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Metabolism Clinical and Experimental 54 (2005) 72–78

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Does the type of hormone replacement therapy affect lipoprotein (a), homocysteine, and C-reactive protein levels in postmenopausal women?

Hanna Bukowska[a], Stanisław Stanosz[b], Ewa Żochowska[b], Barbara Millo[a], Krzysztof Sieja[b], Kornel Chełstowski[a], Marek Naruszewicz[a,*]

[a]*Department of Clinical Biochemistry and Laboratory Diagnostics, Pomeranian Medical University, PL-70-111 Szczecin, Poland*
[b]*Independent Laboratory of Menopause and Andropause, Pomeranian Medical University, PL-70-111 Szczecin, Poland*

Received 9 February 2004; accepted 27 July 2004

Abstract

Background: The results of studies evaluating the effect of hormone replacement therapy (HRT) on the cardiovascular risk raise many controversies. This may be related to both the type of treatment used and the disregard of additional risk factors.
Objective: The objective of the study was to evaluate the effect of natural estrogens taken transdermally and synthetic estrogens taken orally on the concentrations of lipoprotein (a) [Lp(a)], homocysteine, and C-reactive protein (CRP) in healthy women in the early postmenopausal period.
Material: The study was conducted on 61 healthy women with average age of 52.3 ± 4.1 years, in the postmenopausal period, who were randomly assigned to 3 groups depending on the type and route of administration of the products. Group I (n = 24) was administered transdermal estrogens (micronized 17β-estradiol; Systen, Janssen-Cilag, Switzerland) and progesterone in the second phase of the cycle. Group II (n = 21) was administered oral hormones (Cyclo-Menorette). Group III (n = 16), serving as a control, included women taking placebo in the form of patches. In each group, therapeutic cycles took 22 days and were followed by a treatment-free interval of 7 to 10 days for a 3-month period.
Results: After 3 months of treatment, Lp(a) and homocysteine levels were not significantly different from the baseline, irrespective of the route of administration of estrogens or placebo. Both forms of HRT used indicate significant difference in changes of CRP concentration during 3 months of administration (analysis of variance $P = .0356$). CRP concentration values increased in the group of women using oral HRT from 1.22 to 2.68 mg/L. In the group of women using oral therapy, significantly more cases (61%) of increase in CRP concentration compared with 39% in the transdermal HRT group (χ^2 $P = .015$) were observed.
Conclusions: On the basis of our observations, it appears that in women in the early postmenopausal stage with normal initial concentrations of Lp(a) and homocystein, the form of therapy used has no influence on values of these parameters. The 2 forms of HRT therapy differ in effect, which is expressed as a change in CRP concentration. A tendency to increase CRP values when using oral HRT is observed, while such an effect is not observed in case of transdermal therapy after 3 months.

1. Introduction

In connection with the controversial results of studies on hormone replacement therapy (HRT) and cardiovascular risk [1-3], studies assessing the effects of therapy depending on the type, dosage, form, and route of administration of hormones are still being continued. Moreover, new risk markers, which would help explain and predict the increased incidence of cardiovascular disorders in women on HRT, are being sought. The recognized and independent cardiovascular risk factors include lipoprotein (a) [Lp(a)], homocysteine, and C-reactive protein (CRP) [4-6].

The increased serum level of Lp(a) (>30 mg/dL) increases the relative risk of ischemic heart disease more than twice, and in association with other risk factors, such as high LDL or homocysteine levels, it increases several times the risk of early atherosclerosis and cardiovascular disorders.

Currently, we have only limited possibilities of pharmacological lowering of the Lp(a) level. Estrogens [7], anabolic steroids, nicotine acid, neomycin, and fibrates have a limited effect on Lp(a). In a few studies in which the estrogen

* Corresponding author. Tel.: +48 91 466 14 90; fax: +48 91 466 14 92.
E-mail address: mnarusze@sci.pam.szczecin.pl (M. Naruszewicz).

0026-0495/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.07.015

receptor antagonist tamoxifen was used, a significant reduction of Lp(a) level was also obtained [8].

The results of the studies by Ridker et al [9] conducted on healthy postmenopausal women indicate that cardiovascular risk is increased more than twice in women with moderately elevated homocysteine levels, irrespective of the conventional risk factors. However, it is not clear whether estrogens can intermediately affect homocysteine metabolism. Homocysteine level is higher in postmenopausal women in comparison with those in the premenopausal period [10,11].

