ORIGINAL ARTICLE

Effect of early estrogen replacement therapy on microvascular reactivity in patients after bilateral ovarectomy

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Abstract

OBJECTIVES: The aim of the study was to evaluate skin microvascular reactivity (MVR) measured by laser Doppler flowmetry in women early after bilateral ovarectomy treated with oral and transdermal estrogen replacement therapy (ERT).
DESIGN: Interventional, randomized study with a cross-over design.
PATIENTS AND METHODS: 41 patients (49±6 years, 6–12 weeks after surgical castration) were treated with 17-β-estradiol transdermally (0.05 mg/day) or orally (2 mg/day) for three months and 20 healthy female subjects (47±5 years) served as controls.
RESULTS: Records of laser Doppler flowmetry were blinded prior to the evaluation. Maximal perfusion and velocity of perfusion increase during post-occlusive reactive hyperemia (PORH) were lower before ERT comparing to controls at baseline (36±16 vs. 48±18 PU, $p<0.05$, and 2.8±1.9 vs. 4.2±2.3 PU, $p<0.05$, respectively). Velocity of perfusion increase in PORH decreased after oral ERT compared to baseline and also to transdermal ERT (2.1±1.2 vs. 2.8±1.9 PU.s^{-1}, $p<0.05$, and vs. 3.5±3.2 PU.s^{-1}, $p<0.01$, respectively), nonsignificant increase of this parameter after transdermal ERT led to normalization when comparing to control group (3.6±3.2 vs. 4.2±2.3 PU.s^{-1}, NS). Increase of HDL-cholesterol and decrease of LDL-cholesterol (2.1±0.4 vs. 1.8±0.4 mmol.l^{-1}, $p<0.01$, and 2.5±0.7 vs. 3.1±1.0 mmol.l^{-1}, $p<0.01$) was observed after oral ERT while HDL-cholesterol increase after transdermal ERT was less pronounced (1.96±0.42 mmol.l^{-1}, $p<0.05$). LDL-cholesterol levels did not change. A correlation between HDL-cholesterol and maximal post-occlusive flow expressed in % of basal perfusion was observed in patients before treatment ($r=0.47$, $p=0.002$).
CONCLUSIONS: Microvascular reactivity is impaired in women early after bilateral ovarectomy. No statistically significant improvement of MVR was observed after oral estrogen replacement therapy, normalization of MVR after transdermal ERT was only partial. Changes of MVR and lipid profile differed between oral and transdermal routes of estrogen replacement therapy.

Abbreviations

CHD - coronary heart disease
DCCT - Diabetes Control and Complications Trial
ERT - estrogen replacement therapy
FBG - fasting blood glucose
FSH - follicle stimulating hormone
HbA1C - glycated hemoglobin A1C
HDL - high density lipoprotein
HERS - The Heart and Estrogen/Progestin Replacement Study
IFCC - International Federation of Clinical Chemistry and Laboratory Medicine
LDL - low density lipoprotein
LH - luteinizing hormone
TC - total cholesterol
TG - triglycerides
MVR - microvascular reactivity
PORH - post-occlusive reactive hyperemia
PORHmax - maximal perfusion in post-occlusive reactive hyperemia
PORHmax/t - velocity of perfusion increase during post-occlusive reactive hyperemia
PU - perfusion unit
TH - thermal hyperemia
THmax - maximal perfusion in thermal hyperemia
THmax/t - velocity of perfusion increase during thermal hyperemia

INTRODUCTION

Lower rates of coronary heart disease (CHD) with the use of estrogen in postmenopausal women were reported in several observational studies [23,26]. This was not confirmed in the Women's Health Initiative (WHI) trial where possible increase in CHD incidence in women treated with estrogen and progestin compared to placebo [15,24] and neutral effect of estrogen treatment alone [1] were observed. Also in the Heart and Estrogen/progestin Replacement Study (HERS) and in its follow-up (HERS II) hormone therapy did not reduce risk of cardiovascular events in women with CHD [7].

