Acta Obstet Gynecol Scand 2003: 82: 1107–1111
Printed in Denmark. All rights reserved

Acta Obstetricia et Gynecologica Scandinavica
ISSN 0001-6349

ORIGINAL ARTICLE

Effects of low-dose 17-β-estradiol plus norethisterone acetate and tibolone on fasting plasma homocysteine levels in postmenopausal women

BABUR KALELI[1], BASAK YILDIRIM[1], SULEYMAN DEMIR[2] AND ERKAN ALATAS[1]

From the Departments of [1]Obstetrics and Gynecology and [2]Biochemistry, Medical Faculty, Pamukkale University, Denizli, Turkey

Acta Obstet Gynecol Scand 2003; 82: 1107–1111. © Acta Obstet Gynecol Scand 82 2003

Background. Many postmenopausal women currently receive hormone replacement therapy. The use of low-dose 17β-estradiol plus norethisterone acetate and tibolone for hormone replacement therapy is not uncommon in postmenopausal women. Homocysteine, which is known to be an independent risk factor for the development of cardiovascular disease, is found in increased levels postmenopause. This study compared the effects of low-dose 17β-estradiol plus norethisterone acetate and tibolone on the fasting plasma homocysteine level in healthy postmenopausal women.
Methods. Healthy postmenopausal women ($n = 44$) were enrolled in the study. Women randomly assigned received 1 mg of 17β-estradiol plus 0.5 mg of norethisterone acetate or 2.5 mg tibolone during a period of 12 weeks. Fasting plasma homocysteine levels were measured at baseline, the 4th week, and the 12th week of therapy.
Results. In the 4th week there were no significant changes in plasma homocysteine levels in both groups ($p > 0.05$). However at the end of the 12th week the plasma homocysteine levels were reduced significantly in both groups ($p < 0.05$).
Conclusion. Low-dose 17β-estradiol plus norethisterone acetate and tibolone lower the fasting plasma homocysteine levels in healthy postmenopausal women.

Key words: hormone replacement therapy, menopause and homocysteine, tibolone

Submitted 27 December, 2002
Accepted 28 February, 2003

Many postmenopausal women currently receive hormone replacement therapy (HRT). Besides being a preventive for osteoporosis and climacteric complaints (1), HRT has been said in some epidemiological studies to also protect against cardiovascular diseases (2,3). Recently a World Health Initiative (WHI) study showed that conjugated equine estrogen 0.625 mg plus medroxyprogesterone acetate 2.5 mg had no cardioprotective effects in postmenopausal women (4). Although no randomized controlled study existed for the cardioprotective effects of 17β-estradiol plus norethisterone acetate (NETA) and tibolone, these effects became subject to serious doubt following the WHI study.

Hyperhomocysteinemia is an independent risk factor for occlusive arterial disease and deep venous thrombosis (5,6). Plasma homocysteine levels are lower in premenopausal women than postmenopausal women. These levels are also

Abbreviations:
HRT: Hormone replacement therapy; NETA 17β-estradiol plus norethisterone acetate.

Drugs: 1 mg of 17β-estradiol plus 0.5 mg of NETA (Aktivelle; Novo nordisk); 2.5 mg tibolone (Livial; Organon).

lower in women than in men, and decrease during pregnancy. All of these factors suggest that estrogen may lower plasma homocysteine concentrations (7,8). Elevated homocysteine levels may in part contribute to the increased risk of developing cardiovascular disease after menopause.

The pathogenic role of homocysteine as a cardiovascular risk factor is thought to be related to its influence on the coagulation system and the resistance of the endothelium to thrombosis (9), and it might also interfere with the vasodilator and antithrombotic functions of nitric oxide (10).

Tibolone [7 alpha, 17 alpha-17-hydroxy-t-methyl-19-norpregn-5 (10)-en-20-yn-3-one, Org OD 14, Livial] is a synthetic steroid with estrogenic, androgenic, and progestogenic properties, and is used to prevent climacteric symptoms and postmenopausal osteoporosis (11). It is frequently used as a hormone-like alternative to traditional HRT.