The effect of HRT on homocysteine level was evaluated in many studies. Small transient changes in women with normal baseline homocysteine concentrations and significant lowering in women with higher baseline levels were observed [12-16]. Whether the benefits associated with a reduction of elevated homocysteine levels in women using HRT will be connected with a reduction of cardiovascular events is still unexplained and requires further studies.

The results of epidemiological studies indicate unequivocally the prognostic value of CRP for cardiovascular disorders. C-reactive protein determines the risk, irrespective of other conventional risk factors (both in healthy persons as well as in coronary artery disease patients). In addition, a risk estimation taking into account both CRP as well as serum lipids (and, in particular, the ratio of total cholesterol [CH] to high-density lipoprotein cholesterol [HDL-CH]) is characterized by the highest cardiovascular morbidity prognostic effectiveness [17].

In prospective studies conducted on women participating in the Women's Health Study, Ridker et al [18] demonstrated in these patients a stronger correlation between the risk of cardiovascular events and higher CRP levels than in men. Elevated baseline levels of CRP were associated with a 5-fold increase in the risk of occurrence of acute coronary episodes and with a 7 times more elevated risk of heart attack and stroke. In later extensive studies conducted in healthy postmenopausal women observed over the average period of 3 years, Ridker et al [19] documented that the risk of cardiovascular events was 4.4 times higher for women in the highest quartile of CRP concentrations in comparison with those in the lowest quartile. These researchers emphasize that half of cardiovascular events occurred in women without symptomatic hyperlipidemia. CRP levels were shown to be a significant risk factor even in the subgroup of women with LDL cholesterol level below 130 mg/dL and considered normal. The results of numerous studies indicate an elevation of CRP level in women on HRT [20-24].

2. Objective

The objective of this study is to assess the effect of natural estrogens administered transdermally and synthetic estrogens administered orally on the levels of Lp(a), homocysteine, and CRP in healthy women in the early postmenopausal period.

3. Material and methods

The studies were conducted on 61 women at the average age of 52.3 ± 4.1 years (range, 48-60) in the early postmenopausal period of 3.8 ± 2.2 years with a negative disease history. None of the women had previously used HRT. Participants were randomly assigned to 3 groups, depending on the type and route of administration of the product.

Group I consisted of 24 women who were administered natural estrogens transdermally (Systen, Janssen-Cilag)—micronized 17β-estradiol was released from the patch at increasing-decreasing doses (25, 50, 75, and 50 μg/d), imitating the physiological concentration of estrogens throughout the menstrual cycle [25], with concomitant oral intermittent progesterone (P) administration (lutein made by Polfa, Poland) at doses of 50 to 100 mg for 12 days in the second phase of the cycle. Group II consisted of 21 women using HRT by taking Cyclo-Menorette (estradiol valerate 1 mg + estriol 2 mg + levonorgestrel 0.25 mg; Wyeth Group, Germany) in the form of sugar-coated tablets in the second phase of the cycle. Group III (control) consisted of 16 women who received placebo transdermally in the form of patches made by Janssen-Cilag. In each group, 3 therapeutic cycles were conducted over a 3-month period; each therapeutic cycle lasted 22 days, with a subsequent treatment-free interval from 7 to 10 days.

Table 1
Characteristics of the women studied

Parameters	Groups		
	I (n = 24)	II (n = 21)	III (n = 16)
Age (y)	52.4 ± 4.8	52.3 ± 3.3	52.2 ± 3.9
Menopausal age (y)	48.5 ± 4.2	48.6 ± 2.9	48.5 ± 4.2
Postmenopausal period (y)	3.9 ± 2.9	3.7 ± 1.8	3.7 ± 1.9
Parity	1.5 ± 0.9	1.8 ± 0.8	2.0 ± 0.6
BMI (kg/m^2)	25.3 ± 2.7	23.6 ± 2.9	24.9 ± 3.4
Systolic blood pressure (mm Hg)	120.9 ± 10.8	123.5 ± 13.5	118.4 ± 10.3
Diastolic blood pressure (mm Hg)	77.6 ± 85	79.0 ± 6.4	73.1 ± 6.3
Tobacco smoking (%)	37.5	38.1	18.8

All values of parameters, except tobacco smoking, are mean ± SD.

The characteristics of the women studied are presented in Table 1.

Hormone and biochemical tests were performed before treatment initiation and on days 18 to 20 of the third therapeutic cycle. Blood for tests was drawn from women in a fasting state, and serum was stored, after centrifugation, at −70°C.

