

## Transdermal Estradiol and Lipid Profile: Effects on a Specific Group of Brazilian Postmenopausal Women

Daniel R. Callejon<sup>1</sup>, Danyelle Romana. A. Rios<sup>1</sup>, Sílvia A. Franceschini<sup>2</sup>, Maria Regina T. Toloj<sup>1</sup>

Universidade de São Paulo<sup>1</sup>, São Paulo, SP; Universidade de Ribeirão Preto<sup>2</sup>, Ribeirão Preto, SP

### Abstract

**Background:** In postmenopausal women, significant changes occur that can induce cardiovascular diseases, such as atherogenic lipid profile, due to an increase in total cholesterol and LDL levels, and a decrease in HDL cholesterol levels. The hormone replacement therapy (HRT) can prevent these changes in lipid profile.

**Objective:** Verify the effects of HRT consisting of transdermal estradiol gel associated with medroxyprogesterone acetate on the lipid profile and biochemical parameters in Brazilian postmenopausal women.

**Methods:** This study is an open prospective longitudinal study, in which thirty postmenopausal women received transdermal estradiol gel (1 mg/day) continuously combined with oral medroxyprogesterone acetate (MPA) (5 mg/day) for 12 days/month. The following parameters were determined: total cholesterol, triglycerides, High Density Lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gama glutamyl transferase (GGT) and follicle-stimulating hormone (FSH).

**Results:** The parameters of the lipid profile did not show a significant decrease, while the levels of GGT and FSH had a statistically significant decrease.

**Conclusions:** the treatment with transdermal estradiol gel did not have a significant impact on the lipid profile, thus not resulting in a beneficial effect on cardiovascular disease markers, suggesting that the dose, administration route and the time of treatment were important for these results. Moreover, the treatment using small dose and the transdermal administration route also had a significant hepatic effect in this population. Therefore, this treatment might provide interesting effects on the lipid profile in Brazilian postmenopausal women. (Arq Bras Cardiol 2009; 93(6):571-575)

**Key Words:** Estradiol; lipids; postmenopause; cardiovascular diseases; hormones/administration & dosage.

### Introduction

In menopause, ovarian failure results in a state of hypoestrogenism. With the increase in life expectancy, women live a third of their lives under a state of estrogen deficiency; soon the consequences of this deficiency become relevant for the quality of life of postmenopausal women<sup>1,2</sup>. The characteristic symptoms of the postmenopausal period are hot flashes, sweats, dyspareunia, urogenital atrophies and depression<sup>3,4</sup>. Chronic estrogen deficiency can cause osteoporosis and cardiovascular diseases (CVD), which is the leading cause of death and a major contributor to disability in women<sup>5,6</sup>.

After menopause, significant changes occur that can induce cardiovascular diseases, such as, atherogenic lipid profile,

due to an increase in total cholesterol and LDL levels, and a decrease in HDL cholesterol levels<sup>6,7</sup>. Additionally, other factors can contribute to the development of CVD, such as the increase in fibrinogen<sup>8</sup>, factor VII<sup>9</sup>, PAI-1<sup>10</sup> and glycemia levels<sup>11</sup>.

At present, the hormone replacement therapy (HRT) usually involves estrogen combined with progestogen and has the objective of treating the symptoms of menopause and preventing osteoporosis to increase the quality of postmenopausal women's life<sup>3</sup>.

The prevention of CVD as a result of HRT is a controversial issue, because relevant findings in the Heart and Estrogen/progestin Replacement Study (HERS)<sup>12</sup> showed that HRT, constituted by conjugated equine estrogen (CEE) associated with medroxyprogesterone acetate (MPA), was ineffective in preventing cardiovascular events in postmenopausal women with established CVD. The Women's Health Initiative (WHI)<sup>13</sup>, a randomized, controlled, primary prevention trial that used CEE 0.625 mg plus MPA 2.5 mg, showed that the HRT group had a significant increase in the risk of cardiovascular and thromboembolic events and breast cancer when compared

**Mailing address:** Maria Regina Torqueti Toloj •

Bloco M, sala 327, Av do Café, s/n, Monte Alegre, CEP: 14040-903 - Ribeirão Preto/SP, Brazil.