There have been few studies about the effect of homocysteine in postmenopausal women receiving HRT (12–14). We therefore decided to investigate the effects of 1 mg 17β-estradiol plus 0.5 mg of norethisterone acetate (NETA) and 2.5 mg tibolone on fasting plasma homocysteine levels in healthy postmenopausal women.

Materials and methods

Forty-four healthy non-hysterectomized postmenopausal women were enrolled in the study at the outpatient clinic of the Department of Obstetrics and Gynecology. All women were amenorrhoeic for at least 6 months before enrolling and none were using any supplements of vitamin B, such as folic acid, B6, or B12. In addition, the patients were tested for serum FSH levels >25 IU/l andserum estradiol levels of 30 pg/ml or less. Exclusion criteria included a history or active presence of stroke, deep venous thrombosis, thromboembolic disorders, or any other cardiovascular, gastrointestinal, endocrinologic, or renal pathology. A papanicolau smear, mammography, transvaginal ultrasonography, a complete blood count, and liver function tests were performed.

The women were randomly divided into two groups. Group I was continously treated with 1 mg of 17β-estradiol plus 0.5 mg of NETA (Aktivelle; Novo nordisk, Istanbul, Turkey) once a day for 12 weeks. Group II was continously treated with 2.5 mg of tibolone (Livial; Organon, Altunizade, Turkey) once a day for 12 weeks.

There were three dropouts in the study group, one dropout in the 17β-estradiol plus NETA group because of vaginal bleeding, and two dropouts in the tibolone group because of vaginal bleeding and lack of compliance to the study rules. Thus, this study was based on 41 women randomly selected to either 17β estradiol plus NETA ($n = 21$) or tibolone ($n = 20$) groups.

The women were instructed to follow their normal dietary habits and calcium supplements were allowed. The subjects had refrained from consuming alcohol for 24 h before sampling.

Measurements were taken at baseline and after 4 and 12 weeks of treatment. After at least 10 h of fasting and not smoking, venous blood samples were collected from the antecubital vein and placed into ethylenediamine tetraasetic acid (EDTA) vacuum glass tubes between 08 : 00 and 10 : 00. The subjects were in a supine position and the samples were collected after 15 min of rest. The blood samples were immediately centrifuged at $\times 3000$ g and 4 °C for 30 min. Plasma was stored at −70 °C until analysis.

Plasma homocysteine concentrations were determined by high-performance liquid choromatography with fluorescence detection using a DS30 Hcy Homocysteine Assay Kit (Drew Scientific Limited, Barrow-in-Furness, UK) (15). Briefly, following addition of internal standard, the disulfid bonds in the sample are reduced using the reducing agent. Protein is precipated from the solution, and the thiol groups in the supernatant are then derivatized with a flourescent thiol-specific dye. The flourescent-derivative mixture is then separated using the DS30 Hcy analyzer, which automatically calculates the homocysteine concentration. To avoid interassay variation, all the samples were assessed during a single-assay run. The lower limit of detection was 0.5 μmol/l. The intra-assay coefficient of variation was 4.1%.

Serum FSH and estradiol levels were measured by commercial kits using Immulite one. Serum cholesterol and triglyceride levels were determined by the enzymatic colorimetric method, and HDL levels were determined by a direct nonprecipitating method. Liver function tests and complete blood count were performed in the hospital's central laboratory using commercial kits.

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS 9.0, Pamukkale University, Denizli, Turkey). Data is expressed as means ± standard deviation. For all measured parameters, statistical analyses of between-group differences at baseline were performed using one-way analysis of variance and the Kruskal–Wallis test. Wilcoxon's matched-pairs signed-rank test was used to compare the within-group parameters at baseline with those at 4 and 12 weeks respectively.