Large studies evaluating the effect of estrogen replacement therapy on microvasculature are missing. Number of subjects in related studies is usually small, design varies and the results are often contradictory. For example, in postmenopausal women on estrogen replacement therapy, no difference was observed in baseline skin blood flow at rest and after warming using laser Doppler flowmetry compared to women without estrogen replacement [3]. However, impaired microvascular reactivity (MVR) has been demonstrated in postmenopausal women and its improvement was observed in women treated with hormone replacement therapy using laser Doppler method and iontophoresis of acetylcholine and sodium nitroprusside [14]. Improved endothelial function was also described in a study comparing premenopausal women with postmenopausal women with or without estrogen therapy using the same method. Again, endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilatation in the forearm skin microcirculation was evaluated [2]. On the other hand, no effect on endothelium independent myocardial perfusion was observed after short-term estrogen treatment using positron emission tomography [19].

It has been shown that in a female population with low cardiovascular risk a significant correlation can be observed between the significance of cardiovascular risk factors and the impairment of skin microvascular reactivity assessed by laser Doppler method. This may represent a simple and non-invasive tool to determine and monitor microvascular reactivity in observational and interventional studies [30]. We have used this method successfully previously during monitoring skin microvascular reactivity in patients with endocrine disorders – growth hormone replacement therapy in hypopituitary patients [8], patients with Cushing's syndrome [22], and in diabetic patients [27].

The aim of this study was to evaluate skin microvascular reactivity measured by laser Doppler flowmetry in female patients treated with estrogen therapy early (six to twelve weeks) after bilateral ovarectomy (surgical castration). Effects of estrogen on lipid parameters and difference between oral and transdermal route of ERT administration were evaluated.

PATIENTS AND METHODS

This was an interventional, prospective, randomized study with a cross-over design. 41 patients (mean age 49±6 years, range 32–57 years) early (6 to 12 weeks) after bilateral ovarectomy (surgical castration) were randomized to receive either 17-β-estradiol transdermally (group TD) in dose of 0.05 mg daily (Climara, Schering, Germany) or 2 mg of 17-β-estradiol daily orally (Estrofem, Novo Nordisk, Denmark) – group OR. Patients had no signs of estrogen deficiency prior to the surgical castration. After 12 weeks of treatment with first modality one week wash-out period was followed by treatment with the second modality for next 12 weeks. The examination was performed before the start of treatment and after 12 and 25 weeks after the end of treatment with each modality. Blood samples were taken in fasting patients between 8 to 9 a.m. Serum was separated from venous blood and stored at –80 °C.

For the purpose of microvascular reactivity measurements, twenty healthy, sex, age and BMI matched subjects (mean age 47±5 years, range 31–56 years) served as a control group (group C). The control subjects participated on voluntarily basis and were recruited from hospital workers with no signs of estrogen deficiency. The characteristics of patients and control subjects are shown in Table 1. Study was approved by the Ethics Committee of 1st Faculty of Medicine, Charles University, and all subjects gave their written informed consents.

Biochemical methods

LH, FSH, and estradiol levels were measured by chemiluminiscence immunoassay (Advia Centaur, Bayer, USA). Total serum cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) were measured by pho-

tometric enzymatic method on an automated analyzer (COBAS Mira, Roche). LDL-cholesterol (LDL-C) was calculated using the Friedwald formula. Fasting blood glucose (FBG) was measured by a routine enzymatic method. Glycated hemoglobin HbA_{1C} was analyzed by high-performance liquid chromatography method on Variant II analyzer (BioRad, USA) using IFCC calibration ($HbA_{1C}[DCCT] \approx 0.915 * HbA_{1C}[IFCC] + 2.15$).

Microvascular reactivity

Skin microvascular reactivity (MVR) was measured by laser Doppler flowmetry using a PeriFlux PF 4001 Master laser instrument and a PeriTemp 4001 Heater thermostatic unit manufactured by Perimed (Sweden). Records of laser Doppler flowmetry were blinded prior to the evaluation and the operator had no information about the mode of treatment of evaluated patients. All records were evaluated by a single operator. Instrument settings were as follows: time constant 0.02 s, sampling frequency 32 Hz, averaging from 2 samples. Measurements were done at a room temperature of 22 °C in a sitting position, and all subjects rested for at least 30 minutes in order to acclimatize before examination. Post-occlusive reactive hyperemia (PORH) and thermal hyperemia (TH) tests were performed for the assessment of microvascular reactivity. A single thermostatic probe (type 455, 23 mm diameter, fiber separation 0.25 mm) was used for both tests. Optical fibers in this probe are integrated into the heating plate and thus the entire area of tissue under the probe is heated. The probe was fixed with double-stick discs (3M, USA) to the forearm and its temperature was set to 32 °C for the purpose of skin thermal stabilization during PORH. Temperature of 44 °C was used during TH as the thermal stimulus.

