleeding pattern, as well as the metabolic effects, are ongoing in our department [14].

From the review of the literature and the data from our own studies it can be concluded that the benefit/risk ratio of hormone replacement therapy is in favour of the benefits. Nevertheless, still many aspects of this type of treatment are uptill now unknown, especially the long-term effects. It has to be kept in mind that hormone treatment at present with a tendency toward long-term prescription on a larger scale must take place under regular medical supervision. Only by systematic medical follow up any undesired effect can be diagnosed. This also garantees an adequate registration as a basis for future epidemiological evaluation.

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SUMMARY

The **first chapter** gives a short introduction and describes the aim of the thesis.

The **second chapter** of this thesis reviews some aspects of hormone replacement therapy. The main indications and contra-indications for hormone replacement therapy are discussed, after which the cardiovascular impact of hormone treatment and the possible underlying pathogenetic mechanisms are reviewed. Furthermore, the role of progestogens, and its consequences for patient compliance are considered.

The **third chapter** describes the effects of a sequential 17ß-oestradiol-dydrogesterone therapy on serum lipids and lipoproteins in 27 women. **Part I** of chapter 3 reports on the changes after the first year of this two-year study. The observed changes in lipids and lipoproteins were in favour of the prevention of cardiovascular disease. Furthermore, no deterioration in coagulation parameters or glucose metabolism was found. **Part II** of chapter 3 reports the results after two years of treatment. The changes observed after 6 months remained stable for the remainder of the study period. No differences were found between the oestradiol-only phase and the combined oestradiol-dydrogesterone phase of the last treatment cycle studied. So, the progestogen dydrogesterone did not attenuate the oestrogen induced beneficial changes in serum lipids and lipoproteins.

The sequential conjugated oestrogen-medrogestone treatment was described in **chapter 4**. Also this therapy induced favourable changes in the serum lipids and lipoproteins with respect to the prevention of developing cardiovascular disease, and except for HDL_2-cholesterol, no significant differences were found between the combined oestrogen-progestogen treatment and the unopposed oestrogen treatment, indicating that medrogestone lacks an undesirable effect on the lipid profile.

In **chapter 5** the effect of the sequential 17ß-oestradiol-dydrogesterone treatment on the low-density lipoprotein profile, determined by gradient gel electrophoresis, was described. We found a significant shift within the low-density lipoprotein profile towards a smaller low-density lipoprotein particle size. Although such a shift repeatedly has been associated with an increased cardiovascular risk, this implication is questioned with respect to our finding, since the observed shift in our patients was accompanied with clear favourable changes in the conventional lipid and lipoprotein risk estimators. In the discussion this point of view is illustrated by referring to data based on animal experiments and other experimental studies aimed to gain insight into the effect of hormone supplementation on atherosclerosis. All these studies illustrate the anti-atherogenic effect of hormone supplementation.

In **chapter 6** a possible non-lipoprotein etiology for cardiovascular disease is introduced. Elevated homocysteine concentrations are an independent cardiovascular risk factor. Some studies indicated that in postmenopausal women homocysteine levels are higher than in premenopausal women, which phenomenon may explain part of the increased cardiovascular morbidity and mortality after menopause. During the sequential 17ß-oestradiol-dydrogesterone treatment a significant decrease in mean fasting total serum homocysteine of 11% was demonstrated. Those women with high homocysteine concentrations demonstrated a 17% decrease, while women with low homocysteine concentrations showed no changes. It is suggested that this observation may in part explain the cardioprotective effect of hormone replacement therapy. The importance of more research on homocysteine metabolism in relation to hormonal changes in perimenopausal women and during postmenopausal hormone replacement therapy is advocated.

Safety aspects of the endometrium and the changes in the withdrawal bleeding pattern during the sequential 17ß-oestradiol-dydrogesterone treatment were investigated in **chapter 7**. Histological analysis of endometrial biopsy samples showed no undesirable

hyperplastic or carcinogenic changes in all 27 women studied. The withdrawal bleeds were acceptable, and showed a tendency to become shorter with less blood loss during the two years of treatment. Furthermore, more than 80% of women asked to continue the hormone treatment after the two-year study period. These findings are promising with respect to patient acceptance of the treatment, and this again may be decisive to achieve the long-term treatment benefits for osteoporosis and the cardiovascular system.

In **chapter 8** the results of the present thesis are integrated in the latest developments in the field of postmenopausal research. The literature supplies an increasing amount of evidence about the benefits of hormone replacement therapy. Nevertheless, still many aspects of this kind of treatment are incompletely understood. This chapter emphasizes the need for future research, with special reference to studies towards other possible pathogenetic mechanisms explaining the cardioprotective effects of hormone supplementation, and to studies investigating new treatment regimen that may help to improve patient acceptance and compliance.

SAMENVATTING

In **hoofdstuk 1** wordt een introductie gegeven en de doelstellingen van de thesis worden uiteengezet.

