The impact of six-month tibolone postmenopausal treatment on cell adhesion molecules levels

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Summary

Purpose: The aim of the present study was to evaluate the effects of tibolone on inter-cellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), inter-cellular adhesion molecule-2 (ICAM-2) and P-selectin levels in healthy postmenopausal women. *Methods:* This prospective study included 25 postmenopausal women, complaining of hot flashes, assigned in two groups. Fifteen women received tibolone (dosage of 2.5 mg per day for six months) and ten women did not receive any therapy, according to their personal preference. Basal control included complete medical history, anthropometrics, clinical examination, and blood sampling to perform hormonal, biochemical, hematological testing and ICAM-1, ICAM-2, VCAM-1 and P-selectin measurements. Evaluation was repeated in three and six months. *Results:* There was no significant difference in ICAM-1, VCAM-1, ICAM-2, P-selectin, homocysteine, total cholesterol, HDL, LDL, and triglyceride concentrations between the women of the two groups after either three or six months of treatment. However, a significant reduction in the frequency and intensity of hot flashes was noted in both groups. *Conclusions:* Tibolone does not have any adverse effects on cell adhesion molecule levels which primarily affect atherosclerotic processes or on triglyceride and homocysteine concentrations. These results may support the view that tibolone could be considered a safe treatment, regarding its impact on the endothelium, in healthy postmenopausal women.

Key words: Tibolone; Menopause; Cell adhesion molecules; ICAM-1; ICAM-2; VCAM-1; P-selectin.

Introduction

Atherosclerosis is considered a dynamic and progressive process triggered by endothelial dysfunction and inflammation and leading to cardiovascular disease [1]. Women present an age-dependent and more precisely, an estrogen-dependent cardiovascular disease risk pattern [2]. Estrogens exert a widely favorable effect on endothelium function, as already known [2]. This is reflected on the apparent growth in the number of cardiovascular events after menopause [3]. In these terms, conventional hormone therapy (HT) was thought to reduce the higher cardiovascular risk by replacing the intrinsic lack of estrogen in postmenopausal women [4]. However, the value of hormone replacement therapy as a means of cardiovascular disease risk reduction has been seriously debated since the publication of the first results of the Women's Health Initiative (WHI) study [5].

Tibolone is a synthetic steroid with estrogenic, progesteronic and androgenic effects. It has been used as an alternative treatment for menopausal symptoms and gained popularity after the announcement of the negative impact of conventional HT on the cardiovascular system [5-7]. The positive effects of tibolone on the lipidemic profile are widely studied, in contrast to the direct effects on the endothelium or the indirect effects via independent biomarkers.

An initial step in the atherosclerotic process is the adhesion of white blood cells (WBCs) to endothelium. WBC adhesion is mediated by cell-cell adhesion molecules (CAMs) which are produced by the endothelium and include five superfamilies (the immunoglobulin superfamily, integrins, cadherins, transmembrane proteoglycans, and selectins) [8, 9]. The immunoglobulin superfamily includes more than 70 members, among them ICAM-1, ICAM-2, ICAM-3, VCAM-1 and VCAM-2 which adhere to leucocute surfaces via integrins. Selectins include L-selectin which is expressed on WBCs, E-selectin expressed on activated endothelial cells and P-selectin expressed on both activated platelets and endothelial cells [10]. Selectins mediate the calcium-dependent cell-extracellular matrix binding in blood and, thus, they promote WBCs binding to endothelium as part of an inflammatory response.

In case of endothelial dysfunction, there is derangement of endothelial homeostasis and accumulation of WBCs in the arterial intima, mediated by increase in ICAM, VCAM and especially P-selectin expression. Indeed, several CAMs have been found elevated in patients with established atherosclerosis or with high risk for cardiovascular disease [11]. On the other hand, HT interferes with endothelial dysfunction and ameliorates many cardiovascular risk markers [12]. Tibolone effects on cardiovascular risk markers have been also investigated in human cells cultures [13-15], primates [16] and postmenopausal women [17-20]. However, opinions on the effects of tibolone on endothelium remain divergent as the results of both clinical and in vitro studies are not unanimous. In these terms, the aim of the present study was the evaluation of tibolone effects on ICAM-1, V-CAM-1, ICAM-2 and P-selectin in healthy postmenopausal women compared with controls.