Table I. Descriptive characteristics at baseline

	Groups		
	17β-estradiol plus NETA ($n=20$)	Tibolone ($n=21$)	p
Age (y)	52.0 ± 5.92	50.15 ± 5.06	>0.05
Body mass index	27.2 ± 2.26	26.1 ± 2.77	>0.05
Duration of amenorrhea (months)	21.55 ± 30.17	20.45 ± 28.52	>0.05
Blood pressure			
Systolic (mmHg)	110.25 ± 16.89	106.0 ± 17.88	>0.05
Diastolic (mmHg)	65.75 ± 13.69	65.0 ± 11.92	>0.05

Results shown are mean ± SD.

Results

Descriptive characteristics of the subjects in the study are listed in Table I. Age, body mass index, blood pressure, and duration of amenorrhea were similar between the two groups. There were two smokers in the 17β-estradiol plus NETA group, three in the tibolone group, and none were heavy smokers.

Baseline total cholesterol, triglyceride, and HDL and LDL concentrations were similar between the two groups. After 4 weeks of therapy, there was no significant change in the blood lipid profiles vs. baseline in the two groups. After 12 weeks of 17β-estradiol plus NETA therapy, total cholesterol ($p < 0.000$), triglycerides ($p < 0.000$), and LDL ($p < 0.05$) were significantly reduced vs. baseline, and HDL was similar to baseline ($p > 0.05$) (Table II). Total cholesterol, triglycerides, and HDL were significantly reduced vs. baseline ($p < 0.05$, $p < 0.000$, $p < 0.05$, respectively) and the LDL level was similar to baseline ($p > 0.05$) in the tibolone group (Table III).

After 4 weeks of therapy, homocysteine levels were not different from baseline ($p > 0.05$), but at the end of 12 weeks of 17β-estradiol plus NETA (Table II) and tibolone therapy (Table III), the homocysteine levels were reduced significantly vs. baseline in the two groups ($p < 0.000$, $p < 0.05$, respectively): 21% and 22%, respectively.

Table II. Plasma homocystein and total cholesterol, LDL, HDL and triglyceride levels in the 17β-estradiol plus NETA group ($n=21$)

	Baseline	4th week	12th week
Plasma homocystein (μmol/l)	10.2 ± 3.5	9.5 ± 3.1	8.1 ± 1.9**
Total cholesterol (mg/dl)	212.6 ± 40.4	205.9 ± 36.9	187.9 ± 33.6*
LDL-C (mg/dl)	136.8 ± 25.6	132.7 ± 23.7	119.6 ± 38.6*
HDL-C (mg/dl)	56.8 ± 14.5	53.3 ± 14.1	53.4 ± 11.1
Triglycerides (mg/dl)	150.4 ± 44.4	139.9 ± 39.4	128.4 ± 39.1**

Results shown are mean ± SD.
*$p < 0.05$; **$p < 0.000$.

Table III. Plasma homocystein and total cholesterol, LDL, HDL and triglyceride levels in the tibolone group ($n=20$)

	Baseline	4th week	12th week
Plasma homocystein (μmol/l)	10.1 ± 3.6	8.9 ± 2.9	7.9 ± 2.6*
Total cholesterol (mg/dl)	204.3 ± 44.9	202.8 ± 55.9	196.9 ± 38.7*
LDL-C (mg/dl)	137.5 ± 27.8	137.1 ± 28.6	130.5 ± 26.3
HDL-C (mg/dl)	59.1 ± 12.1	57.85 ± 11.0	55.5 ± 13.1*
Triglycerides (mg/dl)	137.4 ± 27.6	133.6 ± 28.3	125.5 ± 37.3*

Results shown are mean ± SD.
*$p < 0.05$.

Discussion

In this study, low-dose 17β-estradiol plus NETA and tibolone significantly lowered fasting plasma total homocysteine levels in healthy postmenopausal women after 12 weeks of therapy.

