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Oral but not transdermal estrogen replacement therapy changes the composition of plasma lipoproteins

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Abstract

The role of hormone replacement therapy and estrogen replacement therapy (ERT) in cardiovascular disease prevention has not been unambiguously defined yet. The metabolic effects of estrogens may vary depending upon the route of administration. Therefore, we compared the impact of unopposed oral or transdermal ERT on plasma lipids and lipoproteins in 41 hysterectomized women. This was an open-label, randomized, crossover study (with 2 treatments and 2 periods). The 41 hysterectomized women were randomized to receive oral or transdermal 17β-estradiol in the first or second of two 12-week study periods. Plasma lipid and lipoprotein levels were assayed before and after each treatment using standard automated methods. Lipid content of lipoprotein subclasses was assessed by sequential ultracentrifugation. The atherogenic index of plasma (AIP) was calculated as log(triglyceride [TG]/high-density lipoprotein [HDL] cholesterol). The difference between the 2 forms of administration was tested using a linear mixed model. The change from baseline for each of the forms was tested using paired t test. Oral ERT resulted in a significant increase in HDL cholesterol and apolipoprotein A-I levels, whereas it significantly decreased total and low-density lipoprotein (LDL) cholesterol and increased TG concentrations. Transdermal ERT had no such effect. Oral ERT led to a significant TG enrichment of HDL (0.19 ± 0.06 vs 0.27 ± 0.07 mmol/L, $P < .001$) and LDL particles (0.23 ± 0.08 vs 0.26 ± 0.10 mmol/L, $P < .001$) compared with baseline, whereas transdermal therapy did not have any effect on lipoprotein subclasses composition. The difference between the 2 treatments was statistically significant for HDL-TG and LDL-TG (0.27 ± 0.07 vs 0.19 ± 0.05 mmol/L, $P < .001$ and 0.26 ± 0.10 vs 0.22 ± 0.07 mmol/L, $P < .001$, respectively). The transdermal but not oral ERT significantly reduced the AIP compared with baseline (−0.17 ± 0.26 vs −0.23 ± 0.25, $P = .023$), making the difference between the therapies statistically significant (−0.23 ± 0.25 vs −0.18 ± 0.22, $P = .017$). Oral administration of ERT resulted in TG enrichment of LDL and HDL particles. Transdermal ERT did not change the composition of the lipoproteins and produced a significant improvement of AIP. Compared with transdermal ERT, orally administered ERT changes negatively the composition of plasma lipoproteins.

1. Introduction

The composition and size of lipoproteins have been recognized as crucial determinants of biological properties of plasma lipoprotein particles, influencing their interaction with enzymes and receptors as well as other properties determining their atherogenicity. Postmenopausal combined hormone replacement therapy and estrogen replacement therapy (ERT) have been shown to have positive effects on plasma lipid levels but negative impact on cardiovascular outcomes in clinical studies [1-3]. Several explanations of the difference have been proposed (eg, selection bias in the observational studies, inappropriate patient selection of participants in interventional trials, too high hormone doses in study drug formulations; for details, see [4-6]). The hormone replacement therapy–induced changes in lipoprotein quality might contribute to elucidate this discrepancy.

Low-density lipoprotein (LDL) particles size is heterogeneous, and the proatherogenic action of small dense LDL particles (sd-LDL) has been well documented [7,8]. The proportion of these particularly harmful particles increases with increasing plasma triglycerides (TGs); and thus, sd-LDL

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accompanies conditions characterized by TG levels greater than 1.7 mmol/L (eg, metabolic syndrome, diabetes) [9,10].

It has been shown that oral estrogen replacement therapy (ERT) increases sd-LDL concentrations in a dose-dependent manner most likely as an effect secondary to the TG-rising effects of estrogens [11,12]. This negative estrogen-induced modification of lipoprotein composition can counteract the beneficial LDL-lowering effect of ERT. On the contrary, transdermal ERT has had neutral or positive effects on both TG concentrations and LDL particle size in some studies [13-15].

Estrogen replacement therapy substantially impacts also the metabolism of high-density lipoprotein (HDL) particles. Generally, ERT increases HDL cholesterol (HDL-C) levels; and this is, perhaps, the most important antiatherogenic action of estrogens [16]. Adding a progestin can blunt the HDL-rising effect of estrogens [17,18]. Most importantly, ERT alters also the composition and size distribution of HDL particles, which are important determinants of their anti-atherogenic properties. Some studies have demonstrated that oral 17β-estradiol use increases predominantly HDL2 particle concentration, whereas others have shown the opposite [18-20]. Transdermal estradiol administration had a trivial or no effect on HDL levels in some studies, whereas others demonstrated a significant increase in HDL2 particle concentrations [21,22].

