EFFECT OF ESTROGEN REPLACEMENT THERAPY ON METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASES IN HYSTERECTOMIZED POSTMENOPAUSAL WOMEN

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Abstract

Estrogen replacement therapy (ERT) has been associated with favorable effects on risk factors for atherosclerosis. In observational studies ERT was also suggested to reduce the risk of cardiovascular disease in postmenopausal women, but the cardioprotective role of estrogen has been challenged after negative results in randomized trials. However, the mechanisms of estrogen action in atherosclerosis development are only partially known.

In order to investigate the regulation of plasma low-density lipoprotein (LDL) cholesterol in postmenopausal women and the effects of ERT on cholesterol and glucose metabolism and blood pressure, 79 hysterectomized, non-diabetic postmenopausal women were randomized in a double-blind, double-dummy study to receive either peroral estradiol valerate (2 mg/day) or transdermal 17β-estradiol gel (1 mg estradiol/day) for six months.

At baseline the level of LDL cholesterol was related to body mass index, the fractional catabolic rate (FCR) and the production of LDL apolipoprotein B (apo B), but not to cholesterol absorption efficiency. Both peroral and transdermal ERT decreased plasma total and LDL cholesterol, while high-density lipoprotein cholesterol and triglycerides increased only in the peroral group. The LDL-lowering response was related to changes in estrogen levels, which presumably enhance LDL receptor activity shown as an increase in FCR for LDL apo B. In contrast, the determined genetic factors, apo E phenotype, EcoRI and XbaI polymorphisms of the apo B gene and polymorphism of 7α-hydroxylase gene, were not significant in regulation of LDL cholesterol, neither did they modify the response of ERT in these postmenopausal women.

Similar outcomes were observed with both peroral and transdermal ERT as regards glucose metabolism and blood pressure. The overall effect of ERT on glucose tolerance was found to be neutral. Blood pressure decreased among non-hypertensive subjects on both estrogens, which could be related, at least in part, to the alterations in vasoactive peptides.

The data of the present study suggest an overall favorable effect of both peroral and transdermal estrogen on common cardiovascular risk factors. However, the clinical significance of these findings in the prevention of cardiovascular diseases needs to be proven in long-term, randomized trials.

Keywords: atrial natriuretic factor, blood pressure, estrogen replacement therapy, glucose metabolism, hysterectomy, LDL cholesterol, menopause
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Anna Karjalainen
Abbreviations

ACE  angiotensin converting enzyme
ANP  atrial natriuretic peptide
apo  apolipoprotein
BNP  b-type natriuretic peptide
BMI  body mass index
CEE  conjugated equine estrogen
CNP  c-type natriuretic peptide
CRP  C-reactive protein
E1   estrone
E2   estradiol
ER   estrogen receptor
ERT  estrogen replacement therapy
FCR  fractional catabolic rate
FSH  follicle stimulating hormone
GHbA1c glycosylated hemoglobin
HERS Heart and Estrogen/progestin Replacement Study
HRT  hormone replacement therapy
HDL  high-density lipoprotein
IDL  intermediate-density lipoprotein
IGF-I insulin-like growth factor-I
IGFBP-1 insulin-like growth factor binding protein-1
ISI(composite) whole-body insulin sensitivity index
ISIest insulin sensitivity index
LDL  low-density lipoprotein
Lp(a) lipoprotein(a)
MCRest metabolic clearance rate of glucose
NT-proANP N-terminal atrial natriuretic propeptide
PEPI Postmenopausal Estrogen Progestin Intervention
RAS  renin-angiotensin system
SHBG sex hormone binding globulin
VLDL very low-density lipoprotein
WHI  Women’s Health Initiative
List of original papers

The thesis is based on the following four articles, which are referred to in the text by their Roman numerals:


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References
1 Introduction

Atherosclerosis, the principal cause of cardiovascular disease, is a multifactorial, progressive disease characterized by the accumulation of lipids and fibrous elements within the arterial vessel wall (reviewed by Lusis (1) and Koh (2)). Endothelial injury and dysfunction including increased endothelial permeability to lipoproteins precede the formation of atherosclerotic lesions. Stimulated endothelial and smooth muscle cells synthesize cell adhesion molecules, chemotactic proteins and growth factors resulting in recruitment of monocytes to the arterial wall. Some specific cytokines activate inflammatory cells and transform monocytes into macrophages. Modified low-density lipoprotein (LDL) is taken up by macrophages, leading to formation of foam cells. The earliest lesions of atherosclerosis, called fatty streaks, are precursors of fibrous plaques and more advanced lesions, which are characterized by the accumulation of lipid-rich necrotic debris and smooth muscle cell proliferation. The composition and vulnerability of a plaque are suggested to be significant in the development of acute thrombotic events. (reviewed by Lusis (1) and by Ross (3))

Hypercholesterolemia, particularly high LDL cholesterol, is one of the major risk factors in the development and progression of atherosclerosis. Other important determinants are low high-density lipoprotein (HDL) cholesterol, elevated levels lipoprotein(a) (Lp(a)) or triglycerides, hypertension, diabetes mellitus, obesity, positive family history and environmental factors such as cigarette smoking, lack of exercise and high-fat diet. Age, sex and genetic factors are also involved in the regulation of metabolic risk factors. In addition, various risk factors are often clustered, and they also interact with each other. The estimation of the role of a single factor is thus a complex task. (1, 4)

At the moment the most frequent indication for hormone replacement therapy (HRT) is alleviation of menopausal symptoms (5). Prevention of osteoporosis is also well established (6), whereas the efficacy of replacement therapies in cardioprotection has been subject to debate (5).

Estrogen has been suggested to explain the observed gender differences in cardiovascular risk. Prior to the menopause the incidence of atherosclerotic diseases is lower in women compared to men of similar age, but increases along with adverse changes in metabolic risk factors related to estrogen deficiency and menopause (7, 8). Accordingly, the use of estrogen replacement therapy (ERT) has been associated with
antiatherogenic effects on lipid profile such as lower plasma total and LDL cholesterol and Lp(a) and elevated plasma HDL cholesterol, although triglycerides may increase adversely (9, 10). Further, ERT may improve glucose tolerance, lower blood pressure, have antioxidant, fibrinolytic and direct effects on vascular cells and tissues (11), but also unfavorable prothrombotic (12) and inflammatory (13) changes have been reported. Furthermore, the beneficial effects of estrogen on risk factors and atherosclerosis have also been supported by animal studies (14).

Thirty years ago ERT was found to increase the risk of cardiovascular diseases in men after myocardial infarction in the Coronary Drug Project (15), but thereafter data from several observational and epidemiological ERT/HRT studies supported the benefits of estrogen in postmenopausal women (16–18). However, recently both randomized HRT and ERT trials have failed to show a reduction in coronary events (19, 20) or in progression of coronary atherosclerosis (21–23) among older women with established coronary heart disease. Moreover, the first, large randomized primary prevention trial was discontinued prematurely due to increased risk of breast cancer with evidence of greater overall risk than benefit in combined HRT group (24). Instead of providing cardioprotection HRT was suggested to increase the risk of coronary heart disease, especially during the first year after the initiation of HRT, although beneficial changes in lipid profile were observed (24, 25).

Nevertheless, cardiovascular disease, particularly coronary heart disease and stroke, is the leading cause of morbidity and mortality not only in men but also in postmenopausal women (7, 26). Therefore it is important to evaluate the best strategies of cardiovascular prevention for women, whether they differ from men, and also the effects of estrogen. Further studies are needed not only to understand estrogen biology but also the pathogenesis of cardiovascular disease (27, 28). Most previous data concerning the mechanisms involved in controlling the risk factors, such as the regulation of cholesterol metabolism, are mainly based on studies carried out in men (15, 29, 30) or animals (31, 32). The mechanisms resulting in for example elevated LDL cholesterol level after menopause or the various mechanisms of estrogen action are incompletely understood. Finally, most replacement therapy studies have been carried out on oral conjugated estrogens. However, it is important to clarify also the effects of other estrogens, since the type of estrogen, the dose and the route of administration are likely to modify metabolic responses.

The present study was focused on established metabolic cardiovascular risk factors investigating cholesterol metabolism, particularly the regulatory mechanisms of LDL cholesterol, and further the effects of ERT on LDL cholesterol, glucose and insulin metabolism among 79 hysterectomized, non-diabetic postmenopausal women. Furthermore, blood pressure and the roles of natriuretic peptides, renin and aldosterone in the regulation of blood pressure were studied. The significance of the route in estrogen administration was assessed by comparing the responses to peroral estradiol valerate and transdermal 17β-estradiol gel therapies.
2 Review of the literature

2.1 Menopause

The menopause, the final menstrual period, is defined as the permanent cessation of menstruation after one year of amenorrhea resulting from the loss of ovarian follicular function (33, 34). The median age at natural menopause is 51 years (34, 35). The perimenopause (reviewed by Prior (36)), is the period of transition from the reproductive phase to the end of one year after menopause preceding the final menses by between 2 and 8 years and is related with the onset of menstrual irregularities, climacteric symptoms such as hot flushes, sweating, sleep disturbances and vaginal dryness and hormonal changes. The postmenopause is defined as commencing from the time of menopause.

Surgical menopause is due to either removal of the uterus, leaving at least one ovary intact or removal of both ovaries, with or without hysterectomy (33). Only the latter results in a sharp drop in endogenous sex hormone levels. However, some hysterectomized women may have premature menopause in spite of ovarian conservation. Therefore it is advisable to assess the level of follicle stimulating hormone (FSH), if premenopausal women complain of menopausal symptoms after hysterectomy (33, 37).

2.2 Hormones used in replacement therapy

In general, there are three protocols for prescribing HRT: estrogen alone, estrogen with the addition of cyclic progestin, or continuous combined estrogen and progestin therapy. Unopposed estrogen therapy, i.e. estrogen therapy alone without a progestin, is indicated only in hysterectomized women, because estrogen stimulates the endometrium and is therefore associated with increased risk of endometrial hyperplasia and cancer. The stimulation of the endometrium can be prevented in women with intact uterus, when appropriate doses of progestins are used for at least 10 days each month. (34)
2.2.1 Estrogens

Conjugated equine estrogen (CEE), which is the most widely used and studied estrogen in the United States, is a mixture containing estrone sulfate, equilin sulfate, dihydroequilin sulfate and several other estrogen sulfates. Estrone sulfate and equilin sulfate are the most important components according to amount and hormonal effectiveness (38). Natural estradiol, 17β-estradiol, estradiol valerate or mixture of estradiol, estrone and estriol are more commonly prescribed in Europe. Transdermal patches and gels contain 17β-estradiol. In contrast, the synthetic estrogens (ethinylestradiol and mestranol) are used in oral contraceptives, but not recommended in replacement therapy.

2.2.1.1 Routes of estrogen administration

The most frequent route is oral administration. In addition, various non-oral regimens are available, including transdermal patches and percutaneous creams and gels, which are also commonly prescribed. In addition, vaginal administration appears to be useful for the treatment of symptoms of urogenital atrophy, whereas subcutaneous pellets and intramuscular injections are rarely used.

Peroral estradiol is absorbed and metabolized by the intestinal mucosa and the liver during the first hepatic passage. Peroral estrogens stimulate the liver more than non-oral modes of administration, since the hormone concentrations in liver sinusoids are 4–5 times higher than those in peripheral blood (38). This first-pass effect induces the hepatic production and secretion of proteins such as hormone binding globulins, including among others sex hormone binding globulin (SHBG) and insulin-like growth factor binding protein (IGFBP), clotting factors and renin substrate. (39, 40) The principal metabolites of estradiol are estrone and estrone sulfate, which are partly reconverted to estradiol, leading to 3–6 times higher estrone concentrations. (38)

Non-oral administration of estrogen avoids the first-pass effect in liver metabolism. The estrone-estradiol ratio is around one after transdermal application of estradiol in patch or gel (41), which corresponds to physiological values observed in premenopausal women during the follicular phase. However, it is not clear whether the estrone/estradiol ratio has real clinical importance, because estrone is a weak estrogen (38). The rate of absorption depends on the dose and also the area of application when estradiol is administered through the skin (38, 40). Furthermore, there seems to be considerable interindividual variation, and therefore individual dose adjustment may be needed (38, 42).
2.2.2 Progestins

Progestins used for HRT can be divided into the following subgroups in order of decreasing androgenetic activity: C-19 derivatives of nortestosterone (norethisterone and norgestrel), the C-21 derivatives of progesterone (medroxyprogesterone acetate (MPA), dydrogesterone, medrogestone and megesterol acetate), and natural progesterone and similar compounds (34, 43). Peroral, transdermal and intrauterine progestin regimens are available in HRT. The dose of the progestin and the degree of androgenicity should be taken into account when combined replacement therapy is prescribed so as not to attenuate the potentially beneficial effects achieved by estrogen.

2.3 Action of estrogen

Multiple mechanisms are involved in the actions of estrogen in different tissues, recently reviewed by Mendelsohn & Karas (11) and Gruber et al. (44). The effects of estrogen are mediated by estrogen receptors, estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β). Estrogen receptors are expressed not only in reproductive tissues but they are also found in myocardial, endothelial and vascular smooth muscle cells, liver, breast, brain and bone. These receptors are targets for both endogenous and exogenous estrogens and pharmacological estrogen agonists, but they can also be activated by growth factors. Similar mechanisms for estrogen action have been described in different cells, the action can however be mediated by different signaling pathways. The classic signaling pathway for estrogen action is the ligand-dependent receptor activation pathway in which activated estrogen receptors are transcription factors that alter gene expression and increase protein synthesis (44). The ligand-independent activation of estrogen receptors for example by growth factors also results in nuclear actions. In addition to these transcriptional mechanisms the non-nuclear estrogen-signaling pathway through cell-membrane estrogen receptors is suggested to be involved in rapid responses to estrogen (44). Molecular actions of estrogens are determined by the structure of the hormone, the subtype or isoform of the estrogen receptor involved, the target gene promotor, and the balance of coactivators and corepressors that modulate the transcriptional activity (44).

2.4 Cardiovascular diseases in women

2.4.1 Effect of menopause

Cardiovascular diseases are rare in premenopausal women compared to men and postmenopausal women. The difference between the sexes diminishes with aging, and gradually after menopause cardiovascular disease becomes the most common cause of
morbidity and mortality in women as well. In the Framingham cohort study, in which 5,209 men and women have been followed since 1948, women lagged behind men by 10 years in the incidence for coronary heart disease and by 20 years for myocardial infarction and sudden death (45).

Loss of ovarian function and subsequent deficiency of endogenous estrogens after either natural or particularly surgically induced menopause have been associated with an increased cardiovascular risk in several studies, although there are some inconsistencies (7, 46, 47). Age at menopause has also been suggested to be a risk factor, thus a higher risk for cardiovascular mortality was observed among women with early menopause compared to those with late menopause (48). The Nurses Health Study, which is a large, prospective cohort of 121,700 female nurses who were 30 to 55 years old at baseline and have been followed since 1976, reported an elevated risk of coronary heart disease among postmenopausal women with bilateral oophorectomy, whereas hysterectomy alone or with unilateral oophorectomy did not raise the risk compared with premenopausal women (46). Further, no appreciable increase in the risk of coronary heart disease was related to natural menopause after adjustment for age and smoking.

2.4.2 Effect of hormone replacement therapy on cardiovascular diseases

2.4.2.1 Observational studies

Most epidemiological studies have observed a protective effect of estrogens on morbidity and mortality from coronary heart disease. These convincing findings have also been supported by angiographic studies showing lower incidence of coronary artery disease or milder disease (49), whereas data involving progression after coronary intervention (50, 51), carotid atherosclerosis (52–55) or risk for stroke (56) are more controversial. Moreover, the risk of venous thrombosis seems to be increased during estrogen therapy, especially oral contraceptives, containing mainly synthetic estrogens and progestins, and a high dose of estrogen, but also the lower doses of estrogen in ERT and HRT may be slightly thrombogenic (12). Further, according to previous meta-analyses, postmenopausal women using estrogens may have a 30–50% lower risk for coronary heart compared with nonusers (16–18) (Table 1). All these studies were conducted in women taking peroral estrogen, which in most studies was CEE alone, whereas only few studies have assessed the effect of combined HRT. Seven HRT studies were included in the meta-analysis, which found a 34% reduction in the relative risk for coronary heart disease among ever-users compared with never-users (18). Furthermore, the impact of nonoral treatments is unclear. A recently published population-based case-control study evaluating the relationship between HRT use and the incidence of first myocardial infarction suggested that transdermal therapy might have similar cardioprotective effects as oral therapy (57). However, these conclusions were based on a small number of subjects. (Table 1)
<table>
<thead>
<tr>
<th>Study</th>
<th>No of subjects (on ERT or HRT)</th>
<th>Follow up (years)</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Relative risk, hazard or odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson et al. 1985 (58)</td>
<td>1234 (302)</td>
<td>8</td>
<td>ERT, less than 5% on HRT</td>
<td>Cardiovascular disease</td>
<td>1.76 (total)</td>
</tr>
<tr>
<td>Nurses Health Study</td>
<td></td>
<td></td>
<td></td>
<td>Coronary heart disease</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cerebrovascular</td>
<td>2.27</td>
</tr>
<tr>
<td>Primary prevention 2000 (60)</td>
<td>70533 (current users 32.8%, past users 22.9%)</td>
<td>20</td>
<td>ERT or HRT</td>
<td>Coronary heart disease</td>
<td>0.61 (0.52–0.71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Current use</td>
<td>0.82 (0.72–0.94)</td>
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<td></td>
<td></td>
<td></td>
<td>Past use</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All stroke</td>
<td>1.13 (0.94–1.35)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Current use</td>
<td>1.02 (0.85–1.24)</td>
</tr>
<tr>
<td>Secondary prevention 2001 (63)</td>
<td>2489 (current users 29%, past users 32.4%)</td>
<td>20</td>
<td>ERT or HRT</td>
<td>Coronary heart disease</td>
<td>0.65 (0.45–0.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Current use</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Short-term use</td>
<td>1.25 (0.78–2.00)</td>
</tr>
<tr>
<td>Rosenberg et al. 1993 (69)</td>
<td>858 (176/205 on ERT)</td>
<td>case-control study</td>
<td>ERT or HRT</td>
<td>Myocardial infarction</td>
<td>0.8 (0.4–1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Recent use</td>
<td>0.9 (0.7–1.3)</td>
</tr>
<tr>
<td>Sidney et al. 1997 (70)</td>
<td>876 (55.5%)</td>
<td>case-control study</td>
<td>ERT or HRT</td>
<td>Myocardial infarction</td>
<td>0.96 (0.66–1.40)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Current use</td>
<td>1.07 (0.72–1.58)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Past use</td>
<td></td>
</tr>
<tr>
<td>Varas-Lorenzo et al. 2000 (57)</td>
<td>1031 (22%)</td>
<td>case-control study</td>
<td>ERT or HRT</td>
<td>Myocardial infarction</td>
<td>0.72 (0.59–0.88)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Current/recent use ERT</td>
<td>0.52 (0.38–0.78)</td>
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<td>Current/recent use HRT</td>
<td>0.79 (0.59–1.08)</td>
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<td></td>
<td>Current/recent use Oral</td>
<td>0.66 (0.50–0.88)</td>
</tr>
<tr>
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<td></td>
<td>Current/recent use Transdermal</td>
<td>0.75 (0.47–1.21)</td>
</tr>
<tr>
<td>Sourander et al. 1998 (61)</td>
<td>7944 (988 current users, 757 former users)</td>
<td>6</td>
<td>ERT or HRT</td>
<td>Cardiovascular disease mortality occurring mortality</td>
<td>Current vs former use 0.21 vs 0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coronary heart disease mortality occurring mortality</td>
<td>1.07 vs 1.11</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Stroke mortality occurring mortality</td>
<td>1.05 vs 1.23</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Coronary heart disease mortality occurring mortality</td>
<td>0.16 vs 1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stroke mortality occurring mortality</td>
<td>0.86 vs 1.08</td>
</tr>
</tbody>
</table>