E-mail: torqueti@fcrp.usp.br

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to the placebo group and the study was interrupted early (follow-up of 5.2 years).

However, many observational epidemiological studies have demonstrated that HRT may have beneficial effects on CVD. These beneficial effects include favorable changes in lipid levels, because HRT may decrease total cholesterol, LDL, Lp(a) levels and increase HDL levels and thereby the lipid profile becomes non-atherogenic<sup>14-16</sup>.

However, these effects are dependent on the type of estrogen used, doses and administration route. The effect of the first passage in the liver seems to have an important effect on the lipid profile and hemostatic parameter changes, because both are produced in the liver. The high levels of estrogen administered by oral route induce beneficial changes in the lipid profile, but increases the hemostatic factors, and therefore, it also increases the risk of venous thromboembolism (VET)<sup>17,18</sup>. In the estrogen administration through the transdermal route, low levels of estrogen are used; therefore, this type of HRT does not have the effect of the first passage in the liver, and consequently has a smaller impact on the lipid profile and hemostatic parameters when compared to the oral route<sup>19</sup>.

This study has the objective of determining the effects of HRT consisting of transdermal estradiol gel associated with medroxyprogesterone acetate on the lipid profile and biochemical parameters in Brazilian postmenopausal women.

## Methods

### Subjects

The selection of patients was conducted by the Health Service of the University of São Paulo. Women with an intact uterus who had been amenorrheic for one year or more, presented follicle-stimulating hormone (FSH) levels >30 mIU/dl and Body Mass Index (BMI) < 32 kg/m<sup>2</sup> were enrolled in this study.

Exclusion criteria included: use of estrogens or progestogen within 3 months prior to the study (washout), abnormal bleeding, side effects of estrogen or progestogen use, previous use of medications that could interfere with lipid levels, skin disease, history of or current thromboembolic disorders, immunological disease, clinically relevant abnormalities in hemostatic, hepatic or endocrine functions, breast cancer or hormone-dependent cancer. Written informed consent was obtained from each patient before study enrollment and this study was approved by the Ethics Committee on Research of the School of Pharmaceutical Sciences of Ribeirão Preto.

### Study protocol

Eligible women were treated with daily administrations of transdermal estradiol gel containing 1 mg of estradiol in 1 g of hydroalcoholic gel. To avoid endometrial hyperplasia, 5 mg of oral medroxyprogesterone acetate (MPA) was administered on 12 consecutive days during each month. Hormonal therapy was given for 6 months.

Fasting venous blood samples were taken between 08:00 and 10:00 am. Subjects remained in a sitting position for

10 minutes before venipuncture. The blood samples were centrifuged for 20 min at 2500 g. Venous blood samples were collected from the volunteers before treatment and after six consecutive cycles of treatment.

### Laboratory assays

Lipid Parameters: total cholesterol [BE-PAK Cholesterol (Fast Color), Bayer], HDL-cholesterol (High Density Lipoprotein) [HDL READS, Labetest Diagnostical], triglycerides [BE-PAK Triglycerides (Fast Color), Bayer]. These parameters were determined by enzymatic and colorimetric methods in an opeRA<sup>®</sup> Bayer autoanalyzer. LDL levels (low density lipoprotein) were calculated by the formula of Friedewald: [LDL-cholesterol] = [total Cholesterol] - [HDL-cholesterol] - [Triglycerides]/5. VLDL-cholesterol (very low density lipoprotein) levels were calculated by the formula: [VLDL-cholesterol] = [triglycerides]/5<sup>20</sup>. Biochemical parameters: Glucose [BE-PAK Glucose, Bayer], AST (aspartate aminotransferase) [T01-1746-85, Bayer], ALT (alanine aminotransferase) [T01-1756-85, Bayer], GGT (gamma glutamyl transferase) [T01-1913-01, Bayer]. All the above parameters were determined by enzymatic and colorimetric methods in an opeRA<sup>®</sup> Bayer autoanalyzer. FSH (Follicle-Stimulating Hormone) [Immulite FSH, DPC Medlab] levels were determined by the chemiluminescent method at an Immulite-1<sup>®</sup>DPCMedline autoanalyzer. Pre- and post-treatment laboratory analyses were performed concomitantly in order to minimize inter-assay variations.