In **hoofdstuk 2** van dit proefschrift worden een aantal aspecten betreffende hormonale suppletie therapie samengevat. De belangrijkste indicaties en contra-indicaties voor hormoon suppletie worden besproken, waarna de cardiovasculaire impact van hormoon behandeling en de mogelijk hieraan ten grondslag liggende pathogenetische mechanismen worden toegelicht. Tevens wordt ingegaan op de rol van progestagenen, en de consequenties hiervan voor de therapie compliance en de acceptatie door de patiënt.

Hoofdstuk 3 beschrijft de effecten van een sequentiële 17ß-oestradiol-dydrogesteron behandeling op de serum lipiden en lipoproteïnen in 27 vrouwen. In **deel I** van hoofdstuk 3 worden de veranderingen genoemd die werden gevonden na het eerste jaar van deze 2 jaar durende behandeling. De gevonden veranderingen in lipiden en lipoproteïnen waren gunstig met betrekking tot de preventie van het ontstaan van cardiovasculaire ziekten. Tevens werd geen verstoring gevonden van de stollings parameters of het glucose metabolisme. **Deel II** van hoofdstuk 3 beschrijft de resultaten na 2 jaar behandeling. De geconstateerde veranderingen in lipiden and lipoproteïnen traden reeds op na 6 maanden, en bleven hierna onveranderd gedurende 2 jaar. Er werden geen verschillen gevonden tussen de oestradiol-alleen fase en de gecombineerde oestradiol-dydrogesteron fase van de laatste behandelcyclus. Dus, het progestageen dydrogesteron had geen nadelige invloed op de gunstige veranderingen in de lipiden en lipoproteïnen, teweeggebracht door het oestrogeen.

Een sequentiële geconjugeerde oestrogenen-medrogeston behandeling wordt beschreven in **hoofdstuk 4**. Ook deze behandeling veroorzaakte gunstige veranderingen in

de serum lipiden en lipoproteïnen met betrekking tot de preventie van het ontstaan van cardiovasculaire ziekten. Met uitzondering van het HDL_2-cholesterol werden er geen significante verschillen gevonden tussen de gecombineerde oestrogeen-progestageen behandeling en de oestrogeen-alleen behandeling, dus medrogeston heeft geen nadelige effecten op het lipiden profiel.

De effecten van de sequentiële 17ß-oestradiol-dydrogesteron behandeling op het low-density lipoproteïne profiel, bepaald met gradiënt gel electrophorese, worden beschreven in **hoofdstuk 5**. Er werd een significante verschuiving binnen het low-density lipoproteïne profiel waargenomen in de richting van een kleinere partikel grootte van het low-density lipoproteïne. Alhoewel een dergelijke verschuiving in de literatuur is geassocieerd met een toegenomen cardiovasculair risico, wordt deze associatie betwijfeld met betrekking tot onze bevindingen, aangezien de waargenomen verschuiving wordt vergezeld door uitgesproken gunstige veranderingen in de conventionele lipiden en lipoproteïnen. In de discussie wordt dit standpunt toegelicht aan de hand van experimentele studies welke het effect van hormoon suppletie op atherosclerose onderzocht hebben. Al deze studies demonstreren een anti-atherogeen effect van hormonale suppletie therapie.

In **hoofdstuk 6** wordt een niet-lipoproteïne etiologie van cardiovasculaire ziekten geïntroduceerd. Verhoogde homocysteïne concentraties zijn een onafhankelijke cardiovasculaire risicofactor. Sommige studies hebben bij postmenopauzale vrouwen hogere homocysteïne concentraties aangetoond dan bij premenopauzale vrouwen. Dit fenomeen zou voor een deel het gestegen cardiovasculaire risico na de menopauze kunnen verklaren. Tijdens de sequentiële 17ß-oestradiol-dydrogesteron behandeling werd een significante daling van 11% gemeten in de gemiddelde concentratie van het nuchter totaal serum homocysteïne. Vrouwen met een hoog homocysteïne hadden een daling van 17%, terwijl vrouwen met een laag homocysteïne geen verandering ondergingen. Er wordt gesuggereerd dat deze bevinding mogelijk een deel van het cardioprotectieve effect van

hormonale suppletie therapie zou kunnen verklaren. Het belang van meer onderzoek op het gebied van het homocysteïne metabolisme in relatie met de hormonale veranderingen rond de menopauze en tijdens postmenopauzale hormoon suppletie wordt aanbevolen.

De veiligheidsaspecten met betrekking tot het endometrium en de veranderingen in het bloedingspatroon tijdens de sequentiële 17ß-oestradiol-dydrogesteron behandeling worden beschreven in **hoofdstuk 7**. Histologisch onderzoek van endometrium, verkregen door middel van microcurettage, in 27 vrouwen vertoonde geen tekenen van hyperplasie of maligniteit. De onttrekkingsbloedingen waren acceptabel, en tijdens de 2 jaar durende behandeling werd een trend geconstateerd naar een kortere bloeding met minder bloedverlies. Meer dan 80% van de deelneemsters wenste de hormoon behandeling na afloop van het onderzoek voort te zetten. Deze bevindingen zijn van groot belang met betrekking tot de acceptatie van de behandeling, en dit is wederom van doorslaggevende betekenis voor het kunnen bereiken van de voordelen van lange-termijn behandeling inzake osteoporose en het cardiovasculaire systeem.

