Mechanisms Regulating LDL Metabolism in Subjects on Peroral and Transdermal Estrogen Replacement Therapy

Anna Karjalainen, Jorma Heikkinen, Markku J. Savolainen, Ann-Christine Bäckström, Y. Antero Kesäniemi

Abstract—To study the mechanisms of low density lipoprotein (LDL) cholesterol lowering by peroral and transdermal estrogen replacement therapy (ERT), 79 hysterectomized postmenopausal women aged 48 to 62 years were randomized in a double-blind double-dummy trial to receive either peroral estradiol valerate (2 mg/d) or transdermal estradiol gel (1 mg/d) for 6 months. Plasma LDL cholesterol decreased from 4.19±0.83 (mean±SD) to 3.39±0.78 mmol/L (P<0.001) in the peroral group and from 4.11±0.86 to 3.72±0.78 mmol/L (P<0.001) in the transdermal estrogen group. Peroral estrogen did, but transdermal treatment did not, enhance the fractional catabolic rate (FCR) and production of LDL apolipoprotein B (apoB). However, the decrease of LDL cholesterol was related to an increase in FCR for LDL apoB on both peroral and transdermal ERT (r=-0.645, P<0.001 and r=-0.627, P<0.001, respectively). These changes were associated with changes in the serum estrogen level. Both therapies reduced absorption of dietary cholesterol by 6% to 10% (P<0.05). The effects of estrogen were not modified by the polymorphisms of apoE and apoB or cholesterol 7α-hydroxylase. In conclusion, the ERT-induced LDL cholesterol–lowering effect is related to changes in estrogen level, which presumably enhance LDL receptor activity, which is manifested as an increase in FCR for LDL apoB. The small decrease in the absorption efficiency of dietary cholesterol does not seem to contribute largely to the cholesterol lowering on either transdermal or peroral ERT. (Arterioscler Thromb Vasc Biol. 2000;20:1101-1106.)

Key Words: estrogen replacement therapy ■ LDL cholesterol ■ menopause ■ lipids

According to observational studies, up to 50% of cardiovascular mortality in postmenopausal women could be prevented by postmenopausal hormone replacement therapy (HRT). However, a recent randomized secondary prevention study was not able to confirm these results. At any rate, menopause is related to unfavorable alterations in lipids and lipoproteins. The beneficial changes in lipids and lipoproteins observed during HRT in several previous studies have been assumed to explain 25% to 50% of the reduced cardiovascular risk. Peroral estrogens decrease total and LDL cholesterol and Lp(a) and increase HDL cholesterol but also triglycerides, whereas less marked reduction in total and LDL cholesterol and no change in Lp(a), HDL cholesterol, and triglyceride levels have been observed during transdermal estrogen treatments.

The underlying mechanisms of action of estrogen administered via various routes are poorly known. The serum estrogen level is varied by the estrogen regimen and the route of estrogen administration. After peroral administration, high levels of estradiol are catabolized into estrone, which induces protein synthesis in the liver. When the transdermal route is used, induction of hepatic first-pass metabolism can be avoided, and a more physiological estrone/estradiol ratio is achieved. Mainly based on animal studies or the use of pharmacological doses of estrogen in prostate cancer, the explanation for the LDL cholesterol–lowering effect of estrogens has been the enhanced LDL receptor activity in the liver. The present study was designed to investigate whether the commonly used doses of estrogen in replacement therapy might also affect LDL clearance. Potential additional mechanisms of LDL lowering, such as changes in cholesterol absorption, were also studied. In addition, the influence of the polymorphisms of important regulatory proteins, eg, apoE, apoB, and cholesterol 7α-hydroxylase, on the lipid response was evaluated. Also, the importance of the route of administration was elucidated by comparing peroral estradiol valerate treatment with transdermal estradiol gel therapy.