The levels of estradiol (E_2), progesterone (P), and prolactin (PRL) were determined by radioimmunoassay using test kits made by bioMerieux (France), and estrone (E_1) level was determined using a kit made by Diagnostic System Laboratory (Germany). The levels of lipid parameters triglyceride (TG), total cholesterol (CH), and high-density lipoprotein cholesterol (HDL-CH) levels were determined by enzymatic methods using test kits made by Roche (Germany). The HDL fraction was obtained using precipitation method. Serum Lp(a) level was measured by turbidimetric immunoassay using test kits made by Dialab (Austria). Homocysteine level was determined using high-performance liquid chromatography with fluorescence detection (Hewlett-Packard) using test kits made by Bio-Rad. C-reactive protein was assayed by high-sensitivity enzyme-linked immunoassay using a test made by Euro-immun Medizinische Labordiagnostica AG.

Statistical calculations were performed using Statistica 6.188 PL package made by StatSoft (Tulsa, OK). The results were presented in the form of arithmetic means and standard deviations. The normal distribution of the variables analyzed was checked using Kolmogorov-Smirnoff test.

Logarithmic transformation has been performed for symmetrization of distribution of Lp(a) and CRP. Groups were compared using analysis of variance (ANOVA) for the set of 2 factors: study group (transdermal HRT, oral HRT, and placebo) and time (before initial administration of the drug and after 3 months of the therapy) with repeated measurements. Interaction of these factors has been assessed using a post hoc Bonferroni test. In addition, to compare mean values of CRP before and after treatment in the oral HRT group, Student t test for paired data has been used. Level of significance was established at $P < .05$. Analysis of frequency in CRP changes in treatment groups was performed with the χ^2 Pearson test.

4. Results

The baseline levels of hormones studied did not differ significantly in the groups of women on using various methods of HRT (Table 2). After 3 months of treatment, a significant, almost 5-fold increase in E_2 and 11-fold increase in E_1 levels in comparison with the baseline values was found in the group of women using oral HRT (Cyclo-Menorette). Prolactin level increased significantly in this group. In women receiving transdermal 17β-estradiol (System) and lutein in the second phase of the cycle, E_2 level increased twice but these changes were insignificant, E_1 level remained unchanged, but P level increased significantly after 3 months of treatment. No significant changes in the levels of the hormones studied were observed in the control group. After 3 months of treatment, E_2 and E_1 concentrations were significantly higher in the group of women taking oral hormones in comparison with the control group and E_1 concentrations were also significantly higher in comparison with the group of women taking transdermal estrogens. Progesterone concentration was significantly higher in the group of women receiving transdermal estrogens with concurrent intermittent P administration, in comparison with the group on oral HRT and the control group.

The values of mean levels of the basic lipid parameters, Lp(a), homocysteine, and CRP are presented in Table 3.

Table 2
The values (mean ± SD) of mean levels of hormones before and after 3 months HRT in postmenopausal women

Parameters	Groups			ANOVA P		
	I (n = 24)	II (n = 21)	III (n = 16)	Group	Time	Group × Time
E_2 (pg/mL)						
Before	31.6 ± 37.5	25.5 ± 18.9	15.5 ± 11.9			
After	61.2 ± 79.2	124.8 ± 71.0*	22.0 ± 19.4	.0359	.0001	.0342
E_1 (pg/mL)						
Before	33.2 ± 37.4	21.8 ± 10.1	18.5 ± 11.0			
After	34.1 ± 29.1	238.7 ± 161.0*	32.3 ± 40.9	.0193	.0094	.0031
P (ng/mL)						
Before	0.44 ± 0.24	0.48 ± 0.20	0.48 ± 0.15			
After	3.72 ± 3.80*	0.66 ± 0.74	0.48 ± 0.16	.0111	.0068	.0094
PRL (ng/mL)						
Before	9.90 ± 4.10	12.5 ± 6.6	10.2 ± 5.0			
After	10.3 ± 4.9	18.9 ± 12.9**	12.2 ± 8.1	NS	NS	NS

NS indicates not significant.

Bonferroni test for comparison mean values of hormones between groups: E_2, $P < .001$ oral HRT vs control after 3 months; E_1, $P < .001$ oral HRT vs control after 3 months and oral HRT vs transdermal HRT after 3 months; P, $P < .001$ transdermal HRT vs oral HRT after 3 months and transdermal HRT vs control after 3 months.

* Bonferroni test for paired data for comparison mean values before and after 3 months therapy in each group, $P < .001$.

