

## GENERAL

CONTROVERSIES OVER HORMONE REPLACEMENT THERAPY.  
HRT AS A RISK FACTOR OF BREAST CANCER  
IN POSTMENOPAUSAL WOMEN

BARBARA E. LICZNERSKA and WANDA BAER-DUBOWSKA

University of Medical Sciences in Poznań, Department of Pharmaceutical Biochemistry  
6 Grunwaldzka Str., 60-780 Poznań

**Abstract:** Hormone replacement therapy (HRT) was introduced in order to overcome the problems associated with tissue catabolism to which postmenopausal women are particularly prone. However, it is widely accepted that estrogen is essential for breast tumorigenesis. Therefore, the question is raised as to whether HRT in a long perspective can increase the risk of breast cancer development. Moreover, the results of many observational studies do not fully support the protective effects against e.g., cardiovascular diseases. This mini review summarizes the results of recent studies indicating the benefits and risks related to HTR.

**Keywords:** hormone replacement therapy; cardioprotection; osteoporosis; HRT cancer risk factor

About five million menopausal and postmenopausal women live currently in Poland and this number is growing (1). The menopause is complete with the loss of ovarian estrogen production. However, substantial amounts of a weak estrogen, esteron, are produced by a peripheral tissue e.g., the aromatization of androstenedione (Figure1) (2, 3). The levels of esteron in circulation are not sufficient to suppress pituitary gonadotropin levels; thus, marked increases of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are characteristic of the postmenopausal years. Gonadotropin-releasing hormone (GnRH) may also be involved at the onset of menopause. Postmenopausal women are particularly prone to two problems associated with tissue catabolism. Esteron is not always able to prevent the atrophy of secondary sex tissues, particularly the epithelium of the lower urinary tract and vagina. Osteoporosis is a major health problem for older individuals while women with the most severe decrease in bone mass have lower than normal estrogen levels. Another effect of reduced estrogen production is changes in the cardiovascular system (4-6).

Prevention of all these negative changes in postmenopausal women's bodies was the reason for implementing exogenous estrogens for therapeutic purposes. Hormone replacement therapy (HRT) has developed over the decades from the simple (estrogen alone) to the complex (cyclic or continuous regimens with estrogens and progestins). The type(s), doses, and forms of hormone are essential to ratio-

nalize HRT. The most common treatment regimen includes: natural estrogens (i.e., 17 $\beta$ -estradiol, estrin, and conjugated equine estrogens) in a combination with one progestin (e.g., norethisteron, lynestrenol, medroxyprogesterone acetate, retroprogesterone, didrogesteron, duphaston, micronised progesterone) (1, 5, 7).

Despite the presumed advantages, however, observational studies and clinical trials do not give a clear answer about the absolute safety of HRT usage and do not fully support their protective effects. The therapy seems to have many side effects, which include e.g., vaginal bleeding, a higher clots probability, an increased risk of breast and uterine cancer; thus, a critical evaluation of the HRT benefits and risks is still necessary.

### Endogenous estrogens

In premenopausal women, estrogens are mainly produced in and secreted from gonads. However, a number of extragonadal sites for estrogen synthesis exist, which in men and in postmenopausal women become a major source of estrogens which includes adipose tissue, skin, bones, vascular endothelium, aortic smooth muscle cells, brain, breast and endometrial cancer tissues (8-10). Gonadal and extragonadal sites differ substantially: 1) an estrogen synthesis beyond gonads acts at the local tissue level and only when it escapes the local metabolism are its products released to the circulation; 2) extragonadal sites are incapable of converting cholesterol