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Materials and Methods

From January 2006 to December 2007, 25 healthy postmenopausal women, referred to the Menopause Infirmary of the First Department of Obstetrics and Gynecology because of severe hot flashes that affected their quality of life, were enrolled voluntarily in the present study. The protocol and procedures were approved by the Institutional Ethics Committee. None of the authors had any conflict of interest.

The main inclusion criterion was the presence of severe hot flashes affecting the patient's quality of life. All women had had their last menstrual episode at least one year before in cases of natural menopauses and four months before in cases of surgical menopause, and presented serum FSH levels higher than 40 IU/ml. Furthermore, all participants had similar lifestyles and body mass index (BMI). Exclusion criteria included: history of thromboembolism, arterial hypertension, cardiovascular disease, liver or kidney disease, thyroid dysfunction, diabetes mellitus, coagulation disturbance, estrogen-dependent tumor, prior hormone replacement therapy or tibolone intake and excessive smoking.

All 25 women completed the protocol. It should be noted that the women were also selected based on similar lifestyles and were advised to continue the same lifestyle throughout the study period. The 25 participants were divided in two groups. Group A included 15 women who received tibolone (Livial, Organon, Holland) at a dosage of 2.5 mg per day for six months early in the morning. Group B included ten women who did not receive any therapy. All subjects were assigned to the groups according to their personal preference, after being fully informed.

Complete medical history, anthropometrics, clinical examination and Pap smear test, ultrasound of the internal genitalia, mammography, and dual energy X-ray absorptiometry (DEXA) were carried out. Blood samples were collected for evaluation of ICAM-1, ICAM-2, VCAM-1 and P-selectin concentrations, and furthermore for hormonal FSH levels, biochemical levels (glucose, urea, creatinine, uric acid, K, Na, SGOT, SGPT, GT, LDH, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, homocysteine, bilirubin, total proteins, PT, αPTT, and fibrinogen) and hematological tests. The evaluation was repeated after three and six months.

Blood samples were collected in noncitrated tubes between 8 and 9 a.m. after an overnight fast. To assess CAM concentrations, blood samples were centrifuged at 3000 rpm for 10 min. The serum was aspirated and kept in special aliquots at -22°C. All samples were assayed together.

ICAM-1, ICAM-2 and VCAM-1 were measured by sandwich ELISA (Diaclone, France). P-selectin was also measured by sandwich ELISA (R&D Systems, Inc., Mineapolis, MN) according to the manufacturer's instructions. The sensitivity of the ICAM-1 assay was lower than 0.1 ng/ml. Intra- and inter-assay variation coefficients (CVs) were 3.56% and 8.76%, respectively. The sensitivity of the ICAM-2 assay was lower than 0.2 U/ml. Intra- and inter-assay CVs were 3.20% and 7.15%, respectively. The sensitivity of the VCAM-1 assay was lower than 0.6 ng/ml. Intra- and inter-assay CVs were 3.34% and 5.94%, respectively. The sensitivity of the P-selectin assay was lower than 0.5 ng/ml. Intra- and inter-assay CVs were 5.80% and 8.90%, respectively.

SPSS software v.16.0 (SPSS inc, Chicago, IL) was used for data analysis. Normality of the distribution was checked by the Kolmogorov-Smirnov test. T-test and a general linear model for repeated measurements were used for the assessment of differences in significance between the mean levels of all study parameters of the two groups for changes in time and between groups. All tests were 2-tailed at a significance level of 0.05.

Results

At baseline (time-t = 0), there was no significant difference in the mean values of age, BMI and time since menopause between the women of the two groups. Similarly, there was no significant difference in the mean values of HDL, LDL and triglycerides. Also, the mean homocysteine, ICAM-1, VCAM-1, ICAM-2 and P-selectin levels were not different between the two study groups. Only, the mean total cholesterol concentration was significantly higher in women of group B ($p = 0.004$) (Table 1). After completion of three (t = 3 months) and six months (t = 6 months) of treatment, all measurements were repeated. There was no significant difference between the two groups regarding the mean concentrations of total cholesterol, HDL, LDL, triglycerides, homocysteine, ICAM-1, VCAM-1, ICAM-2, and P-selectin after either three or six months of treatment (Table 1). Regarding hot flashes, all women reported improvement in frequency as well as intensity both at three and six months after initiation of the study.