** Bonferroni test for paired data for comparison mean values before and after 3 months therapy in each group, $P < .05$.

Table 3
The values of mean levels of the basic lipid parameters, Lp(a), homocysteine, and CRP before and after 3 months HRT in postmenopausal women

Parameters	Groups			ANOVA *P*		
	I (n = 24)	II (n = 21)	III (n = 16)	Group	Time	Group × Time
TG (mmol/L)						
Before	1.49 ± 0.33	1.40 ± 0.75	1.23 ± 0.51			
After	1.36 ± 0.72	1.08 ± 0.39	1.58 ± 0.66	NS	NS	NS
CH (mmol/L)						
Before	6.05 ± 1.09	6.26 ± 1.55	5.51 ± 0.67			
After	5.87 ± 1.22	5.33 ± 1.06	5.51 ± 1.16	NS	NS	NS
HDL-CH (mmol/L)						
Before	1.32 ± 0.26	1.27 ± 0.16	1.19 ± 0.34			
After	1.29 ± 0.29	1.14 ± 0.18	1.19 ± 0.31	NS	NS	NS
Lp(a) (mg/dL)						
Before	5.72 ± 6.03	7.39 ± 7.32	6.60 ± 7.61			
After	4.89 ± 6.35	6.52 ± 5.08	6.79 ± 7.76	NS	NS	NS
HCY (μmol/L)						
Before	11.2 ± 2.9	11.9 ± 3.6	11.9 ± 4.6			
After	11.2 ± 2.8	13.0 ± 9.1	11.8 ± 5.0	NS	NS	NS
CRP (mg/L)						
Before	3.08 ± 3.35	1.22 ± 0.87	1.94 ± 2.07			
After	2.33 ± 1.89	2.68 ± 3.07*	1.65 ± 2.07	NS	NS	.0356

NS indicates not significant; HCY, homocysteine.
Student *t* test for paired data for comparison mean values CRP before and after 3 months of therapy in oral HRT groups.
* $P = .0413$.

Changes in concentration of TG and CH as well as in the HDL fraction after 3 months of hormonal therapy were not observed.

In our studies, the baseline Lp(a) levels were within the normal range—below 30 mg/dL—and they did not differ significantly between the groups after 3 months of treatment, irrespective of the method of estrogens administration. Only 1 woman studied had baseline Lp(a) level of 34.0 mg/dL. After 3 months of treatment with Cyclo-Menorette, this level fell in her case to 22.0 mg/dL.

Homocysteine concentrations were contained within the range of 6.2 to 27.4 μmol/L, and in 21 (34.5%) of the women studied were higher than the 12-μmol/L level considered normal. Mean homocysteine level values did not change after 3 months of treatment, irrespective of the type of hormones taken and of the route of their administration.

Women with baseline CRP $\geq$ 10 mg/L, which might indicate an inflammation, were excluded from the study program. In the final test of our program, no CRP increase above 10 mg/L was found in any of the women examined.

The 2 types of hormone therapy used (transdermal and oral) differ significantly by their effect, which is expressed as CRP level changes at 3 months of administration (ANOVA $P = .0356$). An increase of mean CRP levels values from 1.22 to 2.68 mg/L (Student *t* test, $P = .0413$) is observed in the group of women using oral HRT, and such effect is not observed in the group of women taking estrogens transdermally and lutein in the second phase of