Table 1. ERT and HRT and the risk of cardiovascular disease. Results of observational studies including the studies of good-quality, a Finnish cohort study and 5 meta-analyses.
In the Framingham study postmenopausal ERT was associated with increased cardiovascular mortality, particularly in women who were smokers (58), but generally observational data suggest that women with high risk may benefit more than those with lower risk (59). In addition, current users of ERT/HRT seem to have lower cardiovascular

<table>
<thead>
<tr>
<th>Study</th>
<th>No of subjects (on ERT or HRT)</th>
<th>Follow up (years)</th>
<th>Treatment Outcome</th>
<th>Relative risk, hazard or odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meta-analyses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stampfer &amp; Colditz 1991 (16)</td>
<td>6 case-control, 13 cohort studies with internal controls</td>
<td>ERT (mainly) or HRT</td>
<td>Coronary heart disease</td>
<td>0.76 (0.61–0.94) 0.58 (0.48–0.69)</td>
</tr>
<tr>
<td>Grady et al. 1992 (17)</td>
<td>32 studies</td>
<td>ERT (mainly) or HRT</td>
<td>Coronary heart disease</td>
<td>0.63 (0.55–0.72) 0.65 (0.59–0.71) 0.96 (0.82–1.13)</td>
</tr>
<tr>
<td>Barret-Connor &amp; Grady 1998 (18)</td>
<td>25 ERT studies, 7 HRT studies</td>
<td>ERT</td>
<td>Coronary heart disease</td>
<td>0.70 (0.65–0.75) 0.66 (0.53–0.84)</td>
</tr>
<tr>
<td>Humphrey et al. 2002 (71)</td>
<td>Primary prevention studies of good- or fair-quality included (21 studies and 1 meta-analysis)</td>
<td>ERT or HRT (any use)</td>
<td>Cardiovascular disease</td>
<td>0.75 (0.42–1.23) 1.28 (0.86–2.00)</td>
</tr>
<tr>
<td>Nelson et al. 2002 (56)</td>
<td>Studies of good- or fair-quality including 11 case-control and 10 cohort studies, 1 small trial and a review of HERS and WHI trials</td>
<td>ERT or HRT</td>
<td>Cardiovascular disease</td>
<td>0.75 (0.42–1.23) 1.28 (0.86–2.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coronary heart disease</td>
<td>0.62 (0.40–0.90) 0.74 (0.36–1.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mortality current use all use incidence</td>
<td>0.80 (0.68–0.95) 0.97 (0.82–1.16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>adjusted current use all use</td>
<td>0.88 (0.64–1.21) 1.12 (1.01–1.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stroke Thromboembolism Overall, current use First year</td>
<td>2.14 (1.64–2.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.49 (2.33–5.59)</td>
</tr>
</tbody>
</table>
mortality compared to never-users, while the risk reduction appears to diminish after discontinuation of the treatment (59–61). Thus the advantage of ERT/HRT may decrease if past users are included in the assessment. Even though HRT has been suggested to lower also overall mortality, recent data including randomized studies have emphasized increased risks (19, 24). In addition to an excess of venous thromboembolic events (62), an increased risk of recurrent cardiac events has been reported within the first months after starting replacement therapy (63–65), whereas the risk of breast cancer has been related to long-term use of estrogen (66).

On the other hand, observational studies have used a cohort, case-control or cross-sectional study design that may overestimate the extent of advantages due to selection bias, since the women taking estrogen may have lower risk for cardiovascular disease already before starting ERT. Thus, compared to non-users, they tend to be younger, leaner, better educated and more health conscious, and have higher socioeconomic status and a healthier lifestyle with less heart disease, diabetes or hypertension at baseline (67, 68). Accordingly, the cardioprotective effect of ERT/HRT seemed to diminish in observational studies (69, 70) and latest meta-analyses (56, 71) after adjustment of confounding major cardiovascular risk factors and socioeconomic status (Table 1).

2.4.2.2 Randomized studies

In contrast to consistent epidemiological evidence and promising findings in animal model studies and clinical trials, the results of randomized studies, not available until recent years, have not been as positive as expected (Table 2). A pooled data of 22 small, mostly short-term studies that evaluated other effects of HRT showed an insignificant increase in the risk of cardiovascular events among women who were randomized to receive HRT (72). In these trials cardiovascular events had been reported incidentally as drop-outs or adverse effects, but thereafter also larger, long-term randomized studies have failed to confirm a favorable effect of ERT or HRT on cardiovascular diseases. (Table 2)

The first randomized, prospective, placebo-controlled trial (the Heart and Estrogen/progestin Replacement Study, HERS) was not able to find a reduction in cardiovascular risk during 4.1 years of therapy with oral 0.625mg of CEE plus continuous 2.5 mg of MPA among 2,763 postmenopausal women with established coronary disease and an average age of 66.7 years (19). In fact, coronary events were more frequent in the HRT group compared to placebo during the first year, and although an insignificantly less frequent trend was observed thereafter, it was not sustained in follow-up (73) (Table 2). In addition, no significant effect on the risk of stroke or progression of peripheral artery disease was found among these women, but the incidence of thromboembolic events and gallbladder disease was increased in the HRT group (73–75).
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects/ follow-up (y)</th>
<th>Mean age (range)</th>
<th>Treatment</th>
<th>Results</th>
<th>Cardiovascular risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary prevention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled data of 22 trials, 1997 (72)</td>
<td>41/28/34</td>
<td></td>
<td>Various forms of ERT/HRT or placebo or other therapy</td>
<td>Cardiovascular events: ods ratio 1.35 (95% CI 0.48—3.55)</td>
<td>NS</td>
</tr>
<tr>
<td>Angris et al. 2001 (79)</td>
<td>74/48 weeks</td>
<td></td>
<td>Percutaneous 17β-estradiol (1mg) + gelsemine (2 different doses) effect placebo</td>
<td>No effect in slowing progression of clinical atherosclerosis measured by carotid intima-media thickness</td>
<td>NS</td>
</tr>
<tr>
<td>Estrogen and Prevention of Atherosclerosis (EFAT), 2001 (78)</td>
<td>22/2</td>
<td>(44–80)</td>
<td>Percutaneous 17β-estradiol (1mg) or placebo</td>
<td>Slower progression (carotid intima-media thickness, estrogen vs placebo 0.017mm vs 0.036mm, p&lt;0.05)</td>
<td>Decreased</td>
</tr>
<tr>
<td>Women’s Health Initiative (WHI). 2002 (24,25)</td>
<td>16/20</td>
<td>(50–79)</td>
<td>Percutaneous 0.625mg + MPA (2.5 mg) or placebo</td>
<td>Coronary heart disease events: Hazard ratio 1.26 (95% CI 0.97–1.60), first year 1.81 (1.09–3.01), Stroke 1.41 (0.48–4.31), Thromboembolic events 2.11 (1.26–3.55)</td>
<td>Increased</td>
</tr>
<tr>
<td><strong>Secondary prevention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart and Estrogen/progestin Replacement Study (HERS), 1998 (19,73,75)</td>
<td>27/5</td>
<td></td>
<td>Percutaneous 0.625mg + MPA (2.5 mg) or placebo</td>
<td>Coronary heart disease events: Relative hazard 0.59 (95% CI 0.84—1.17), first year 1.52 (1.01–2.92), Stroke 1.49 (0.85–1.35), Peripheral artery disease 0.87 (0.70–1.08), Thromboembolic events 2.03 (1.23–3.41)</td>
<td>NS</td>
</tr>
<tr>
<td>Estrogen Replacement and Atherosclerosis Trail (ERA), 2000 (21)</td>
<td>30/3</td>
<td></td>
<td>Percutaneous 0.625mg + or placebo 0.625mg + or placebo</td>
<td>No differences in the progression of angiographic changes or cardiovascular events</td>
<td>Increased</td>
</tr>
<tr>
<td>Women’s Estrogen for Stroke Trial (WES), 2001 (77)</td>
<td>66/2</td>
<td></td>
<td>Percutaneous 17β-estradiol (1mg) or placebo</td>
<td>Recurrent nonfatal stroke RR 1.0 (95% CI 0.7–1.4); death 1.2 (C 0.8–1.8); fatal stroke 2.0 (C 0.8–5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Papworth HRT Atherosclerosis Study, 2002 (76)</td>
<td>25/1</td>
<td></td>
<td>Transdermal 17β-estradiol or cyclic estradiol + placebo</td>
<td>Coronary heart disease events RR 1.49 (95% CI 0.93–2.34)</td>
<td>NS</td>
</tr>
<tr>
<td>Women’s Angiographic Vitamin and Estrogen Trial, 2002 (22)</td>
<td>42/3</td>
<td></td>
<td>Percutaneous 0.625mg + MPA (2.5 mg) or placebo</td>
<td>Some angiographic progression (NS). Cardiovascular events: 1.5 (95% CI 0.57–3.3)</td>
<td>Slightly increased</td>
</tr>
<tr>
<td>Estrogen in Prevention of Reinfarction Trial (ESPRIT), 2002 (20)</td>
<td>10/7</td>
<td>(56–69)</td>
<td>Percutaneous estradiol (2mg) or placebo</td>
<td>Reinfarction or cardiac death RR 0.99 (95% CI 0.70–1.41)</td>
<td>NS</td>
</tr>
<tr>
<td>Women’s Estrogen-Fragment Lipid-Lowering Hormone Atherosclerosis Trial (WELL-HART), 2003 (23)</td>
<td>22/3</td>
<td></td>
<td>Percutaneous 17β-estradiol (1mg) or cyclic MPA (5mg) or placebo</td>
<td>No significant effect on the progression of atherosclerosis measured by quantitative coronary angiography</td>
<td>NS</td>
</tr>
</tbody>
</table>
In agreement, neither oral conjugated estrogen (0.625 mg/d) or 17\(\beta\)-estradiol alone, nor combined estrogen and progestin treatment seemed to prevent angiographic progression of coronary atherosclerosis (Table 2) (21, 23). Instead, a potential harm has been reported with combination therapy (22). Further, no difference in cardiac events was found during unopposed ERT (estradiol valerate 2 mg/day) compared to placebo for 2 years (20). Similarly, the results from a placebo-controlled study with transdermal 17\(\beta\)-estradiol alone or in combination with cyclic noretidrone revealed no benefit (76). Finally, ERT (17\(\beta\)-estradiol 1 mg/day) for three years was ineffective in reducing the risk of cardiac events, stroke or mortality in postmenopausal women with a recent cerebrovascular event (77). (Table 2)

A slower progression of subclinical atherosclerosis assessed by carotid artery intima-media thickness was observed by ultrasound in healthy postmenopausal women without preexisting cardiovascular disease using unopposed ERT (micronized 17\(\beta\)-estradiol 1 mg/d) compared to placebo, but not in those women who were taking lipid-lowering drugs (78). In contrast, combined HRT with 17\(\beta\)-estradiol plus a standard or lower dose of gestodene did not slow the progression of subclinical atherosclerosis (79). (Table 2)

Finally, not only randomized secondary prevention studies but also the first, large randomized controlled trial of HRT and primary prevention, Women’s Health Initiative (WHI) study, including 16,608 healthy menopausal women failed to show a beneficial cardiovascular effect with continuous combined HRT (Table 2) (24). In contrast, the risk of coronary heart disease was reported to increase slightly during the first year (25). The risk of thromboembolic events was also increased. The HRT part of this study was recently stopped after a mean of 5.2 years of follow-up because of increased risk for breast cancer. Further, a global disease index suggested risks exceeding benefits. However, the part of the study comparing estrogen alone with placebo in hysterectomized women continues.

### 2.5 Cardiovascular risk factors in women

Major risk factors for atherosclerosis including dyslipidemia, diabetes mellitus, hypertension, smoking and overweight appear to be similar in both sexes, although the magnitude of their relative significance may differ. (4, 7, 80, 81)

Elevated levels of total cholesterol and specifically LDL cholesterol are clearly related to cardiovascular morbidity and mortality in both sexes (4, 7). Women appear to have generally higher levels of protective HDL cholesterol than men. In fact, some studies have suggested that low HDL cholesterol, particularly high total/HDL ratio or low HDL-2 subfraction, could be more a significant risk factor for females than males, whereas total cholesterol would be less important for women (7, 8, 82, 83). Further, the role of triglycerides is controversial, but elevated triglycerides have been suggested to be a stronger predictor of cardiovascular diseases in women than in men (80, 84). Finally, high Lp(a) levels have been associated with an increased risk both in women and men (85).
Not only diabetes, but also impaired glucose tolerance and hyperinsulinemia have been associated with an increased risk for atherosclerosis (8, 86). Moreover, diabetes mellitus appears to almost eliminate the gender difference in coronary heart disease mortality (7, 87).

Adverse changes in cholesterol and glucose metabolism, in insulin action, body fat distribution, blood pressure, hemostatic system and vascular function have been related to menopause. However, it is not ascertained whether these findings are attributed to the decline in endogenous estrogens, because similar alterations in risk factors are, at least to some extent, associated with aging. (81, 88–91)

In addition to age and gender, genetic, dietary factors, smoking, alcohol consumption, physical activity, socioeconomic status and medication are known to modify metabolic risk factors and the risk of cardiovascular diseases (4, 92). Both menopause and increasing age are often accompanied with changes in lifestyle and a tendency to weight gain with abdominal fat distribution (93). Thus, different factors tend to be interdependent. For example overweight, especially abdominal obesity, predisposes to unfavorable lipid changes, such as low HDL, high LDL/HDL ratio and triglyceride, hyperinsulinemia, impaired glucose tolerance, diabetes and elevated blood pressure (94–97).

2.6 Overview of lipid metabolism

2.6.1 Lipids and lipoproteins

The major lipids in human plasma are cholesterol, cholesteryl esters, triglycerides and phospholipids. Triglyceride and cholesteryl ester molecules, which are insoluble in aqueous solutions, are carried in the core of spherical macromolecular complexes, called lipoproteins (reviewed by Ginsburg (98) and Bachorik et al (99)). In lipoprotein particles the hydrophobic lipid core is surrounded by an amphipathic monolayer of phospholipids, free cholesterol and specific apolipoproteins. Lipoproteins share common lipid and apolipoprotein components, but apolipoproteins and the amounts of cholesterol, triglyceride, phospholipids vary between lipoprotein particles. Consequently, lipoproteins can be identified based on particle size, chemical composition, physicochemical and flotation characteristics or electrophoretic mobility. The main lipoproteins are commonly classified according to their hydrated densities as follows: chylomicrons (density <0.95 g/ml), very low-density lipoprotein (VLDL, density 0.95–1.006 g/ml), intermediate-density lipoprotein (IDL, density 1.006–1.019 g/ml), low-density lipoprotein (LDL, density 1.019–1.063 g/ml) and high-density lipoprotein (HDL, density 1.063–1.210 g/ml). Further, specific subfractions of these lipoproteins can be determined. For example, HDL cholesterol consists of heterogeneous group of particles. The main apolipoproteins (apo) of HDL cholesterol are apo A-I and apo A-II. HDL subfractions are commonly defined
either by density to HDL2 and HDL3 subfractions, or by apolipoprotein content to lipoprotein (Lp) apoA-I and apoA-I/apoA-II particles, containing either apoA-I without apoA-II or both apoA-I and apoA-II, respectively.

Furthermore, there is a LDL-like particle, Lp(a) (density 1.045–1.080 g/ml), which has been modified by binding of apo (a) via disulfide bridge to apo B. Due to structural homology with LDL and also with plasminogen, Lp(a) is suggested to be proatherogenic and thrombogenic.

Chylomicrons and VLDL particles are mainly transporting triglycerides, whereas cholesterol is carried by LDL and HDL particles. Lipids serve as a source of energy, as a component of cell membranes, as a precursor for steroid hormones and bile acids. Apolipoproteins are important for structural stability of lipoproteins, in binding these particles to specific receptors, and they also act as cofactors for enzymes.

The lipid transport can be divided into the exogenous pathway, which refers to the metabolism of intestinally derived lipoproteins, and the endogenous pathway, which refers to hepatic-derived lipoproteins. In the plasma lipid transport is regulated by specific apolipoproteins, lipoprotein receptors, lipolytic enzymes and transfer proteins. The liver has a central role in the regulation of lipoprotein metabolism. The following review focuses on LDL cholesterol metabolism.

### 2.6.2 Regulation of LDL cholesterol

Plasma levels of LDL cholesterol are mainly determined by the production of apo B, the apolipoprotein of LDL cholesterol, by the conversion of VLDL to LDL, and by LDL-receptor mediated clearance. In addition, the cholesterol absorption efficiency, enterohepatic cholesterol metabolism and genes encoding various proteins involved in LDL metabolism modify LDL cholesterol concentrations.

#### 2.6.2.1 Cholesterol absorption

The diet and the bile are the major sources of intestinal cholesterol, thus the cholesterol absorbed from the intestine is a mixture of exogenous and endogenous cholesterol, reviewed by Wilson & Rudel (100). In fact, dietary cholesterol represents only about one-third of total intestinal cholesterol pool, while endogenous sources, the bile and to some extent intestinal mucosal cholesterol account for the remaining two-thirds. Only free cholesterol appears to be absorbed. Lipolytic pancreatic enzymes and solubilizing properties of bile salts are needed before dietary fat is absorbed.