There are differences in homocysteine levels in pregnancy, during intake of oral contraceptives, and during menopause (8,16,17). It is reported that plasma homocysteine levels increase with natural menopause, which strengthens the hypothesis that estrogens influence homocysteine levels (17).

The previous studies generally reported significant reductions in total homocysteine levels in postmenopausal women using HRT. Mijatovic et al. and Van der Mooren et al. reported that a HRT regimen with a combination of micronized 17 B E2 dydrogesteron lowered homocysteine levels significantly (13,18). In another study Van der Mooren used conjugated estrogen-medrogesteron as HRT, which lowered homocysteine levels (19). However, those studies were uncontrolled. In a randomized controlled study, Mijatovic found that plasma fasting homocysteine concentrations were lowered by E2-didrogesteron therapy in postmenopausal women (20). Van Baal has shown that the fasting total homocysteine concentration was significantly reduced by combined estradiol-progesteron replacement in a placebo-controlled study (12). Ventura reported that combined oral HRT with 2 mg of estradiol plus 1 mg of NETA reduces homocysteine levels

(21). Evio et al. demonstrated that neither an oral nor transdermal combination of sequential estradiol and NETA caused a significant change in the plasma homocysteine level in Finnish postmenopausal women (22). But as their patients had normal levels of homocysteine, these levels might not have been affected by HRT, and genetic or dietary factors may have been responsible for this discrepancy. Our results appear to be in conflict with the Celik et al. who reported that tibolone had no significant effect on the homocysteine level in postmenopausal women (14). Divergence may be a result of the differing ethnicity and differences in nutrition between different geographic parts of Turkey, and therefore ethnicity and nutrition may contribute to the different effects of HRT on homocysteine.

The mechanism underlying the observed HRT-induced decrease in plasma homocysteine is still unknown. Homocysteine is a sulfur amino acid whose metabolism is at the intersection of two metabolic pathways, remethylation and transsulfuration, and it results from the demethylation of methionine (23). Elevated homocysteine levels in plasma may be a result of genetic factors and nongenetic factors such as folate, renal, or liver failure (24). Hormone replacement therapy is reported to increase the flow to the remethylation pathway and can be hypothesized as a mechanism in the reduction of homocysteine (25). Hormone-induced changes in the transamination of methionin could also be a potential mechanism by which HRT can lower the homocysteine concentration (26). Hormone replacement therapy may affect folate homeostasis. Fasting homocysteine concentration was found to be negatively and significantly correlated with the serum folate concentration in postmenopausal women (27). Another mechanism to explain the hormone-induced reductions of homocysteine is the changes in methylene tetrahydrofolate reductase and betain-homocysteine methyltransferase; two other enzymes involved in the remethylation of homocysteine to methionine. The increase in the activity of cystathionine Beta synthase could be another mechanism (12).

Several mechanisms have been reported to explain how homocysteine may promote both the development of atherosclerosis and the formation of thrombi, which may finally result in clinical diseases. Tawakol et al. demonstrated that elevation in the serum homocysteine concentration in humans is associated with impaired endothelial-depended vasodilation (28). Although the precise mechanism is not known, homocysteine may decrease the bioavailability of nitric oxide and in addition homocysteine increases lipid peroxidation, which then impairs the expression of nitric oxide synthase (29,30). Harper reported that it may inhibit the protein C anticoagulant pathway, interfere with heparan sulfate proteoglycans, which modulate antithrombin III activity, inhibit tissue plasminogen activator receptor function, decrease endothelial ADPase activity, and enhance the fibrin binding and tissue factor stimulating activity of lipoprotein a (31). Homocysteine also potentates the autooxidation of low-density lipoprotein cholesterol (32).

Menopause is associated with a higher atherogenic lipid profile than that of the premenopausal state. The increase in total cholesterol, observed after menopause, is mainly attributable to an increase in LDL, as HDL tends to be reduced (33). After menopause unfavorable changes in lipid occur and significantly increase the risks for cardiovascular disease in women (34).