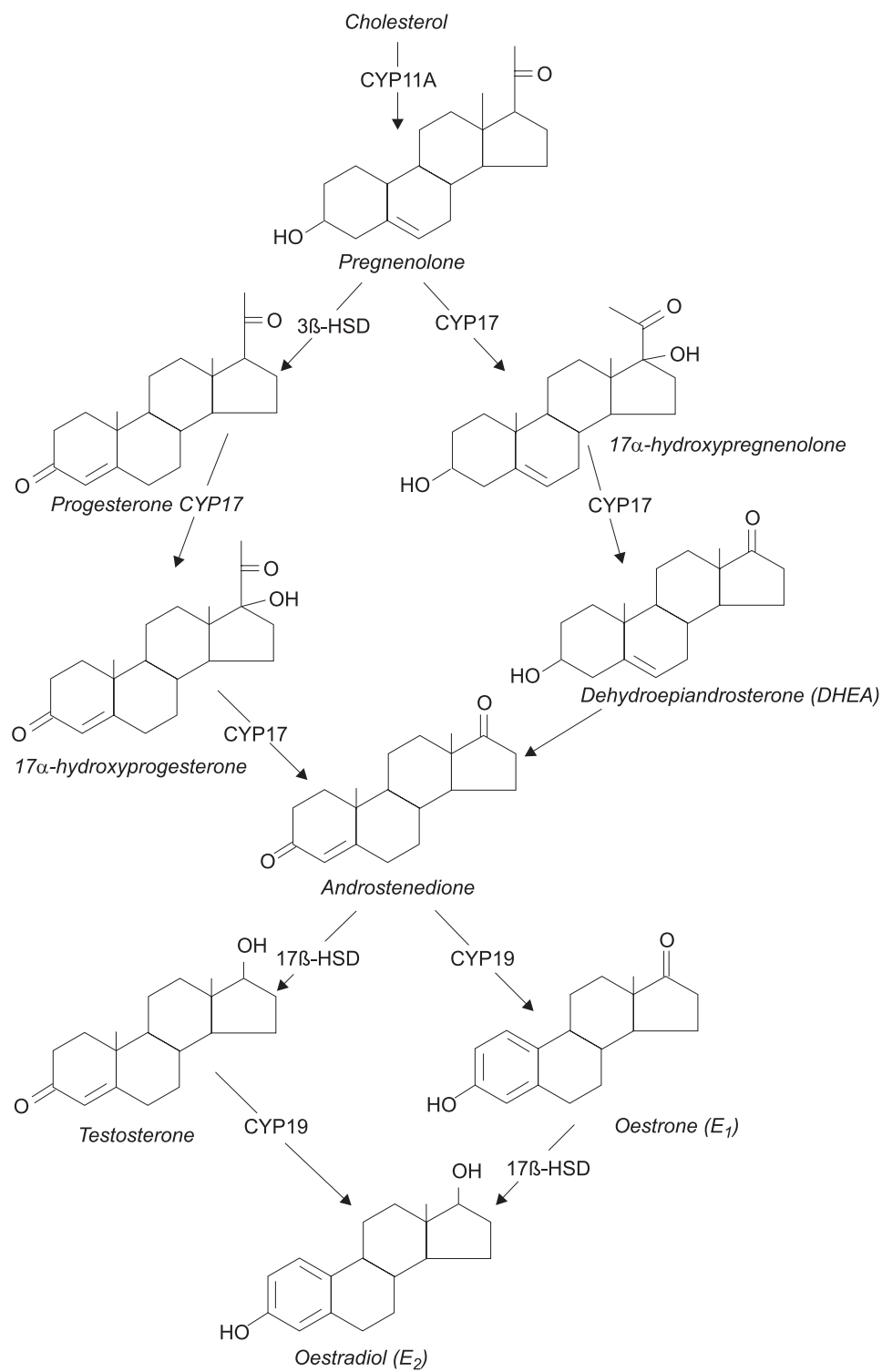


Figure 1. Biosynthesis of estrogens (37).

to the C<sub>19</sub> steroids; thereby an extragonadal biosynthesis of estrogens depends on an external source of precursors: testosterone (in men), androstenedione, dehydroepiandrosterone (DHEA), DHEA sulphate (DHEAS), and estrone sulphate (in women, mainly postmenopausal ones).

Estrogen acts in an intracrine, paracrine, or endocrine manner, wherever estrogen receptors exist. Estrogens stimulate the development of tissues involved in reproduction. Under estrogen stimulation, the vaginal and uterine epithelium proliferates and differentiates. Estrogens are responsible for the development of secondary sex characteristics in females (4). Estradiol also has an anabolic effect on bones and cartilages providing high bone mineral density. Estrogen production in bones appears to be vital for the prevention of osteoporosis (11). By affecting peripheral blood vessels, estrogens cause a rapid and long-term vasodilatation by a nitric oxide (NO) release with or without altering gene expression. Thus, premenopausal women are relatively protected against cardiovascular diseases in comparison with men. Besides, estrogens play a major role in body homeostasis by influencing the electrolyte- and water balance, and temperature regulation. An extragonadal synthesis in several brain sites has an influence on sexual behaviour, and may have a role in the maintenance of cognitive function (4, 12). Thus, estrogens play several important physiological roles and the hormonal supplementation seems to be a reasonable alternative to diminished natural production.