After absorption the intestinally derived cholesterol is packaged into chylomicrons within enterocytes and transported via the lymph to the circulation. The chylomicrons are triglyceride-rich lipoproteins containing apo B48 that is necessary for the initial assembly and secretion of these particles. In the plasma, the chylomicrons acquire different classes of apo A and apo C, and also apo E. Apo C II is an activator of lipoprotein lipase that stimulates the hydrolysis of triglycerides in chylomicrons resulting in the formation of
remnant particles, whereas phospholipids, free cholesterol and apo C are transferred to HDL. The cholesterol-rich chylomicron remnants are removed from the circulation to the liver by remnant or LDL receptors, which recognize apo E. (100)

Significant interindividual variation has been observed in cholesterol absorption, the values ranging from 15% to 80% (101–103). In men, dietary cholesterol absorption was found to correlate positively with plasma total and LDL cholesterol concentration (101), but among subjects consuming a low-cholesterol low-fat diet the efficiency of intestinal cholesterol absorption and the amount of dietary cholesterol absorbed were not related to plasma cholesterol and LDL cholesterol (103). In addition, individual variation in responses to the amount and the quality of dietary fat has been observed both in animal and human studies, suggesting genetic control of cholesterol absorption (103). Agents inhibiting cholesterol absorption include among others dietary fiber such as guar gum and plant sterols (β-sitosterol and sitostanol) and ezetimibe, a new selective inhibitor of intestinal cholesterol absorption (104, 105). Cholesterol homeostasis is partly balanced by dietary cholesterol absorption, while dietary cholesterol modifies other regulatory mechanisms inducing down-regulation of hepatic cholesterol synthesis and LDL receptors (106).

2.6.2.2 Production and clearance of LDL cholesterol

LDL is formed mainly by the catabolism of VLDL and IDL, but to some extent also by hepatic secretion. Triglyceride-rich VLDL particles are synthesized in the liver and they transport triglycerides to adipose tissue and muscle. When VLDL is hydrolyzed by lipoprotein lipase, smaller VLDL remnants, IDL particles are generated and surface lipids and apolipoproteins are transferred to HDL as in hydrolysis of chylomicrons. Remnant particles are partially removed by the liver via receptor-mediated pathway, or IDL is further converted to LDL by hepatic lipase. Transfer proteins (cholesterol ester transfer protein, CETP and phospholipid transfer protein, PLTP) modify the size and the composition of lipoproteins by exchanging lipids between lipoprotein particles. LDL primarily transports cholesterol to peripheral target cells, whereas HDL cholesterol, mainly HDL2 subfraction, is an antiatherogenic lipoprotein. Thus it is responsible for the reverse transport of cholesterol from peripheral tissues to circulation and to the liver for degradation. (reviewed by Ginsberg (98))

Apo B is the major apolipoprotein of LDL and has a central role in LDL cholesterol metabolism. Apo B is produced by the liver and secreted in VLDL. Availability of lipids regulates whether apo B is secreted or degraded. Moreover, production of apo B is correlated with LDL cholesterol level (107).

The clearance of LDL cholesterol from plasma is primarily mediated by LDL receptors (108, 109). In addition, some of LDL may be removed by scavenger receptors, which are responsible for the clearance of modified LDL, or by non-receptor-mediated pathways (106). The LDL receptor is a single-chain transmembrane glycoprotein that specifically binds two proteins, apo B and apo E. LDL receptors are mainly expressed in the liver, but they are also present on the surface of nearly all normal cells where the uptake of plasma LDL provides cholesterol for membrane synthesis and other
requirements of these cells. After binding the lipoprotein-receptor complex is internalized by endocytosis. LDL dissociates from the receptor, which returns to the cell surface and is again able to bind lipoproteins. Within lysosomes, cholesterol esters of LDL are converted to free cholesterol by acid lipase and apolipoproteins are degraded to amino acids. Free cholesterol is delivered to the cytoplasm. Inverse correlation has been observed between LDL cholesterol and fractional catabolic rate (FCR) for LDL apo B that reflects receptor-mediated clearance of LDL (106, 109).

2.6.2.3 Enterohepatic cholesterol metabolism

In the liver cholesterol is re-esterified by acyl coenzyme A:cholesterol acyltransferase (ACAT) and stored as cholesteryl esters, reused for lipoprotein synthesis and resecreted into the plasma or excreted into the bile either as free cholesterol or after conversion to bile acids. Cholesterol 7α-hydroxylase is the rate-limiting enzyme in bile acid synthesis. After secretion biliary cholesterol and bile acids are either reabsorbed from the intestine or excreted as fecal neutral and acidic sterols. (106) Finally, hepatic cells may also synthesize cholesterol from acetate by microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-limiting enzyme of cholesterol biosynthesis. The cholesterol content of hepatocytes regulates the number of LDL receptors. Excess of cellular cholesterol, for example after high intake of dietary saturated fat and cholesterol, down-regulates LDL receptors, which lowers the uptake of LDL and consequently results in elevated plasma LDL cholesterol levels and accumulation of cholesterol in peripheral cells. Cholesterol excess also induces suppression of HMG CoA reductase and cellular cholesterol biosynthesis is decreased. Conversely, cholesterol-lowering treatments with bile acid-binding resins or statins, inhibitors of HMG CoA reductase, and other causes of low cellular cholesterol stimulate the expression of hepatic LDL receptors and decrease plasma LDL cholesterol levels. (109)

2.6.2.4 Genetic factors

In addition to environmental factors, interindividual variation in plasma LDL cholesterol concentrations is partly controlled by genetic factors. Genes encoding various proteins involved in cholesterol metabolism such as apolipoproteins, lipoprotein receptors, lipid transfer proteins and enzymes modify plasma LDL cholesterol levels. Familial hypercholesterolemia caused by several different mutations in the gene encoding LDL receptor and other genetic defects are quite rare. In contrast, the common polymorphisms of some regulatory proteins, such as apo E, apo B and 7α-hydroxylase, have been reported to mainly explain the heritable variation of plasma LDL cholesterol levels in the general population (110–113). Concerning this polymorphism the data on apo E are the most comprehensive. Three common alleles (ε2, ε3, ε4) code for three major isoforms (apo E2, apo E3 and apo E4) resulting in six different phenotypes (E2/2, E2/3, E2/4, E3/3, E4/3, E4/4). Apo E polymorphism has been estimated to account for about 5–10% of
the variance in plasma LDL cholesterol. In addition, an association between apo E phenotypes and cholesterol absorption efficiency, synthesis and clearance of LDL cholesterol has been observed in men (29, 114, 115). The subjects with the ε2 allele have lower total and LDL cholesterol levels, cholesterol absorption and a lower LDL apo B production rate, and higher FCR for LDL apo B than those who are homozygous for the ε3 allele, while the subjects with at least one ε4 allele tend to have higher levels of total and LDL cholesterol, cholesterol absorption and LDL production and lower LDL clearance than the others (29).

2.7 Lipid and lipoprotein metabolism in postmenopausal women

2.7.1 Effect of menopause

Atherogenic alterations in lipid and lipoprotein profiles have been found in studies of surgically induced menopause (91, 116, 117), epidemiological studies comparing premenopausal women with menopausal and postmenopausal women (89, 90, 118–121) and also confirmed by longitudinal trials (88, 122, 123). Gradual changes in lipoproteins were actually seen within two years preceding the menopause in a longitudinal study (122). An increase of both total and LDL cholesterol concentrations has been observed during menopause. Additionally, a shift to smaller, denser and potentially more atherogenic LDL particle sizes has been related to menopause (116, 124). However, data on HDL cholesterol have been inconsistent, as HDL cholesterol has been reported to remain unaffected (7), but also a decline of HDL cholesterol, particularly HDL2 subfraction, with a rise of HDL3 cholesterol has been observed, which may result in unfavorable net effect (122, 125). An increase of plasma triglycerides and Lp(a) concentrations has also been reported after menopause (90).

However, there has been controversy about the importance of endogenous estrogens, because age and menopause are closely related. In fact, the influence of menopause on lipoprotein changes varies between different studies (90), and it may diminish after adjustment with age and other confounding factors (88, 89, 121). Therefore it is important that menopausal status is adequately determined, a sufficient number of women are included and the age range is wide enough when the effects of menopause are evaluated. Total, LDL and VLDL cholesterol as well as triglycerides seem to increase with age in both sexes. These cholesterol levels tend to be significantly higher in men compared with women before menopause, but the gender difference diminishes particularly in VLDL cholesterol and triglyceride levels during postmenopausal years. (7) In contrast, women tend to have higher levels of HDL cholesterol than men also after menopause.
2.7.2 Effect of estrogen replacement therapy

2.7.2.1 Lipids and lipoproteins

The effects of estrogens on serum lipids and lipoproteins are most widely studied regarding cardiovascular risk factors. Favorable changes in the lipoprotein profile have previously been estimated to account for 25–50% of the cardioprotective effects observed in postmenopausal women during ERT (126). Lipid and lipoprotein responses to ERT depend on the type and dose of estrogen and the route of administration. A number of studies have shown that peroral ERT results in a significant decrease of total and LDL cholesterol concentrations, an increase of HDL cholesterol, but also of triglycerides, whereas a less marked reduction in total and LDL cholesterol with a modest, if any, change in HDL cholesterol and triglyceride levels have been observed during transdermal estrogen treatments (9, 127–133). In HRT the concomitant progestin may alter and partly counteract the effects of estrogen according to the degree of androgenicity of the progestin (10, 133). To some extent the effect of progestin may be dose-dependent. Progestins may attenuate the increase of HDL cholesterol and triglycerides, but they do not modify significantly the effect on total and LDL cholesterol. Lp(a) concentrations appear to be largely genetically determined and resistant to most forms of environmental modification, including diet and lipid-lowering drugs with the exception of niacin. However, both peroral estrogen alone and in combination with progestin has been reported to lower Lp(a), whereas transdermal ERT appears not to affect Lp(a) levels (134–137).

A pooled analysis of prospective HRT studies published between 1974–2000 (133) revealed that the route of estrogen administration and the type of progestin determine differential effects of HRT on lipid and lipoprotein levels. According to that analysis peroral estrogens alone, both CEE (1.25 mg/d or 0.625 mg/d) and estradiol 17-β or estradiol valerate at doses of 2 mg/d, induced comparable decreases in total and LDL cholesterol levels. Pooled mean of percentage changes was about –3–4 % and –11%, respectively. Estradiol regimens increased HDL cholesterol by 10–11% and triglycerides by 3–11%, whereas the effect of CEE seemed to be more profound and dose-dependent, particularly in triglycerides, resulting in a 15–18.5 % increase in HDL cholesterol level and a 19–28.5 % increase in triglycerides. In contrast, synthetic estrogens such as ethinyl estradiol may increase serum triglycerides even more. However, synthetic estrogens are no more used in postmenopausal ERT.

Both types of transdermal ERT, patches and gel (the latter is often called percutaneous ERT), contain 17-β estradiol and appear to have similar effects on lipids and lipoproteins. These parenteral regimens of ERT were reported to decrease total and LDL cholesterol on average by 2–7 % and by 4–7 %, respectively, and to increase HDL cholesterol by 0–6 %. In contrast to peroral estrogens the transdermally administered estradiol seemed not to affect or slightly reduced triglyceride levels (mean percentage change –8–0 %) (133).
Despite similar treatment there was, however, considerable variation between the studies in the pooled analysis. Particularly changes in triglycerides differed substantially. Various factors in study design and quality, such as size and composition of study population, different treatment durations, differences in laboratory methods and lack of placebo controls, may explain differences.

2.7.2.2 Regulation of lipoprotein metabolism

Effect of estrogen on VLDL and LDL metabolism. According to kinetic studies peroral estrogens increase VLDL cholesterol by inducing the hepatic production rate of light VLDL (127, 138). Further, IDL concentration was not altered by estrogen since no change in overall production or FCR of IDL was observed (138). A simplified presentation about the effects of estrogen on lipoprotein metabolism is shown in Fig. 1.

Decreased LDL receptor activity has been observed in hypercholesterolemic postmenopausal women (139), whereas estrogen has been shown to increase the rate of LDL catabolism (30, 127, 138). In rats estrogen induced the expression of hepatic LDL receptors (31, 32, 140). Kinetic studies have shown that not only high doses of estrogen used in treatment for prostatic cancer enhance LDL -receptor mediated uptake of LDL cholesterol and decrease plasma LDL concentration (30, 141), but also peroral ERT increases both the production rate and especially the FCR of LDL resulting in reduction in LDL cholesterol and apo B concentrations (Fig. 1) (127, 138). Further, the effect of estrogen on hepatic cholesterol metabolism has been reported to be dose-dependent (142).

In addition, part of estrogen-induced LDL lowering could be attributed to increased apo B catabolism by LDL-receptor-independent pathways (143) or removal of desialylated LDL by transcytosis (144). Estrogen has also been reported to accelerate the conversion of hepatic cholesterol to bile acids (145, 146). A dose-dependent increase of plasma triglyceride levels seems to be related to the enhanced hepatic production of large triglyceride-rich VLDL particles, not to impaired VLDL catabolism (127). (Fig. 1)

Finally, in contrast to peroral administration, transdermal estradiol did not induce a significant effect on VLDL or LDL cholesterol metabolism in postmenopausal women (127).

In addition, estrogen may induce qualitative changes in LDL. Estrogen has been reported to decrease more large LDL particles because the production rate of light LDL is increased by a lesser amount than the FCR, whereas small and dense LDL particles remain unchanged due to similar increases in production and FCR. (138) These smaller particles may be more atherogenic and more susceptible to oxidative modification than the larger ones. Nevertheless, both peroral and transdermal estrogen have been reported to inhibit the susceptibility of LDL to oxidative modification in vitro (147, 148). Further, the antioxidative effect of estrogen has been suggested to depend on the changes in triglycerides during the ERT, because the increase of triglycerides is associated with the decrease of LDL particle size. (147, 149)
**ERT and HDL metabolism.** In a kinetic study HDL cholesterol levels were primarily determined by HDL size, apo AI and apo AII levels in normolipidemic females (150). HDL size was inversely correlated with hepatic lipase activity, BMI and triglycerides. Apo AI transport rate was the major determinant of apo AI and correlated also with HDL cholesterol level, whereas FCR of apo AI did not. Menopause had no significant effect on HDL cholesterol levels or HDL turnover. (150)

Oral but not transdermal ERT has been reported to increase HDL cholesterol and apo AI, particularly HDL2 and Lp A-I subfractions. In metabolic studies oral estrogen raised HDL cholesterol by increasing the production of apo AI and by reducing hepatic lipase activity, while lipoprotein lipase activity and FCR of Lp A-I and Lp A-I/A-II were unchanged (Fig. 1) (151, 152). In contrast, transdermal estradiol for six weeks had no significant effect on HDL levels or metabolic rates (153). Similarly, estrogen has been shown to increase hepatic apoA-I but not apoA-II mRNA transcription *in vitro*, resulting in a selective increase of apoA-I containing HDL particles (154).
2.7.2.3 Response to ERT and genetic factors

Genetic factors may modify lipoprotein response to ERT, although thus far the findings including apo E and ER-α polymorphisms are inconsistent and limited. Apo E genotype has been suggested to contribute to changes of LDL (155) or HDL cholesterol (156) or triglycerides during HRT (157). Further, an augmented response of HDL cholesterol to estrogen has been found in postmenopausal women with established coronary artery disease who have the ER-α IVS1-401 C/C genotype, or several other closely linked intron 1 polymorphisms (158).

2.8 Glucose and insulin metabolism

2.8.1 Effects of menopause

Both menopause and aging have been associated with an increase in plasma glucose and insulin levels resulting in impaired glucose tolerance and increased insulin resistance (88, 159, 160). Reduction in the secretion of C-peptide and lower elimination of insulin was observed in intravenous glucose tolerance test among postmenopausal women compared with premenopausal women (160). According to PEPI trial BMI and waist-to-hip ratio account for nearly all the explained variance of glucose and insulin levels among healthy postmenopausal women who were not taking estrogen (161). However, about two thirds of the variance remained unexplained.

2.8.2 Effect of ERT

In epidemiological studies, postmenopausal estrogen use has been associated with reduced fasting glucose and insulin levels (9, 162). Further, a population-based study reported also lower GHbA1c levels among current HRT users compared with non-users, and the difference was independent of confounders such as age, BMI and lifestyle factors (163, 164). Moreover, a tendency to lower incidence of diabetes (165) or even significant reduction in diabetes (166–168) has been related to current use of estrogen, but the association was not significant after adjustment for age and obesity (165, 166).

Both the type and dose of estrogen and also the route of administration appear to modify the response to ERT. In addition, some discrepancy seems to be related to different methods that have been used for assessment of carbohydrate metabolism and insulin resistance, including fasting values of glucose and insulin, oral glucose tolerance test, insulin tolerance test, intravenous glucose tolerance test and hyperinsulinemic euglycemic clamp. Thus, the results of ERT studies are partially controversial (169–174).

The effects of menopause and the commonly used estrogen regimens, peroral CEE, estradiol (including estradiol valerate and 17β-estradiol) and transdermal estradiol on
glucose metabolism reported during the past twenty years have been summarized in Table 3. Further, in HRT the addition of certain progestins may alter the glucose balance and insulin sensitivity (reviewed by Godsland (174)).

Synthetic estrogens including ethinyl estradiol and mestranol (169, 170, 175), but also higher dose of CEE (1.25 mg/day) (172) may impair glucose tolerance and insulin sensitivity. In general, the effect of peroral estrogen on glucose metabolism seems to be rather neutral (170, 176), though slightly beneficial responses have been attributed to a lower dose (0.625 mg/day) of CEE (171, 173, 177, 178) or estradiol (179).

No significant changes were observed during 3 months’ treatment with CEE 1.25 mg/day in glucose metabolism assessed by OGTT and hyperinsulinemic euglycemic clamp (176), whereas the same dose decreased insulin sensitivity (172) measured by disappearance of glucose in an insulin tolerance test. A reduction in fasting glucose and/or insulin (171, 173, 178), and enhanced insulin sensitivity in insulin tolerance test has been reported on CEE 0.625 mg/day (177, 180). In a placebo-controlled trial peroral 17β-estradiol decreased fasting glucose, insulin and C-peptide levels, but the change in glucose was not significant compared to a placebo group and, further, there was no change in GHbA1c concentration (181). A decrease in fasting glucose and in the incremental insulin area for insulin, and an increase in hepatic insulin uptake were found in intravenous glucose tolerance test in a study of peroral 17β-estradiol combined with cyclical oral noretidrone acetate (179). This resulted in improved insulin sensitivity during the estrogen-alone phase, which was reversed during the combination phase.