Methods

Subjects
The study design and the subjects have been described in detail previously. Briefly, 79 hysterectomized postmenopausal women seeking hormone substitution therapy for climacteric symptoms were randomized according to age and body mass index (BMI) to receive either peroral estradiol or transdermal estradiol gel for 6 months. Because the study was focused on the effects of estrogen alone,
hysterectomized women were chosen to avoid the need of progesterone for endometrium protection. In this controlled, double-blind, double-dummy trial, the peroral estrogen group (n=39) received a daily tablet containing 2 mg estradiol valerate (Orion Pharma). The transdermal estrogen group (n=40) applied estradiol gel (Divigel, Orion Pharma; Sandrena, NV Organon), which was packed as daily dose units of 1.0 g in stick-pack sachets containing 1.0 mg 17β-estradiol (0.1% gel). Placebo tablets were taken by the subjects in the gel group, and placebo gel was applied in the peroral group. The dose of estradiol chosen for the study was based on findings from earlier pharmacokinetic and clinical studies showing that 1 g of estradiol gel is sufficient to control postmenopausal symptoms in the majority of the patients. 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 2 other women dropped out because of itching and discomfort. One woman in the gel group withdrew because of weight gain, whereas 1 participant in both groups discontinued for personal reasons without having side effects. These dropouts were excluded from the statistical analysis.

The criteria for inclusion were as follows: 45 to 65 years of age, a previous hysterectomy with at least 1 remaining ovary, serum follicle-stimulating hormone >30 IU/L, fasting blood glucose <6.7 mmol/L, and BMI <30 kg/m². Women having contraindications to estrogen therapy or any diseases or medication interfering with lipid metabolism were excluded. The participants were able to keep their lifestyle and concomitant medication during the study.

Height, weight, and waist and hip circumferences were measured with the subjects wearing lightweight clothes without shoes. BMI (weight in kilograms/height in meters squared) and waist-to-hip ratio (waist circumference in centimeters/hip circumference in centimeters) were used to estimate generalized and abdominal obesity, respectively.

Oral and written information was given to the participants, and written informed consent was obtained from all subjects. The study was approved by the ethics committees of the Oulu University and Oulu Deaconess Institute.

**Laboratory Analyses**

Blood samples were drawn into EDTA-containing tubes in the morning after an overnight fast. Plasma was separated by centrifugation at 1200g (2600 rpm) for 15 minutes (4°C). Total plasma cholesterol and triglyceride levels were determined by enzymatic colorimetric methods. VLDL, IDL, and LDL were isolated by repeated ultracentrifugations according to density, as described in the Manual of Laboratory Operations of the Lipid Research Clinics Program. HDL cholesterol was determined from VLDL-free plasma after precipitation of LDLs with heparin-manganese. LDL cholesterol was also calculated by the Friedewald formula,17 and these values were used in the LDL response analyses.

The protein content of lipoproteins was measured by the method of Lowry et al,18 and the amount of apoB was determined after isoelectric precipitation.20 The plasma total apoA1 and apoB concentrations were determined by using a commercial kit with a specific selective chemistry analyzer (KONE Instruments Corp).

LDL turnover assessment was carried out as described previously.20,21 In short, 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,22 and the LDL protein was labeled with iodine 125 by use of the iodine monochloride method of McFarlane,23 as modified by Bilheimer et al.24 Radiolabeled LDL was injected in the morning on the day after idination. Blood samples were collected at 0, 15, 30 minutes and at 1, 2, and 3 hours and thereafter 3 times a week for 14 days after the injection. The radioactivity of total plasma was measured in each sample. The fractional catabolic rate (FCR) was calculated from the plasma decay curves by using the method described by Matthews.25 The production rate of LDL apoB was measured in each sample. The fractional catabolic rate (FCR) was calculated from FCR, pool volume, and apoB concentration and expressed as milligrams of LDL apoB produced per day normalized for body weight.