Basal perfusion (PORHb) was measured for 2 minutes before the PORH test. The brachial artery was then occluded by a sphygmomanometer cuff inflated to a suprasystolic pressure for 3.5 minutes. The cuff was applied around the arm before the procedure started in order to avoid any extra manipulation with the extremity during the test. PORH was recorded after 3.5 minutes of arterial occlusion. Maximal perfusion during hyperemia was recorded (PORHmax) as well as the time needed for reaching this maximal perfusion (PORHt). The velocity of the perfusion increase (PORHmax/t) was calculated as PORHmax/PORHt. Relative hyperemia (PORH%) was calculated as a percent increase above the baseline (PORH%=(PORHmax/PORHb–1) * 100). Thermal hyperemia was measured 10 minutes later than the PORH test, at the same location. The probe temperature was set to 44 °C and parameters THmax, THt, and THmax/t were recorded or calculated similarly as those in the PORH test. TH% was calculated using formula TH%=(THmax/PORHb–1) * 100. Perfusion is given in arbitrary perfusion units (PU). Perisoft for DOS 5.10C2 and Perisoft for Windows 2.5 software were used for recording and evaluating perfusion data. The intra-individual coefficient of variation of the laser Doppler method in 5 healthy subjects measured ten times on ten consecutive days varied from 17 to 24% in TH and 19 to 25% in PORH, depending on the analyzed parameter.

We did not repeat the examination of MVR in the control group because it was demonstrated that the effect of time difference in healthy subjects is negligible – about 0.55–0.88 flow units per year, i.e. estimated 0.4–0.7% decrease in both resting and stimulated skin blood flow [20].

Statistical evaluation

Statistical evaluation was performed by Statistica for Windows 6.0 software. Basic descriptive statistics was calculated for presented parameters. ANOVA, Student's t-test or Wilcoxon's test, Mann-Whitney and Kolmogorov-Smirnov tests were used for comparing data between groups. Tests were selected depending on normality of data distribution. Pearson's and Spearman's correlations were used for analysis of relationships between measured parameters. Data are expressed as mean±SD if not stated otherwise.

RESULTS

Before ERT, microvascular reactivity was significantly lower in patients early after bilateral ovarectomy comparing to healthy controls in two parameters during post-occlusive reactive hyperemia (PORHmax and PORHmax/t). PORHmax/t decreased nonsignificantly after oral ERT and increased nonsignificantly after transdermal ERT compared to baseline, however, the difference between oral and transdermal route of ERT was statistically significant. THmax/t decreased nonsignificantly after both oral and transdermal ERT compared to baseline values. PORHmax after both oral and transdermal ERT and PORHmax/t after oral ERT remained statistically significantly lower than in control group, difference between PORHmax/t after transdermal ERT and control group was not statistically significant. Results of microvascular reactivity are shown in Table 2.

Table 1. Baseline characteristics of patients and control subjects.

	Patients before treatment (n = 41)	**Control subjects** (n = 20)
Age (years)	49.0±5.5	47.5±5.1
BMI ($kg.m^{-2}$)	26.80±4.97	26.20±3.84
FBG ($mmol.l^{-1}$)	4.89±0.47	4.76±0.44
HbA1C (%) [IFCC]	3.87±0.81	3.62±0.63
SBP (mm Hg)	113±16	116±14
DBP (mm Hg)	77±9	78±8

BMI - body mass index, HbA1C - glycated hemoglobin A1C, SBP - systolic blood pressure, DBP - diastolic blood pressure, FBG - fasting blood glucose

Table 2. Hormone and lipid levels and microvascular reactivity in patients after bilateral ovarectomy at baseline, after oral (OR) and transdermal estrogen therapy (TD), and in control subjects (C).