Transdermal administration has been suggested to improve glucose tolerance and insulin sensitivity (171, 172, 182), but in most studies the findings are quite neutral (176, 183–185). Both fasting insulin and AUC for insulin decreased and AUC for C-peptide increased in OGTT, while fasting blood glucose remained unchanged after three months on transdermal estradiol 50 µg/day (171). Consequently, hepatic insulin clearance, estimated as the C-peptide-to-insulin ratio, increased. These findings were suggested to indicate a beneficial effect of estradiol on glucose metabolism. Comparable results were revealed in two other studies with transdermal estradiol 50 µg/day in OGTT (182, 184), but there were methodological differences, since no changes were observed in the glucose, insulin or C-peptide levels during intravenous glucose tolerance test (184), while the other study noticed enhanced insulin sensitivity by hyperinsulinemic euglycemic clamp (182). In a small study with seven subjects receiving transdermal estradiol 100 µg/day, enhanced insulin clearance and glucose disappearance suggesting improved insulin sensitivity were observed during insulin tolerance test (172). Furthermore, a cross-over study with CEE (1.25 mg/day) and transdermal estradiol (100 µg/day) for three months revealed no significant changes in glucose tolerance or insulin sensitivity assessed by OGTT and hyperinsulinemic euglycemic clamp (176).
Table 3. Effects of menopause and after menopause therapy on glucose metabolism.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No of subjects</th>
<th>Duration of estrogen</th>
<th>Fasting glucose</th>
<th>Fasting insulin</th>
<th>AUC insulin</th>
<th>AUC C-peptide</th>
<th>Insulin sensitivity</th>
<th>GHbA1c</th>
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<td>(88) 130</td>
<td>↑</td>
<td>↑</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(160) 158</td>
<td>↑ (↑)</td>
<td>↑ (↑)</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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<tr>
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<td></td>
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<tr>
<td>CEE 1.25mg</td>
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<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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</tr>
<tr>
<td></td>
<td>(170) 10 3 months</td>
<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(176) 9 3 months</td>
<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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</tr>
<tr>
<td></td>
<td>(171) 15 3 months</td>
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<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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<td>(183) 30 3 months</td>
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<td>2. phase ↑</td>
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<td></td>
<td>(177) 5 6 months</td>
<td>N</td>
<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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<tr>
<td></td>
<td>(178) 29 12 months</td>
<td>↓</td>
<td>↓</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(173) 97 36 months</td>
<td>↓</td>
<td>↓</td>
<td>N (N)</td>
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<td>N (↓)</td>
<td>N (↑)</td>
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<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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</tr>
<tr>
<td></td>
<td>(170) 10 3 months</td>
<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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<tr>
<td></td>
<td>(169) 20 6 months</td>
<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
<td>N (↑)</td>
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<tr>
<td></td>
<td>(181) 67 6 months</td>
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<tr>
<td></td>
<td>(179) 19 46 weeks</td>
<td>↓</td>
<td>N</td>
<td>N (N)</td>
<td>N (N)</td>
<td>N (↓)</td>
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<th>Fasting insulin</th>
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<th>AUC for insulin</th>
<th>AUC for C-peptide</th>
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<td>N</td>
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<td>(183)*</td>
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<td>3 months</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
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<tr>
<td>(179)#</td>
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<td>↓</td>
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<table>
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<th>Fasting insulin</th>
<th>AUC for glucose</th>
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<td></td>
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<td>N</td>
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</tr>
<tr>
<td>(270)</td>
<td>14</td>
<td>2 months</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↓</td>
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<tr>
<td>(188)</td>
<td>20</td>
<td>6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fasting ↑</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>(189)</td>
<td>25</td>
<td>2 months ↓</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fasting ↓</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>(186)</td>
<td>19</td>
<td>6 months</td>
<td>N</td>
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<td>N</td>
<td>N</td>
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</tbody>
</table>

AUC, area under the curve; CEE, conjugated equine estrogen; N, no statistically significant change; ↑=increase, ↓=decrease

* The women received combination therapy; these are the results of glucose metabolism studies at the end of the estrogen-alone phase.

* Decrease of insulin in oral glucose tolerance test, no change in intravenous glucose tolerance test.

Values in parenthesis show results after adjustment.
Most ERT studies involving glucose metabolism are small and short-term. The PEPI trial (173) is actually the only controlled study that has reported long-term effects of peroral CEE therapy (0.625 mg/day) decreased slightly fasting glucose and insulin levels. A modest increase in postchallenge glucose concentration was observed in OGTT, which may indicate delayed glucose clearance. However, a reduction in fasting glucose was most apparent among women with the highest baseline concentrations of fasting insulin and 1-hour postprandial glucose. Thus it seems that ERT/HRT does not impair glucose tolerance in those predisposed to the development of glucose intolerance. In the PEPI trial glucose responses to combined replacement therapies did not differ significantly from ERT. (173)

Estrogen seems not to impair glycemic control in diabetics, thus no change (186, 187) or a reduction in GHbA1c has been observed during ERT/HRT (188, 189). In addition, the increased suppression of hepatic glucose production by insulin during short-term peroral estradiol therapy (188) and the lowering of fasting glucose and C-peptide on combined hormone replacement therapy (189) may suggest improved glycemic control and insulin sensitivity in diabetics.

IGF-I, insulin-like growth factor, and its binding protein, IGFBP-1, are associated with carbohydrate metabolism and are regulated by sex hormones (190). Peroral ERT induces liver metabolism and production of hepatic proteins such as SHBG and also IGFBP-1, while no significant changes have been observed with transdermal ERT (191–193). The increase in IGFBP-1 obtained by peroral ERT is suggested to be beneficial, since enhanced IGFBP-1 levels are associated with reduced risk factors for cardiovascular diseases (194).

### 2.9 Blood pressure

#### 2.9.1 Natriuretic peptides and the renin-angiotensin-aldosterone system in regulation of blood pressure

The natriuretic peptide family includes three peptides: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) (195). ANP is released primarily in response to atrial wall stretching and intravascular volume expansion. At the time of secretion, the cleavage of proANP releases ANP and the biologically inert N-terminal fragment of proANP (NT-proANP). Thus NT-proANP and ANP are produced and secreted in equimolar amounts into the circulation. BNP is mainly secreted by the ventricles, while CNP is found predominantly in the brain. Furthermore, CNP can also be synthesized by vascular endothelial cells. Natriuretic peptides are cleared by an enzyme called neutral endopeptidase or by binding to clearance receptors. (195, 196)
The natriuretic peptides defend against excess salt and water retention, inhibit the production and action of vasoconstrictor peptides, promote vascular relaxation and inhibit sympathetic outflow. Both ANP and BNP have natriuretic, diuretic and vasodilatatory activity. They also inhibit the renin-angiotensin-aldosterone system (RAS) by decreasing renin secretion, inhibiting aldosterone secretion and suppressing angiotensin converting enzyme (ACE) activity. Natriuretic peptides also lower blood pressure. CNP acts as a vasodilator, especially on the venous side of the circulation, and it has antiproliferative effects on vascular smooth muscle. CNP also inhibits aldosterone, but it has limited effects on natriuresis and diuresis or blood pressure. (195)

2.9.2 Blood pressure in menopause

The influence of menopause on blood pressure is controversial (88, 197–199). High blood pressure is more prevalent in men than in women and also more common in older women compared with younger females, suggesting that sex hormones may affect blood pressure (200). Cross-sectional studies (120, 197, 198, 201) have reported higher systolic and diastolic blood pressures in postmenopausal women, whereas no increase in blood pressure has been observed in longitudinal studies (88, 199, 202, 203). On the other hand, the rise in blood pressure after menopause has been related to weight gain and aging (94, 198, 203). An increase in plasma ANP observed in menopause, however, has been suggested to be related to age rather than the physiological changes in sex hormones (204). Moreover, one cross-over study has suggested that the uterus may play a role in the regulation of blood pressure, because hysterectomized women with ovarian preservation have been shown to have higher blood pressure compared to age-matched women who have not undergone hysterectomy (205). The pathogenesis of this finding is not known.

2.9.3 Effect of ERT

Oral contraceptives may increase blood pressure (206), but the effect of ERT and HRT is more controversial. Lower blood pressure has been reported among postmenopausal women using ERT or HRT compared with nonusers (119, 207, 208), but also an increase (200) in blood pressure has been noticed during hormone replacement therapy, whereas the largest cross sectional study found no difference in blood pressure after adjustment for multiple confounders (9). Blood pressure has also remained unchanged in most long-term studies (209, 210) including the placebo controlled PEPI trial (10) with follow-up of three years. However, some studies have reported a decrease in blood pressure during peroral (211, 212) or transdermal (213–216) ERT. Moreover, HRT is suggested to be safe in hypertensive women, as neither systolic nor diastolic blood pressure increased in women with mild or moderate hypertension (217–220).

Only limited data are available about the effects of estrogen on natriuretic peptides, and the findings are mainly from animal studies focusing on ANP (221). Further, it is not known whether estrogen alters ANP production directly or indirectly (Fig. 2). In
experimental studies, decreased atrial ANP mRNA transcripts have been found after ovariectomy and orchietomy in rats, while substitution of sex hormones increased atrial mRNA expression (221). On the other hand, oral contraceptives have been found to enhance the ANP concentration (222). Only few studies have measured ANP during HRT revealing controversial results (222–224). A decrease in ANP level was obtained in one study (223), while another one found no significant change (222). Finally, the latest study reported an increase in both ANP and BNP levels after three months of combined HRT with transdermal estrogen (224).

Fig. 2. Interactions between estrogen, natriuretic peptides and the renin-angiotensin system (RAS) and the effect on blood pressure. Estrogen may alter blood pressure by affecting natriuretic peptide levels (1) or through effects on RAS (2). Estrogen has also rapid vasodilatory effects mediated by nitric oxide (3). Broken arrows indicate the effects of natriuretic peptides, while solid arrows are used between other interactions. The plus sign indicates stimulatory effect and the minus sign indicates inhibitory effect. (1) Estrogen may increase natriuretic peptides by inducing directly gene expression and release or in response to volume expansion. The increased secretion of natriuretic peptides reduces plasma volume and blood pressure by increasing diuresis and natriuresis, decreasing water intake and salt appetite, aldosterone and renin concentrations and ACE activity and inducing vasodilatation. (2) In general, the activation of RAS increases angiotensin II and aldosterone levels, and plasma volume. Estrogen can activate RAS by enhancing angiotensinogen synthesis and reducing ACE activity, leading to a decrease in the conversion of angiotensin I to angiotensin II and to an increase in angiotensinogen and renin levels. Estrogen-induced changes in RAS have been related to hypertension, but the results are controversial.
Estrogens have been shown to induce fluid retention and activate the RAS, but only limited and partially controversial data are available (221). Experimental data indicate that estrogen activates RAS by enhancing angiotensinogen synthesis, reducing ACE mRNA (225) and activity (226), and augmenting the tissue and plasma levels of angiotensinogen and renin. A decrease in AT1 receptor gene expression and density has also been observed (227). Oral estrogens, especially synthetic estrogens, stimulate dose-dependently the synthesis of hepatic proteins, including SHBG and the renin substrate, angiotensinogen, while nonoral estrogens do not seem to affect the renin-angiotensin-aldosterone system (129, 228, 229). In a population-based study, renin suppression was seen with oral and transdermal ERT, while the increase of angiotensinogen was limited to the women taking oral estrogens, and serum ACE activity and aldosterone or blood pressure were not significantly altered by ERT. (230) Interactions between estrogen, natriuretic peptides, the renin-angiotensin-aldosterone-system and blood pressure are presented in Fig. 2.

2.10 Other potential mechanisms of estrogen action

Additional favorable effects of estrogen on the vasculature have been described, including short-term vasodilatation caused by increased release of nitric oxide or changes in ion-channel function (nongenomic effects), and long-term effects on the response to vascular injury and on atherosclerosis involving changes in gene expression (genomic effects). (2, 11, 126)

Estrogens cause a rapid, transient vasodilatation by ER-α mediated activation of endothelial nitric oxide synthase enzyme, resulting in increased formation and release of nitric oxide without altering gene expression. Vasodilatation may also be partly due to the activation of potassium channels or the inhibition of calcium currents. (11, 126)

Long-term administration of estrogen has been reported to increase acetylcholine-mediated vasodilatation in experimental studies as well as in healthy postmenopausal women and in women with coronary artery disease (11). In addition to rapid effects on nitric oxide, estrogen may increase the gene expression of vasodilatory enzymes, nitric oxide synthases and prostacyclin synthase. Part of estrogen-induced vasorelaxation could be related to beneficial changes in lipoproteins, such as an increase of HDL cholesterol (2, 231). A decrease of endothelin-1 level, a vasoconstrictor, has also been observed during ERT (2, 232). In animal models or cell culture estrogen has been shown to reduce collagen and elastin synthesis and enhance their degradation in arterial wall, inhibit the migration and proliferation of smooth muscle cells, inhibit apoptosis and to promote angiogenesis. (2, 11)

Estrogen has complex effects on hemostatic factors (12, 233). Moreover, different studies have provided slightly inconsistent results. Oral estrogens result in both the activation of coagulation and enhanced fibrinolysis. Procoagulant changes include increases of factor VII, prothrombin fragments 1 and 2, activated protein C resistance and decrease of antithrombin III and protein S, whereas an increased D-dimer and a reduction in fibrinogen, tissue plasminogen activator and plasminogen activator inhibitor-1 levels have been observed, suggesting increased fibrinolytic activity (233–235). In addition,
ERT has been reported to lower plasma viscosity (236). Favorable changes in prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, have been observed, but it remains unclear how platelet reactivity is altered by estrogen (233). In contrast to peroral ERT, the effects of transdermal estrogen on hemostatic factors appear to be insignificant (235, 237). However, both oral ERT with CEE or estradiol and also transdermal ERT are associated with an increased risk of venous thrombosis (238). The risk is highest during the first year of use (56) and among women with coagulation abnormalities (233). Accordingly, HRT is associated with an early increase of venous and arterial thrombotic events in postmenopausal women with established coronary artery disease (63–65).

The influence of ERT and HRT on inflammation is also conflicting. In postmenopausal women peroral ERT/HRT has been shown to elevate C-reactive protein (CRP) levels (13, 239, 240), a marker of inflammation associated with increased risk of future cardiovascular events (241, 242), whereas a reduction of soluble cell adhesion molecules (including E-selectin, intercellular and vascular cell adhesion molecules), chemokines and cytokines (such as interleukin-6 and tumor necrosis factor-α) suggests reduced inflammation and protection against atherosclerosis (2). Generally transdermal estrogen has no effect on markers of inflammation, although a lowering of CRP levels has been reported (2, 237).

Thus, estrogen may modify the risk for atherosclerosis by various mechanisms. Finally, the net effect of different factors determines whether the outcome is antiatherogenic or proatherogenic.
3 Purpose of the present research

The present study was designed to investigate the regulation of LDL cholesterol metabolism in postmenopausal women before and after six months of ERT. In addition, the response of carbohydrate metabolism and blood pressure to ERT was determined. The following questions were specifically addressed:

1. What are the major factors associated with plasma LDL levels and by what mechanisms are the plasma LDL levels regulated in postmenopausal females? (I)
2. How does ERT affect plasma lipids and lipoproteins, particularly the regulatory mechanisms of LDL cholesterol? (II)
3. Does ERT alter glucose and insulin metabolism? (III)
4. What is the role of plasma natriuretic peptides, renin and aldosterone in the regulation of blood pressure during ERT? (IV)
5. Are the effects of ERT modified by the route of administration? (II–IV)
4 Subjects and methods

4.1 Subjects

Postmenopausal women seeking hormone substitution therapy for relief of climacteric symptoms were recruited by one gynecologist at Oulu Deaconess Institute. Including only hysterectomized women allowed us to study the effects of estrogen alone without the need to combine progestogen for endometrium protection.

The criteria for inclusion were as follows: 45–65 years of age, a previous hysterectomy with at least one remaining ovary, postmenopausal status confirmed by serum FSH >30 IU/l, BMI <30 kg/m², fasting blood glucose <6.7 mmol/l to exclude diabetic subjects (243), serum creatinine <120 μmol/l, serum alkaline phosphatase <275 IU/l and alanine aminotransferase <50 IU/l and a mammography without any evidence of malignancy performed within six months. No estrogen or progestin treatment was allowed within the preceding two months. Women with uncontrolled hypertension (systolic blood pressure >160 or diastolic blood pressure >95 mmHg), congestive heart failure, untreated thyroid or other endocrine diseases, any disease requiring medication that might confound analysis of the estrogen effect, a history of malignancy or contraindications for ERT were excluded. Heavy smokers (>20 cigarettes/day) were also excluded. None of the hypercholesterolemic women were on lipid-lowering drugs, or had any clinical evidence of familial hypercholesterolemia (FH). Additionally, the two most common LDL receptor gene mutations in Finland (FH-Helsinki and North Karelia) were determined to rule out FH if total cholesterol was >7.0 mmol/l.

Altogether 105 women were screened and 79 of them met the inclusion criteria and volunteered to participate in the metabolic studies. All subjects gave written informed consent, and the study was approved by the ethics committees of Oulu University and Oulu Deaconess Institute.
4.2 Randomization and treatments

Participants were randomly assigned to receive either peroral or transdermal estrogen therapy for six months. The women were stratified by age and BMI within the treatment groups using two age groups (45–54 and 55–65 years) and two BMI groups (19–25 and 26–30 kg/m²). Double-dummy design was used to keep the study double blind. Thirty-nine women received peroral estradiol valerate 2 mg/day (the estradiol valerate tablet from Divitren®, Orion Pharma) with placebo gel, while 40 women were treated with transdermal 17β-estradiol 1 mg/day in gel (Divigel®, Orion Pharma; Sandrena®, NV Organon) and placebo tablets. The women were instructed to take one tablet and to apply the gel once daily on the skin of the lower abdomen or thighs, preferably in the evening at bedtime. The site of application was to be varied every day. The dose of the gel chosen was based on earlier pharmacokinetic and clinical studies showing that this dose was sufficient to control postmenopausal symptoms in most women.