Estrogen-induced changes of HDL-C and LDL cholesterol (LDL-C) concentrations were shown as the most important predictors of intima-media thickness in the Estrogen in the Prevention of Atherosclerosis Trial [23].

Apparently, there are important differences between oral and transdermal routes of estrogen administration in their impact on quality and quantity of plasma lipoprotein subclasses. There are certain limitations of previous studies comparing the 2 ERT regimens, for example, type of estrogen used for comparison (conjugated equine estrogens vs 17β-estradiol), dose of estrogens, concomitant progestin use, long period between the onset of menopause and ERT initiation, and small sample size.

Therefore, we have designed a randomized, open-label, crossover study comparing a low-dose, unopposed, oral 17β-estradiol treatment with an equal dose of 17β-estradiol delivered transdermally in a group of 45 women, 6 to 12 weeks after hysterectomy and oophorectomy. The TG and cholesterol concentrations in lipoprotein subclasses were assessed, and the atherogenic index of plasma (AIP) was calculated. The AIP is a sensitive marker of plasma lipoprotein subclasses size and thus reflects the overall lipoprotein-associated atherogenicity of plasma [24]. It has been shown in several clinical studies to be a powerful predictor of cardiovascular events [25-27].

2. Patients and methods

Forty-five healthy women, 6 to 12 weeks after hysterectomy and oophorectomy, were enrolled into the study. Before the surgery, none of them had climacteric syndrome and all had regular menstrual periods. The exclusion criteria were as follows: cardiovascular disease, diabetes mellitus, smoking, dyslipidemia, uncontrolled hypertension, body mass index >35 kg/m^2, current use of hypolipidemic or hormonal therapy, drinking of more than 1 U of alcohol daily (eg, 0.05 L of spirits, 0.2 L of wine, or 0.33 L of beer), and common contraindications of ERT (eg, untreated hormone-dependent malignancy, namely, breast and endometrial carcinomas, acute liver disease, untreated hypertension, acute tromboembolism). Study subjects were instructed to keep their diet (including alcohol consumption) and physical activity pattern unchanged throughout the study.

All study participants signed the informed consent before the study. The study was approved by the Ethical Committee of the General Teaching Hospital in Prague, and its conduct conformed to the principles of the Helsinki Declaration.

Four participants did not complete the study (3 because of loss of follow-up and 1 withdrew the informed consent); the data collected on 41 subjects who completed the study were used for the analysis.

The average age of the study group was 49 ± 6 years. Using the crossover design, the study subjects were randomized to receive oral or transdermal ERT administered for 12 weeks with a 1-week washout period between the treatments. The patients were administered 2 mg of 17β-estradiol (E2) daily orally (Estrofem tablet; Novo Nordisk, Bagsvaerd, Denmark) or 0.05 mg of E2 in a transdermal therapeutic system (Climara emplastrum; Schering, Weimar, Germany). The bioequivalence of the 2 estrogen doses has been documented previously [28].

Laboratory assays were performed at baseline and in weeks 12 and 25 of the study. Blood was collected after at least 12 hours' fast into Vacutainer EDTA tubes (Becton Dickinson, Plymouth, UK) and immediately used for the analysis.

Concentrations of total and HDL-C and of TGs were assayed enzymatically on automated analyzer (COBAS Mira; Roche, Basel, Switzerland). The LDL-C concentrations were calculated using the Friedewald equation. Apolipoprotein (apo) B and A-I levels were determined using immunonephelometry kits (Beckman-Coulter, Fullerton, CA).

Cholesterol and TG were measured also in lipoprotein subfractions (HDL, LDL, intermediate-density lipoprotein [IDL], and very low-density lipoprotein [VLDL]) separated by sequential ultracentrifugation [29].

The AIP was calculated as log(TG/HDL-C) [24].

Data were analyzed as a 2-period, 2-treatment crossover design. Each outcome measure was analyzed according to the intention-to-treat principle, and the analyses included all patients who received both treatments and for whom complete data on outcome measures were available for both periods. The difference between the 2 treatments (2 forms of administration) was tested using a linear mixed model (SAS procedure MIXED; SAS, Cary, NC). The

change from baseline for each of the forms was tested using paired *t* test.