The general linear model analysis indicated a non significant main effect of time on total cholesterol ($F = 1.632$, $p = 0.209$), HDL ($F = 2.048$, $p = 0.151$), LDL ($F = 0.207$, $p = 0.763$), triglycerides ($F = 2.364$, $p = 0.110$), homocysteine ($F = 0.627$, $p = 0.494$), ICAM-1 ($F = 0.701$, $p = 0.483$), VCAM-1 ($F = 0.369$, $p = 0.649$), ICAM-2 ($F = 1.708$, $p = 0.197$) and P-selectin ($F = 1.566$, $p = 0.221$) levels. Furthermore, the pattern of the main effect of time was not significantly different between the two groups with regards to the mean values of total cholesterol ($F = 0.450$, $p = 0.622$), HDL ($F = 1.699$, $p = 0.201$), LDL ($F = 0.680$, $p = 0.510$), triglycerides ($F = 2.683$, $p = 0.084$), homocysteine ($F = 0.426$, $p = 0.597$), ICAM-1 ($F = 0.170$, $p = 0.815$), VCAM-1 ($F = 0.208$, $p = 0.766$), ICAM-2 ($F = 1.308$, $p = 0.279$) and P-selectin ($F = 1.291$, $p = 0.284$) over the whole study period.

Discussion

This study demonstrates that tibolone intake for a period of six months had no impact on ICAM-1, VCAM-1, ICAM-2, P-selectin, total cholesterol, HDL, LDL, trigycerides and homocysteine levels in the serum of healthy postmenopausal women complaining of hot flashes. As described above, women receiving one tablet (2.5 mg) of tibolone per day did not present any difference from controls who did not receive any therapy throughout the whole study period. Importantly, all women included in this study were outpatients complaining of hot flashes, without any prior history of cardiovascular diseases, in an effort to ensure a homogeneous sample and compensate for the small sample size. Additionally, all participants were of similar age and BMI, as these parameters exert a direct or even indirect influence on the cardiovascular system. All women had the same nationality and similar lifestyle.

A limitation point is the small number of women included in the study as well as the lack of randomization.

Table 1. — *Anthropomentrics and serum lipids and CAM levels in the women of the two study groups at baseline (t=0), 3 and 6 months*.

	Baseline Group A n = 15	Group B n = 10	*p* values	t = 3 months Group A	Group B	*p* values	t = 6 months Group A	Group B	*p* values
Age (years)	50.40 ± 0.73	49.50 ± 0.67	0.403						
TSM (months)	10.86 ± 2.03	15.30 ± 2.58	0.188						
BMI (Kg/m^2)	25.56 ± 0.50	24.41 ± 0.62	0.166						
Total cholesterol (mg/dl)	207.20 ± 5.23	233.3 ± 6.41	0.004	205.00 ± 6.21	224.30 ± 7.601	0.062	202.80 ± 6.15	219.70 ± 7.53	0.096
HDL (mg/dl)	50.66 ± 3.86	55.90 ± 4.72	0.400	51.40 ± 3.53	53.40 ± 4.37	0.726	50.40 ± 3.47	51.20 ± 4.25	0.885
LDL (mg/dl)	134.00 ± 6.65	155.60 ± 8.14	0.052	144.66 ± 7.35	152.30 ± 9.03	0.518	149.40 ± 8.40	149.40 ± 8.40	0.348
Triglycerides (mg/dl)	102.73 ± 10.14	95.60 ± 12.43	0.661	87.26 ± 10.22	102.70 ± 12.52	0.350	81.13 ± 12.40	94.20 ± 12.54	0.428
Homocysteine (mmol/l)	10.03 ± 0.62	10.33 ± 0.76	0.764	10.06 ± 0.55	10.53 ± 0.68	0.598	10.04 ± 0.54	10.30 ± 0.66	0.769
ICAM-1 (ng/ml)	690.99 ± 34.93	665.37 ± 42.78	0.520	692.98 ± 43.42	701.54 ± 53.18	0.952	631.99 ± 51.02	657.65 ± 62.49	0.225
VCAM-1 (ng/ml)	915.06 ± 88.28	890.45 ± 108.12	0.089	920.63 ± 84.56	899.44 ± 103.57	0.992	891.46 ± 100.35	907.24 ± 122.90	0.820
ICAM-2 (U/ml)	462.61 ± 23.41	429.08 ± 39.70	0.647	466.44 ± 40.00	470.25 ± 48.99	0.982	459.55 ± 46.01	368.88 ± 56.35	0.753
P-selectin (ng/ml)	134.32 ± 9.48	107.69 ± 11.62	0.862	125.06 ± 9.25	124.92 ± 11.33	0.612	111.17 ± 8.63	108.04 ± 10.54	0.922