### Potential benefits of HRT

#### Cardiovascular system

Cardiovascular disease (CD) is the major cause of death among postmenopausal women (11). Most epidemiological studies from the last decade of the 20<sup>th</sup> century have indicated the primary prevention of cardiovascular disease among current HRT users (5, 11). However, these studies (usually on surrogates' endpoints, like lipid profile) were not completely reliable. The need for prospective, randomized, controlled studies was obvious. Therefore, the data from the Heart and Estrogen/Progestin Replacement Study (HERS) were surprising (13). The HERS was the first large randomized, placebo-controlled, secondary prevention trial that monitored 2763 women with established coronary heart disease (CHD) for an average of 4.1 years of HRT (conjugated estrogens plus medroxyprogesterone acetate oral tablets) to determine the risk of further CHD events. The results suggested no cardioprotective effects in women with established

CHD and even showed a potential for increased CHD events during the first year of therapy. The findings of the HERS II trial, the unblinded continuation of HERS (reported in 2002, with 2321 participants, an additional follow-up average of 2.7 years, the total average follow-up of 6.8 years) showed no differences between HRT and the placebo in terms of primary and secondary CHD events (14). In 2000, the Women's Health Initiative (WHI) Project found a small, increased risk of cardiovascular disease, and the Estrogen Replacement and Atherosclerosis (ERA) Studies showed no effect on the atherosclerosis risk after three years (15, 16). Also, other reports indicated on increasing rather than a decreasing tendency among HRT users and a risk of coronary artery disease (17). Contradictory, however, in the clinical trial of Madsen et al. (18), were the beneficial effects of HRT shown on the blood fibrinolytic capacity. Similarly, Cerquetani et al. (19) presented data indicating that HRT improved the endothelial function and reduced the plasma levels of endothelin-1 in postmenopausal women at risk of cardiovascular disease. To sum up, the influence (positive and negative) of HRT on cardiac events requires critical evaluation. The results of current studies do not support the application of HRT for protection against cardiovascular diseases.

#### Bone structures

The vasomotor symptoms of menopause and the negative impact on bone mineral density (BMD) due to chronic estrogen deficiency were two major indications for hormone therapy during and after menopause in the last decade of the last century (5, 11). Low BMD is an important fracture risk of the vertebra and hip (13). The results of epidemiological studies supported the use of HRT in order to prevent osteoporotic changes after menopause. Clinical trials were in the minority, and the results in favour of HRT were unreliable (too small and poorly analyzed) (20). Recent studies on HRT (15, 20-22) suggested that: 1) the estrogen supplementation must be continued (10 years after HRT cessation, the BMD and fracture risk seemed to be similar to the never-users of HRT); 2) low-dose HRT is safe and effective for the prevention of postmenopausal bone loss; 3) the prevention is not related to the age of the patient; 4) there are many alternatives to HRT for bone loss prevention. It has to be noticed that the WHI (15) study was the first large (27 000 women), randomized, placebo-controlled trial to determine the absolute risks and benefits of estrogen therapy in healthy, postmenopausal women.

### Dementia

The prognosis for 2020 estimates 35 million Alzheimer's disease sufferers (1). When estrogen receptors have been found in numerous sites in the brain, estradiol becomes potentially relevant to the pathogenesis and treatment of Alzheimer's disease, the most common cause of dementia. The observation of the disease mostly among female patients supported the statement. Unfortunately, at present, there is no evidence that at least one-year HRT (namely estrogens) prevents memory loss or slows the progression of dementia (11, 20).

### Insulin sensitivity

The menopause period is undoubtedly associated with body fat distribution changes with preference for the abdomen; besides, decreased insulin sensitivity might be observed (23) and may predict type 2 diabetes. Recently, Sites et al. (6), regardless of their previous hypothesis, found a 17 % decrease in insulin sensitivity in the HRT group by 6 months persisted for two years. Fortunately, this reduction was reversible after one year of HRT discontinuing. It was a preliminary trial while the patients group was relatively small, they used only one regimen (conjugated estrogens plus medroxyprogesterone acetate), and all the women were early postmenopausal. Interestingly, no significant affect on body composition or body fat distribution was found. Thus, diabetes prevention by HRT should be the subject of more detailed studies.

