ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 1, 167–171
www.aaem.pl

Effect of transdermal hormone therapy on platelet haemostasis in menopausal women

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Stachowiak G, Pertyński T, Pertyńska-Marczewska M. Effect of transdermal hormone therapy on platelet haemostasis in menopausal women. Ann Agric Environ Med. 2015; 22(1): 167–171. 10.5604/12321966.1141389

Abstract

Introduction. Despite the undeniably positive effect on the quality of life of menopausal women, menopausal hormone therapy (HT) also has negative side-effects, which include, among others, thromboembolic complications.

Objective. To assess the effect of a popular type of this therapy – transdermal HT on platelet hemostasis, which plays a significant role in intravascular coagulation.

Materials and method. The study group consisted of 92 postmenopausal women: 1) group G1 (n=30), treated with transdermal HT (17β-estradiol 50 μg/day plus NETA 170 μg/day); 2) group G2 (n=31), treated with the above transdermal HT and low dosage of acetylsalicylic acid (ASA); 3) control group P (n=31). All the women qualified for the study had two or more risk factors for arterial thrombosis, such as: smoking, hypertension, visceral obesity, hypercholesterolaemia, hypertriglyceridaemia, elevated levels of PAI-1, and increased fibrinogen, increased activity of coagulation factor VII.

Results. After three months of therapy, in the G1 group there was a decrease in platelet count ($p = 0.004$) and a decrease in GP IIb/IIIa – a platelet receptor for fibrinogen ($p = 0.022$). In the G2 group, no changes in the tested parameters were observed.

Conclusions. 1) Transdermal HT in the form of combined, estrogen-progestogen patches favourably modifies platelets haemostasis, reversing the adverse effects that occur after menopause. 2) The use of low ASA doses as a thromboprophylaxis in short-term transdermal HT is not necessary.

Key words

menopause, transdermal hormone therapy, platelets, GP IIb/IIIa

INTRODUCTION

The use of menopausal hormone therapy (HT) is associated with a number of side- effects and potential complications, such as breast cancer or venous thromboembolism [1, 2, 3, 4].

The menopausal hormone therapy risk is difficult to assess, as a large part of women who require such a treatment due to premature menopause, severe climacteric symptoms, postmenopausal osteoporosis, and symptoms associated with urogenital atrophy, are also subject to risk factors for cardiovascular diseases, including thromboembolic events in the arterial system [5]. This may result in restricted availability of HT to a large part of the menopausal population.

Therefore, the impact of HT used by women during the menopausal period affecting their risk of vascular diseases, especially when risk factors for thromboembolic complications are already present, remains a very important issue [6].

In the light of current knowledge, this problem has not been fully resolved because researchers who study vascular problems in HT, surprisingly often ignore the issue of the risks of thromboembolic events.

In the presented study, women qualified for HT were younger than in large RCTs (randomized controlled trials), such as the WHI (Women's Health Initiative), which reduced the potential risk of atherogenic events in the arterial system. Indeed, the population of women in their fifties who suffer from severe climacteric symptoms, are the target group in which HT should be used.

In this study, HT was used in a group of patients with risk factors for arterial thrombosis, as there is a clear concern and resistance to HT in these women. Since the risk of thromboembolic events is highest in the first months of HT, the observation period was limited to the first three months of treatment.

Transdermal therapy was used which, according to the available data, is safer in terms of thromboembolic events [7]. In some patients, an additional thromboprophylaxis was introduced.

The study focuses on the examination of platelets, since these eucariotic, cytoplasmic fragments of bone marrow megakaryocytes play two important functions in haemostasis: 1) creation of vascular plugs – so-called primary haemostasis; 2) involved in further coagulation reactions, known as secondary haemostasis. Both have a clear impact on the potential risk of thromboembolic events [8].

Objective. The aim of the study was to evaluate the effect of transdermal HT and thromboprophylaxis used in menopausal women on platelet haemostasis.

MATERIALS AND METHOD

The study comprised 92 postmenopausal women, of whom 61 women formed the study group and the remainder (n = 31)

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Received: 09 November 2012; Accepted: 23 September 2013

constituted the control group. All the women at baseline (T_0) had a gynecological examination performed, combined with a palpable breast examination, mammography and transvaginal ultrasound (TVU – endometrial thickness assessment, assessment of vascular flow in the uterine arteries – PI, RI, S/D). Fasting glucose, leukocytosis, C-reactive protein, erythrocyte sedimentation rate (ESR), serum ALT, AST and bilirubin were determined at the same time.