Absorption of dietary cholesterol was measured by the peroral double-isotope continuous-feeding method described by Crouse and Grundy.26 Absolute absorption of dietary cholesterol was calculated by multiplying the daily cholesterol intake with the percentage absorption of dietary cholesterol. Seven-day food records were analyzed by a dietitian with the Finnish Food Database Program, Nutrica.27

ApoE phenotype was determined after delipidation with isoelectric focusing and immunoblotting techniques28,29 that made use of commercial antibodies. The EcoRI and XbaI polymorphisms of the apoB gene and the cholesterol 7α-hydroxylase (CYP7) genes were determined by polymerase chain reaction as described previously.30,31

**Statistical Analysis**

Data analyses were performed with the software packages SAS (version 6.08) or SPSS for Windows (6.01). The results for continuous variables are presented as mean±SD. The changes from baseline to 6 months were analyzed by paired-sample t test, and the changes between treatments were compared by independent-sample t test. The effects of estrogen regimens are presented as mean changes with 95% CI, except the changes of triglycerides, which are presented as medians (95% CI). ANOVA with Bonferroni adjustment was used in the group comparison of apoE phenotypes and the polymorphisms of apoB and CYP7 genes. In addition, the effect of apoE allele e4 was studied by comparing the apoE4-negative (including apoE phenotypes 2/3 and 3/3) with the apoE4-positive (including apoE phenotypes 4/2, 4/3, and 4/4) subjects. Because of the skewed distribution of triglycerides, a nonparametric Mann-Whitney U test and Wilcoxon signed rank test were used, as appropriate. Spearman correlation coefficients were calculated to indicate the associations between variables. Stepwise multiple regression analysis was performed to estimate the independent factors contributing to the change in LDL cholesterol and FCR. A value of P<0.05 (2-sided) was considered to indicate statistical significance.

**Results**

The mean age of the women in the peroral group (53.1±2.9 [mean±SD] years) was similar to that in the transdermal group (54.1±2.8 years). Table 1 summarizes the body weights, waist-to-hip ratios, and plasma lipid levels for both groups of women. The body weight and waist-to-hip ratio remained notably constant during the 6-month treatment on the peroral and the transdermal therapies. Only a minor insignificant increase of body weight was observed in the transdermal group.

As reported previously,15 serum estrone was increased more in the peroral group (n=35, from 176±92 to 2176±1156 pmol/L) than in the gel group (n=38, from 224±139 to 418±196 pmol/L; P<0.001 between the treatments). Serum estradiol was increased from 87±133 to 352±213 pmol/L with peroral therapy and from 111±177 to 264±161 pmol/L with the gel therapy (P<0.05 between the treatments).

The LDL cholesterol level decreased by 19%, from 4.19±0.83 (mean±SD) to 3.39±0.78 mmol/L, in the peroral group and by 9%, from 4.11±0.86 to 3.72±0.78 mmol/L, in the gel group. Quite similar decreases of total, VLDL, and IDL cholesterol levels were seen in the study groups, whereas only peroral estrogen increased HDL cholesterol (12%) and total triglycerides (10%) and decreased plasma total and LDL apoB (~12% and ~9%, respectively).

Fractional cholesterol absorption was reduced by 10% in the peroral group (P<0.05) and by 6% in the transdermal group (P<0.05, Table 2). Absolute absorption of dietary cholesterol also decreased by 18% (P<0.01) and 9% (P<0.05) for peroral and transdermal therapies, respectively. No correlation was observed between the changes in the serum estrone levels and cholesterol absorption. The change of total and LDL cholesterol was positively related to the
TABLE 1. Plasma Lipids and Lipoproteins at Baseline and Change After 6 mo on Peroral and Transdermal Estradiol Therapy