### Cancer risk related to HRT

It is clear that hormones affect carcinogenesis both by epigenetic and genetic mechanisms (24, 25). The possible effect of HRT on cancer induction is considered the main risk factor related to this treatment. The main targets are endometrial, ovarian, cervical, colon, and breast cancer. The pathogenesis of all these carcinomas seems to be connected more or less with changes in estrogens level. Most observations have shown that appropriate HRT regimens (i.e., progestins for at least 10 days in each cycle) can reduce the risk of endometrial hyperplasia (5, 20, 26-28). In the case of ovarian and cervical cancer there is no epidemiological or clinical evidence that HRT can be carcinogen (26-28). In contrast, some observational studies have shown a reduced risk of colon cancer in women treated with HRT (25). The breast cancer risk among HRT users is still contradictory.

### Breast cancer etiology

The human breast is undergoing changes throughout life, both as part of the normal menstrual cycle

and in pregnancy. During each estrous cycle, proliferation occurs, followed by apoptosis of the epithelial cells. It is thought that this intrinsic dynamic ability of breast cells to be continuously influenced and remodeled makes them especially susceptible to carcinogenesis (29). Specific pathological findings and genetic hereditary or somatic alternations seem to be major factors in developing breast cancer. The molecular mechanisms underlying the development of mammary carcinoma are not completely understood. Beckmann et al. (30) suggested a hypothetical model of breast cancer to show how complicated the evolution and the interaction of the genetic predisposing factors of the disease could be. For now, it is well known that up to 60% of premenopausal and up to 75% of postmenopausal breast cancer patients have estrogen-dependent carcinomas (31). A large amount of data implicates estrogens in the etiology of human breast cancer (32), thereby an increased cumulative endogenous (e.g., early age at menarche (<12 years), late age at menopause, late first full-term pregnancy) and exogenous (i.e., hormone replacement therapy and oral contraceptives) exposure to hormones being the fundamental risk factor of the disease. Estrogens, like the other hormones, affect breast cancer development by the epigenetic mechanism such as the stimulation of cell proliferation and reprogramming of cellular differentiation. In addition, estrogens can also cause genetic alterations by mechanisms not involving the classic estrogen receptor. As tumor promoters, the effects of estrogens are related to the duration and timing of exposure (25). In contrast, the action of estrogens as initiators is more controversial. Nevertheless, estrogen-induced carcinogenesis in the mammary gland is complex and requires both receptor-mediated and genotoxic events for neoplastic development (24, 25). Only a weak carcinogenic activity is to be expected, because all estrogens are endogenous hormones at low picomolar levels and a strong carcinogenicity would have provided a disadvantage to humans. A natural estrogenic hormone exerts genotoxicity most likely *via* metabolic activation to catechol estrogens (4-hydroxylation at the A ring by the product of *CYP1B1* to 4-hydroxyestron), and the subsequent induction of specific gene mutations. The metabolism of estrogens mostly occurs in the liver with 2-hydroxylation as the major breakdown process. Other metabolising tissues include kidney, intestines, breast, and uterus, where 4-OHE<sub>2</sub> is the dominant catechol estrogen (13, 33, 34). The 4-OHE<sub>2</sub> has been shown to be a strong carcinogen that was in an animal model, and a significant increase of this metabolite has been reported in human breast

cancer (35, 36). In contrast, 2-OHE<sub>2</sub> does not exhibit any carcinogenic activity reported.

Numerous factors contributing to breast cancer have been described so far, sex and age, the family history of the disease, some genetic factors (i.e., germ-line mutations in *BRCA1* and *BRCA2*) which are one of the few identified as directly associated with an increased risk of breast carcinoma, and life-style factors such as physical activities, cigarette smoking, alcohol consumption, diet habits, and socioeconomic status (37-40).