The women who took part in the study presented two or more of risk factors for arterial thrombosis, such as: smoking, hypertension, visceral obesity, hypercholesterolaemia, hypertriglyceridaemia, elevated levels of PAI-1, increased fibrinogen, and increased activity of coagulation factor VII. Indications for the use of HT in the study group were intensified climacteric symptoms, mostly in the form of severe and frequent hot flashes, accompanied by excessive night sweats.

In order to assess the severity of the above symptoms, a 51-points Blatt-Kupperman scale was used, based on 11 parameters. Women who qualified for HT had a score greater than 20 points on the scale. Among women enrolled for HT, the following groups were distinguished:

1) G1 (n=30) – which used transdermal hormone therapy in the form of patches, consisting of 17β-estradiol (E_2) in a dose of 50 μg/day plus norethisterone acetate (NETA) at a dose of 170 μg/day;
2) G2 (n=31) – where together with the above transdermal HT (the same patches – 50 μg E_2 + 170 μg NETA) low-doses of acetylsalicylic acid (ASA) were administered.

The observational period lasted three months. The control group P (n = 31) included age-matched menopausal women who were not enrolled for HT. The general characteristics of the studied women population is presented in Table 1.

Table 1. General characteristics of the studied women population

Group	G1	G2	P	*p-value*
Numbers	30	31	31	>0.05
Age [years]	54.3 ±5.58	56.4±4.75	55.4±4.31	0.17
Menopausal age [months]	56.0±49.22	76.2±56.70	65.8±50.51	0.16
BMI [kg/m^2]	26.5±4.27	26.7±3.49	26.3±3.98	0.88
WHR	0.83±0.05	0.82±0.06	0.82±0.05	0.77
Waist circumference [cm]	82.2±4.9	81.2±5.3	81.3±5.1	0.72
Fasting glucose [mg%]	87.8±15.73	82.4±9.04	84.5±11.04	0.24
Cholesterol [mg%]	225.4±32.48	228.1±21.74	226.6±27.85	0.76
Triglycerides [mg%]	107.6±48.16	109.6±51.15	107.3±47.88	0.89
CRP [mg/dl]	0.60±0.02	0.58±0.08	0.61±0.05	0.87
Leukocytes[10^3/ml]	5681±1622	5569±1351	5574±1557	0.80
ESR [mm/h]	12.4±10.68	9.9±4.51	11.3±77.46	0.32
Endometrial thickness [mm]	3.8±0.73	3.6±0.70	3.5±0.71	0.52
PI-av. uterine artery	1.90±0.62	1.82±0.53	1.83±0.55	0.66
RI-av. uterine artery	0.77±0.08	0.75±0.07	0.75±0.08	0.33
SD-av. uterine artery	5.3±2.05	4.5±1.67	4.9±1.87	0.18
RR sys [mmHg]	131±17.7	137±16.70	133±16.68	0.34
RR dias [mmHg]	81±10.52	84±8.86	83±8.92	0.28
Kupperman Index	27.9±8.26	28.5±9.38	28.1±9.11	0.84
Smoking [%]	16.6	16.1	16.1	>0.05

Parameter values expressed as mean ± standard deviation (SD); p-value – between G1 and G2; there were no statistically significant differences (p> 0.05) between groups G1 and G2 and group P.

Platelet count (PLT) and the expression of platelet fibrinogen receptor – GP IIb/IIIa were determined twice in the blood of each patient: at the baseline (T_0) and after 3 months (T_3). Platelet count were determined using an analyzer Baker 810. For the assessment of GP IIb/IIIa flow cytometry was used (FACSCalibur Instrument [Becton Dickinson]. analysis in Lysis II software).

Statistics. Distributions of the parameters before hormone therapy is as an arithmetic mean. together with its standard deviation. To evaluate density distribution. the kernel density estimator with Gaussian density function was applied. The smoothing parameter selection was performed by use of the Sheater and Jones' method. The effect of hormone therapy after three months was assessed by comparing selected parameters between the two groups using a linear regression model. In this model. initial values of the studied parameters were taken into account to correct the differences between the two groups before the onset of HT. For all regression models. a sensitivity analysis of outliers. identified by the analysis of residuals. was performed. All statistical calculations were made using the RR package Development Core Team [9].

RESULTS

In the G1 group. after three months the applied hormonal therapy resulted in a decrease in platelet count (p = 0.004). as well as a decrease in the expression of GP IIb/IIIa (p=0.022). In the G2 group. where the above transdermal HT was used in combination with low-dose ASA. there were no statistically significant changes in the studied haemostatic parameters.