### Statistical analysis

All statistical data are expressed as mean ± SD. Comparison between pre- and post-treatment values was performed using the parametric Student *t*-test for paired data. Values of *p* < 0.05 were considered significant.

## Results

Table 1 shows the physical and clinical characteristics of the 30 women who were selected for the study.

During the treatment period, no significant changes were found in BMI, systolic and diastolic blood pressure. Four patients were smokers. None of the patients used any medication that could interfere with the lipid and biochemical assays.

Table 2 shows the lipid profile and biochemical parameters of 30 patients who received daily administrations of 1 mg of transdermal estradiol gel, combined with 5 mg of oral medroxyprogesterone acetate on 12 consecutive days during each month. All parameters were determined both before treatment (T0) and at the end of the 6-month treatment period (T1).

Regarding the lipid profile, there were no significant decreases in any of the parameters. The decrease in the serum levels of total cholesterol was of 4.6%, triglycerides of 8.3%, LDL of 2.6%, VLDL of 11.3% and HDL of 5.0% in comparison with T0.

After treatment, no significant change was observed in glucose, AST and ALT levels. However GGT and FSH presented

a significant decrease, of 34.2% ( $p=0.0170$ ) and 36.6% ( $p<0.0001$ ), respectively.

## Discussion

The results showed that all of the parameters of the lipid profile (total cholesterol, HDL, LDL, VLDL, triglycerides), presented a decrease; however, this decrease was not statistically significant, so the treatment did not result in a beneficial effect on cardiovascular disease markers. However, there was a significant decrease in hepatic enzymes; these results suggest that in spite of the dose and administration route, estradiol can have an impact on the liver.

Several studies have shown that the cardiovascular protection promoted by HRT has an estrogen effect on the lipid profile. The estrogen decreases the total cholesterol and LDL levels and increases the HDL levels, makes the lipid profile less atherogenic, reverting the atherogenic changes in the lipid profile during menopause<sup>14-16</sup>. However, this beneficial effect is dependent on two main factors of HRT: administration route and progestogen.

Some studies have shown that the oral administration of estrogen has a marked impact on the lipid profile, as it uses high levels of estrogens in comparison to other administration routes due to bioavailability problems, such as transformation of estrogen in estrone in the gut and the effect of the first passage in the liver. These estrogen levels increase the activity of the lipase hepatic enzymes and, consequently, the levels of total cholesterol and LDL will decrease and the synthesis of HDL, mainly the HDL<sub>2</sub> fraction, will be stimulated, thus decreasing CVD risk<sup>21,22</sup>. With the transdermal route, the estrogen dose is smaller than in the oral route, preventing this effect of the first passage in the liver and so, the effect of the transdermal route on the lipid profile is delayed and somewhat

attenuated<sup>23-25</sup>. Hence, the effects of the transdermal route on lipids only become apparent after prolonged therapy.

However, the main side effect of oral HRT, which is venous thromboembolism (VTE), is lower with the transdermal route. Studies have shown that the VTE risk is higher in oral HRT users than in non-users, or transdermal HRT users<sup>19,26</sup>. Therefore, transdermal HRT can promote the beneficial effect on the lipid profile with a smaller risk of VTE.