	Baseline (n=41)	**OR** (n=41)	**TD** (n=41)	**C** (n=20)
LH ($U.l^{-1}$)	42.71±21.39	31.19±16.92^{b}	34.58±18.74	–
FSH ($U.l^{-1}$)	63.58±25.06	29.78±16.38^{c}	41.30±20.91^{c,r}	–
Estradiol ($mmol.l^{-1}$)	0.15±0.14	0.45±0.31^{c}	0.31±0.18^{c,q}	–
TC ($mmol.l^{-1}$)	5.54±1.07	5.33±0.90	5.52±1.17	5.57±1.02
HDL-C ($mmol.l^{-1}$)	1.84±0.38	2.09±0.42^{c}	1.96±0.42^{a,s}	1.56±0.46
LDL-C ($mmol.l^{-1}$)	3.06±0.97	2.52±0.71^{c}	3.00±1.00^{s}	3.16±0.86
Triglycerides ($mmol.l^{-1}$)	1.39±0.79	1.61±0.80^{b}	1.29±0.68^{s}	1.48±0.62
PORHmax (PU)	35.6±16.3^{x}	34.3±13.3^{x}	36.8±15.5^{x}	48.1±18.0
PORH% (%)	482.6±277.4	462.7±220.9	456.9±262.8	511.9±288.1
PORHmax/t ($PU.s^{-1}$)	2.82±1.86^{x}	2.10±1.20^{a,z}	3.58±3.24^{r}	4.19±2.27
THmax (PU)	106.8±42.1	99.6±43.5	102.9±42.4	111.7±41.43
TH% (%)	1637.3±853.1	1576.3±905.6	1503.6±795.6	1374.5±784.4
THmax/t ($PU.s^{-1}$)	1.29±1.15	1.01±0.55^{z}	1.13±0.79^{y}	1.72±0.85

LH - luteinizing hormone, FSH - follicle stimulating hormone, TC - total cholesterol, HDL-C - HDL cholesterol, LDL-C - LDL cholesterol. Statistical significance between basal values and groups OR and TD $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$; between groups OR and TD $^{q}p<0.05$, $^{r}p<0.01$, $^{s}p<0.001$; between basal values, group OR, TD and control group (C) $^{x}p<0.05$, $^{y}p<0.01$, $^{z}p<0.001$.