#### HRT and breast cancer

Most researchers and physicians supported the statement that a high exogenous estrogen supplementation was associated with an increased risk of breast cancer incidence among postmenopausal women (20, 28, 41-44). Surprisingly, lower grade, less aggressive tumors, with a lower probability of large tumor diagnosis, negative axillary lymph nodes have been found simultaneously at the time of diagnosis (5, 42, 44, 45). Regardless of the majority of such statements, trials with contradictory results were also presented (37, 46). Moreover, the density of the breast tissues (a well-known breast cancer risk factor) among HRT users was significantly higher compared to never-users (42, 47). Mostly, a higher estrogen level in the HRT regimen was announced as less favorable to the prevention of breast carcinoma. Besides, the longer (>10-15 years in different studies) the treatment, the higher the risk of cancer (38, 42, 43). Although the effect was found reversible, and no increase in the risk of breast cancer was shown five and more years after the cessation of HRT among former-users in comparison to never-users (43, 48). An additional dilemma brought the role of progestagens on the breast tissue to the fore. Initially, protection against the development of breast cancer (like in the case of endometrial carcinoma) was hypothesized. Later, no connection between progestins and the disease's appearance was suggested (42) and, finally, the most recent studies of the WHI and the MWS brought opposite conclusions for HRT (15, 49). The WHI project was started in the year 1995 in the USA, but the estrogen/progestin part of the trial was prematurely stopped after 5.2 years (the planned duration was 8.5 years), because the weighed risks outweighed the benefits (the risk of cardiovascular disease). The WHI combined HRT trial reported a slightly increased risk of breast cancer on a continuous, combined estrogen (conjugated equine estrogens) and progestin (medroxyprogesterone) regimen after up to 5.2 years of treatment. Only women who received hormone therapy for 5 or 10 years before

entering the study and, then, an additional 5.2 years of treatment in the study had a significant increase in the risk (50). In contrast, more recent WHI Estrogen Only Study found no increased risk of breast carcinoma related to therapy (51), showing the possible harmfulness of progestins to breast tissue. The Million Women Study (MWS), a retrospective/prospective epidemiological cohort study, showed basically similar results (49). The study found that women using any type of HRT (combined or estrogen alone regimen – no differences) for more than 1 year had a relative risk of breast cancer of 1.45 when compared to non-users. However, the findings of the MWS could be overestimated by the fact that the population included in this screening program (only volunteers – usually women concerned about their health) was at a higher risk than the standard population.

Another issue associated with HRT is the treatment of breast cancer survivors. Common sense has suggested the absolute contraindication of HRT after breast cancer diagnosis. Usually, doctors would not take the risk of HRT treatment. Firstly, there seemed to be the possibility of a recurrence induced by exogenous hormones. Secondly, the interaction between the HRT component and antiestrogenic drugs was pointed out. However, estrogen-deficiency effects in breast cancer survivors are often more serious and persist for longer as a result of a natural menopause or subsequent side effects of their treatment (e.g., ovarian ablation, chemotherapy, tamoxifen) (44). According to the recent analyses, it should be noted that none of the studies demonstrated an increase in cancer progression among breast cancer survivors treated with HRT (46, 52-54). It is widely suggested that before a breast cancer survivor with severe menopausal symptoms would start HRT, an alternative treatment should be used and the patient must be aware of the possible risk of HRT therapy (44, 53-55).

#### SUMMARY AND CONCLUSIONS

The basic weak sides of the trials on HRT reported so far are: 1) the small number of patients (although HRT up to 2-3 years is very common, the long-term treatment is quite rare; breast cancer survivors with HRT usage are even fewer) (20, 52); 2) usually observational studies, the clinical ones in a minority; 3) selection criteria varied considerably. The facts mentioned above make the comparison of different studies very difficult and unreliable.

It is highly probable that the estrogen (and its metabolites) replacement could be related to the promotion of pre-existing breast cancer, rather than be-

ing involved in direct *de novo* carcinogenesis (7, 28). Although no synergism between some reproductive risk factors and HRT was found (43), all genetic and environmental factors influencing estrogen homeostasis should be taken into consideration.

In general, hormone replacement therapy was first applied in order to prevent and control menopausal symptoms (hot flushes; night sweats; climacteric, depressive mood; atrophy processes of genitourinary tract – vaginal dryness, urogenital infections) and improve the quality of life (11). Besides, numerous benefits were announced and new indications for HRT usage are shown (e.g., osteoporosis, increased cardiovascular disease risk, susceptibility of dementia). Nevertheless, the final conclusion from recent observational and clinical trials brought only very limited indications for HRT. Serious vasomotor symptoms of menopause reducing the quality of life and osteoporosis resistant to other types of treatment remain the only adequate application. Short-term treatment (<5 years) was associated with only a low risk of breast cancer in most studies, and it seems to be quite safe. But the long-term therapy was connected with a slight but significant increase in the risk of breast cancer development, and such regimens (>5 years) should always be discussed with a patient to estimate the risk-benefit ratio. Ideally, the withdrawal of HRT should be attempted after 4-5 years of therapy. Despite some benefits such as an increased bone mineral density, and a decreased risk of fracture and colorectal cancer, most recent data suggest that the risks of HRT usage outweigh the benefits (17, 57, 59).