In **hoofdstuk 8** worden de resultaten van dit proefschrift geïntegreerd binnen de laatste ontwikkelingen op het gebied van postmenopauze research. In de literatuur worden vele gegevens aangedragen ten gunste van hormoon suppletie. Desalniettemin blijkt onze kennis ten aanzien van vele aspecten van hormonale suppletie therapie onvoldoende. Dit hoofdstuk benadrukt het belang van meer onderzoek, in het bijzonder onderzoek naar mogelijke nieuwe pathogenetische mechanismen die het cardioprotectieve effect van hormoon suppletie kunnen verklaren, en verder onderzoek naar nieuwe behandelschema's die acceptatie van de behandeling en de compliance kunnen verbeteren.

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CURRICULUM VITAE

Jan van der Mooren werd op 26 april 1959 geboren in Oosterbeek. Het VWO-B examen werd in 1980 behaald aan het Christelijk Atheneum te Arnhem. Vanaf 1981 werd de geneeskunde opleiding gevolgd aan de Katholieke Universiteit Nijmegen alwaar het arts-examen werd behaald op 13 januari 1989.

Na als AGNIO chirurgie werkzaam te zijn geweest op twee lokaties van het Rijnstate Ziekenhuis te Arnhem werd hij in april 1990 aangesteld als arts-onderzoeker binnen de afdeling Obstetrie & Gynaecologie van het Academisch Ziekenhuis Nijmegen Sint Radboud, alwaar onder leiding van Prof.dr. R. Rolland een aanvang werd gemaakt met de onderzoeken die de basis vormen van dit proefschrift. Deze werkzaamheden werden tijdelijk afgewisseld door een AGNIO aanstelling binnen de kliniek.

Tijdens de afronding van dit proefschrift werd een aanvang gemaakt met meerdere nieuwe trials, zowel op het gebied van postmenopauzale hormoonsuppletie als op het gebied van de ontwikkeling van nieuwe orale anticonceptiva. Hij zal voorlopig zijn onderzoeksaktiviteiten binnen de afdeling voortzetten.

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Stellingen

behorende bij het proefschrift

Hormone Replacement Therapy
Metabolic and Endometrial Changes, and the Role of Progestogens

1. Tijdens sequentiële hormoonsuppletie met de combinatie van zowel gemicroniseerd 17ß-oestradiol en dydrogesteron, als van geconjugeerde oestrogenen en medrogeston, treden gunstige veranderingen op in de serum concentraties van lipiden en lipoproteïnen. *(dit proefschrift)*

2. Tijdens hormoonsuppletie neemt de partikel-grootte van de low-density lipoproteïnen af. De betekenis van deze verandering is nog geenszins duidelijk. *(dit proefschrift)*

3. Er zijn aanwijzingen dat het homocysteïne metabolisme een rol speelt in de preventieve effecten van hormoonsuppletie bij postmenopauzale vrouwen. *(dit proefschrift)*

4. Tijdens sequentiële hormoonsuppletie met 17ß-oestradiol in combinatie met maandelijks dydrogesteron wordt binnen 2 jaar behandeling de onttrekkingsbloeding korter van duur en lichter van intensiteit. *(dit proefschrift)*

5. In de discussie betreffende het al dan niet natuurlijk zijn van hormoonsuppletie dient men zich te realiseren dat het afraden danwel onthouden van een "onnatuurlijke" therapie kan leiden tot een verhoogde kans op gezondheidrisico's samenhangend met de "natuurlijk" optredende oestrogeendeficiëntie.

6. Bij therapietrouw speelt tijd een essentiële rol, waarbij echter niet alleen aan de tijd van de patiënt dient te worden gedacht.

7. De ontwikkeling van orale contraceptiva met natuurlijke oestrogenen biedt wellicht de mogelijkheid reeds tijdens de premenopauzale jaren te profiteren van de geconstateerde preventieve effecten hiervan op hart- en vaatziekten.

8. Met het oog op de beperkte financiële middelen die er bestaan voor het verrichten van medisch wetenschappelijk onderzoek verdient samenwerking met de farmaceutische industrie een positieve benadering.

9. Bij de behoefteraming voor gynaecologen/obstetrici tot het jaar 2005 zijn demografische ontwikkelingen, deeltijdwerk, afname van de werkweek en een toename van niet-patiëntgebonden activiteiten betrokken*. De recente ontwikkelingen inzake zwangerschapsbevorderende technieken bij perimenopauzale vrouwen dienen hierbij in de toekomst niet miskend te worden.

(Hingstman L, et al. Ned Tijdsch Geneesk 1994;138:969-973)*

10. Een vloeiende overgang is niet per definitie een probleemloze.

Nijmegen, 13 oktober 1994 Marius Jan van der Mooren