TSM: time since menopause; BMI: body mass index; *Statistical difference ($p = 0.004$) at baseline mean levels between the two groups.

However, replacement therapy is seen under the prism of profound scepticism on the part of women and even the gynecologists in Greece. Prescription of replacement therapy is significantly lower in comparison to other European countries, especially after the publication of WHI and HERS [5-7] Greek women often exclude the possibility of receiving therapy for menopausal symptoms rendering, thus, the performance of a blind study very difficult, as already stated by other researchers [20].

The main findings of the study are the almost stable serum concentrations, without significant decrease, of ICAM-1, VCAM-1, ICAM-2 and P-selectin throughout the six-month treatment with tibolone. Previous clinical studies have shown a significant reduction in ICAM-1 concentrations after tibolone treatment [17-19]. The reduction was presumably yielded to the observed effects of tibolone on myocardial ischemia and cardiac flow [17-21] or even an intrinsic cardiovascular protective, lipid-independent potential through WBC suppression [18]. Concerning the mechanism, it was suggested that the estrogen receptor could directly or indirectly interact with transcription factors as none of the promoter regions for the CAM genes contain hormone-responsive elements [17].

The approximate reduction of 15-20% in ICAM-1 and VCAM-1 observed in the study of Cicinelli *et al*. was similar to what was reported after estrogen therapy [22, 23]. This was the only study including postmenopausal women not complaining of vasomotor symptoms in order to exclude the interference of hot flashes and sweating on endothelial function [19]. However, the authors highlighted the importance of individual and ethnic genetic variability that could interfere with steroid secretion and metabolism, and the avoidance of generalizations.

On the other hand, in vitro studies with human endothelial cells failed to show any significant decrease in ICAM-1 [13, 14]. As for ICAM-2, there are no data about the impact of tibolone or other replacement therapy in postmenopausal women. VCAM-1 has been found to be reduced after hormone therapy in a clinical [19] and an in vitro study [13]. However, our results do not confirm the latter study probably because of the small number of patients or the different study time length. Regarding the levels of P-selectin, there is only one recent publication referring to non significant alterations of P-selectin serum levels after eight weeks of oral tibolone intake [18].

The non significant changes in homocysteine levels in the present study are supportive of the lack of negative impact of this drug on the endothelium. Homocysteine serum levels have also been found to be unaffected in previous studies of tibolone administration during either three or 18 months [24, 25]. Although inflammatory activation of the endothelium has been shown during concurrent transient hyperhomocysteinemia, no correlation between homocysteine and CAM or P-selectin levels has ever been reported [26].

As for the impact of tibolone on the lipid profile, a reducing trend in total cholesterol, triglycerides, LDL and also HDL is generally indicated [20, 27-31]. In the present study, no significant difference in any of the lipids was found. This discrepancy could be attributed to the sample size, though comparable with some previous studies, or even to genetic variability and dietary habits. The fact that the treatment did not reduce HDL levels could be regarded as positively for the use of the drug, although there was no difference in total cholesterol, LDL, and triglycerides.

All women enrolled in this study initially complained of hot flashes but afterwards they all referred improvement in the frequency and intensity of hot flashes and, furthermore, general amelioration of mood and quality of life. Although this has been a subjective judgment that was not systemically evaluated, it is an important case in point that should be considered for the treatment choice, at least for this population of patients, as already pointed out [32, 33].