In this study, we showed that the transdermal administration of estradiol gel did not result in a significant effect on the lipid profile, but there was a decrease of all of the analyzed parameters. These results are probably associated with factors such as the transdermal route of administration, the short-term therapy and progestogen.

Hirvonen et al<sup>27</sup> compared the effect of transdermal and oral estradiol associated with MPA on the lipid profile in postmenopausal women. The results showed that the oral HRT resulted in a significant reduction in total cholesterol after 12 months of treatment. The transdermal HRT with 1 mg of estradiol showed a small, but statistically significant decrease in total cholesterol and triglyceride levels during the progestogenic phase after 12 months, whereas the group

**Table 1 - Subject Characteristics**

Parameter	Mean $\pm$ SD	Range
Age (years)	49,07 $\pm$ 4,7	39 - 61
BMI (kg/m <sup>2</sup> )	27,01 $\pm$ 4,0	22 - 31
Systolic blood pressure (mmHg)	120 $\pm$ 15,0	100 - 160
Diastolic blood pressure (mmHg)	80 $\pm$ 11,0	60 - 100

BMI - Body Mass Index. Data are expressed as mean  $\pm$  standard deviation (SD)

**Table 2 - Changes in Lipid and Biochemical parameters**

Parameters	Baseline (T0) n = 30	After 6 Months (T1) n = 30	p Value*	Reference Value
Total cholesterol (mg/dl)	213.80 $\pm$ 39.46	203.87 $\pm$ 36.81	0.1013	< 200
Triglycerides (mg/dl)	123.80 $\pm$ 59.13	113.47 $\pm$ 45.60	0.3735	< 150
LDL (mg/dl)	126.73 $\pm$ 36.40	123.37 $\pm$ 31.76	0.5666	< 130
VLDL (mg/dl)	25.39 $\pm$ 14.34	22.51 $\pm$ 8.94	0.2765	< 30
HDL (mg/dl)	60.43 $\pm$ 12.69	57.43 $\pm$ 11.12	0.1037	> 45
Glucose (mg/dl)	80.47 $\pm$ 11.14	83.03 $\pm$ 11.08	0.1905	70 - 110
AST (U/l)	19.43 $\pm$ 11.00	17.37 $\pm$ 7.50	0.3447	5 - 40
ALT (U/l)	24.90 $\pm$ 19.60	24.50 $\pm$ 12.71	0.3055	5 - 40
GGT (U/l)	37.23 $\pm$ 26.10	24.50 $\pm$ 12.71	0.0170**	13 - 47
FSH (mIU/ml)	63.52 $\pm$ 36.71	40.28 $\pm$ 30.21	< 0.0001**	42-126

LDL - Low density lipoprotein; VLDL - Very low density lipoprotein; HDL - High density lipoprotein; Aspartate Amino-Transferase (AST); Alanine Amino-Transferase (ALT); Gamma Glutamyl Transferase (GGT); Follicle-Stimulating Hormone (FSH). Data are expressed as mean  $\pm$  standard deviation (SD) \*Determined by paired t test. \*\* $p < 0.05$ .

that received 2 mg of estradiol gel showed no significant changes in lipid levels. Karjalainen et al<sup>28</sup> compared the effect of transdermal (estradiol gel 1.0 mg) and oral (estradiol valerate 2.0 mg) HRT on the lipid profile. Transdermal therapy consisted of estradiol gel and the oral therapy of valerate estradiol. The hysterectomized postmenopausal women underwent treatment for 6 months and the results showed that both types of treatment resulted in anti-atherogenic changes in the lipid profile, demonstrated by a decrease in the levels of total cholesterol and LDL; however, the oral HRT group presented an increase in the levels of triglycerides and HDL.