Comparison of haemostatic parameters after three months (T_3) between the groups G1. G2 and the control group P – (values adjusted by initial levels of studied parameters (T_0) is shown in Figures 1 and 2.

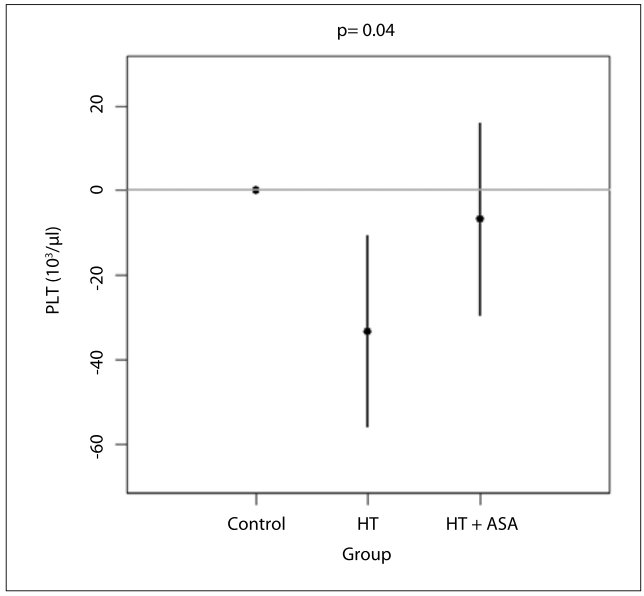
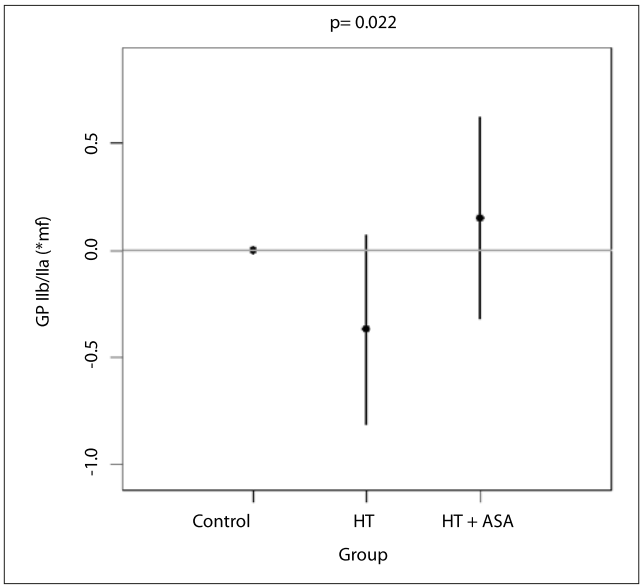


Figure 1. Changes in platelets count (PLT) after 3 months (T_3) – comparison between the groups G1. G2 and the control group P



*mf – mean fluorescence

Figure 2. Changes in GP IIb/IIIa after 3 months (T_3) – comparison between groups G1, G2 and control group P

DISCUSSION

The issue of safe hormone therapy to address menopausal disorders is complex and requires the physician to have comprehensive knowledge of the medical sciences, including pharmacology, endocrinology, oncology, internal medicine and cardiology. This is particularly important in the context of potential thromboembolic complications in the cardiovascular system of menopausal women [10, 11, 12].

The menopause, in addition to a number of common complaints, such as hot flushes, night sweats or depressive disorders, comes with a range of adverse changes in haemostasis [13, 14].

The available data, including the authors' previous studies, show that postmenopausal women are subject to a number of adverse changes in the coagulation and fibrinolytic systems. In comparison to younger women (30-years-old), women after their 50s have higher concentrations of fibrinogen, increased activity of coagulation factor VII, as well as higher concentrations of plasminogen activator inhibitor -1 (PAI-1), and lower concentrations of tissue plasminogen activator (t-PA). This can be defined as activation of coagulation with concurrent impaired fibrinolysis, or otherwise as prothrombotic alterations in haemostasis [15]. A decrease in platelet count and a decrease in the expression of GP IIb/IIIa, which was observed in the current study, proved to have beneficial effects of the applied HT on platelet haemostasis.