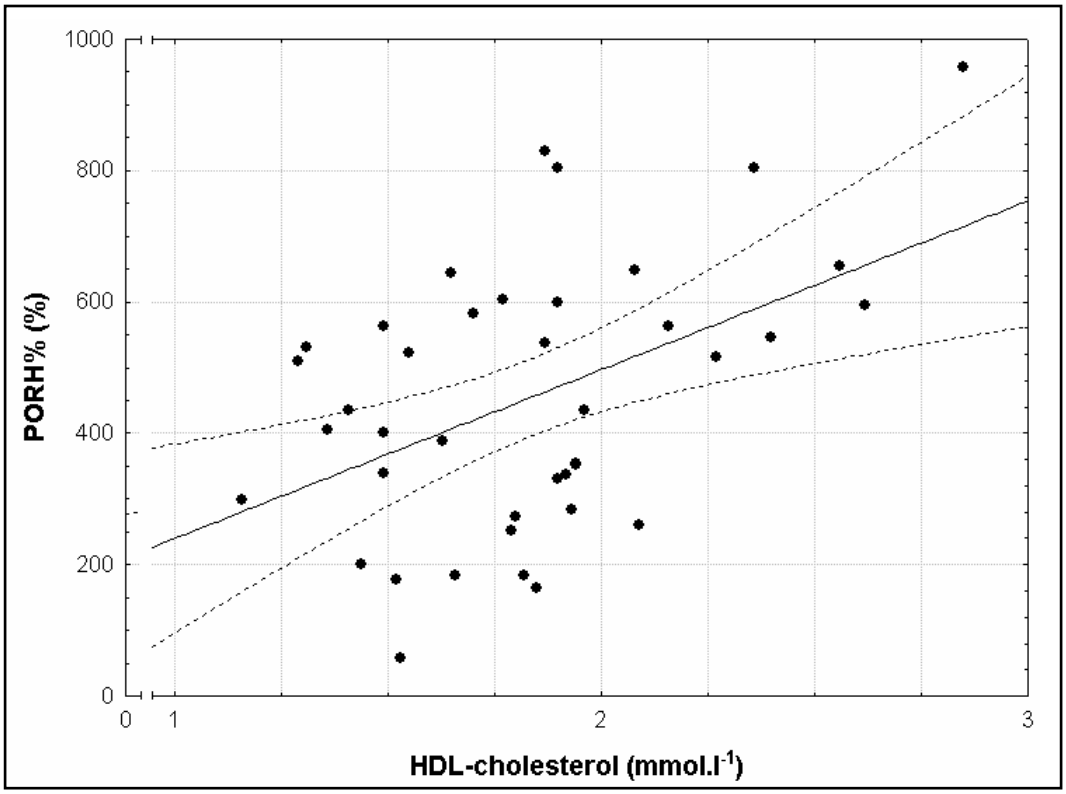

Figure 1. The correlation between HDL-cholesterol and post-occlusive reactive hyperemia (expressed in % of basal skin blood flow) in women early after surgical castration before estrogen treatment (y=–17.43+257.06 * x, r=0.47, p=0.002).

Lipid parameters were comparable between patients and control group at baseline. Total cholesterol levels were not changed after ERT. Triglycerides increased significantly after oral ERT. Differences between oral and transdermal form of ERT were significant because of nonsignificant lowering of triglycerides after transdermal ERT. The changes of HDL-cholesterol and LDL-cholesterol levels after oral ERT were significantly more favourable than after transdermal ERT. LDL levels decreased after oral ERT in comparison with the nonsignificant decline after transdermal ERT. Correlation between HDL-cholesterol and post-occlusive reactive hyperemia (expressed in % of basal skin blood flow – PORH%) was observed in patients before ERT at baseline only (r=0.47, p=0.002, Figure 1). No correlations between microvascular reactivity and lipid parameters or estradiol concentration were found in patients after both oral and transdermal ERT. Results of lipid parameters are shown in Table 2 as well as results of LH, FSH and estradiol.

DISCUSSION

Estrogen replacement therapy should be initiated based on an individual assessment of risks and benefits for each patient. Benefits of ERT should outweigh its known risks when low dose estrogen replacement therapy is started early after menopause as was done in our study [5,17,31]. Impaired microvascular reactivity has been described in postmenopausal women previously [14], and its presence was confirmed in our study, too. After estrogen replacement, no statistically significant improvement of MVR was observed after oral route of estrogen replacement. In fact, a decrease in the velocity of perfusion increase in post-occlusive reactive hyperemia was observed after this mode of ERT. The same parameter increased nonsignificantly after transdermal estrogen and normalized in comparison to control group. However, this was the only improvement of MVR observed in our study. Thus, normalization of MVR after transdermal ERT was only partial.

Different effect of oral and transdermal estrogen replacement on MVR may be explained by known differences between peroral and transdermal ERT that have been described previously [12,28]. In transdermal ERT, plasma estrogen levels are more steady and the metabolic load of liver during the "first-pass effect" is missing. During oral estrogen administration, the concentration of estradiol in the liver is several times higher than in the systemic circulation. Different effect of oral and transdermal route of estrogen replacement therapy can be also partially explained by the differences in metabolism of estrogens and their pharmacokinetics [18,21,25]. Transdermal ERT is thought to be metabolically more favourable [6,16].

Microvascular function can be affected by cholesterol levels [4,13]. In this study, no influence of lipid parameters on MVR was observed despite their substantial changes during estrogen replacement. These changes included increase of HDL-cholesterol and decrease of LDL-cholesterol after oral ERT and statistically significant difference between these parameters between oral and transdermal ERT. The only observed improvement in transdermal ERT (PORHmax/t) was not related to lipid parameters, either.

Direct, lipid-independent actions of estrogen on the blood vessel wall are mediated by specific receptors and may modify vascular function [11,29]. However, measurement of estradiol has only a limited value due to high variability of its serum concentrations during replacement therapy [10]. Accordingly, no correlation was found between respective hormone levels and parameters of lipid metabolism or microvascular reactivity.