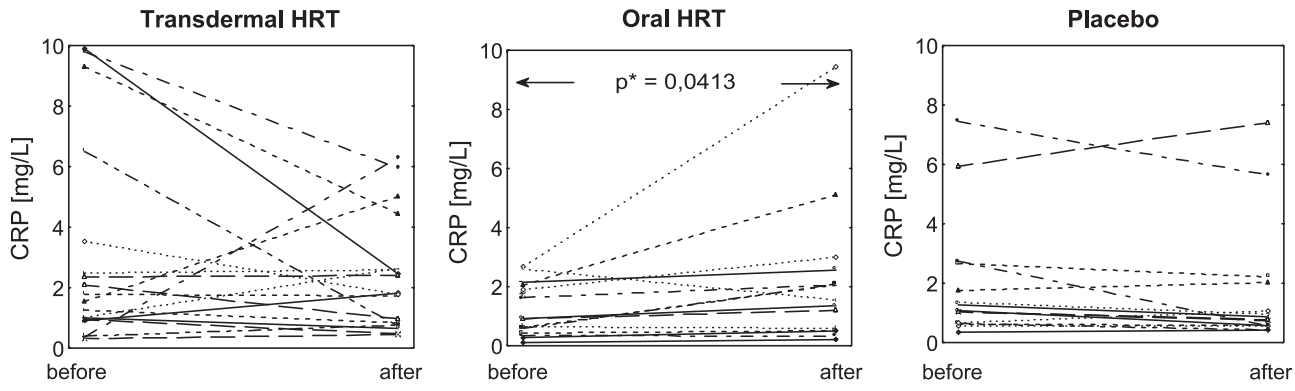

Fig. 1. CRP levels before and after 3 months transdermal HRT, oral HRT and placebo groups in postmenopausal women. Increase mean values CRP after 3 months oral HRT (p = 0,0413) t – Student's test. Significantly more -61% cases of increase CRP concentration in oral HRT as compared to 39% in transdermal therapy group ($p^{Chi2} = 0.015$) Chi^2 Pearson test.

the cycle. In this group, the CRP levels even slightly, although statistically insignificantly, lowered.

In Fig. 1, individual changes in CRP concentration for study groups have been shown. On the basis of performed analysis with χ^2 Pearson test for frequency of changes in CRP in the group of women using oral therapy, significantly more cases (61%) of increase in CRP concentration as compared with 39% in transdermal therapy group (χ^2 P = .015) were observed.

5. Discussion

In the randomized Postmenopausal Estrogen/Progestin Interventions study [26], and also in many other studies, a beneficial effect of HRT on lipoprotein metabolism (decrease of CH and LDL fraction cholesterol and increase of HDL-CH) was observed. In our studies, no significant changes in basic lipid parameters (TG, CH, and HDL-CH) were observed after 3 months of oral and transdermal hormonal therapy.

In our studies, the average values of Lp(a) levels were contained within the range of 5.72 to 7.39 mg/dL, and no significant changes were observed after 3 months of treatment in any of the study groups. In the Heart and Estrogen/progestin Replacement Study, having divided the participants into groups on the basis of their baseline Lp(a) level, Shlipak et al [7] concluded that women whose Lp(a) level was equal or lower than the mean value of 25.3 mg/dL are especially at risk for coronary artery disease within the first year after the initiation of oral HRT. On the other hand, in women with above-mean baseline Lp(a) levels, a drop in these levels was observed, and a significant reduction of the coronary disease risk was noted within 2 to 5 years of follow-up. Thus, women with elevated Lp(a) levels benefited from the use of HRT.

Similar mean values of homocysteine levels, as in our studies (11.5 μmol/L), were observed in postmenopausal women in the studies of Hak et al [10]; they were higher than in premenopausal women (10.7 μmol/L). This difference did not depend on the body mass index (BMI), cigarette smoking, alcohol use, and creatinine level. The results of the Women's Health Study conducted by Ridker et al [27] on healthy American women without coronary disease and cancer in the postmenopausal period showed that cardiovascular risk was increased more than twice in women with moderately elevated homocysteine levels (14.1 vs 12.4 μmol/L), irrespective of the conventional risk factors.

Results of the study show that HRT causes reduction of homocystein concentration in women with higher initial levels and small transient decrease in women with normal initial levels [12,13].

In randomized controlled studies, Hak et al [14] found that HRT significantly decreased the homocysteine level after 6 months of use in women in the perimenopausal period without coronary disease. This decrease was lower (−10.1%) in comparison with placebo in women using oral (conjugated equine estrogen) and norgestrel and was −6.3% in comparison with placebo in women using oral micronized 17β-estradiol and desogestrel. Chiantera et al [16] assessed the effect of oral and transdermal HRT on homocysteine level in healthy postmenopausal women for 24 months. After 6 months of treatment, homocysteine level lowered significantly in both actively treated groups in comparison with the baseline values and the control, but no significant changes were observed in the further phases of the study. The average lowering of homocysteine level was 13.6% in women taking oral estrogens and 8.9% in women taking transdermal estrogens. Women with higher baseline homocysteine levels obtained a greater reduction.

In our studies, no significant changes of homocysteine level were noted, irrespective of the types of medicinal products used and the route of administration of estrogens.

The results of studies by Hak et al [28] indicate that CRP levels do not differ between healthy women in the pre- and postmenopausal period, even after correction for age and BMI, and are 0.62 and 0.69 mg/L, respectively. In our study, the baseline CRP levels were higher and were 3.08, 1.22, and 1.94 mg/L in groups I, II, and III, respectively. In the analysis of results, we disregarded baseline CRP values $\geq$10.0 mg/L, which could indicate an acute inflammatory condition.