<table>
<thead>
<tr>
<th></th>
<th>Peroral Estrogen (n=35)</th>
<th>Transdermal Estrogen (n=38)</th>
<th>(Difference Between Therapies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change After 6 mo</td>
<td>Baseline</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>68.3±7.1</td>
<td>0.0 (−0.7−0.8)</td>
<td>68.9±7.6</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.81±0.06</td>
<td>0.00 (−0.00−0.01)</td>
<td>0.79±0.05</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.36±0.94</td>
<td>−0.53 (−0.74−0.33)*</td>
<td>6.31±0.92</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.39±0.23</td>
<td>−0.02 (−0.07−0.03)</td>
<td>0.45±0.27</td>
</tr>
<tr>
<td>IDL</td>
<td>0.25±0.12</td>
<td>−0.03 (−0.06−0.00)†</td>
<td>0.26±0.13</td>
</tr>
<tr>
<td>LDL</td>
<td>4.19±0.83</td>
<td>−0.80 (−1.01−0.59)*</td>
<td>4.11±0.89</td>
</tr>
<tr>
<td>HDL</td>
<td>1.60±0.35</td>
<td>0.20 (0.14−0.26)*</td>
<td>1.58±0.38</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.25±0.50</td>
<td>0.12 (0.05−0.23)*</td>
<td>1.38±0.57</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.55±0.30</td>
<td>0.06 (−0.04–0.07)</td>
<td>0.62±0.37</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.94±0.35</td>
<td>0.03 (−0.06–0.12)</td>
<td>1.90±0.40</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.07±0.30</td>
<td>−0.13 (−0.18–−0.07)*</td>
<td>1.10±0.31</td>
</tr>
</tbody>
</table>

Baseline values are mean±SD. Changes are expressed as mean (95% CI), except for triglycerides, which are expressed as median (95% CI). NS indicates not significant. *P<0.001 and †P<0.05 vs baseline.

change in fractional absorption of dietary cholesterol in the peroral estradiol group (r = 0.427, P<0.05 and r = 0.431, P<0.05, respectively), whereas no significant correlation was observed in the transdermal group (r = −0.115, P = NS and r = −0.014, P = NS, respectively).

FCR for LDL apoB increased significantly in the peroral estradiol group (18%), from 0.294 to 0.345 pools per day, but only a minor insignificant increase (2%) was observed in the transdermal group (Table 2). However, the change in LDL cholesterol was associated with the change in FCR for LDL apoB in both study groups (Figure 1). LDL apoB production was raised by 9% and plasma LDL apoB concentration was lowered by 6% on the peroral therapy but not on the transdermal gel therapy (Table 2). The change in FCR for LDL apoB correlated with the change in serum estradiol (r = 0.503, P<0.01 for the change in estrone; r = 0.381, P<0.05 for the change in estradiol) in the peroral group (Figure 2), but no significant correlation was observed in the gel group. However, the change in the production of LDL apoB was related to the change of serum estrone on the peroral and transdermal treatments (r = 0.380, P<0.05 and r = 0.362, P<0.05, respectively), whereas no correlation was observed for the change in serum estradiol levels. The decrease of LDL apoB was related to the increase in LDL apoB production (r = 0.637, P<0.001) and FCR for LDL apoB (r = −0.571, P<0.01) for the group on the peroral estradiol replacement therapy (ERT) compared with the corresponding relations for the group on transdermal therapy (r = 0.634, P<0.001 and r = −0.241, P = NS, respectively).

The apoE phenotype distribution was slightly different in the study groups: there was 1 subject with apoE2/3 in each group, none with apoE4/2 in the peroral group, and 1 with apoE4/2 in the transdermal estradiol group, whereas 26 subjects in the peroral and 20 in the transdermal group had the apoE3/3 phenotype, 5 and 14 subjects had the apoE4/3 phenotype, and 3 and 2 subjects had the apoE4/4 phenotype.
respectively. When the subjects were analyzed according to their apoE phenotypes, significant decreases of total and LDL cholesterol were observed in all apoE phenotypes, and no differences in response to the treatments were found between the phenotypes. Also, the changes in other lipids and lipoproteins, LDL turnover, and cholesterol absorption were quite equal for the different apoE phenotypes (data not shown). To study whether the effect of ERT is modified by the $e_4$ allele of apoE, the subjects were divided into apoE4-negative (phenotypes apoE2/3 and apoE3/3) and apoE4-positive (phenotypes apoE4/2, apo4/3, and apo4/4) groups. Although the influence of both estrogen therapies on serum lipids and cholesterol absorption, FCR, and production for LDL apoB varied to some extent between the apoE4-negative and the apoE4-positive subjects, the differences did not reach statistical significance (data not shown).