3. Results

Compared with baseline, oral estradiol administration resulted in a significant increase of plasma TG (1.4 ± 0.8 vs 1.6 ± 0.8 mmol/L, $P = .003$), HDL-C (1.9 ± 0.4 vs 2.1 ± 0.4 mmol/L, $P < .001$), and apo A-I (1.5 ± 0.2 vs 1.6 ± 0.2 g/L, $P < .001$) concentrations accompanied by a significant decrease of LDL-C (3.1 ± 1.0 vs 2.5 ± 0.7 mmol/L, $P < .001$) and apo B (1.1 ± 0.4 vs 1.0 ± 0.3 mmol/L, $P = .01$). Transdermal estradiol did not have a significant impact on any of the lipid variables. Oral E2 treatment increased TG (1.6 ± 0.8 vs 1.3 ± 0.7 mmol/L, $P < .001$), HDL-C (2.1 ± 0.4 vs 2.0 ± 0.4 mmol/L, $P = .006$), and apo A-I levels (1.6 ± 0.2 vs 1.5 ± 0.2 g/L, $P < .001$) significantly more than transdermal E2. Oral treatment was also significantly more effective in lowering LDL-C levels (2.5 ± 0.7 vs 3.0 ± 1.0 mmol/L, $P < .001$), but not apo B (1.0 ± 0.3 vs 1.0 ± 0.4 g/L, $P = .07$), than transdermal E2. The differences between the sum of cholesterol and TGs in different lipoprotein subclasses and those measured in the whole plasma were caused by loss during ultracentrifugation. The results are summarized in Table 1.

Analysis of lipoprotein composition revealed that oral E2 resulted in a significant TG enrichment of HDL (0.19 ± 0.06 vs 0.27 ± 0.07 mmol/L, $P < .001$) and LDL particles (0.23 ± 0.08 vs 0.26 ± 0.10 mmol/L, $P < .001$) compared with baseline, whereas no differences in cholesterol or TG content were observed in the other lipoprotein subclasses. Transdermal therapy did not have any effect on lipoprotein subclasses composition; and thus, the difference between the 2 treatments was statistically significant for HDL-TG and LDL-TG (0.27 ± 0.07 vs 0.19 ± 0.05 mmol/L, $P < .001$ and 0.26 ± 0.10 vs 0.22 ± 0.07 mmol/L, $P < .001$, respectively). The compositional changes are summarized in Table 2.

The transdermal but not oral ERT (data not shown) significantly reduced the AIP compared with baseline (−0.17 ± 0.26 vs −0.23 ± 0.25, $P = .023$). Thus, transdermal ERT was significantly more effective in AIP lowering than oral ERT (−0.23 ± 0.25 vs −0.18 ± 0.22, $P = .017$).

Table 1
Lipid, lipoprotein, and apolipoprotein levels at baseline and after oral and transdermal estradiol

	Baseline	Oral	Transdermal
TC (mmol/L)	5.5 ± 1.1	5.3 ± 0.9 *	5.5 ± 1.2
TG (mmol/L)	1.4 ± 0.8	1.6 ± 0.8 *	1.3 ± 0.7 †
HDL-C (mmol/L)	1.9 ± 0.4	2.1 ± 0.4 *	2.0 ± 0.4 †
LDL-C (mmol/L)	3.1 ± 1.0	2.5 ± 0.7 *	3.0 ± 1.0 †
Apo B (g/L)	1.1 ± 0.4	1.0 ± 0.3 *	1.0 ± 0.4
Apo A-I (g/L)	1.5 ± 0.2	1.6 ± 0.2 *	1.5 ± 0.2 †

TC indicates total cholesterol.
* Significantly different from baseline, $P < .01$.
† Significantly different between treatments, $P < .01$.

Table 2
Cholesterol and TG content in ultracentrifugally isolated lipoprotein subclasses after oral and transdermal ERT

	Baseline	Oral	Transdermal
HDL-C (mmol/L)	1.78 ± 0.48	1.94 ± 0.49 *	1.63 ± 0.45 †
HDL-TG (mmol/L)	0.19 ± 0.06	0.27 ± 0.07 *	0.19 ± 0.05 †
IDL-C (mmol/L)	0.19 ± 0.15	0.15 ± 0.0	0.15 ± 0.09
IDL-TG (mmol/L)	0.10 ± 0.06	0.10 ± 0.05	0.08 ± 0.04
LDL-C (mmol/L)	2.78 ± 0.93	2.21 ± 0.69	2.58 ± 0.82
LDL-TG (mmol/L)	0.23 ± 0.08	0.26 ± 0.10 *	0.22 ± 0.07 †
VLDL-C (mmol/L)	0.43 ± 0.27	0.34 ± 0.20	0.35 ± 0.26
VLDL-TG (mmol/L)	0.93 ± 0.57	0.83 ± 0.51	0.83 ± 0.65

* Significantly different from baseline, $P < .001$.
† Significantly different between treatments, $P < .001$.

4. Discussion

Our study demonstrated that the effects of low-dose ERT, initiated early after the menopause, on the composition and quality of plasma lipoproteins significantly differ between oral and transdermal routes of administration. Whereas oral ERT was accompanied by a significant enrichment of HDL and LDL particles with TGs, transdermal estrogens did not affect the lipoprotein composition. The differential impact of the 2 routes of estrogen administration was confirmed by an indirect measure of HDL particle quality, the AIP that was lowered significantly by the transdermal but not oral ERT.