However, up-to-date knowledge of sex steroids and HT effects on platelet hemostasis is not consistent:

- the use of low-dose, oral HT (1 mg E_2 + 0.5 mg NETA per day) in postmenopausal women (n=26) with elevated cholesterol, resulted in a decrease of serum P-selectin levels ($p<0.0001$) and a weak growth trend ($p=0.13$) for the expression of P-selectin on platelet surface [16];
- the use of sex steroids in healthy postmenopausal women for eight weeks resulted in an increase in the concentration of thromboxane B_2 (TXB_2), with no effect on the expression of P-selectin in the group receiving continuous oral HT (n=8; E_2+norethisterone). In the group treated with E_2 alone (n=9), decreased serum levels of TXB_2 were observed. There were no statistically significant changes in the expression of P-selectin [17];
- both oral estrogen therapy (ET, n=16) as well as oral, sequential HT (E_2 with trimegestonone, n=14 or E_2 with dydrogesterone, n=14), used for twelve weeks in postmenopausal women, resulted in increased platelet activation measured by increased P-selectin and GP 53 expression on the platelets surface [18];
- after six months of continuous oral HT (2 mg E_2+1 mg NETA/day) in postmenopausal women, no significant changes in the parameters of platelet aggregation were noticed. There was no effect of endogenous, platelet derived nitric oxide (NO) on the aggregation parameters [19];
- after six weeks of cyclic oral HT, at a daily dose of 0.625 mg CEE (conjugated equine estrogens) + 75 mg of levonorgestrel in the second phase, a decrease in fatty acids content in the cell membrane of platelets (arachidonic acid by 17.8%, linolenic acid by 8.1%), together with a small (statistically insignificant) increase in their blood concentration and an increase in the average PLT volume was observed, which may indicate their increased reactivity during HT [20].

There is little available data on the effects of transdermal HT. In the case of cyclic transdermal E_2 (50 mcg/day) combined with oral medroxyprogesterone acetate (MPA) in postmenopausal women, a decrease in free radical production (and lipid oxidation reduction) in platelet cell membrane was noticed during the first 25 treatment days. Such an effect was also observed when MPA was administrated alone at a dose of 20 mg/day [21].

In another study, where the effects of oral (2 mg E_2 /day) and transdermal ET (50 mcg E_2/day) on platelet activation parameters were compared, it was found that both routes of estrogen administration, affected neither the expression of P-selectin, nor the Ca^{2+} metabolism in unstimulated platelets. However, after thrombin stimulation, platelet activation (measured by increased expression of P-selectin) were more pronounced in the group treated with transdermal E_2. Also, an increase in the concentration of cytosol Ca^{2+} (a feature of activated platelets) in platelets stimulated by ADP (adenosine diphosphate) occurred only in the group where E_2 (in this case, combined with progesterone) was administered transdermally, not orally [22]. In an Italian study, transdermal ET caused a positive increase in platelet NOS (nitrogen oxide synthase) activity in healthy postmenopausal women, which was also observed in the group of type 2 diabetic postmenopausal women, although to a lesser extent [23].

In the presented study, the use of thromboprophylaxis (low ASA doses) did not show any changes in haemostatic parameters during three months of the treatment, which indicates that there is no need for the above thromboprophylaxis during transdermal HT in postmenopausal women with risk factors for arterial thrombosis. There have been no cases of thrombosis, neither arterial nor venous, during the treatment time.

However, the observed, beneficial effect of transdermal HT (50 µg E_2 + 170 µg NETA) on platelet haemostasis, with no adverse effects on coagulation and fibrinolytic systems, nor on vascular endothelium, is contradictory to the results of the

WHI or HERS (Heart and Estrogen-Progestin Replacement Study), where the observed increased risk of thromboembolic events (especially in the arterial system) was the reason for a clear retreat from HT [24, 25].

But is this opposing nature of the above studies so clear and still relevant? The basic elements of patients qualification for HT (bearing in mind primarily the safety of therapy), such as patient's age, menopausal age, or the presence of other thromboembolic risk factors, are not only essential in the daily clinical practice, but also have a decisive effect on the final results of several studies focusing on menopausal HT.

A thorough analysis performed by Grodstein [26] a participant in the NHS (Nurses Health Study), showed that in both NHS and WHI, when HT was initiated in younger women (in NHS up to four years after menopause, in the WHI study, up to ten years after menopause – a relatively small group), the risk of a heart attack did not increase, but inversely – in the NHS was lower by 34%, and reduced by 11% in the WHI (it should be stressed that in both studies. HT unfavourably increased the risk of cardiovascular complications in the group of older women) [26].