All concomitant medication remained unchanged throughout the study. Sixteen women had regular antihypertensive medication and five of them used a combination of two antihypertensive drugs. Thiazide diuretics were taken by five subjects, beta-blockers by seven, five women used calcium antagonists and four used ACE inhibitors. In addition, one woman took diuretics to prevent swelling of the ankles and four participants used beta-blockers or calcium-blockers for symptoms of angina pectoris or extrasystolies. Inhaled corticosteroids were used for bronchial asthma by four women, but none were using systemic steroids.

4.3 Methods

4.3.1 Clinical examination

Metabolic studies were carried out as out-patient visits at baseline and after 3 and 6 months of ERT in the research unit of the Department of Internal Medicine at Oulu University Hospital. The studies evaluating the regulation of LDL cholesterol metabolism and oral glucose tolerance test (OGTT) were performed at baseline and after six months, while other variables were also determined at the 3-month visit. Height, weight, and waist and hip circumferences were measured with subjects wearing light clothing and no shoes. BMI, calculated as the weight in kilograms divided by the square of the height in meters, was used as an estimate of obesity. The waist was measured at the minimum circumference, and the hips at the level of maximum hip circumference (244). The waist-to-hip ratio was calculated and used as an estimate for abdominal obesity.
Blood pressure and heart rate were measured with an automatic, oscillometric, microprocessor-controlled device (Critikon Dinamap 1846 SX/P; Critikon Inc., Tampa, FL) from the right arm after at least 15 minutes’ rest. Three measurements were performed in sitting and two in standing position at one-minute intervals, and the average of both sitting and standing measurements was analyzed separately.

### 4.3.2 Assessment of lifestyle factors

Lifestyle habits including diet, smoking, alcohol intake and physical activity were documented using standardized questionnaires. Seven-day food records were kept during cholesterol absorption study and analyzed by a dietitian with the Finnish Food Database Program, Nutrica (245). According to smoking habits the subjects were divided into non-smokers and smokers, and the number of cigarettes smoked per day was registered. Alcohol intake was assessed by asking the subjects about the consumption of wine, beer and strong alcoholic beverages in a week, and the amount of alcohol was calculated as grams of absolute alcohol consumed per week. Physical activity was classified for work and leisure time activities as none, light, moderate and heavy by interview and accordingly scored from 1 to 4. The participants were advised to continue with their regular diet and customary physical activity and not to change their smoking habits or alcohol consumption during the study.

### 4.3.3 Laboratory analyses

Before enrollment in the study the analyses of serum creatinine, alkaline phosphatase and alanine aminotransferase were carried out in the routine laboratory of Oulu Deaconess Institute using standard methods. Blood samples for assessment of sex hormone levels collected at the baseline screening visit and after 6 months of ERT were analyzed in the same laboratory. Serum FSH was measured by fluoroimmunometric method (DELFIA, Wallac, Turku, Finland). Serum estrone, estradiol and SHBG were determined by radioimmunoassays using the commercial kits of Orion Diagnostica, Espoo, Finland.

Venous blood samples for all metabolic assays were obtained in EDTA-containing tubes after an overnight fast. Plasma was separated by centrifugation at 1200 x g (2600 rpm) for 15 minutes at +4°C. The specimens for IGF-I, IGFBP-1, natriuretic peptides, aldosterone and renin determinations were stored at –20°C until analyzed.
4.3.3.1 Lipids and lipoproteins

Total plasma cholesterol and triglyceride concentrations were determined by enzymatic colorimetric methods (kits from Boehringer Diagnostica, Mannheim, Germany). VLDL,IDL and LDL cholesterol were isolated by repeated ultracentrifugations according to their densities (246). HDL cholesterol was determined from VLDL-free plasma after precipitation of low-density lipoproteins with heparin-manganese chloride (246). LDL cholesterol level was also estimated by using the Friedewald equation (247), and these values were used in the final response analyses. Lipid and lipoprotein values both at baseline and after six months of ERT are the average of the four measurements taken at the first visit and three times during the subsequent LDL turnover study, while at three months there was only one follow-up visit when blood samples were also collected.

The protein content of the lipoproteins was measured by the method of Lowry et al (248), and the plasma LDL apo B level was determined after isopropanol precipitation (249). Plasma total apoA1 and apo B concentrations were measured by immunoprecipitation methods (commercial kits of Kone Instruments, Espoo, Finland).

4.3.3.2 Cholesterol absorption

Absorption of dietary cholesterol was measured by the peroral double-isotope continuous-feeding method described by Crouse and Grundy (250). The subjects received $^{14}$C-cholesterol and $^3$H-betasitosterol (or $^3$H-betasitostanol) three times a day with the major meals for seven days. Stool collections were performed on the last three days. Absolute absorption of dietary cholesterol was calculated by multiplying the daily cholesterol intake, with the percentage absorption of dietary cholesterol and expressed as milligrams per kilogram of body weight. The cholesterol intake was obtained from seven-day food records kept during the absorption study.

4.3.3.3 Clearance and production of LDL apo B

In the LDL turnover study, 100 ml of fasting blood was drawn for the isolation of LDL, which was carried out according to the method described by Lindgren et al (251), and the LDL protein was labeled with $^{125}$I by use of the iodine monochloride method of McFarlane (252) as modified by Bilheimer et al (253). Radiolabeled LDL was injected in the morning on the day after iodination. Blood samples were collected at 0, 15 and 30 minutes and at 1, 2 and 3 hours, and thereafter three times a week for 14 days after the injection. The radioactivity of total plasma was measured in each sample. Finally, the FCR was calculated from the plasma decay curves by using the method described by Matthews (254).
The production rate of LDL apo B was calculated from FCR of LDL, pool volume and LDL apo B concentration and expressed as milligrams of LDL apo B produced per day normalized for body weight.

4.3.3.4 Determination of the apo E phenotype and polymorphisms of the apo B and 7α-hydroxylase genes

The apo E phenotype was determined after delipidation with isoelectric focusing and immunoblotting techniques that made use of commercial antibodies (111, 255). The EcoRI and XbaI polymorphisms of the apo B gene (256, 257) and the cholesterol 7α-hydroxylase (CYP7) genes (113) were determined by polymerase chain reaction as described previously.

4.3.3.5 Carbohydrate metabolism, oral glucose tolerance test

A standard 75 g oral glucose tolerance test with assessments of fasting and postchallenge blood glucose, serum insulin and C-peptide at 30, 60 and 120 minutes was performed at baseline and after six months of ERT. The area under the curve (AUC) of these variables during the glucose tolerance test was also calculated. Blood glucose concentration was measured with the glucose dehydrogenase method (Merck, Darmstadt, Germany). Serum C-peptide level was assessed by a commercial double-antibody radioimmunoassay (Double Antibody C-peptide, EURO/DPC Ltd., Llanberis, United Kingdom). Serum insulin concentration was determined by two-site immunoenzymometric assay (AIA-PACK IRI, Tosoh Corporation, Tokyo, Japan). GHbA1c level was determined by liquid chromatography. All these measurements of glucose metabolism were carried out in the central laboratory of Oulu University Hospital.

4.3.3.6 Indices for insulin sensitivity and insulin release

Insulin sensitivity indices and insulin secretion obtained from OGTT were calculated as described by Matsuda & DeFronzo (258) and Stumvoll et al (259). A whole-body insulin sensitivity index (ISI(composite)) was calculated as follows: 10 000/square root of (Gluc0 * lnS0 * Gluc_mean * Ins_mean ). Gluc0 and Ins0 represent the glucose and insulin concentrations, respectively, at time t. Gluc_mean and Ins_mean represent the mean glucose and insulin concentrations, respectively, during the OGTT. The metabolic clearance rate of glucose (MCRest) was calculated as 18.8–(0.271*BMI)–(0.0052*Ins120)–(0.27*Gluc90) and insulin sensitivity index (ISIest) was calculated as 0.226–(0.0032*BMI)–(0.0000645*Ins120)–(0.0037*Gluc90). First-phase (1st PH) and second-phase (2nd PH) insulin release were calculated as follows: 1st PH=1283+(1.829*Ins30)−
(138.7*Gluc_{30})+(3.772*Ins_{0}) and 2nd PH= 287+(0.4164*Ins_{30})–(26.07*Gluc_{30})+(0.9226*Ins_{0}). The calculated mean value of blood glucose determined at 60 and 120 min was used as Gluc_{90}, because Gluc_{90} was not determined in the present study. SI units were used in the calculations, i.e. mmol/l for glucose and pmol/l for insulin.

### 4.3.3.7 Determination of IGFBP-1 and IGF-I

Plasma IGFBP-1 was measured with an immunoenzymometric assay (IEMA TEST, Medix Biochemica, Kauniainen, Finland). Plasma IGF-I was determined by a commercial standard double-antibody disequilibrium radioimmunoassay (Incstar Stillwater, MN, USA) after an acid extraction procedure.

### 4.3.3.8 Determination of natriuretic peptides

The plasma samples for measurements of natriuretic peptides (ANP, NT-proANP and BNP), aldosterone and renin were stored at \(-20^\circ\) C until all samples were analyzed together.

ANP and BNP were extracted from plasma using SepPak C_{18} cartridges (Waters, Milford, MA), while NT-proANP was analyzed directly from unextracted plasma. Plasma ANP, NT-proANP and BNP concentrations were determined with specific radioimmunoassays as described previously (260–262).

### 4.3.3.9 Determination of plasma renin and aldosterone

Plasma aldosterone level was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) and plasma renin by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) in the central laboratory of Oulu University Hospital.

### 4.3.4 Statistical analysis

Data were analyzed using the software packages SPSS for Windows (6.01) or SAS (version 6.08). Sample size calculation was based on expected difference of 18 percentage units between treatment groups in the changes of triglycerides. A sample of 80 subjects (40 in both treatment groups) had an 80% power to detect a significant difference at the levels of P=0.05, allowing a 20% drop-out rate. Furthermore,
retrospective power analyses were calculated for the differences in LDL cholesterol, FCR for LDL apo B and LDL apo B production between low and high LDL groups (I), for the changes of GHbA1c (III) and blood pressure (IV).

Results for continuous variables are presented as mean ± standard deviation (SD) (I–II) or standard error (SE) of the mean (III–IV). In baseline data analysis the subjects were also divided into two groups according to plasma LDL cholesterol concentration and BMI for estimation of the mechanisms involved in regulation of LDL cholesterol and the effect of overweight, respectively. The cut point to low and high LDL cholesterol was 4.05 mmol/l in study I, which is considerably high and therefore in this review the data were reanalyzed by using 3.5 mmol/l as a cut point. The cut point to low and high BMI was 26 kg/m². The differences in these dichotomous analyses (I) as the differences between the two treatment groups at baseline (II–IV) and the changes between the treatments were compared with the independent samples of t-test (II–III). The changes from baseline to six months were analyzed with paired samples t-test (II–III). The differences between the two groups and the mean changes induced by ERT are partially expressed with 95% confidence intervals (CI). Analysis of variance with Bonferroni adjustment was performed in the group comparison of apo E phenotypes and the polymorphisms of apo B and 7α-hydroxylase genes (I–II) and when the analysis also included variables measured at three months (II, IV). In case of a skewed distribution, natural logarithmic transformation was performed or non-parametric Mann-Whitney U-test and Wilcoxon signed rank test were used, as appropriate. Pearson’s or Spearman’s correlation coefficients were calculated to indicate the associations between the variables. Finally, stepwise multiple regression analysis was performed to assess the independent factors contributing to the changes in LDL and FCR levels (II). Two-sided P values less than 0.05 were considered to indicate statistical significance.
5 Results

5.1 Clinical characteristics

The baseline characteristics of the subjects according to treatment group assignment are shown in Table 4. The two study groups did not differ significantly with regard to age, BMI, menopausal status or lifestyle factors.

Table 4. Baseline characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Peroral estradiol (n=39)</th>
<th>Transdermal estradiol (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.7 (2.9)</td>
<td>54.7 (2.8)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 (2.8)</td>
<td>25.8 (2.3)</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.81 (0.06)</td>
<td>0.79 (0.05)</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
<td>5.2 (2.8)</td>
<td>5.9 (3.4)</td>
</tr>
<tr>
<td>Earlier ERT, yes/no</td>
<td>27/12</td>
<td>25/15</td>
</tr>
<tr>
<td>Duration of earlier ERT (years)</td>
<td>4.7 (2.9)</td>
<td>4.5 (2.9)</td>
</tr>
<tr>
<td>S-FSH (IU/l)</td>
<td>69 (25)</td>
<td>61 (19)</td>
</tr>
<tr>
<td>Estrone (pmol/l)</td>
<td>176 (92)</td>
<td>224 (139)</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>87 (133)</td>
<td>111 (177)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>48 (24)</td>
<td>45 (16)</td>
</tr>
<tr>
<td>Current smokers, yes/no</td>
<td>5/34</td>
<td>11/29</td>
</tr>
<tr>
<td>Smoking (cigarettes/day)</td>
<td>10 (6)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>Alcohol intake (g/week)</td>
<td>19 (23)</td>
<td>22 (29)</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>light/moderate</td>
<td>21/18</td>
<td>20/20</td>
</tr>
<tr>
<td>leisure time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>none/light/moderate/heavy</td>
<td>1/6/21/11</td>
<td>1/7/22/10</td>
</tr>
</tbody>
</table>

Values are mean (SD)
Thirty-five women in the peroral group and 38 in the gel group completed the study. One subject in the peroral group discontinued because of breast tenderness, and two other women dropped out because of itching and discomfort. In the gel group one woman withdrew because of weight gain. Furthermore, one participant in both groups discontinued because of personal reasons without having any side effects related to the treatment. These drop-outs are included in baseline data analysis, but excluded from treatment response analysis.

The baseline hormone values are shown in Table 4 and responses to estrogen treatments in Fig. 3. The peroral ERT induced an over 10-fold increase in serum estrone level in six months (p<0.001), while the increase was 2-fold (p<0.001; between the treatments p<0.001) with the gel regimen. The difference between the estradiol therapies was less profound in serum estradiol concentrations (p<0.05). Serum SHBG was more increased in the peroral group (p<0.001), while only a minor increase was observed during transdermal ERT (p<0.05). (Fig. 3)

A slight, though insignificant, weight gain (from 68.9±7.6 kg to 69.5±8.1 kg, p=NS) resulting in an increase in BMI (from 25.9±2.3 kg/m² to 26.1±2.5 kg/m², p=NS) was observed in the transdermal group, but not in the peroral group after six months of ERT. The waist to hip ratio remained at the baseline level in both study groups.

According to repeated analyses of seven-day food records and a life style questionnaire, there were no significant changes in diet, physical activity, smoking or alcohol consumption in either of the study groups during the trial.
Fig. 3. Serum estrone (top), estradiol (middle) and SHBG (bottom) concentrations (mean±SE) at baseline and after 6 months of ERT.
5.2 Cholesterol metabolism

5.2.1 Plasma lipids and lipoproteins (I–II)

Before starting estrogen therapy the plasma LDL cholesterol of the study subjects ranged from 2.20 to 6.15 mmol/l with a mean value of 4.09 mmol/l. The mean total cholesterol, 6.26 mmol/l (range, 3.9–8.80 mmol/l), was also over the recommendation levels, while the mean HDL cholesterol was 1.58 mmol/l (0.85–2.56 mmol/l) and total triglycerides 1.31 mmol/l (0.61–3.26 mmol/l). The women with high LDL cholesterol had also higher levels of total, VLDL and IDL cholesterol and triglycerides (Table 5). Further, LDL cholesterol was inversely related to HDL cholesterol ($r = -0.243$, $p < 0.05$), although no difference was observed in HDL cholesterol between the high and low LDL groups when the cut point to LDL grouping was 3.5 mmol/l. Overweight was related to atherogenic lipid profile, for example the mean LDL cholesterol was 4.39±0.73 mmol/l among the women with BMI $\geq 26$ kg/m$^2$, compared to 3.79±0.90 mmol/l in the group with BMI <26 kg/m$^2$. The obese women had also higher plasma total, VLDL and IDL cholesterol, total and VLDL triglyceride concentrations than the nonobese subjects, whereas no significant difference was observed in HDL cholesterol.

Table 5. Age, BMI, plasma lipids, lipoproteins, cholesterol absorption and the fractional catabolic rate (FCR) and production rate values for LDL apo B at baseline among all study subjects and the comparison of subjects with low and high LDL cholesterol.

<table>
<thead>
<tr>
<th></th>
<th>All (n=79)</th>
<th>LDL &lt; 3.5 (n=20)</th>
<th>LDL &gt;3.5 (n=59)</th>
<th>Mean difference between LDL groups (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54.2 (2.9)</td>
<td>53.2 (2.8)</td>
<td>54.5 (2.9)</td>
<td>-1.3 (-2.8, 0.2)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.1 (2.5)</td>
<td>25.0 (2.5)</td>
<td>26.4 (2.5)</td>
<td>-1.4 (-2.6, -0.1)*</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.26 (0.96)</td>
<td>5.09 (0.48)</td>
<td>6.66 (0.72)</td>
<td>-1.57 (-1.91, -1.22)**</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.42 (0.25)</td>
<td>0.32 (0.19)</td>
<td>0.45 (0.26)</td>
<td>-0.13 (-0.26, -0.01)*</td>
</tr>
<tr>
<td>IDL</td>
<td>0.25 (0.13)</td>
<td>0.17 (0.10)</td>
<td>0.28 (0.13)</td>
<td>-0.11 (-0.17, -0.05)**</td>
</tr>
<tr>
<td>LDL</td>
<td>4.09 (0.87)</td>
<td>3.02 (0.41)</td>
<td>4.46 (0.65)</td>
<td>-1.44 (-1.75, -1.13)**</td>
</tr>
<tr>
<td>HDL</td>
<td>1.58 (0.36)</td>
<td>1.59 (0.28)</td>
<td>1.57 (0.38)</td>
<td>0.02 (-0.17, 0.20)</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.31 (0.54)</td>
<td>1.07 (0.37)</td>
<td>1.40 (0.57)</td>
<td>-0.32 (-0.55, -0.10)**</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.59 (0.34)</td>
<td>0.48 (0.26)</td>
<td>0.62 (0.36)</td>
<td>-0.14 (-0.29, 0.01)</td>
</tr>
<tr>
<td>Plasma apo A1 (g/l)</td>
<td>1.92 (0.38)</td>
<td>1.85 (0.34)</td>
<td>1.94 (0.39)</td>
<td>-0.09 (-0.28, 0.11)</td>
</tr>
<tr>
<td>Plasma apo B (g/l)</td>
<td>1.09 (0.30)</td>
<td>0.83 (0.17)</td>
<td>1.17 (0.29)</td>
<td>-0.34 (-0.45, -0.24)**</td>
</tr>
<tr>
<td>LDL apo B (g/l)</td>
<td>0.94 (0.26)</td>
<td>0.69 (0.12)</td>
<td>1.01 (0.24)</td>
<td>-0.32 (-0.41, -0.23)**</td>
</tr>
<tr>
<td>Fractional absorption of dietary cholesterol (%)</td>
<td>50 (14)</td>
<td>50 (13)</td>
<td>50 (14)</td>
<td>0 (-7, 7)</td>
</tr>
<tr>
<td>Absolute absorption of dietary cholesterol (mg/kg/d)</td>
<td>2.0 (1.0)</td>
<td>1.8 (0.9)</td>
<td>2.0 (1.0)</td>
<td>-0.2 (-0.7, 0.4)</td>
</tr>
<tr>
<td>FCR for LDL apo B (pools/d)</td>
<td>0.303 (0.043)</td>
<td>0.348 (0.026)</td>
<td>0.291 (0.039)</td>
<td>0.057 (0.040, 0.074)**</td>
</tr>
<tr>
<td>LDL apo B production (mg/kg/d)</td>
<td>12.5 (2.7)</td>
<td>10.7 (1.8)</td>
<td>13.0 (2.7)</td>
<td>-2.3 (-3.8, -0.9)**</td>
</tr>
</tbody>
</table>

Baseline values are expressed as mean (SD), *$p<0.05$, **$p<0.01$, ***$p<0.001$. 