The results of the present study were similar to those obtained by Hirvonen et al<sup>27</sup>, although they used a longer period of treatment than the one used in our study, while Karjalainen et al<sup>28</sup> verified a beneficial effect on the lipid profile using unopposed estradiol. Therefore, it suggests that progestogen use can interfere with the effects of estrogen on the lipid profile, decreasing the beneficial effects that the estrogen can generate on the lipid profile, mainly in transdermal HRT, of which beneficial effect is very attenuated.

The interference of the progestogens on the estrogen effects on the lipid profile is dependent on the progestogen androgenicity. The testosterone-derived progestogens result in a higher interference of the estrogen effects on the lipid profile, annulling or decreasing the beneficial estrogen effect<sup>29</sup>. However, the progesterone-derived progestogens result in a lower interference on the lipid profile<sup>30</sup>.

In our study, we could not verify the MPA effect on the lipid profile, because a treatment group was not enrolled to receive unopposed estrogen. However, it is necessary to say that MPA might have interfered with the estrogen-induced changes in the lipid profile.

Pang et al<sup>31</sup> compared the treatment with unopposed transdermal estradiol gel and the one associated with MPA for 2 years. The results showed that total cholesterol levels decreased significantly with the two regimens, whereas LDL levels significantly decreased in the group that used MPA and the levels of HDL, VLDL and triglycerides did not significantly change with any of the regimens. Therefore, the treatment with MPA had a more positive impact than the treatment with unopposed estradiol. The Postmenopausal Estrogen/Progestin Interventions (PEPI)<sup>15</sup> compared the treatment with unopposed conjugated equine estrogen (CEE), associated with MPA and with micronized progesterone for 3 years of treatment. The results showed that the HDL levels increased significantly in the group that used unopposed CEE and CEE associated with micronized progesterone in relation to the other groups; the LDL levels decreased, but it was a non-significant decrease. Levels of triglycerides increased in all of the groups treated with HRT and the total cholesterol levels decreased in the groups that used CEE associated with MPA. Therefore, the treatment with unopposed estrogen and that associated with progestogen might have induced an improvement in the lipid profile; however, the unopposed CEE and the treatment associated with micronized progesterone had better results in relation to HDL levels.

Therefore we can suggest that the treatment with transdermal estradiol gel may result in a beneficial effect on the lipid profile, as long as two important factors are observed: the time of treatment and the associated progestogen use.

The glucose and insulin levels are associated with the risk of cardiovascular diseases. The studies with oral contraceptives (OC) showed a negative effect on the carbohydrate tolerance; however, the progestogens of OC are responsible for this effect. Studies verified a beneficial effect of HRT on the metabolism represented by the decrease of glycemia and insulinemia<sup>14,32</sup> and this reduction can contribute to the decrease of the CVD; however, more studies are necessary to establish this possible positive effect of HRT on the carbohydrate metabolism.

The hepatic enzyme profile consisting of the ALT, AST and GGT enzymes showed a decrease in all the enzymes, but the GGT levels showed a significant decrease. These results suggest that the hepatic effect of the first passage in the liver is lower with the transdermal route when compared with the oral route, in addition to having a beneficial effect on the decrease of GGT. Lox et al<sup>33</sup> showed that the treatment with tamoxifen for breast cancer in postmenopausal women significantly increased AST, ALT and GGT levels. However, this increase was within the normal range, suggesting that the treatment did not induce liver failure. Perry and Wisemen<sup>34</sup> verified the effect of estradiol valerate combined with norethisterone in postmenopausal women. The results showed that AST and ALT levels decreased significantly after 3 years of treatment, but these changes were small and their clinical significance was uncertain.

Therefore, in this study, we showed that the treatment with transdermal estradiol gel did not have a great impact on cardiovascular disease markers of the lipid profile, suggesting that the dose and the time of treatment were account for these results. The hepatic effects of this treatment demonstrated an impact on the liver, in spite of the low estradiol dose and transdermal route of administration, but this impact has uncertain clinical significance.

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## Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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## Study Association

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