No effects of the EcoRI and XbaI polymorphisms of the apoB gene on the regulation of LDL and cholesterol metabolism were observed during ERT (data not shown). Also, no effect of 7a-hydroxylase polymorphism on lipoprotein or cholesterol metabolism was observed among the subjects on either treatment (data not shown).

In stepwise multiple regression analysis, the changes in FCR and the production of LDL apoB 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.

### Discussion

In the present study, transdermal estradiol gel and peroral estradiol therapies decreased total, IDL, and LDL cholesterol. The changes in total and IDL cholesterol were similar in the 2 groups, whereas LDL cholesterol was lowered more extensively with the peroral treatment. HDL cholesterol and triglycerides increased in only the peroral estrogen group, whereas VLDL cholesterol was reduced by transdermal ERT. Overall, our results are in agreement with the previous studies.\textsuperscript{7,9,32–34} The magnitude of the lipid changes seemed to be related to the estrogen regimen: peroral treatment has more profound effects than transdermally delivered treatments, such as patches or gel, which were used in the present study. The quality of estrogen is also important, because synthetic estrogens in particular tend to enhance serum triglycerides.\textsuperscript{35} Different effects on liver metabolism have been suggested as an explanation for the differences in response, because hepatic first-pass metabolism and the induction of hepatic protein synthesis are avoided in transdermal administration.\textsuperscript{36} Moreover, transdermal estrogen seems to act slowly; hence, 3 to 6 months of treatment is required to reach a maximum effect.\textsuperscript{7,33} In fact, some short-term studies have failed to reveal any effect on lipid metabolism. The present study of 6-month duration was long enough to elucidate the effects of both therapies. We also found that regardless of the estrogen regimen, the higher the baseline LDL cholesterol, the more effectively was LDL cholesterol decreased ($r=-0.381$, $P=0.001$), which is in accord with a previous study suggesting that hypercholesterolemia could be an indication for ERT.\textsuperscript{37}

Could the changes in body weight and fat distribution during the study be responsible for the changes in lipoprotein levels? A slight insignificant weight gain (0.6 kg on average) and an increase in BMI with an unchanged waist-to-hip ratio were noticed in the gel group, but these changes can hardly have any adverse effects on lipid metabolism. In the peroral estrogen group, no change in body weight, BMI, or waist-to-hip ratio was observed. In fact, previous studies\textsuperscript{34,38} have suggested that HRT may prevent weight gain and the tendency of central fat distribution associated with the menopause.\textsuperscript{39}