In conclusion, the growing need for an individualization of an appropriate regimen of HRT (the aim of the application, dose, route of administration, and regimen) is quite obvious. Similarly, there is a further, striking need for a more complex, clinical, big cohort trial in order to dispel the controversies over hormone replacement therapy. Truly, little do we know about hormones and their functions. Finally, it is well established that in the process of breast carcinogenesis caused by estrogens, the major factor is the local production of hormones, and the level of estrogens in the blood is the second issue (9, 10). Thus, the problem which should be solved is the effect of "exogenous estrogens" from blood circulation on mammary carcinoma development.

## REFERENCES

1. Sikorski R.: Nowa Medycyna 111, 3 (2001).
2. Purohit A., Reed M. J.: Steroids 67, 979 (2002).
3. Pawlicki M.: in Rak piersi-nowe nadzieje i możliwości leczenia, Pawlicki M., pp. 10-15, 21-29, 47-49, 57-59, 124-137,  $\alpha$ -medica press, Bielsko-Biała 2002.
4. Brueggemeier R. W., Miller D. D., Dalton J. T.: in Foy's Principles of Medical Chemistry, Fifth Edition, Williams D. A., Lemke T. L.(eds.), pp. 653-684, Lippincott Williams Wilkins, New York 2002.
5. Kenemans P., van Unnik G. A., Mijatovic V., van der Mooren M. J.: Maturitas 38, S41 (2001).
6. Sites C. K., L'Hommedieu G. D., Toth M. J., Brochu M., Cooper B. C., Fairhurst P. A.: J. Clin. Endocrinol. Metab. doi: 10.1210/jc. 2004 (2005).
7. Sitruk-Ware R., Plu-Bureau G.: Maturitas 49, 58 (2004).
8. Simpson E. R.: J. Steroid. Biochem. Mol. Biol. 86, 225 (2003).
9. Simpson E. R.: J. Mammary Gland Biol. Neoplasia 5, 251 (2000).
10. Simpson E. R., Clyne C., Rubin G., Chin Boon W., Robertson K., Britt K., Speed C., Jones M.: Annu. Rev. Physiol. 64, 93 (2002).
11. Genazzani A. R., Gambacciani M.: Maturitas 32, 11 (1999).
12. Meinhardt, U., Mullis P. E.: Semin. Reprod. Med. 20, 277 (2002).
13. Hulley S., Grady D., Bush T., Furberg C., Herrington D., Riggs B. et al.: J. Am. Med. Assoc. 280, 605 (1998).
14. Grady D., Herrington D., Bitter V. et al.: J. Am. Med. Assoc. 288, 49 (2002).
15. Writing Group for the Women's Health Initiative Investigators: J. Am. Med. Assoc. 288, 321 (2002).
16. Herrington D., Reboussin S. J., Brosnihan K. B. et al.: N. Engl. J. Med. 343, 522 (2000).
17. Biscup P.: Am. J. Health-Syst. Pharm. 60, 1419 (2003).
18. Madsen J. S., Kristensen S. R., Gram J., Bladbjerg E. M., Henriksen F. L., Christensen K., Jespersen J.: J. Thromb. Haemost. 1, 1984 (2003).
19. Cerquetani E., Vitale C., Mercuro G., Fini M., Zoncu S., Rosano G. M.: Gynecol. Endocrinol. 18, 291 (2004).
20. Barrett-Connor E., Struening C.: Int. J. Epidemiol. 30, 423 (2001).
21. Compston J. E.: J. Musculoskelet. Neuronal Interact. 4, 187 (2004).
22. Yasui T., Uemura H., Takikawa M., Irahara M.: J. Med. Invest. 50, 136 (2003).