Data on lipid, lipoprotein and apolipoprotein values at baseline, 3 and 6 months are shown in Table 6 and the mean percentage changes during six-month ERT in Fig. 4. No significant differences in any of these variables were observed between the treatment groups at baseline. The main responses to ERT were seen already after the first 3 months and appeared to sustain to the end of the six-month study (Table 6). The LDL cholesterol concentration was lowered significantly, on average by 19 % (p<0.001) from baseline, among the women receiving peroral estradiol, and by 9 % (p<0.001) in the gel group (Fig. 4). Decreases of total and IDL cholesterol were similar in the study groups. The VLDL cholesterol level was also slightly decreased from baseline in the gel group. The HDL cholesterol and total triglycerides, however, increased only in the peroral ERT group (+13 %, p<0.001 and +10 %, p<0.001, respectively). Mean apo B concentration decreased by 12 % (p<0.001) in the peroral group, but was not changed in the gel group. The mean apoA1 level did not differ significantly from baseline in either treatment. (Table 6, Fig. 4)

Table 6. Lipids and lipoproteins at baseline, after 3 and 6 months of peroral and transdermal ERT.

<table>
<thead>
<tr>
<th></th>
<th>Peroral estradiol (n=35)</th>
<th>Transdermal estradiol (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 months</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>6.36 (0.94)</td>
<td>5.72 (0.94)***</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.39 (0.23)</td>
<td>0.33 (0.21)</td>
</tr>
<tr>
<td>IDL</td>
<td>0.25 (0.12)</td>
<td>0.22 (0.11)*</td>
</tr>
<tr>
<td>LDL</td>
<td>4.19 (0.83)</td>
<td>3.35 (1.00)***b</td>
</tr>
<tr>
<td>HDL</td>
<td>1.60 (0.35)</td>
<td>1.74 (0.39)**</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>1.25 (0.50)</td>
<td>1.41 (0.64)*</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.55 (0.30)</td>
<td>0.65 (0.40)</td>
</tr>
<tr>
<td>Apo A1 (g/l)</td>
<td>1.94 (0.35)</td>
<td>1.97 (0.35)</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.07 (0.30)</td>
<td>0.96 (0.26)****a</td>
</tr>
</tbody>
</table>

Values are mean (SD).
* p<0.05, ** p< 0.01 and *** p< 0.001 vs baseline.
P for the difference between treatments a, p<0.05; b, p<0.01; c, p<0.001.
Fig. 4. Mean percentage changes (±SE) of plasma lipids and lipoproteins during peroral and transdermal ERT in 6 months. TC, total cholesterol; TG, triglycerides. * p<0.05, ** p<0.01, *** p<0.001 for the change from baseline and a, p<0.05; b, p<0.01; c, p<0.001 for the difference in percentage change between treatments.
5.2.2 Regulation of LDL metabolism (I–II)

5.2.2.1 Cholesterol absorption

At baseline cholesterol absorption efficiency varied among the subjects from 16 % to 83 % with an average value of 50 %, and the mean absolute absorption of cholesterol was 2.0 mg/kg/d, ranging from 0.4 mg/kg/d to 4.9 mg/kg/d. Plasma triglycerides and VLDL cholesterol were negatively associated with fractional cholesterol absorption ($r = -0.324$, $p<0.01$ and $r = -0.232$, $p<0.05$, respectively), whereas no other correlations were observed between cholesterol absorption and lipid or lipoprotein values. Furthermore, the high and low cholesterol groups did not differ as regards cholesterol absorption (Table 5). The obese subjects (BMI ≥26 kg/m$^2$), however, had lower rates of cholesterol absorption than the non-obese women. The mean fractional cholesterol absorption of the obese women
was 46±14 % and of the non-obese women 54±14 % (p<0.01 between the groups), and
the mean absolute absorption of dietary cholesterol was 1.7±0.9 mg/kg per day and
2.3±1.0 mg/kg per day, respectively (p<0.01 between the groups).

During ERT the fractional cholesterol absorption was reduced by 10 % (p<0.05) in the
peroral and by 6 % (p<0.05) in the transdermal group (Table 7). Absolute absorption of
dietary cholesterol also decreased by 18% (p<0.01) and 9 % (p<0.05) on both therapies,
respectively. The change of total and LDL cholesterol was positively related to the change
in fractional absorption of dietary cholesterol in the peroral estrogen group (r= 0.427,
p=0.05 and r= 0.431, p<0.05, respectively), while no significant correlation was found in
the transdermal group. Further, no association was observed between the changes in
cholesterol absorption and estrogen levels.

Table 7. Cholesterol absorption and clearance and production of LDL apolipoprotein B
(apo B) at baseline and the change in 6 months on peroral and transdermal estradiol
therapy.

<table>
<thead>
<tr>
<th></th>
<th>Peroral estradiol</th>
<th>Transdermal estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Baseline</td>
<td>Change in 6 months</td>
</tr>
<tr>
<td>Fractional absorption</td>
<td>32 52</td>
<td>–5</td>
</tr>
<tr>
<td>of dietary cholesterol</td>
<td>(12; 35–82)</td>
<td>(–9, –1)*</td>
</tr>
<tr>
<td>Absolute absorption</td>
<td>29 2.07 (1.04;</td>
<td>–0.44</td>
</tr>
<tr>
<td>of dietary cholesterol</td>
<td>0.72–4.91)</td>
<td>(–0.74, –0.14)**</td>
</tr>
<tr>
<td>Fractional catabolic</td>
<td>30 0.294 (0.037;</td>
<td>0.051</td>
</tr>
<tr>
<td>rate of LDL apo B</td>
<td>(0.036, 0.066)***#</td>
<td>(0.036, 0.066)***#</td>
</tr>
<tr>
<td>(mg/kg/d)</td>
<td>30 12.37 (3.09;</td>
<td>0.89</td>
</tr>
<tr>
<td>LDL apo B production</td>
<td>7.28–20.18)</td>
<td>(0.15, 1.62)*</td>
</tr>
<tr>
<td>(mg/kg/d)</td>
<td>30 0.95 (0.26;</td>
<td>–0.08</td>
</tr>
<tr>
<td>Plasma LDL apo B</td>
<td>0.56–1.66)</td>
<td>(–0.16, –0.01)*</td>
</tr>
<tr>
<td>(g/l)</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Baseline values are mean (SD; range). Changes are expressed as mean (95% CI). * p<0.05, ** p< 0.01 and ***
p< 0.001 vs baseline. P for the difference between treatments # p<0.001.

5.2.2.2 Clearance and production of LDL apo B

The baseline FCR values for LDL apo B ranged from 0.183 pools/d to 0.418 pools/d with
a mean value of 0.303 pools/d, and the LDL apo B production rates ranged from 7.3 mg/
kg/d to 20.2 mg/kg/d with a mean value of 12.5 mg/kg/d. LDL cholesterol had an strong
association with FCR for LDL apo B (r= –0.757, p<0.001) and was also correlated with
LDL apo production (r=0.531, p<0.001). A significant difference was also observed
between the low and high LDL cholesterol groups (Table 5). In the analysis of BMI
subgroups a higher LDL apo B production rate was noticed among the obese subjects, but
no differences was observed between the two groups in FCR for LDL apo B.
Peroral estradiol increased significantly FCR for LDL apo B (18 %), whereas only a minor insignificant increase (2 %) was observed in the transdermal group (Table 7). However, the change in LDL cholesterol was associated with the change in FCR for LDL apo B in both study groups (Fig. 5). LDL apo B production was raised by 9 % and plasma LDL apo B concentration was lowered by 6% on peroral therapy, but not on transdermal gel therapy (Table 7). The increase in FCR for LDL apo B correlated with the increase in serum estrogens ($r=0.503$, $p<0.01$ for the change in estrone and $r=0.381$, $p<0.05$ for the change in estradiol) in the peroral group, whereas no significant correlation was observed in the gel group. However, the increase in the production of LDL apo B was related to the change of serum estrone, but not estradiol, with both peroral and transdermal treatment ($r=0.380$, $p<0.05$ and $r=0.362$, $p<0.05$, respectively). Furthermore, among all the subjects the decrease in LDL cholesterol was also associated with an increase in serum estrone ($r=0.367$, $p<0.01$) and estradiol ($r=0.288$, $p<0.05$).

In stepwise multiple regression analysis the changes in FCR and production of LDL apo B explained 60 % ($R^2 0.604$, $p<0.001$) of the reduction in LDL cholesterol in the peroral ERT group and 80% ($R^2 0.798$, $p<0.001$) in the gel group.

### 5.2.2.3 Genetic factors

**Apo E phenotype.** The apo E phenotypes were distributed as follows: four apo E2 (including phenotypes apo E2/2, E2/3 and E2/4), 49 apo E3 (apo E3/3) and 26 apo E4 (including apo E4/3 and E4/4). Apo E2 seemed to be related to lower total (5.93±1.11 mmol/l) and LDL cholesterol (3.54±1.21 mmol/l) and higher VLDL (0.53±0.21 mmol/l), IDL (0.34±0.10 mmol/l) and HDL (1.81±0.34 mmol/l) cholesterol levels compared with the women having apo E3 or E4, while the women with apo E4 tended to have the highest total (6.5±0.88 mmol/l) and LDL (4.27±0.77 mmol/l) cholesterol and the lowest HDL cholesterol (1.47±0.39 mmol/l) levels. Also, an increasing trend in the fractional (42±8; 50±14; 52±14 %, apo E2, E3, E4, respectively) and absolute absorption of cholesterol (1.4±0.5; 1.9±1.0; 2.2±1.1 mg/kg/d) and the LDL apo B production rate (10.3±4.1; 12.6±2.5; 12.6±2.9mg/kg/d) and a decreasing trend in the FCR values for LDL apo B (0.322±0.084; 0.308±0.037; 0.293±0.049 pools/d) could be observed between the apo E phenotypes 2, 3 and 4, respectively. However, these differences between the apo E groups were not statistically significant.

In response analysis significant decreases of total and LDL cholesterol were observed in all apo E groups, and no difference was found between the phenotypes. Also, the changes in other lipids and lipoproteins, LDL turnover and cholesterol absorption were quite similar. In addition, the significance of apo E allele ε4 was studied by comparing the apo E4-negative (including apo E2/3, E3/3) with the apo E4-positive (including apo E4/2, E4/3 and E4/4) subjects. Although the effect of both estrogen therapies on serum lipids and cholesterol absorption, FCR and production for LDL apo B varied to some extent between the apo E4-negative and the apo E4-positive subjects, the differences did not reach statistical significance.
Polymorphisms of the apo B and 7α-hydroxylase genes. The EcoRI and XbaI polymorphisms of the apo B gene had no significant effect on cholesterol metabolism among these postmenopausal women. Similarly, there were no significant differences in lipid and lipoprotein values, cholesterol absorption efficiency, FCR and production of LDL apo B in relation to 7α-hydroxylase polymorphism. Further, the effects of estrogen were not modulated by these polymorphisms of apo B or cholesterol 7α-hydroxylase.

5.3 Glucose and insulin metabolism (III)

Table 8 presents data on values of glucose and insulin metabolism for both the peroral and the transdermal estradiol group at baseline and at the end of 6 months’ treatment. The fasting and postchallenge concentrations of glucose, insulin and C-peptide, GHbA_1c and the calculated indices reflecting insulin sensitivity, insulin release and metabolic clearance rate of glucose were comparable in the two groups at baseline. Moreover, the responses in both estrogen regimens were also similar. Compared with the baseline values, the AUC for C-peptide decreased by 8% both in the peroral group (p<0.05) and in the gel group (p<0.01) as fasting and 30-minute C-peptide values declined significantly in both groups, and in the transdermal group also the 60-minute value. In contrast, the fasting and postchallenge glucose and insulin levels were not significantly altered. A significant reduction in the GHbA_1c concentration was observed during both peroral (p<0.05) and transdermal estrogen therapy (p<0.05). However, the calculated indices of glucose and insulin metabolism were not altered by either treatment.

The mean IGFBP-1 increased from 4.5±0.7 to 9.2±1.3 nmol/l (p<0.001), and the mean IGF-I decreased from 6.5±0.4 to 5.7±0.3 nmol/l (p<0.05) in the peroral estradiol group, while the changes (from 5.7±0.6 to 6.4±0.3 nmol/l, respectively) observed in the transdermal group were insignificant (p= Ns). Thus the difference between the modes of ERT was notable for the changes in the IGFBP-1 (p<0.01) and IGF-I (p<0.05) levels.

At baseline, significant associations were found between glucose and fasting C-peptide values, insulin sensitivity index and GHbA_1c. The fasting and AUC values for glucose, C-peptide and insulin correlated highly with one another and also with the calculated indices and IGFBP-1, but not with IGF-I. (Table 9)
Table 8. Effect of peroral and transdermal estradiol on glucose and insulin metabolism.

<table>
<thead>
<tr>
<th></th>
<th>Peroral estradiol (n=35)</th>
<th>Transdermal estradiol (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 6 months</td>
<td>Baseline 6 months</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>4.4 (0.1)</td>
<td>4.5 (0.1)</td>
</tr>
<tr>
<td>2 h</td>
<td>4.9 (0.2)</td>
<td>5.0 (0.3)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>62 (4)</td>
<td>59 (5)</td>
</tr>
<tr>
<td>2 h</td>
<td>299 (35)</td>
<td>257 (34)</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>0.64 (0.03)</td>
<td>0.63 (0.04)</td>
</tr>
<tr>
<td>2 h</td>
<td>2.16 (0.13)</td>
<td>2.02 (0.11)</td>
</tr>
<tr>
<td>AUCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l*h)</td>
<td>10.8 (0.3)</td>
<td>11.3 (0.5)</td>
</tr>
<tr>
<td>Insulin (pmol/l*h)</td>
<td>755 (53)</td>
<td>640 (51)</td>
</tr>
<tr>
<td>C-peptide (nmol/l*h)</td>
<td>4.17 (0.17)</td>
<td>3.84 (0.16)</td>
</tr>
<tr>
<td>GHbA1c (%)</td>
<td>5.0 (0.1)</td>
<td>5.1 (0.1)</td>
</tr>
<tr>
<td>ISI (composite)</td>
<td>15.6 (1.3)</td>
<td>19.8 (2.2)</td>
</tr>
<tr>
<td>ISI est</td>
<td>0.10 (0.00)</td>
<td>0.11 (0.00)</td>
</tr>
<tr>
<td>MCRest</td>
<td>8.7 (0.3)</td>
<td>9.0 (0.3)</td>
</tr>
<tr>
<td>1st PH</td>
<td>1470 (79)</td>
<td>1312 (67)</td>
</tr>
<tr>
<td>2nd PH</td>
<td>370 (18)</td>
<td>335 (15)</td>
</tr>
</tbody>
</table>

AUC, area under the curve; ISI (composite) and ISIest, insulin sensitivity indices; MCRest, metabolic clearance rate of glucose; 1st PH and 2nd PH, first-phase and second-phase insulin release. Values are mean (SE)

*p<0.05, **p<0.01, ***p<0.001 vs baseline.

In response analysis to ERT the decrease of AUC for C-peptide was found to be associated with the decrease of fasting C-peptide (r=0.528, p=0.001) and the decrease of AUC for insulin (r=0.797, p<0.001) in the peroral group, and similar associations were also found in the gel group, r=0.429, p<0.01 and r=0.624, p<0.001, respectively. In the peroral estrogen group the increase in IGFBP-1, but not the reduction in IGF-I, correlated with the increases of estrone (r= 0.350, p<0.05), estradiol (r= 0.484, p<0.01) and SHBG (r=0.393, p<0.05), while no relationship was seen with the changes in glucose metabolism. In the transdermal group, no association was found between the changes of IGFBP-1 or IGF-I and estrogens, but a negative correlation was observed with the decrease of AUC for C-peptide and the increase of IGFBP-1 (r= –0.423, p<0.01). Moreover, the reduction in the GHbA1c level was not related to the insulin sensitivity indices or the changes in the fasting, postchallenge or AUC values for glucose, C-peptide and insulin or sex hormones.
Table 9. Relationships (r-values) between fasting blood glucose, C-peptide, insulin, AUC values for glucose tolerance test, GHbA1c, insulin sensitivity and insulin release indices, IGFBP-1 and IGF-I at baseline among all the study subjects (n=79).