Hypertriglyceridemia results in generation of small dense and highly atherogenic LDL particles [30-32]. Such particles easily penetrate into the vascular wall; and they are more susceptible to oxidation and other modifications that predispose them to interact with the macrophage scavenger receptors, thus forming foam cells and promoting atherosclerosis [33-35]. Therefore, oral ERT–induced increase of TG levels can be considered a proatherogenic change with a negative impact on LDL particle size as has been shown previously [12,16]. Our observation of changes of apo B/LDL-C ratio associated with oral estrogens is in keeping with the presumed shift of LDL particle size toward sd-LDL. Whereas oral estrogen lowered LDL-C significantly, levels of apo B remained unchanged. As there is only one apo B molecule in each LDL particle, the concentration of apo B accurately reflects the number of LDL (and other atherogenic) particles in the plasma [36]. Therefore, the higher apo B/LDL-C ratio after oral ERT can be considered an indirect evidence of more (smaller) LDL particles. Interestingly, Skeggs and Morton [37] did not note any change of LDL particle size after enrichment with TG. However, in their experimental setting, they described several other changes of LDL particle properties associated with TG enrichment (eg, aberrant regulation of sterol biosynthesis, conformational changes of apo B leading to poor degradation of LDL

particles and thus enhanced foam cells formation) that can promote atherosclerosis.

Analogically to the changes of LDL composition, oral estrogen administration resulted in a significant increase of TG concentration in HDL particles, whereas no change was observed with the transdermal ERT application. Similar results were reported by a Finnish group [38]. They demonstrated a clear TG enrichment of all HDL subclasses after oral estrogens and no such effect when estrogens were administered transdermally. Increased TG to cholesterol ratio in HDL changes the conformation of the particle, leading to a decreased affinity to the receptors and enhanced interaction with enzymes [39,40]. Lamarche et al [41] documented an enhanced clearance of TG-enriched HDL particles from circulation in healthy men. This was further confirmed in an animal experiment where the same authors proved increased apo A-I fractional catabolic rate of TG-enriched HDL particles that was mediated by hepatic lipase (HL), an enzyme that hydrolyzes HDL phospholipids and TGs and participates in the interconversion of HDL particles [42,43]. It has been demonstrated that oral estrogens decrease the transcription of the gene for HL. The concentration of larger and less dense HDL particles, for example, HDL2a and HDL2b, has been shown to correlate negatively with the postheparin plasma HL activity [44,45]. However, Tilly-Kiesi et al [38] found a significant estrogen-induced shift in HDL subclasses toward smaller, denser HDL3. Other studies also showed no association of lowered HL activity with oral estrogen administration and fractional catabolic rate of HDL particles [46]. According to these results, the changes of HL activity could not entirely explain the impact of estrogens on HDL. Thus, the changes in lipoprotein composition associated with oral estrogen administration seem to play an important role. Triglyceride-rich HDL particles serve as a better substrate for HL and accelerate the conversion of HDL2 to HDL3. This effect possibly counteracts the beneficial effects of oral estrogens on HL activity; and therefore, the resulting change of HDL subclasses distribution caused by oral estrogens may be detrimental. These findings are further supported by a study of American authors who have found no effect of oral 17β-estradiol on HDL particle size. Surprisingly, administration of medroxyprogesterone acetate together with either 17β-estradiol or conjugated equine estrogens led to a significant increase of HDL particle size compared with placebo [20]. Different progestins have been shown to have variable, and mostly unfavorable, effects on plasma HDL concentrations; and therefore, the above results are somewhat difficult to interpret [18].

To further assess the impact of different routes of estrogen administration, we have calculated the AIP. The AIP correlates with fractional esterification rate of HDL cholesterol that was demonstrated to be directly associated not only with HDL particle size [24] but also with cardiovascular disease risk [26,27]. The AIP can be easily calculated; and thus, it appears to be a feasible alternative to the fraction esterification rate of HDL cholesterol assay. Recently, Tan et al [47] have shown its usefulness for assessing changes of lipoproteins after pioglitazone treatment. The observed significant decrease of AIP with transdermal estrogen therapy is in keeping with the neutral effect of the treatment on TG concentration in distinct lipoprotein subclasses. In other words, although the transdermal estrogens did not change total HDL concentrations according to AIP, they seem to increase HDL particle size and therefore improve their antiatherogenic properties compared with the oral treatment.

In conclusion, we have demonstrated significant and clinically important differences in effects of oral and transdermal 17β-estradiol treatment on lipoprotein composition in postmenopausal women. Oral administration of ERT resulted in TG enrichment of LDL and HDL particles, which may increase atherogenicity of these lipoprotein subclasses. Transdermal ERT did not change the composition of the lipoproteins and, moreover, produced a significant improvement of AIP, an indirect marker of HDL particle size and quality. These differences should be taken into consideration when therapeutic decisions are made.

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