In the tibolone group a significant decrease was observed in the levels of total cholesterol, HDL, and triglyceride while a tendency for a decrease in LDL was observed. Our study results suggest that in the tibolone group, the homocysteine decrease had a beneficial effect on cardiovascular disease risk while the HDL decrease had negative effects.

In the 17β-estradiol plus NETA group a significant decrease was observed in the level of total cholesterol, LDL, and triglycerides while a tendency for a decrease of HDL was observed. Decrease in the levels of total cholesterol, LDL, and triglycerides has beneficial effects on cardiovascular risks when combined with a decrease of homocysteine.

This study has some potential limitations. Firstly, there was no control group, and secondly, it was of short duration. Future studies addressing this issue should take these points into consideration.

Conclusion

Low-dose 17Beta-estradiol plus NETA and Tibolone lower fasting plasma total homocysteine levels significantly in postmenopausal women.

References

1. Consensus Development Conference. Diagnosis, prophylaxis and treatment of osteoporosis. Am J Med 1993; 94: 646–50.
2. Bush TL. Extrasceletal effects of estrogen and the prevention of atherosclerosis. Osteoporosis Int 1991; 2: 5–11.
3. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a Quantitative assessment of the epidemiologic evidence. Prev Med 1991; 20: 47–63.
4. WHI. Risk and benefits of estrogen plus progestin in healthy postmenopausal women. JAMA 2002; 288: 321–33.

5. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B et al. Hyperhomocystenemia: an independent risk factor for vascular disease. N Engl J Med 1991; 324: 1149–55.
6. Ridker PM, Manson JE, Buring JE, Shih J, Matias M, Hennekes CH. Homocysteine and risk of cardiovascular disease among postmenopausal women. JAMA 1999; 281: 1817–21.
7. Brattstrom LE, Hultberg BL, Hardebo JE. Folic acid responsive postmenopausal homocysteinemia. Metabolism 1985; 34: 1073–7.
8. Anderson A, Hultberg BL, Brattstrom LE, Isaksson A. Decreased serum homocysteine in pregnancy. Eur J Clin Chem Clin Biochem 1992; 30: 377–9.
9. Malinow MR. Homocysteine and arterial occlusive diseases. J Intern Med 1994; 236: 603–17.
10. Stamler JS, Slivka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. Nutr Rev 1996; 54: 1–30.
11. Bjarnason N, Bjarnason K, Haarbo J, Christiansen C. Tibolone: prevention of bone loss in late postmenopausal women. J Clin Endocrinol Metab 1996; 81: 2419–22.
12. Van Baal WM, Smolders RGV, Van der Mooren MJ, Teerlink T, Kenemans P. Hormone replacement therapy and plasma homocysteine levels. Obstet Gynecol 1999; 94: 485–91.
13. Mijatovic V, Kenemans P, Netelenbos C, Jakobs C, Popp-Snijders C, Peters-Muller ER et al. Postmenopausal oral 17B-estradiol continuously combined with dydrogesterone reduces fasting serum homocysteine levels. Fertil Steril 1998; 69: 876–82.
14. Celik H, Ayar A, Tug N, Cikim G, Kýlýc N, Parmaksýz C. Effects of tibolone on plasma homocysteine levels in postmenopausal women. Fertil Steril 2002; 78: 347–50.
15. Zhang M, Gunter EW, Pfeifer CM. Evaluation of the drew scientific d30 homocysteine assay in comparison with the centers for disease control and prevention references HPLC method. Clin Chem 2001; 47: 966–7.
16. Brattstrom L, Israelsson B, Olsson A, Andersson A, Hultberg B. Plasma homocysteine in women on oral estrogen-containing contraceptives and in men with esrogen-treated prostatic carcinoma. Scand J Clin Lab Invest 1992; 52: 283–7.
17. Hak AE, Polderman KH, Westendorp ICD, Jakobs C, Hofman A, Witteman JC et al. Increased plasma homocysteine after menopause. Atherosclerosis 2000; 149: 163–8.
18. Van der Mooren MJ, Wouters MGAJ, Blom HJ, Schellekens LA, Eskes TKAB, Rolland R. Hormone replacement therapy may reduced high serum homocysteine in postmenopausal women. Eur J Clin Invest 1994; 24: 733–6.
19. Blom HJ, Van der Mooren MJ. Hyperhomocystenemia: a risk factor for cardiovascular disease-influence of sex hormones on homocysteine metabolism. Gynecol Endocrinol 1996; 10: 75–9.
20. Mijatovic V, Kenemans P, Jacops C, Van Baal WM, Peters-muller ERA, Van Der Mooren MJ. A randomized controlled study of the effects of 17Beta-estradiol-dydrogesterone on plasma homocysteine in postmenopausal women. Obstet Gynecol 1998; 91: 432–6.
21. Ventura P, Cagnacci A, Malmusi S, Panini R, Baldassari F, Arangino S et al. Continuous combined hormone replacement therapy with oral 17beta-estradiol and norethiterone acetate improves homocysteine metabolism in postmenopausal women. Menopause 2001; 8: 252–8.
22. Evio S, Tiitinen A, Turpeinen U, Ylikorkala O. Failure of the combination of sequential oral and transdermal estradiol plus norethisterone acetate to affect plasma homocysteine levels. Fertil Steril 2000; 74: 1080–3.
23. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulsuration of homocysteine. Am J Clin Nutr 1992; 55: 131–8.
24. D'angelo A, Selhub J. Homocysteine and thrombotic disease. Blood 1997; 99: 1–11.
25. Somekawa Y, Kobayashi K, Tomura S, Aso T, Hamaguchi H. Effects of hormone replacement therapy and methylenetetrahydrofolate reductase polymorphism on plasma folate and homocysteine levels in postmenopausal japanese women. Fertil Steril 2002; 77: 681–6.
26. Blom HJ, Boers GHJ, Van den Elzen PAM, Van Roessel JJM, Trijbels JMF, Tangerman A. Differences between premenopausal women and young men in the transamination pathway of methionine catabolism, and the protection against vascular disease. Eur J Clin Invest 1988; 18: 633–8.
27. Wouters MGAJ, Moorees M, Van der Mooren MJ, Blom HJ, Boers GH, Schellekens LA et al. Plasma homocysteine and menopausal status. Eur J Clin Invest 1995; 25: 801–5.
28. Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. Circulation 1997; 95: 1119–21.
29. Loscalzo J. The oxidant stress of hyperhomocysteinemia. J Clin Invest 1996; 98: 5–7.
30. Liao JK, Shin WS, Lee WY, Clark SL. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide sythase. J Biol Chem 1995; 270: 319–24.
31. Harpel PC, Zhang X, Borth W. Homocysteine and hemostasis: pathogenetic mechanisms predisposing to thrombosis. J Nutr 1996; 126: 1285S–89S.
32. Heinecke JW, Rosen H, Suzuki LA, Chait A. The role of sulfur containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. J Biol Chem 1987; 262: 1098–103.
33. De Aloysio D, Gambacciani M, Meschia M, Pansini F, Bacchi Modena A, Bolis PF et al. The effects of menopause on blood lipids and lipoproteine levels. Atherosclerosis 1999; 147: 147–53.
34. Mosca L, Grundy SM, Judelson D, King K, Limacher M, Oparil S et al. AHA/ACC scientific statement: consensus panel statement. Guide to preventive cardiology in women. Circulation 1999; 99: 2480–4.

Address for correspondence:
Babur Kaleli
Saltak Caddesi 57/7
20100 Denizli
Turkey
e-mail: bkaleli@netscape.net