<table>
<thead>
<tr>
<th></th>
<th>Fasting glucose (mmol/l)</th>
<th>AUC of glucose (mmol/l*h)</th>
<th>Fasting C-peptide (nmol/l)</th>
<th>AUC of C-peptide (nmol/l*h)</th>
<th>Fasting insulin (pmol/l)</th>
<th>AUC of insulin (pmol/l*h)</th>
<th>GHbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC of glucose (mmol/l*h)</td>
<td>0.570 ***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting C-peptide (nmol/l)</td>
<td>0.489 ***</td>
<td>0.438 ***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC of C-peptide (nmol/l*h)</td>
<td>0.430 ***</td>
<td>0.578 ***</td>
<td>0.760 ***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>0.308 **</td>
<td>0.317 **</td>
<td>0.782 ***</td>
<td>0.568 ***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC of insulin (pmol/l*h)</td>
<td>0.388 ***</td>
<td>0.562 ***</td>
<td>0.702 ***</td>
<td>0.889 ***</td>
<td>0.652 ***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GHbA1c (%)</td>
<td>0.295 **</td>
<td>0.263 *</td>
<td>0.295 **</td>
<td>0.217</td>
<td>0.102</td>
<td>0.168</td>
<td></td>
</tr>
<tr>
<td>ISI (composite)</td>
<td>-0.521 ***</td>
<td>-0.667 ***</td>
<td>-0.814 ***</td>
<td>-0.835 ***</td>
<td>-0.816 ***</td>
<td>-0.915 ***</td>
<td>-0.226 *</td>
</tr>
<tr>
<td>IGFBP-1 (µg/l)</td>
<td>-0.309 **</td>
<td>-0.298*</td>
<td>-0.646 ***</td>
<td>-0.564 ***</td>
<td>-0.438 ***</td>
<td>-0.545 ***</td>
<td>-0.072</td>
</tr>
<tr>
<td>IGF-I (nmol/l)</td>
<td>-0.077</td>
<td>-0.073</td>
<td>0.137</td>
<td>0.090</td>
<td>0.062</td>
<td>0.081</td>
<td>0.128</td>
</tr>
</tbody>
</table>

AUC, area under the curve; ISI (composite), insulin sensitivity index. *p<0.05, **p<0.01, ***p<0.001

5.4 Blood pressure (IV)

The blood pressure response to ERT among the non-hypertensive women is shown in Fig. 6. At baseline the mean blood pressure in sitting position was 137/85 mmHg in the peroral group and 137/84 mmHg in the transdermal group and in the standing position 133/86 and 132/86 mmHg, respectively. Both diastolic and systolic blood pressure in sitting position decreased in the peroral estrogen group (n=26), the mean reduction in diastolic blood pressure was –6 mmHg (p<0.01) and in systolic blood pressure –4 mmHg (p=0.07). Similar changes were also induced by the transdermal estrogen (n=27), –6 mmHg (p=0.001) and –7 mmHg (p<0.05), respectively. Comparable decreases in blood pressure were also observed in standing position (Fig. 6). There was no significant difference between the treatment groups, although in the transdermal estradiol group a lowering of the blood pressure was observed earlier, at three months. Furthermore, the heart rate remained unchanged during ERT.
Fig. 6. Effects of peroral (○) (n=26) and transdermal (●) (n=27) ERT on blood pressure in sitting (left) and in standing (right) among non-hypertensive women. Values are mean (±SE). For the change from baseline after Bonferroni correction * p< 0.05, ** p< 0.01, *** p≤ 0.001. The changes were not significantly different between the treatment groups.

Additionally, 15 of the 16 women who had treatment for hypertension at the baseline, completed the study. Eight of them had received peroral estradiol and seven were on transdermal therapy. Their blood pressure remained at the baseline level, except that there was a tendency to systolic blood pressure lowering on both estrogen regimens when the subjects were sitting, but the change was not statistically significant. These women were using heterogeneous antihypertensive drugs. Consequently, detailed analysis of subgroups according to treatments was not possible.

### 5.4.1 Natriuretic peptides, renin and aldosterone

The mean baseline values (±SE) for natriuretic peptides, NT-proANP, ANP and BNP were 212±14, 10.2±0.7 and 3.4±0.2 pmol/l in the peroral estrogen group and 240±17, 10.9±0.9 and 4.4±1.1 pmol/l in the estrogen gel group, respectively. Plasma NT-proANP increased by 25 % on the peroral ERT and by 22% on the transdermal ERT in six months, whereas no significant changes were observed in the plasma ANP and BNP levels (Fig. 7). The sodium intake of the study groups was similar and remained unaltered during the trial. Furthermore, neither of the estradiol therapies changed the plasma active renin and
aldosterone levels. Women using antihypertensive medication were excluded, because these agents affect natriuretic peptide, renin and aldosterone values and medication was too heterogeneous for subgroup analysis.

At baseline a relationship between systolic blood pressure and natriuretic peptides was observed (for NT-proANP $r=0.285$, $p<0.05$; for ANP $r=0.309$, $p<0.05$; for BNP $r=0.345$, $p<0.01$). Further, both systolic ($r=–0.397$, $p<0.01$) and diastolic ($r=–0.336$, $p<0.01$) blood pressure was associated with plasma renin, but not with aldosterone levels. After ERT, however, these associations were diminished.

![Graph of natriuretic peptide levels at baseline and after 3 and 6 months of peroral (open symbols) and transdermal (solid symbols) ERT among non-hypertensive women. NT-proANP, N-terminal fragment of proANP; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide. Values are mean (SE). For the change from baseline after Bonferroni correction * $p<0.05$, ** $p<0.01$, *** $p<0.001$.](image-url)
6 Discussion

6.1 General aspects

The present study was designed to assess the impact of ERT on cardiovascular risk factors including lipid and lipoprotein metabolism, especially the regulation of plasma LDL cholesterol, glucose and insulin metabolism and blood pressure. Mechanisms of estrogen action and potential differences between the routes of administration were investigated by comparing peroral estradiol valerate and transdermal 17β-estradiol gel therapies. As regards cardiovascular prevention, the different effects of estrogen are poorly understood. In HRT the addition of progestin makes the estimation even more complex. A carryover effect of progestin may alter the outcome in HRT trials. The effect of progestin is modified by the dose and mode of the progestin, and further, the degree of androgenicity of the progestin regimen is important (133, 174). Thus, it is important to evaluate first the mechanisms of estrogen action, and thereafter to find out the best choice for estrogen and progestin combination.

The present study used a randomized, parallel, double-blind, double-dummy design, where each subject served as her own control. A washout period of at least two months was required for those women who had earlier used HRT. Totally 79 hysterectomized, postmenopausal women aged 48 to 62 years were randomized to receive either peroral estradiol valerate 2 mg/day or transdermal estradiol gel 1 mg/day for 6 months. The duration of the study was considered long enough to reveal estrogen induced metabolic changes also in subjects on transdermal ERT. Only hysterectomized women were included because they provided a good possibility to focus on the effects of estrogen alone without the need of progestin for endometrial protection.

Randomization succeeded well and compliance was also good throughout the study. The treatment groups were comparable in terms of age, BMI, menopausal status, lifestyle factors and studied variables at baseline. Further, no significant changes in physical activity, diet, scores of alcohol consumption and cigarette smoking or concomitant medication that could have interfered with the results were noticed during the study. Both treatments relieved equally climacteric symptoms. A slight increase in weight observed with transdermal ERT during the first three months was not considered clinically
significant. It was not associated with changes in metabolic factors measured in this study, and furthermore it was diminished during the follow-up. Breast tenderness was the most prevalent side effect, whereas no serious adverse effects were related to the study treatments. In addition, no cardiovascular adverse events were reported during the study. However, a larger study with longer follow-up would have been required to assess the long-term effects of ERT on morbidity and mortality.

A major limitation of the study is the lack of a placebo group, excluding the possibility of placebo effect. Especially the effect of ERT on blood pressure lowering and small changes in glucose metabolism could be criticized. However, the women in the present study had menopausal symptoms and there would presumably have been many drop-outs among the placebo-treated subjects. On the other hand, a cross-over study design might have yielded more information about the differences in the route of estrogen administration and increased the statistical power, but would also have involved problems. The detailed metabolic assays should have been repeated three times for the same subjects, which was considered to be difficult and to result in poor compliance.

Even though the age range of these women was quite wide there were not enough older women to assess the effect of age on the response to ERT. 80% of the subjects were under 56 years, indicating that climacteric symptoms were the main reason for seeking replacement therapy. Further, the study did not allow estimation of the effect of menopause since premenopausal women were not included.

Finally, as most of the women in this study were clinically healthy, these results may not apply to older women or women with risk factors such as obesity, hypertension, diabetes or established coronary heart disease.

6.2 Cardiovascular risk factors and ERT

6.2.1 Plasma lipids and lipoproteins

In agreement with previous studies (133) both peroral and transdermal ERT decreased significantly plasma total, IDL and LDL cholesterol. VLDL cholesterol decreased only on transdermal ERT, while HDL cholesterol and triglycerides increased by peroral, but not by transdermal ERT use (II). Consequently, a decrease of the LDL/HDL ratio was observed on both treatments. However, the lowering of LDL cholesterol and the LDL/HDL ratio was more pronounced in the peroral estrogen group than in the transdermal group.

In general, oral estrogens seem to induce similar decreases in LDL cholesterol, whereas compared with estradiol treatment CEE may increase HDL cholesterol slightly more, and dose-dependent responses have been observed especially in VLDL cholesterol and triglyceride levels (133). Further, the LDL cholesterol response appears to depend on the LDL cholesterol level. Thus, the most profound decrease of LDL has been reported in hypercholesterolemic women (263). Accordingly, in our study the higher the baseline LDL cholesterol, the more effectively it was decreased. In the present study, the overall
lipid response was considered favorable as LDL cholesterol decreased by 19% and HDL cholesterol increased by 12%. Even though peroral ERT increased total triglycerides by 10%, it resulted in hypertriglyceridemia in only a few subjects. Three women in the peroral group and seven in the gel group had plasma triglycerides between 2.0 and 3.0 mmol/l after ERT. However, all of them except for one subject on peroral ERT had a high triglyceride level (> 2.0 mol/l) already at the baseline.

Is it possible that estrogen induced LDL lowering and increase in HDL are insignificant and blunted by the increase of triglyceride levels? Hypertriglyceridemia has been suggested to be an important risk factor for cardiovascular diseases in women, even more significant than in men (80). Furthermore, it is associated with other risk factors in lipid metabolism such as reduced levels of HDL cholesterol and an increase of small dense LDL particles. After all, the modest changes in triglyceride levels, which in most women remain within normal range, probably have no clinical significance. However, the potential benefits of estrogen may disappear resulting in null effect if the increase of triglycerides is connected with unfavorable qualitative changes in LDL and HDL cholesterol. Furthermore, in some subjects or with different estrogens ERT may induce marked hypertriglyceridemia.

The benefit of non-oral estrogen administration has been more controversial. Transdermal ERT may induce minor decreases in triglycerides, total and LDL cholesterol levels as well as a rise in HDL cholesterol concentrations, while some short-term studies have failed to reveal any significant changes (133). However, it should be noticed that at least 3 to 6 months is required to achieve the maximal effect with transdermal ERT (39, 130, 264). Taken together, the effects of transdermal estrogens, patches or gels, on lipids appear to be rather neutral or slightly beneficial as in our study.

### 6.2.1.1 Regulation of LDL cholesterol

To study the regulation of plasma LDL cholesterol among postmenopausal women, FCR and the production rate for LDL apo B, cholesterol absorption and the polymorphisms of some regulatory proteins, such as apo E, apo B and 7α-hydroxylase, were determined (I-II).

At baseline LDL cholesterol level was mainly explained by FCR ($r = -0.757$, $p < 0.001$) and the production ($r = 0.531$, $p < 0.001$) of LDL apo B (I). In agreement, decreased LDL receptor activity has been suggested to explain hypercholesterolemia in postmenopausal women, whereas no difference in the production rate of LDL apo B was observed in that study. (139). An association between LDL cholesterol and FCR for LDL apo B and LDL production rate has also been observed in middle-aged and elderly men (107, 108, 265).

Atherogenic risk factors tend to be clustered, and obesity is often behind unfavorable metabolic changes (89, 94, 96, 120). High LDL cholesterol level is often related to other atherogenic findings in lipid profile, such as high total cholesterol and triglycerides and low HDL cholesterol observed in the present study. Further, LDL cholesterol was associated with BMI ($r = 0.265$, $p < 0.05$) and obese women had increased production of LDL apo B. (I)
Several studies have shown an association between apo E (110, 111), apo B (112, 266) and \(7\alpha\)-hydroxylase (113) polymorphisms and LDL cholesterol concentrations. 5.0 % of the LDL cholesterol level variance is suggested to be explained by apo E polymorphism in postmenopausal women, but only 0.5 % in premenopausal female subjects, the value for men being 1.0 % (267). In men apo E polymorphism was found to affect FCR and production of LDL apo B and cholesterol absorption (29). However, in our study neither cholesterol absorption efficiency nor any of the determined genetic factors were associated with LDL cholesterol, suggesting that these factors may not be important in the regulation of LDL cholesterol in postmenopausal women. Low dietary intake of cholesterol could also be an explanation (103). On the other hand, the power of our study may be too low to find the effect of genetic factors.

During peroral ERT total VLDL cholesterol remained unchanged in the present study, whereas a decrease in IDL cholesterol was observed, suggesting that the overall production was lowered or the clearance rate of IDL was enhanced (II). Previous kinetic studies on peroral estrogen treatment of six weeks have reported to raise VLDL cholesterol by increasing the hepatic production rate of light VLDL (127, 138). However, these VLDL particles were also rapidly removed from circulation. Further, there was some increase in the conversion of light VLDL to dense VLDL, but overall dense VLDL level remained unchanged because both the production and FCR of dense VLDL increased similarly. In addition, there was no change in overall production or FCR of IDL, thus IDL concentration was not altered by estrogen. However, a lesser amount of IDL was formed directly or by conversion of light VLDL to IDL, whereas increased flux of IDL arising from dense VLDL and conversion of IDL to LDL with a lesser amount of direct removal were found during ERT compared with placebo (138). A dose-dependent increase of plasma triglyceride is related to the enhanced production of large triglyceride-rich VLDL particles (127).

Estrogen has been shown to increase the rate of LDL catabolism, suggesting the importance of estrogen in regulation of LDL cholesterol. Estrogen-induced expression of hepatic LDL receptors (31, 32, 140) has been observed both at the protein and mRNA levels (268) in rats. Responses to ERT are probably mediated by estrogen receptors (268). Pharmacological doses of estrogen used in treatment for prostatic cancer also enhanced LDL-receptor mediated uptake of LDL and decreased plasma LDL concentration in men (30, 141). In agreement with our findings, peroral ERT has been shown to increase both the production rate and especially the FCR of LDL, resulting in reduction in LDL cholesterol and apo B concentrations (127, 138). In contrast, no significant effects on VLDL production or LDL catabolism have been observed with transdermal estradiol use (127).

Biphasic dose-dependent effect of 17β-estradiol on hepatic cholesterol metabolism has been observed in female rats. In the liver, 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol \(7\alpha\)-hydroxylase activities were increased at low, but not at high doses of E2, whereas LDL-receptor expression was stimulated at high doses. Apo A-I synthesis increased dose-dependently, while an increase of plasma HDL cholesterol was found with physiological doses of E2. In this study the responses of HDL cholesterol to both oral and subcutaneous administration of estradiol were similar. (142)

Differences between peroral and transdermal estrogen treatments have been explained by different impact on liver metabolism, as peroral estrogen-induced hepatic first pass
metabolism and protein synthesis in the liver is avoided in transdermal administration (39). However, in the present study, estrogen-induced changes in lipid metabolism were associated with an increase in estrogen level. Peroral estrogen did, but transdermal treatment did not enhance FCR and production of LDL apo B. Although the production rate of LDL apo B was increased in the peroral group, the LDL clearance rate was increased more, leading to a decrease in LDL cholesterol. Moreover, the decrease of LDL cholesterol was related to an increase in FCR for LDL apo B on both peroral and transdermal ERT, \( r = -0.645, p < 0.001 \) and \( r = -0.627, p < 0.001 \), respectively. Consequently, the effect on FCR (LDL-receptor-mediated uptake) is suggested to be important in the lowering of LDL cholesterol independent of the route of estrogen administration.

One additional mechanism for LDL lowering by ERT could be altered cholesterol absorption efficiency. In fact, a slight decrease in dietary cholesterol absorption was observed with both treatments (II). However, the changes in cholesterol absorption were not related to the decrease of LDL cholesterol or to the changes in FCR or the production of LDL apo B. After all, it is possible that total absorption of intestinal cholesterol did not change, because dietary cholesterol represents about one third and biliary cholesterol two thirds of the total intestinal cholesterol.

Few studies have previously examined how lipoprotein responses to estrogen could be modulated by genetic factors including apo E (155–157) and estrogen receptor (ER-\( \alpha \)) polymorphisms (158). However, different studies have revealed conflicting findings. Allelic variation of apo E has been suggested to explain LDL response (155) as HRT decreased LDL cholesterol more among apo E4-positive subjects than among apo E4-negative subjects. In another study apo E2 appeared to influence the response of HDL levels to estrogen use. The women with apo E2 using ERT had the highest HDL levels, and the women with apo E2 not using ERT had the lowest HDL levels (156). In addition, one study showed that the women with apo E2 had the highest triglyceride levels, suggesting that apo E polymorphism modulated the effect of estrogen use on triglyceride levels (157). In the present study responses of lipoprotein metabolism to ERT were not altered by apo E polymorphism or the other determined genetic factors (II). However, the finding may be limited by the lack of statistical power. In addition, differences in the study population, the regimens and the durations of treatment may modify the findings.

### 6.2.2 Glucose and insulin metabolism

A slight decrease in \( \mathrm{GHbA_1c} \) was observed on both peroral and transdermal ERT over six months suggesting a long-term beneficial effect on glucose balance. However, the fasting and postchallenge glucose and insulin levels as well as the AUCs for glucose and insulin did not change significantly, whereas endogenous insulin secretion, estimated as AUC for C-peptide, decreased. In addition, the calculated indices predicting metabolic clearance rate of glucose, insulin sensitivity and insulin release were not altered. Consequently, the overall response to ERT seemed to be relatively modest. (III)

Previously transdermal ERT has been suggested to improve glucose tolerance and insulin sensitivity (171), while responses to peroral estrogens, CEE and estradiol, are rather neutral, or peroral CEE at a higher dose (1.25 mg/day) may decrease insulin
sensitivity (172, 174), whereas a lower dose (0.625 mg/day) of CEE (171, 173, 177, 178) as well as estradiol (169, 170, 179, 181, 269) has been reported to decrease fasting glucose or insulin concentrations and enhance insulin sensitivity. A meta-analysis was considered to reach a more balanced overview from previous studies, but due to heterogeneous data it was not possible. Considerable variation exists not only in ERT regimens but also in patient selection and the duration of treatment. Most studies are small, short-term and uncontrolled. In addition, studies are difficult to compare due to methodological differences.