In the 2007 re-analysis of the results of the WHI study, special attention was paid to the role of absolute age and menopausal age of patients who used HT. It was been shown, for example, that in women with a menopausal age of less than ten years, the hazard ratio (HR) for ischemic coronary heart disease (IHD) was 0.76 (95% CI: 0.50–1.16), in the group with 10–19 years after menopause – 1.10 (95% CI: 0.84–1.45), and for the menopausal age of twenty years and above – 1.28 (95% CI: 1.03–1.58); p-value for the trend – 0.02. Calculated absolute increase in the risk of IHD for women up to ten years after menopause was negative (a decrease in risk!) and equalled -6/10.000 persons/year; for other groups of women. 10–19 and ≥ 20 years after menopause, the risk had been already positive, equalling 4/10.000 persons/year and 17/10.000 persons/year respectively. In the age group 50–59 years, the HR for IHD was 0.93 (95% CI: 0.65–1.33), and the absolute risk increase (negative!) was calculated at -2/10.000 persons/year; in the age range of 60 – 69 years. HR and absolute increase in risk was 0.98 (95% CI: 0.79–1.21) and -1/10.000 persons/year; in the group 70–79 years – 1.26 (95% CI: 1.00–1.59) and 19/10.000 persons/year (p-value for the trend – 0.16). The main conclusion of this re-analysis was that women who begin using HT shortly after menopause tend to have a lower risk of IHD, compared to an increased risk in HT women occurring long after menopause [27].

The positive aspect is that the latest reports on HT take the 'vascular odium' off hormone therapy. For gynecologists who prescribe HT in their daily practice, the fact that an increasing number of scientific studies, including those that are large and population-based, report potentially beneficial effects of HT on the cardiovascular system in menopausal women, is extremely encouraging. It is a fact that HT is one of the basic elements of menopausal care that has a positive effect on the women's quality of life (QoL) [28, 29].

However, to provide such an HT effect, it is crucial to choose a hormone therapy that will best suit the needs of individual patient (dose, composition, route of administration), apply it timely once all contraindications had been excluded, and perform a continuous evaluation of the risks associated with this type of treatment. Such are the basic elements of an individually tailored therapy which should be considered in every case [30].

CONCLUSIONS

1) Transdermal hormone therapy, in the form of combined estrogen-progestogen patches, favourably modifies platelet haemostasis, reversing the adverse effects that occur after menopause.
2) The use of low ASA doses, as a thromboprophylaxis in short-term transdermal HT, is not necessary.