Mechanisms of different effect of oral and transdermal ERT are unknown and need further experiments. Two ongoing clinical trials, KEEPS (Kronos Early Estrogen Prevention Study) [9] and ELITE (Early versus Late Intervention Trial with Estradiol, clinicaltrials.gov/show/NCT00114517) may provide additional information about the role of oral and transdermal estrogen replacement in younger postmenopausal women.

CONCLUSIONS

Microvascular reactivity is impaired in women early after bilateral ovarectomy. No statistically significant improvement of MVR was observed after oral estrogen replacement therapy and the normalization of MVR after transdermal estrogen replacement was only partial. Changes of MVR and lipid profile differed between the oral and transdermal routes of estrogen replacement therapy.

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REFERENCES

1 Anderson GL, Limacher M. Effects of Conjugated Equine Estrogen in Postmenopausal Women with Hysterectomy: The Women's Health Initiative Randomized Controlled Trial. Journal of the American Medical Association. 2004; **291**(14): 1701–1712.
2 Arora S, Veves A, Caballaro E, Smakowski P, LoGerfo FW, Donaldson MC, Kaufman JL, Cronenwett JL, McDaniel MD, Whittemore A, Babson WW. Estrogen improves endothelial function. Journal of Vascular Surgery. 1998; **27**(6): 1141–1147.
3 Brooks-Asplund EM, Kenney WL. Chronic hormone replacement therapy does not alter resting or maximal skin blood flow. Journal of Applied Physiology. 1998; **85**(2): 505–510.
4 Bush TL, Barrett-Connor E, Cowan LD. Cardiovascular mortality and noncontraceptive use of estrogen in women: Results from the Lipid Research Clinics Program Follow-up Study. Circulation. 1987; **75**(6): 1102–1109.
5 Ettinger B. Rationale for use of lower estrogen doses for postmenopausal hormone therapy. Maturitas. 2007; **57**(1): 81–84.

6 Fait T., Vrablik M., Cibula D., Masata J., Hill M., Trnkova B. Oral but not Transdermal Estrogen Replacement Therapy Reduced Level of Tissue Factor Pathway Inhibitor: Cross-over Designed Study. Neuro Endocrinol Lett. 2006, **27**(5): 557–61

7 Grady D, Herrington D, Bittner V, Blumenthal R, Davidson M, Hlatky M, Hsia J, Hulley S, Herd A, Khan S, Newby LK, Waters D, Vittinghoff E, Wenger N. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and estrogen/progestin replacement study follow-up (HERS II). Journal of the American Medical Association. 2002; **288**(1): 49–57.

8 Hána V, Prázný M, Marek J, Škrha J, Justová V. Reduced microvascular perfusion and reactivity in adult GH deficient patients is restored by GH replacement. European Journal of Endocrinology. 2002; **147**(3): 333–337.

9 Harman SM, Brinton EA, Cedars M, Lobo R, Manson JE, Merriam GR, Miller VM, Naftolin F, Santoro N. KEEPS: The Kronos Early Estrogen Prevention Study. Climacteric. 2005; **8**(1): 3–12.

10 Järvinen A, Nykänen S, Paasiniemi L. Absorption and bioavailability of oestradiol from a gel, a patch and a tablet. Maturitas. 1999; **32**(2): 103–113.

11 Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation. 1994; **89**(5): 1943–1950.

12 Kawano H, Yasue H, Hirai N, Yoshida T, Fukushima H, Miyamoto S, Kojima S, Hokamaki J, Nakamura H, Yodoi J, Ogawa H. Effects of transdermal and oral estrogen supplementation on endothelial function, inflammation and cellular redox state. International Journal of Clinical Pharmacology and Therapeutics. 2003; **41**(8): 346–353.

13 Khan F, Litchfield SJ, Stonebridge PA, Belch JJF. Lipid-lowering and skin vascular responses in patients with hypercholesterolaemia and peripheral arterial obstructive disease. Vascular Medicine. 1999; **4**(4): 233–238.

14 Lim SC, Caballero AE, Arora S, Smakowski P, Bashoff EM, Brown FM, LoGerfo FW, Horton ES, Veves A. The effect of hormonal replacement therapy on the vascular reactivity and endothelial function of healthy individuals and individuals with type 2 diabetes. Journal of Clinical Endocrinology and Metabolism. 1999; **84**(11): 4159–4164.