Based on controlled studies (173, 176, 181, 185), the effect of ERT/HRT should be considered as being quite minimal and further, the route of estrogen administration may not significantly alter the outcome. In agreement with our findings, a randomized cross-over study comparing peroral CEE 1.25 mg/day to transdermal estradiol 100 µg/day for 12 weeks found no significant difference in glucose metabolism, as assessed by OGTT and hyperinsulinemic euglycemic clamp (176). The changes in glucose and insulin levels were insignificant, although a slight trend towards lower postprandial insulin and glucose in OGTT and a trend towards a higher glucose infusion rate to maintain euglycemia in the clamp were observed during transdermal ERT. Moreover, insulin sensitivity was not altered by estrogen (176). Similar results were obtained also after surgically induced menopause in a placebo-controlled cross-over study, which compared the effects of transdermal estradiol alone and in combination with norethistrone on insulin sensitivity using the hyperinsulinemic euglycemic clamp technique (185). However, the duration of this study was only 6 weeks, which could be too short to detect changes induced by transdermal administration. In contrast, studies analyzing the estrogen alone phase during HRT have suggested a more favorable response to transdermal estrogen than to peroral estrogen (174, 179). Furthermore, the possibility of a carryover effect of progestin is not totally excluded in these trials.

In contrast to our findings, a decrease in C-peptide and GHbA1c concentrations, but no changes in glucose and insulin levels, a placebo-controlled study with unopposed 17β-estradiol treatment reported decreases in fasting insulin and C-peptide levels suggesting improved insulin sensitivity, while a decrease in fasting glucose did not differ from the placebo group and no change was observed in GHbA1c level (181). On the other hand, a decrease in GHbA1c during ERT has been noticed in some small studies including diabetic women (188, 189, 270). Finally, the only placebo-controlled, long-term study (PEPI trial) showed no adverse effects on glucose metabolism with peroral CEE 0.625 mg/day or with HRT over 3 years of treatment (173).

Why are the results conflicting? Moreover, what is the importance of these findings? Some of the differences may be related to methodological differences. The reason for the failure to show any significant changes in glucose, C-peptide and insulin levels may reflect intra-individual variation or poor reproducibility of these measurements. In contrast, GHbA1c is presumably a better estimate of the overall changes in long-term glucose metabolism than the measurements during OGTT. GHbA1c represents glucose levels over the preceding months reflecting long-term glucose tolerance and has also better reproducibility than fasting or postchallenge glucose measurements (271). Additionally, GHbA1c has also been suggested to be a better predictor of cardiovascular disease in women without diabetes (271).
It is not possible to assess insulin action \textit{in vivo} by fasting insulin level, although it has been used as an estimate of insulin sensitivity, especially in epidemiological studies. The euglycemic hyperinsulinemic clamp uses the amount of exogenous glucose required to maintain euglycemia under controlled hyperinsulinemic conditions as an index of insulin sensitivity. It is a reproducible and reliable method, the golden standard for assessment of glucose metabolism. Mathematical modeling analysis of the intravenous glucose tolerance test (IVGTT) and insulin tolerance tests have also been used to evaluate glucose tolerance, insulin sensitivity, secretion and elimination. In our study measurements of serum insulin and C-peptide levels in fasting state and following standardized OGTT were used to assess indirectly the presence of insulin resistance. In addition, the metabolic clearance rate of glucose, insulin sensitivity and insulin secretion indices obtained from OGTT were calculated. (258, 259) These indices have a good correlation with variables obtained by the euglycemic hyperinsulinemic clamp and are more appropriate for large studies and repeated measurements than the complex clamp method.

Our results in OGTT, i.e. a decrease of C-peptide concentrations, could reflect reduced insulin secretion, alteration in the ratio of C-peptide and insulin secretion, diminished insulin clearance or enhanced rate of C-peptide clearance. However, the reduction of fasting and AUC for C-peptide was moderate, while no changes were observed in insulin and glucose levels. Further, insulin sensitivity remained unchanged according to the calculated indices. These findings with the slight reduction in GHbA1c suggest that no adverse effects on glucose tolerance and insulin sensitivity are related to either peroral or transdermal ERT.

In agreement with the previous studies (191–193) peroral ERT decreased IGF-I levels and increased IGFBP-1 significantly, whereas no significant changes were found in the transdermal group. IGF-I and IGFBP-I are regulated by sex hormones and associated with carbohydrate metabolism (190). The alterations in IGF-I and IGFBP-1 seem to reflect the induction of first-pass liver metabolism, since the increase of IGFBP-1 was also associated with the increase of SHBG and estrogen levels on peroral estrogen. However, these findings were not associated with the changes in glucose and insulin metabolism, suggesting that IGF-I and IGFBP-1 have a minor role in the regulation of glucose metabolism during ERT. (III)

\subsection{6.2.3 Blood pressure and role of natriuretic peptides, renin and aldosterone}

Both peroral and transdermal ERT resulted in similar decreases in systolic and diastolic blood pressure in nonhypertensive women during the present study (IV). In previous studies the effect of oral estrogens on blood pressure, especially conjugated estrogen regimens, have tended to be neutral (10, 209, 210, 212), whereas natural (211, 212) and transdermal estrogens (213–216) may lower the blood pressure. Studies with ambulatory 24-hour blood pressure recordings have revealed only minor changes in daytime blood pressure, while a greater decrease in blood pressure has been reported in the nighttime than in the daytime (214–216). However, it should be noted that regardless of treatment
regimen blood pressure might increase in some subjects during ERT. A significant increase in blood pressure was observed in some women in the ambulatory blood pressure study, although the mean blood pressure of the study group was not increased (214). An increase in blood pressure was also observed in some subjects on both ERT regimens in our study, although the blood pressure of these nine women still remained at normotensive level. In general, most long-term studies, also ones including women with mild or moderate hypertension, have reported no change in blood pressure (217–220). Accordingly, the mean systolic or diastolic blood pressure did not change among women using antihypertensive medication in the present study. Nevertheless, blood pressure should be controlled after starting ERT, because not only contraceptives (206), but also ERT may increase blood pressure in some women (214).

The interaction of natriuretic peptides and RAS in the regulation of blood pressure during ERT is poorly understood (221). Since natriuretic peptides have natriuretic and vasodilatory activity and also inhibit the renin-angiotensin-aldosterone system and lower the blood pressure, it was hypothesized that the changes in blood pressure caused by ERT might be mediated via changes in natriuretic peptides. In the present study plasma NT-proANP was similarly elevated on both estrogen treatments indicating an activation of the natriuretic peptide system, but plasma ANP, BNP, renin and aldosterone remained unchanged (IV). Could this finding explain part of the blood pressure lowering during ERT?

It is unclear whether estrogen alters ANP production directly or indirectly. Since a slight, insignificant, tendency towards weight gain was observed in our study, it is possible that estrogen, which is known to increase plasma volume (211), resulted in mild volume retention, which enhanced the secretion of natriuretic peptides, as reflected by the increase in the plasma NT-proANP levels (196). Blood pressure decreased during our ERT trial, which diminishes the atrial load and could be expected to decrease ANP. However, no significant changes in the levels of ANP were detected, which further suggests increased secretion of ANP. Similar amounts of NT-proANP and ANP are secreted but the plasma half-lifes and also the elimination of these natriuretic peptides differ. (196) Our inability to detect changes in ANP could be explained by its rapidly fluctuating levels. Due to longer half-life the concentration of NT-proANP is higher and more stable than the levels of ANP (or BNP). Further, ANP is eliminated by neutral endopeptidase-mediated proteolysis and by binding to clearance receptors, while NT-proANP is mainly eliminated via the kidneys. (195, 196) It remains unknown how estrogen alters these clearance mechanisms of natriuretic peptides.

Previously, ANP and BNP have been determined only in a few small studies (204, 222–224) including postmenopausal women, whereas NT-proANP has not been measured. Moreover, the results are inconsistent. No change in plasma ANP was observed in women receiving CEE (n=17) (222), when the samples were taken at the end of the estrogen alone phase, whereas plasma ANP decreased during the estrogen phase in a study with either peroral (n=5) or transdermal (n=8) estradiol in combination HRT for two months (223). In another study, however, both ANP and BNP increased significantly after 3 months of HRT with transdermal estradiol (n=22) (224). No change in blood pressure was observed in these studies.

Peroral ERT, but not transdermal, is known to induce hepatic synthesis of proteins (209, 213). However, plasma renin and aldosterone did not change in the present study.
Further, responses to peroral and transdermal ERT were similar. Compared with other studies these differences may be related to the estrogen regimens, the study population or the variation and the sensitivity of the measurement. (230) For example, the measurements of plasma renin and aldosterone may not have been sensitive enough to detect small changes in these hormones among normotensive women in the present study because the samples were taken fasting, but not strictly resting at early morning. On the other hand, an elevation of ANP levels induces renin suppression (196). Blood pressure changes were not associated with different responses in natriuretic peptides, aldosterone or renin levels. In addition, other mechanisms that may be involved in the ERT-related decrease of blood pressure are calcium antagonist properties of estrogen (272), a decrease in endothelin (232, 273), an increase in nitric oxide (273), endothelium-dependent vascular relaxation, arteriolar distensibility and baroreceptor sensitivity (274).

Antihypertensive drugs are known to alter natriuretic peptide, renin and aldosterone levels (196). Thus it would have been interesting to assess whether the effect of ERT was modified by antihypertensive treatment. However, this was not possible in the present study, due to heterogeneous medication resulting in an insufficient number of subjects in the subgroups. Taken together, ERT-induced lowering in blood pressure seems to be modest, and more comprehensive, controlled studies are needed to understand the role of natriuretic peptides and RAS.

6.3 Prevention of cardiovascular disease in postmenopausal women

Coronary heart disease is the leading cause of death and a significant cause of morbidity in postmenopausal women. It has been estimated that a 50-year-old white woman has a 46% lifetime probability of developing coronary heart disease and a 31% risk of death due to coronary heart disease, whereas the estimated percentages for stroke are 20% and 8%, for hip fracture 15% and 1.5% and for breast cancer 10% and 3%, respectively (17). Thus, an important question is how to reduce the risk of cardiovascular disease in women. Could estrogen be the answer?

Increased risk of cardiovascular and thrombotic events has previously been reported in men using high doses of estrogen for treatment of prostatic cancer (30). Similar results were obtained in the Coronary Drug Project when men with previous myocardial infarction were treated with CEE (2.5 or 5 mg/day) in the early 1970s (15). In contrast, lower doses of estrogen used in postmenopausal replacement therapy resulted in opposite effects. Based on findings from observational studies supported by beneficial responses to estrogen in cardiovascular risk factors, ten years ago ERT/HRT was recommended for cardiovascular prevention (275). Especially women with coronary heart disease or women at increased risk for coronary heart disease, e.g. having elevated LDL cholesterol, were suggested to benefit. Different mechanisms of estrogen action have been widely studied to explain the preventive effect. Antiatherogenic changes in the lipid profile, including the reduction in LDL cholesterol and Lp(a) and the increase of HDL cholesterol, beneficial vascular effects, fibrinolysis, lower levels of fasting glucose and insulin as well as decrease of blood pressure have been related to ERT (2, 11). In contrast, there is also evidence that peroral estrogens may increase triglyceride and CRP levels and
thrombin generation. However, the results of large randomized studies, indicating null effect (19) or even some increase rather than decrease in cardiovascular risk (19, 24, 25), were unexpected.

How to explain divergent results between the observational and the randomized studies? Differences in study designs, the characteristics of study subjects, outcome measurements, compliance and other methodological factors may modify the results (28, 276). Further, confounding factors could be an important reason. In fact, according to a recent meta-analysis current use of HRT was associated with a reduction in coronary heart disease incidence and mortality (relative risk 0.80 and 0.62, respectively), but after adjustment for socioeconomic factors the risk for incidence was not reduced (relative risk 0.97) (56). Similar results were obtained in the adjusted meta-analysis of HRT and the primary prevention of cardiovascular disease (71). In addition to selection bias observational studies are more likely to suffer from other biases including prevention, compliance, survivor and prevalence-incidence bias. Interestingly, observational and randomized data are more consistent regarding the relationships between hormone replacement and stroke, venous thromboembolism, osteoporosis and cancer (277). Thus, not only confounding factors but also biological explanations may account for the different findings.

Our study and several others have shown consistently that ERT may induce potentially beneficial changes in lipids and lipoproteins, which are modified by the route of estrogen administration (133). ERT also appears to protect against LDL oxidation and to diminish penetration and retention of LDL in the arterial wall (2, 278). Is it possible that these alterations including the lowering of LDL cholesterol are insignificant in cardiovascular risk reduction among women?

Consistent evidence from statin trials, however, has shown that lowering cholesterol, especially LDL cholesterol, reduces the progression of coronary atherosclerosis and prevents recurrent cardiovascular events both in men and women (279–281). In addition, statins provide greater LDL lowering compared with ERT, suggesting that estrogen alone may not be sufficient to achieve desired LDL target levels (281–284). In contrast, estrogens seem only to reverse the menopause-associated 10–12 % increase of LDL cholesterol (122). On the other hand, it remains unclear what is the importance of the estrogen-induced qualitative changes in LDL and increase of VLDL triglycerides, which are suggested to be atherogenic (149).

Further, the participants of the randomized studies were older and therefore more likely to have subclinical coronary disease than women who usually initiate replacement therapy at menopause. Experimental data suggest reduced benefit in established atherosclerosis (14, 285, 286). However, the timing of replacement therapy seemed not to be the reason for negative results in the WHI trial since the risk of the women at 50 to 59 years of age was not lower compared with older women (24).

The results of the present study suggest that peroral and transdermal estradiol replacement therapy could induce beneficial changes in the lipid profile, while no adverse effects were observed on glucose metabolism or blood pressure. However, the clinical significance of these estrogen-induced alterations in the prevention of atherosclerosis remains unclear. It is possible that other mechanisms of estrogen action such as changes in plaque stability, hemostatic or inflammatory factors may counteract observed benefits (233). Additionally, we should remind ourselves that an association between a risk factor
and the risk of a disease does not mean that a drug-induced beneficial change in that factor results in consequent risk reduction. If we could understand how estrogen, which improves several risk factors, fails in cardiovascular prevention, we would probably understand better the pathogenesis of atherosclerosis. (27) Thus, further studies are important.

The negative results of the randomized studies, HERS and WHI, were obtained by continuous combined oral CEE (0.625 mg/day) with MPA (2.5 mg/day). The effects of other HRT regimens or other dosages as well as unopposed estrogen or different routes of administration are not established. To date neither estrogen alone nor other estrogen-progesterin combinations have been proven to prevent the progression of coronary atherosclerosis (21, 23) or cardiovascular events (20, 22, 76, 77) in randomized trials. The findings from randomized studies do not exclude the possibility that some women could be more likely to benefit from replacement therapy, whereas others might have an even higher risk. Genetic diversity, for example, may modify individual responses (27). However, until these factors are identified ERT or HRT should not be initiated or continued for primary or for secondary prevention of cardiovascular disease (277, 287).

The recommendations for reducing the risk of cardiovascular disease in women include management of lifestyle factors, risk factors and pharmacologic treatments (26). Counseling on lifestyle factors such as smoking avoidance, regular physical activity, proper nutrition and reduction of overweight is indicated in all women. Medication for dyslipidemia, blood pressure and diabetes are indicated if target levels are not met with lifestyle interventions. In addition, for women with coronary heart disease antiplatelet agents/anticoagulants, β-blockers and ACE inhibitors should be considered (287, 288).

Is there a role for postmenopausal hormone therapy today after randomized data do not suggest overall benefit and raise the possibility of harm in cardiovascular prevention? As regards the prevention and treatment of osteoporosis other effective agents are available. Further, HRT has been suggested to prevent cognitive decline and dementia, but there are methodological limitations in these trials and HRT is not recommended (289). The effect of HRT on health-related quality of life appears to be modest and associated with vasomotor symptoms (290, 291). The benefits and risks should be considered individually. At present short-term treatment of menopausal symptoms remains the only indication for ERT/HRT (276, 277, 292).
7 Conclusions

1. At baseline high LDL cholesterol level was related to other atherogenic findings in lipid profile and obesity. FCR for LDL apo B, LDL apo B production rate and BMI were the main determinants of LDL metabolism among these postmenopausal women. In contrast, the absorption of dietary cholesterol or the determined genetic factors regulating LDL cholesterol level, i.e. apo E phenotype, EcoRI and XbaI polymorphisms of the apo B gene and polymorphisms of 7α-hydroxylase gene appeared not to have a significant role.

2. Both peroral and transdermal ERT resulted in significant decreases of total, IDL and LDL cholesterol. Peroral estrogen also increased plasma HDL cholesterol and triglyceride levels, whereas no change was observed during transdermal ERT. The lowering of LDL cholesterol and LDL/HDL ratio was more pronounced on peroral ERT. The overall effect of both peroral and transdermal ERT on lipid profile was suggested to be beneficial during this six-month study, although triglycerides were slightly increased during peroral ERT.

   Peroral ERT enhanced both the production rate of LDL apo B and the FCR resulting in a decrease of LDL cholesterol, since the LDL clearance rate was increased more than the production. Neither the production nor the FCR for LDL apo B were significantly altered in the estradiol gel group. However, on both treatments the ERT-induced LDL cholesterol lowering was related to the increases in estrogen level and in FCR for LDL apo B, reflecting enhanced LDL receptor activity, suggesting that the achieved estrogen level and the increase of FCR are important in the regulation of LDL level during ERT. In contrast, a slightly lowered absorption efficiency of dietary cholesterol induced by both therapies was not associated with the decrease of LDL cholesterol. Furthermore, the determined genetic factors were not found to modify the effects of ERT.

3. A reduction in the GHbA1c concentration observed on both modes of ERT could suggest a long-term improvement in the glucose balance. However, the clinical significance of this finding is uncertain, since the changes were modest and no other favorable effects on glucose metabolism and insulin sensitivity were observed.
4. Both peroral and transdermal ERT lowered diastolic and systolic blood pressure among non-hypertensive women, which was accompanied by an increase in plasma NT-proANP, indicating an activation of the natriuretic peptide system. Natriuretic peptides might have a role in the regulation of blood pressure during ERT, although no significant changes were observed in plasma ANP, BNP, aldosterone and renin levels.

5. Peroral ERT induced more profound changes in lipoprotein metabolism than the transdermal therapy, whereas the route of estrogen administration did not alter the effects of ERT on glucose metabolism and blood pressure.
References

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