VLDL cholesterol decreased and VLDL triglycerides tended to decrease on gel therapy, whereas total triglycerides...
remained unchanged. Walsh et al\textsuperscript{40} have suggested that the increased production of triglyceride-rich VLDL causes the rise of plasma triglyceride levels on peroral ERT. Similar to findings in the present study, peroral estrogen was shown to increase FCR more than LDL apoB production, resulting in a decrease of plasma LDL levels.\textsuperscript{30} These metabolic changes were observed in light LDL particles, whereas the clearance and production of dense LDL were increased equally.\textsuperscript{41} Arca et al\textsuperscript{42} have suggested that hypercholesterolemia in postmenopausal women is caused by decreased LDL receptor activity. Previously, pharmacological doses of estrogens used in animal studies\textsuperscript{13,43} or in the treatment of patients with prostate cancer\textsuperscript{14,44} have also been shown to increase LDL receptor activity. However, there have been only limited data concerning the doses commonly used in replacement therapy. In accord with previous smaller studies,\textsuperscript{40,41} the present study showed that the serum estrogen levels usually achieved by ERT also decrease LDL cholesterol, mainly by increasing FCR for LDL apoB, and that the effect is related to the change in serum estrogen. Indeed, the large increases in serum estrogen levels on peroral treatment seemed to result in larger reductions in LDL cholesterol levels, and a correlation between the change in serum estradiol and estrone and the change in LDL cholesterol has been noticed.\textsuperscript{15} The relation between the increase of FCR and the reduction of LDL cholesterol on both treatments indicates that the change of FCR is important. Although the production rate of LDL apoB was increased in the peroral group, the increase of FCR was more profound, leading to a decrease of LDL cholesterol. Overall, the data of the present study suggest that FCR is the most important factor for the lowering of LDL cholesterol independent of the route of administration and at the current therapeutic doses of estrogen. Previous studies with higher doses of estrogen in men and animals have shown that estrogen stimulates hepatic LDL receptor expression,\textsuperscript{13,14,44} probably mediated by estrogen receptors.\textsuperscript{35,46} This is also the most likely explanation for the increased FCR seen in the present study with lower doses of estrogen. On the other hand, estrogen-stimulated transcytosis of LDL has recently been suggested to have some importance in cholesterol lowering when desialylated forms of LDL are removed by asialoglycoprotein receptors.\textsuperscript{47}

One additional mechanism for the LDL lowering by estrogen therapy could be the altered cholesterol absorption. A slight but significant decrease in dietary cholesterol absorption was found with both regimens of ERT, suggesting that part of the beneficial effect noticed in lipids and lipoproteins could be mediated by the diminished absorption of cholesterol. It is possible, however, that the overall absorption of intestinal cholesterol (dietary plus biliary) may not have changed. We determined the absorption of dietary cholesterol, which represents about one third of the total intestinal cholesterol pool. At any rate, the changes in cholesterol absorption were not associated with the changes in FCR or the production of LDL apoB, factors that seemed to have a major role in the regulation of LDL cholesterol.

The polymorphisms of some regulatory proteins, such as apoE, apoB, and 7a-hydroxylase, have been reported to affect the plasma lipid and lipoprotein levels.\textsuperscript{29–31,48} Also, some studies have suggested that the response to hyperlipidemic therapies could be related to apoE polymorphism,\textsuperscript{49,50} even though there are also contradictory findings.\textsuperscript{51,52} The present study did not reveal any such effects of either ERT treatment among these postmenopausal subjects. Recently, the LDL cholesterol levels in apoE4-negative subjects were reported to respond more favorably to HRT than did the levels in apoE4-positive subjects.\textsuperscript{53} The differences between that study and our trial could be due to a number of factors. The study by Heikkinen et al\textsuperscript{45} used a combination therapy of estradiol and ciproterone acetate and introduced a long-term dietary therapy, both of which might affect the final outcome. Patient selection could also be important, even though it is unlikely that the hysterectomy in our study patients could have affected the lipid response. The differences in the duration of estrogen treatment might also be one factor explaining the different results. It must also be noticed that because the 73 subjects of the present study were subdivided by treatment type and genetic polymorphisms, the numbers in each group became quite small. Therefore, the power of the present study to find differences between the apoE, apoB, and 7a-hydroxylase polymorphisms is probably low.

In conclusion, ERT-induced changes in lipids and lipoproteins are related to the achieved estrogen level on peroral and transdermal therapy. The increase of FCR for LDL apoB seems to be the most important factor in the regulation of the LDL level on both treatments, and although peroral ERT slightly enhanced LDL production, the LDL clearance rate was increased more, with the net effect being a decrease in LDL levels. The small decrease in the absorption of dietary cholesterol does not seem to be important for the reduction in serum cholesterol by peroral and transdermal ERT.

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References


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