23. Toth M. J., Tchhernof A., Sites C. K., Poehlman E. T.: *Int. J. Obes. Relat. Metab. Disord.* 24, 226 (2000).
24. Liehr J. G.: *Endocr. Rev.* 21, 40 (2000).
25. Clarke R., Liu M. C., Bouker K. B., Gu Z., Lee R. Y., Zhu Y., Skaar T. C., Gomez B., O'Brien K., Wang Y., Hilakivi-Clarke L. A.: *Oncogene* 22, 7316 (2003).
26. Persson I.: *Maturitas* 23, S37 (1996).
27. Schneider H. P.: *Maturitas* 33, S65 (1999).
28. Schneider H. P.: *Maturitas* 43, S35 (2002).
29. Howard B. A., Gusterson B. A.: *J. Mammary Gland Biol. Neoplasia* 5, 119 (2000).
30. Beckmann M. W., Niederacher D., Schurch H.-G., Gusterson B. A., Bender H. G.: *J. Mol. Med.* 75, 429 (1997).
31. Ray S., Rastogi R., Kumar A.: *Prog. Drug Res.* 59, 201 (2002).
32. Henderson B. E., Spenser Feigelson H.: *Carcinogenesis* 21, 427 (2000).
33. Mettlin C.: *CA-A Cancer J. Clinic.* 49, 138 (1999).
34. Grodstein F., Stampfer M. J., Colditz G. A. et al.: *New Eng. J. Med.* 336, 1769 (1997).
35. Ettinger B., Friedman G. D., Budh T., Quesenberg C. P.: *Obestet. Gynecol.* 87, 6 (1996).
36. Antoine C., Liebens F., Carly B., Pastijn A., Rozenberg S.: *Endocr. Relat. Cancer* 11, 523 (2004).
37. Mitrinen K., Hirvonen A.: *Mutat. Res.* 544, 9 (2003).
38. Hulka B. S., Moorman P. G.: *Maturitas* 38, 103 (2001).
39. Baum M., Saunders C., Meredith S.: in *Rak piersi. Poradnik dla kobiet i lekarzy*, Baum M., Saunders C., Meredith S.(eds), pp. 9-18, 25-27, 34-37, 43-47, 62-67, 93-117. Springer PWN, Warsaw 1995.
40. Olson H.: in *Cancer and Aging*, Macieira-Coelho A., Nordenskjöld B. (eds), pp. 109-115, CRC Press, Boston 1990.
41. Scheele F., Burger C. W., Kenemans P.: *Maturitas* 33, 191 (1999).
42. Marsden J.: *Maturitas* 34, S11 (2000).
43. Jacobs H. S.: *Endocr. Relat. Cancer* 7, 53 (2000).
44. Verheul H. A. M., Coelingh-Bennink H. J. T., Kenemans P., Atsma W. J., Burger C. W., Eden J. A., Hammar M., Marsden J., Purdie D. W.: *Maturitas* 36, 1 (2000).
45. Grodstein F., Stampfer M. J., Colditz G. A. et al.: *New Eng. J. Med.* 336, 1769 (1997).
46. Ettinger B., Friedman G. D., Budh T., Quesenberg C. P.: *Obestet. Gynecol.* 87, 6 (1996).
47. Lundstrom E., Wilczek B., von Palffy Z., Soderquist G., von Schoultz B.: *Climacteric* 5, 92 (2001).
48. Collaborative Group on Hormonal Factors for Breast Cancer: *Lancet* 350, 1047 (1997).
49. Beral V., Million Women Study Collaborators: *Lancet* 362, 419 (2003).
50. Chlebowski R. T., Hendrix S. L., Langer R. D. et al., WHI Investigators: *J. Am. Med. Assoc.* 289, 3243 (2003).
51. Anderson G. L., Limacher M., Assaf A. R. et al.: *J. Am. Med. Assoc.* 291, 1701 (2004).
52. Biglia N., Gadducci A., Ponzzone R., Roagna R., Sismondi P.: *Maturitas* 48, 333 (2004).
53. Seifert M., Galid A., Kubista E.: *Maturitas* 32, 63 (1999).
54. Meurer L. N., Milwaukee S. L.: *J. Fam. Pract.* 51, 1056 (2002).
55. Clover A., Ratsey D.: *Homeopathy* 91, 75 (2002).
56. Sitruk-Ware R., Plu-Bureau G.: *Maturitas* 49, 58 (2004).
57. Baber R. J., O'Hara J. L., Boyle F. M.: *MJA* 178, 630 (2003).
58. Deady J.: *J. Manag. Care Pharm.* 10, 85 (2004).

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