REFERENCES

1. The 2012 Hormone Therapy Position Statement of the North American Menopause Society. Menopause 2012; 19(3): 257–271.
2. Bojar I, Biliński P, Boyle P, Zatoński W, Marcinkowski JT, Wojtyła A. Prevention of female reproductive system cancer among rural and urban Polish pregnant women. Ann Agric Environ Med. 2011; 18: 183–188.
3. Krzyżak M, Maślach D, Juczewska M, Lasota W, Rabczenko D, Marcinkowski JT, Szpak A. Differences in breast cancer incidence and stage distribution between urban and rural female population in Podlaskie Voivodship. Poland in years 2001–2002. Ann Agric Environ Med. 2010; 17: 159–162.
4. Krzyżak M, Maślach D, Bielska-Lasota M, Juczewska M, Rabczenko D, Marcinkowski JT, Szpak A. Breast cancer survival gap between urban and rural female population in Podlaskie Voivodship, Poland, in 2001–2002. Population study. Ann Agric Environ Med. 2010; 17: 277–282.
5. Stachowiak G, Pertyński T. Najnowsze doniesienia dotyczące bezpieczeństwa hormonalnej terapii zastępczej dla układu sercowo-naczyniowego. Prz Menopauzalny. 2012; 1: 1–4 (in Polish).
6. Rosano G, Vitale C, Spoletini I, Fini M. Cardiovascular health in the menopausal woman: impact of the timing of hormone replacement therapy. Climacteric 2012; 15: 299–305.
7. Pertyński T, Stachowiak G. Przezskórna terapia okresu menopauzy – state of the art in 2010. Prz Menopauzalny 2010; 2: 71–77 (in Polish).
8. Plow EF, Ginsberg MH. The molecular basis for platelet function. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein P, McGlave P. Hematology. Basic principles and practice. Churchill Livingstone. New York, Edinburgh, Londyn. Philadelphia, San Francisco, 2000.pp.1741–1752.
9. A language and environment for statistical computing (2007). R Foundation for Statistical Computing. Vienna. Austria http://www.R-project.org (access: 2102.11.09).
10. Stachowiak G, Pertyński T. Bezpieczeństwo kardiologiczne terapii hormonalnych okresu menopauzy. Prz Menopauzalny. 2009; 6: 315–319 (in Polish).
11. Paszkowski T, Sikorski R, Jakiel G. Medycyna menopauzalna u progu trzeciego tysiąclecia. Ginekol Pol. 2002; 72(12A): 1370–1376 (in Polish).
12. Rzymski P, Opala T. Elastography as a new diagnostic tool to detect breast cancer – evaluation of research and clinical applications. Prz. Menopauzalny. 2011; 5: 357–362.
13. Stachowiak G, Faflik U, Stetkiewicz F, Pertyński T. Cardiovascular diseases in women – impact of the menopausal period. Prz Menopauzalny. 2006; 6: 382–387.
14. Humeniuk E, Bojar I, Owoc A, Wojtyła A, Fronczak A. Psychosocial conditioning of depressive disorders in post-menopausal women. Ann Agric Environ Med. 2011; 18(2): 441–445.
15. Stachowiak G, Połać I, Jędrzejczyk S, et al. Postmenopausal status, coagulation and fibrinolysis. Pol J Gynaecol Invest. 2001; 3: 97–100.
16. Peverill RE, Smolich JJ, Malan E, et al. Comparison of effects of pravastatin and hormone therapy on soluble P-selectin and platelet P-selectin expression In postmenopausal hypercholesterolemic women. Maturitas 2006; 53: 158–165.
17. Oliveira RL, Aldrighi JM, Gebara OE, et al. Postmenopausal hormone replacement therapy increases plasmatic thromboxane beta 2. Int J Cardiol. 2005; 99: 449–454.
18. Thijs A, van Baal WM, van der Mooren MJ, et al. Effects of hormone replacement therapy on blood platelets. Eur J Clin Invest. 2002; 32: 613–618.
19. Teede HJ, McGrath BP, Turner A, et al. Effects of oral combined hormone replacement therapy on platelet aggregation In postmenopausal women. Clin Sci (Lond). 2001; 100: 207–213.
20. Ranganath LR.Christofides J. Semple MJ. Increased mean platelet volume after estrogen replacement therapy. Ann Clin Biochem. 1996; 33: 555–560.

21. Traquilli AL, Mazzanti L, Cugini AM, et al. Transdermal estradiol and medroxyprogesterone acetate in hormone replacement therapy are both antioxidants. Gynecol Endocrinol. 1995; 9: 137–141.
22. Garcia-Martinez MC, Labios M, Hermenegildo C, et al. The effect of hormone replacement therapy on Ca2+ mobilization and P-selectin (CD62P) expression in platelets examined under flow cytometry. Blood Coagul Fibrinolysis. 2004; 15: 1–8.
23. Martina V, Bruno GA, Origlia C, et al. Transdermal oestradiol replacement therapy enhances platelet constitutive nitric oxide synthase activity In postmenopausal women with type 2 diabetes mellitus. Clin Endocrinol (Oxf). 2002; 57: 371–375.
24. Hulley S, Grady D, Bush T, et al. For the Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 1998; 280: 605–613.
25. Writing group for the women's health initiative investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Principal results from the women's health initiative randomized controlled trial. JAMA 2002; 288: 321–333.
26. Grodstein F, Manson JE, Stampfer MJ. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. J Womens Health. 2006; 15: 35–44.
27. Rossouw JE, Prentice RL, Mason JE, et al. Postmenopausal Hormone Therapy and Risk of Cardiovascular Disease by Age and Years Since Menopause. JAMA 2007; 297: 1465–1477.
28. Skrzypulec V, Drosdzol A, Ferensowicz J. Ocena jakości życia kobiet w okresie klimakterium. Ginekol Pol. 2004; 75(5): 373–381 (in Polish).
29. Żołnierczuk-Kieliszek D, Kulik TB, Jarosz MJ, Stefanowicz A, Pacian A, Pacian J, Janiszewska M. Quality of life in peri- and post-menopausal Polish women living in Lublin Province – differences between urban and rural dwellers. Ann Agric Environ Med. 2012; 19(1): 129–133.
30. Stachowiak G, Tomasz Pertyński T. Bezpieczeństwo zakrzepowo-zatorowe przezskórnej terapii hormonalnej. Prz Menopauzalny. 2008; 6: 285–290 (in Polish).