Such a criterion has also been used in other studies [21,28,32]. Statistically significant interaction (ANOVA P = .036) indicates that the 2 HRT forms used (oral and transdermal) show significant difference in changes of CRP concentration during 3 months of therapy. Mean increase of CRP concentration in women using oral HRT is significant (P = .0413) when Student t test for paired data was used and is weak (P = .093) when post hoc Bonferroni test was used. Observed tendency, however, is worth mentioning. In that group, significantly more cases (61%) of increase in CRP concentration as compared with 39% in transdermal HRT group (χ^2 P = .015) were observed.

The increase in synthesis of CRP in the liver may be associated with disturbed metabolism of estrogens in women using oral HRT. In this group of women, a high increase in E_2 and E_1 serum levels was found after 3 months of treatment; these levels were significantly higher than in the group of women on transdermal estrogens and in the control group.

Estrogens administered transdermally avoid first-pass enterohepatic metabolism, resulting in no increase of CRP. Small decrease of CRP concentration in the group of women taking 17β-estradiol transdermally and lutein in the second phase of therapeutic cycle may be related to significantly higher concentration of P, which shows anti-inflammatory properties [29].

Results of many studies on HRT use also indicate an increase in CRP levels. In the prospective study by Ridker et al, average CRP values were higher in women using HRT in comparison with those who did not use hormones (2.7 vs 1.4 mg/L). This difference was maintained in all the subgroups

of women using only estrogen and estrogen combined with P. In the multivariate analysis, the association between the use of HRT and CRP remained significant after the age, BMI, hypertension, hyperlipidemia, tobacco smoking, and alcohol consumption were taken into account [20]. Walsh et al studied the effect of HRT and raloxifen—a selective modulator of estrogens receptor—on the levels of homocysteine and CRP in healthy postmenopausal women for 6 months. Both HRT as well as raloxifen at 2 different doses significantly reduced homocysteine level by 7%, 8%, and 6%, respectively. Combined conjugated equine estrogen and medroxyprogesterone treatment significantly increased CRP levels, whereas raloxifen had no significant effect on CRP. On this basis, it was suggested that HRT in postmenopausal women was associated with an increase in inflammatory response which may trigger acute coronary events [21].

In the PEPI study, Cushman et al [22] observed a significant increase in CRP level (85%) at 12 months in all groups of women using hormones (estrogen in monotherapy and combined with P, at various doses) in comparison with a control group (in postmenopausal women); this increase was consistently sustained for 3 years. The concentration of soluble E-selectin was lowered by 18%, which may indicate a possible anti-inflammatory effect. Silvestri et al assessed a wider spectrum of vascular inflammation markers (CRP, soluble forms of endothelial adhesion molecules—soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1, and E-selectin, interleukin 6, s-thrombomodulin) in postmenopausal women at an increased cardiovascular risk. In comparison with the baseline values, CRP level increased significantly in women on HRT (from 0.9 ± 0.2 to 1.6 ± 0.4 mg/L), and the values of other vascular inflammation markers were reduced. On the other hand, no significant changes in the value of any of the parameters measured were noted in women who did not use HRT. This disparity between the CRP level increase and lowering of the level of all other inflammation markers suggests that the increase in CRP after HRT may be associated with hepatic metabolism activation and not with the acute phase response [23].

Our results are similar with those of other studies comparing the influence of HRT in the form of orally and transdermally administered estrogens on CRP concentration. Oral therapy resulted in increase of CRP, and such an effect was not observed for transdermal therapy [30-32]. Vongpatanasin et al [33] showed that oral estrogen replacement therapy results in more than double increase of CRP and significant decrease of anti-inflammatory growth factor insulin-like growth factor 1, while transdermal administration of estrogens does not result in the effect in the same group of women after 8 weeks. Administration route of estrogens has no influence on cytokines responsible for CRP synthesis, such as interleukin 6, interleukin 1β, and tumor necrosis factor α.

On the basis of our observations, it appears that in women in the early postmenopausal stage with normal initial concentrations of Lp(a) and homocystein, the form of therapy used has no influence on the values of these parameters. The 2 forms of HRT therapy differ in effect, which is expressed as a change in CRP concentration. The tendency to increase CRP values when using oral HRT is observed, while such effect is not observed in case of transdermal therapy after 3 months.

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