In conclusion, the six-month tibolone treatment did not influence cell adhesion molecule levels. However, the fact that CAM levels remained stable, as well as lipid and

homocysteine concentrations, may support the view that tibolone could be considered as a safe treatment regarding its impact on the endothelium in healthy postmenopausal women. Additional investigations concerning the role of tibolone on endothelial activity would further enlighten the impact of this drug.

References

[1] Ribeiro F., Alves A.J., Teixeira M., Ribeiro V., Duarte J.A., Oliveira J.: "Endothelial function and atherosclerosis: circulatory markers with clinical usefulness". *Rev. Port. Cardiol*., 2009, *28*, 1121.

[2] Bechlioulis A., Naka K.K., Calis K.A., Makrigiannakis A., Michalis L., Kalantaridou S.N.: "Cardiovascular Effects of Endogenous Estrogen and Hormone Therapy". *Curr. Vasc. Pharmacol*., 2010, *8*, 249.

[3] Karkanaki A., Vavilis D., Traianos A., Kalogiannidis I., Panidis D.: "Hormone therapy and asymmetrical dimethylarginine in postmenopausal women". *Hormones*, 2010, *9*, 127.

[4] Barrett-Connor E.: "Fortnightly review: hormone replacement therapy". *Brit. Med. J*., 1998, *317*, 457.

[5] Rossouw J.E., Anderson G.L., Prentice R.L., LaCroix A.Z., Kooperberg C., Stefanick M.L.: "Writing group for the Women's Health Initiative investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women". *J. Am. Med. Assoc*., 2002, *288*, 2210.

[6] Million Women Study Collaborators. Patterns of use of hormone replacement therapy in one million women in Britain, 1996-2000. *Br. J. Obstet. Gynecol*., 2002, *109*, 1319.

[7] Hulley S., Grady D., Bush T., Furberg C., Herrington D., Riggs B., Vittinghoff E.: "Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group". *J. Am. Med. Assoc*., 1998, *280*, 605.

[8] Lodish H., Berk A., Zipursky S.L., Matsudaira P., Baltimore D., Darnell J. (eds): "Molecular cell biology, 4th ed. W.H. New York: Freeman and Company", 2001, 970.

[9] Rosenfeld M.E.: "Cellular mechanisms in the development of atherosclerosis". *Diabetes Clin. Pract*., 1996, *30*, 1.

[10] Adams D.H., Shaw S.: "Leucocyte-endothelial interactions and regulation of leucocyte migration". *Lancet*, 1994, *343*, 831.

[11] Pai J.K., Pischon J.M., Ma J., Manson J.E., Hankinson S.E., Joshipura K. *et al*.: "Inflammatory markers and the risk of coronary heart disease in men and women". *N. Engl. J. Med*., 2004, *351*, 2599.

[12] Garnero P., Jasmin C., Benhamou C.L., Pelissier C., Roux C.: "Effects of tibolone and combined 17 -estradiol and norethisterone acetate on serum C-reactive protein in healthy post-menopausal women: a randomized trial". *Hum. Reprod*., 2002, *17*, 2748.

[13] Simoncini T., Genazzani A.R.: "Tibolone inhibits leukocyte adhesion molecule expression in human endothelial cells". *Mol. Cell. Endocrinol*., 2000, *162*, 87.

[14] Mueck A.O., Lippert C., Seeger H., Wallwiener D.: "Effects of tibolone on human breast cancer cells and human vascular coronary cells". *Arch. Gynecol. Obstet*., 2003, *267*, 139.

[15] Seeger H., Kloosterboer H.J., Studen M., Wallwiener D., Mueck A.O.: "In vitro effects of tibolone and its metabolites on human vascular coronary cells". *Maturitas*, 2007, *58*, 42.

[16] Williams J.K., Hall J., Anthony M.S., Register Th.C., Reis S.E., Clarkson T.B.: "A comparison of tibolone and hormone replacement therapy on coronary artery and myocardial function in ovariectomized atherosclerotic monkeys". *Menopause*, 2002, *9*, 41.

[17] Egarter C., Sator M., Huber J.: "Effects of tibolone on cells adhesion molecules in postmenopausal women". *Menopause*, 2003, *10*, 218.

[18] Sator K., Sator M.O., Sator P.G., Egarter C., Huber J.C.: "Effects of tibolone on selectins in postmenopausal women". *Maturitas*, 2006, *53*, 166.