15 Manson JAE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R, Strickland OL, Wong ND, Crouse JR, Stein E, Cushman M. Estrogen plus progestin and the risk of coronary heart disease. New England Journal of Medicine. 2003; **349**(6): 523–534.

16 Menon DV, Vongpatanasin W. Effects of transdermal estrogen replacement therapy on cardiovascular risk factors. Treatments in Endocrinology. 2006; **5**(1): 37–51.

17 Naessen T, Lindmark B, Lagerström C, Larsen HC, Persson I. Early postmenopausal hormone therapy improves postural balance. Menopause. 2007; **14**(1): 14–19.

18 O'Connell MB. Pharmacokinetic and pharmacologic variation between different estrogen products. Journal of Clinical Pharmacology. 1995; **35**(9 SUPPL.).

19 Peterson LR, Eyster D, Dávila-Román VG, Stephens ALP, Schechtman KB, Herrero P, Gropler RJ. Short-term oral estrogen replacement therapy does not augment endothelium-independent myocardial perfusion in postmenopausal women. American Heart Journal. 2001; **142**(4): 641–647.

20 Petrofsky J, Lee S. The effects of type 2 diabetes and aging on vascular endothelial and autonomic function. Med Sci Monit. 2005; **11**(6): CR247–254.

21 Powers MS, Schenkel L, Darley PE. Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17β-estradiol: Comparison with conventional oral estrogens used for hormone replacement. American Journal of Obstetrics and Gynecology. 1985; **152**(8): 1099–1106.

22 Prázný M, Ježková J, Horová E, Lazárová V, Hána V, Kvasnička J, Marek J, Pecen L, Škrha J, Kršek M. Impaired microvascular reactivity and endothelial function in patients with Cushing's syndrome: influence of arterial hypertension. Physiological Research. 2008, [in press].

23 Psaty BM, Heckbert SR, Atkins D, Lemaitre R, Koepsell TD, Wahl PW, Siscovick DS, Wagner EH. The risk of myocardial infarction associated with the combined use of estrogens and progestins in postmenopausal women. Archives of Internal Medicine. 1994; **154**(12): 1333–1339.

24 Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SAA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. Journal of the American Medical Association. 2002; **288**(3): 321–333.

25 Scott J, Ross B, Anderson C, Archer DF. Pharmacokinetics of percutaneous estradiol: A crossover study using a gel and a transdermal system in comparison with oral micronized estradiol. Obstetrics and Gynecology. 1991; **77**(5): 758–764.

26 Sidney S, Petitti DB, Quesenberry CP, Jr. Myocardial Infarction and the Use of Estrogen and Estrogen-Progestogen in Postmenopausal Women. Ann Intern Med. 1997; **127**(7): 501–508.

27 Škrha J, Prázný M, Haas T, Kvasnička J, Kalvodová B. Comparison of laser-Doppler flowmetry with biochemical indicators of endothelial dysfunction related to early microangiopathy in Type 1 diabetic patients. Journal of Diabetes and its Complications. 2001; **15**(5): 234–240.

28 Vehkavaara S, Silveira A, Hakala-Ala-Pietilä T, Virkamäki A, Hovatta O, Hamsten A, Taskinen MR, Yki-Järvinen H. Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. Thrombosis and Haemostasis. 2001; **85**(4): 619–625.

29 Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells: A potential mechanism for steroid hormone regulation of endothelial function. Circulation. 1996; **94**(4): 727–733.

30 Vuilleumier P, Decosterd D, Maillard M, Burnier M, Hayoz D. Postischemic forearm skin reactive hyperemia is related to cardiovascular risk factors in a healthy female population. Journal of Hypertension. 2002; **20**(9): 1753–1757.

31 Wakatsuki A, Ikenoue N, Shinohara K, Watanabe K, Fukaya T. Effect of Lower Dosage of Oral Conjugated Equine Estrogen on Inflammatory Markers and Endothelial Function in Healthy Postmenopausal Women. Arterioscler Thromb Vasc Biol. 2004; **24**(3): 571–576.