[19] Cicinelli E., Ranieri G., Maffei S., Colafiglio G., Ria R., Bellavia M., Schonauer M.M.: "Long-term effects of tibolone on circulating levels of vascular cell adhesion molecules and E-selectin in postmenopausal women". *Fertil. Steril*., 2006, *86*, 899.

[20] Bianco V., Murina F., Roberti P., Valente I.: "Tibolone in the treatment of menopause: compliance, efficacy and safety in a ten year experience". *Minerva Ginecol*., 2006, *58*, 335.

[21] Lloyd G.W., Patel N.R., McGing E.A., Cooper A.F., Kamalvand K., Jackson G.: "Acute effects of hormone replacement with tibolone on myocardial ischaemia in women with angina". *Int. J. Clin. Pract*., 1998, *52*, 155.

[22] Goudev A., Georgiev D.B., Koycheva N., Manasiev N., Kyurkchiev S.: "Effects of low dose hormone replacement therapy on markers of inflammation in postmenopausal women". *Maturitas*., 2002, *43*, 49.

[23] Oger E., Alhenc-Gelas M., Plu-Bureau G., Mennen L., Cambillau M., Guize L. *et al*.: "Association of circulating cellular adhesion molecules with menopausal status and hormone replacement therapy. Time-dependent change in transdermal, but not oral estrogen users". *Thromb. Res*., 2001, *101*, 35.

[24] Barnes J.F., Farish E., Rankin M., Hart D.M.:" Effects of two continuous hormone therapy regimens on C-reactive protein and homocysteine". *Menopause*, 2005, *12*, 92.

[25] Christodoulakos G., Panulis C., Lambrinudaki I., Dendrinos S.G., Rizos D.A., Creatsas G.C.: "Effect of hormone replacement therapy and tibolone on serum total homocysteine levels in postmenopausal women". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 2004, *112*, 74.

[26] Mansoor M.A., Seljeflot I., Arnesen H., Knudsen A., Bates C.J., Mishra G., Larsen T.W.: "Endothelial cell adhesion molecules in healthy adults during acute hyperhomocysteinemia and mild hypertriglyceridemia". *Clin. Biochem*., 2004, *37*, 408.

[27] Vassalle C., Cicinelli E., Lello S., Mercuri A., Battaglia D., Maffei S.: "Effects of menopause and tibolone on different cardiovascular biomarkers in healthy women". *Gynecol. Endocrinol*., 2011, *27*, 163.

[28] Skouby S.O., Sidelmann J.J., Nilas L., Gram J., Jespersen J.: "The effect of continuous combined conjugated equine estrogen plus medroxyprogesterone acetate and tibolone on cardiovascular metabolic risk factors". *Climacteric*, 2008, *11*, 489.

[29] Daher R., Al-Amin H., Beaini M., Usta I.: "Effect of tibolone therapy on lipids and coagulation indices". *Clin. Chem. Lab. Med*., 2006, *44*, 1498.

[30] Christodoulakos G.E., Lambrinoudaki I.V., Economou E.V., Papadias C., Panoulis C.P., Kouskouni E.E. *et al*.: "Differential effect of hormone therapy and tibolone on lipids, lipoproteins, and the atherogenic index of plasma". *J. Cardiovasc. Pharmacol*., 2006, *47*, 542.

[31] Osmanağaoğlu M.A., Osmanağaoğlu S., Osmanağaoğlu T., Okumu B., Bozkaya H.: "Effect of different preparations of hormone therapy on lipid and glucose metabolism, coagulation factors, and bone mineral density in overweight and obese postmenopausal women". *Fertil. Steril*., 2005, *84*, 384.

[32] Onalan G., Onalan R., Selam B., Akar M., Gunenc Z., Topcuoglu A.: "Mood scores in relation to hormone replacement therapies during menopause: a prospective randomized trial". *Tohoku J. Exp. Med*., 2005, *207*, 223.

[33] Inan I., Kelekci S., Yilmaz B.: "Psychological effects of tibolone and sequential estrogen-progestogen therapy in perimenopausal women". *Gynecol. Endocrinol*., 2005, *20*, 64.

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