

# Effect of Daily Estradiol Hemihidrate and Norethisterone Acetate on Carotid and Renal Arterial Blood Flow in Postmenopausal Women

Tevfik YOLDEMİR, Başak BAKSU, İnci DAVAS, Atif AKYOL, Ahmet VAROLAN, Ali YAZGAN

Şişli Etfal Training and Research Hospital, 2<sup>nd</sup> Gynecology and Obstetrics Clinic, İstanbul, Turkey

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## Abstract

**Objective:** To determine short term effects of daily combined 2 mg estradiol hemihidrate and 1 mg norethisterone acetate on internal carotid artery and renal artery in postmenopausal women.

**Materials and Methods:** Fifty postmenopausal women who applied to our menopause outpatient clinic were recruited into the study. Daily combined 2 mg estradiol hemihidrate and 1 mg norethisterone acetate were given for 6 months. Internal carotid and renal artery pulsatility indices were assessed by color flow pulsed doppler. Paired t test was used for statistical analysis.

**Results:** There were no significant differences in weight, arterial blood pressure and pulsatility indices before and after treatment ( $P>0.05$ ). Pulsatility indices before and after treatment were  $0.87\pm 0.17$  and  $0.89\pm 0.13$  for right internal carotid artery and  $0.84\pm 0.12$  and  $0.86\pm 0.11$  for left internal carotid artery respectively. Pulsatility indices before and after treatment were  $0.98\pm 0.17$  and  $1.01\pm 0.14$  for right renal artery and  $0.98\pm 0.16$  and  $0.98\pm 0.15$  for left renal artery respectively.

**Conclusion:** Daily combined 2 mg estradiol and 1 mg norethisterone acetate use has no favourable short term effects on carotid and renal arterial perfusion that supply the two main end-organs, the brain and the kidneys.

**Keywords:** menopause, Doppler, ultrasonography, hormone replacement therapy

## Özet

### Östradiol Hemihidrat ve Noretisteron Asetatın Postmenopozal Kadınlarda Karotis ve Renal Arter Kan Akımına Etkisi

**Amaç:** Postmenopozal kadınlarda günlük kombine 2 mg estradiol hemihidrat ve 1 mg noretisteron asetat kullanımının internal karotis arter ve renal arter üzerine kısa dönemdeki etkilerinin incelenmesi.

**Materyal ve Metot:** Menopoz polikliniğimize başvuran postmenopozal dönemdeki 50 kadın çalışmaya alındı. Günlük kombine 2 mg estradiol hemihidrat ve 1 mg noretisteron asetat 6 ay boyunca uygulandı. İnternal karotis ve renal arter pulsatilite indeksleri 'color flow pulsed Doppler' ile incelendi. İstatistiksel incelemede 'Paired t' testi kullanıldı.

**Sonuçlar:** Çalışma öncesi ve 6 aylık tedavi sonrası hastaların kilo, arter kan basıncı ve pulsatilite indeksleri arasında anlamlı fark saptanmadı ( $P>0.05$ ). İnternal karotis arter pulsatilite indeksleri sağ taraf için tedavi öncesi ve sonrasında sırası ile  $0.87\pm 0.17$  ve  $0.89\pm 0.13$ ; sol taraf için tedavi öncesi ve sonrasında sırası ile  $0.84\pm 0.12$  ve  $0.86\pm 0.11$  idi. Renal arter pulsatilite indeksleri sağ taraf için tedavi öncesi ve sonrasında sırasıyla  $0.98\pm 0.17$  ve  $1.01\pm 0.14$ ; sol taraf için tedavi öncesi ve sonrasında sırasıyla  $0.98\pm 0.16$  and  $0.98\pm 0.15$  idi.

**Tartışma:** Postmenopozal dönemde günlük kombine 2 mg estradiol hemihidrat ve 1 mg noretisteron asetat kullanımının beyin ve böbrek gibi iki 'hedef-organ'ı besleyen karotis ve renal arter perfüzyonuna kısa dönemde olumlu etkisi yoktur.

**Anahtar sözcükler:** menopoz, Doppler, ultrasonografi, hormon replasman tedavisi

**Corresponding Author:** Dr. Başak Baksu  
Nato Yolu Cad. Doktorlar Sitesi, A9 D9 Çengelköy  
34850 İstanbul, Türkiye  
Phone : +90 216-329 61 06  
+90 542 435 74 93  
Fax : +90 212-259 10 50  
E-mail : basakbaksu@yahoo.com

## Introduction

At first glance, cardiovascular disease seems to affect men more than women. Women are protected from cardiovascular disease until menopause (1). Coronary artery disease develops on average 10-15 years later in women than in men (2). The incidence of arterial disease increases dramatically in the postmenopausal period and the relative risk of cardiovascular disease in women equals that of men between 6<sup>th</sup> and 7<sup>th</sup> decades (3). In women older than 60 years of age, cardiovascular disease is the first cause of mortality, leaving behind deaths from all malignancies (4). It is possible that the hypoestrogenic state contributes to this observation. Epidemiological studies have consistently found that women using hormone therapy (HT) are at a substantially 35% to 50% lower risk of developing coronary artery disease, with a reduction in the incidence of ischaemic heart disease and cerebrovascular disease (5,6).

The data of Women's Health Initiative Study published in 2002 caused great concerns about the risks/benefits of postmenopausal hormone therapy because of non-statistically significant increased risk of cardiovascular events and breast cancer in the combination of 0.625 mg conjugated equine estrogen and 2.5 mg medroxyprogesterone acetate arm and increased rate of thromboembolic events and stroke in both estrogen+progestin and estrogen-only arms (7). Of course, these results should be evaluated with caution since the results of this study should not apply to other combinations and types of estrogens and progestogens or other routes of administration (8,9).

Even though clinical trials show an excess of thrombotic events with hormone therapy, many experimental and epidemiological studies suggest that estrogen may have beneficial effects on endothelial function and atherosclerosis (10). Hormone therapy improves several risk factors of coronary artery disease, especially the favorable changes in lipid profile, reducing low density lipoprotein and increasing high density lipoprotein levels (11). However, this is estimated to account for about 30% of the observed reduction in cardiovascular risk (12). As well as inhibiting atheroma formation, estrogen appears to affect fibrinolytic, coagulation and antioxidant systems and produces vasoactive molecules like nitric oxide and prostaglandins. Long-term actions of estrogens are by modulating changes in gene expression (13).

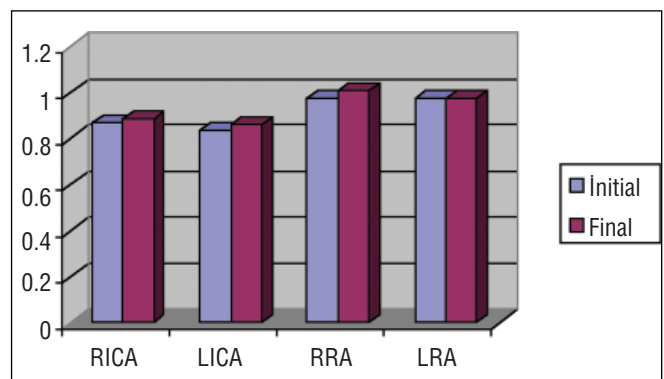
Several studies have reported vasodilatory effect of estrogen on both central and peripheral circulation (1,14,15). Doppler ultrasound is a noninvasive method for investigating the arterial circulation. By measuring pulsatility index (PI), that probably represents blood flow impedance down stream to the point of sampling, gives a measure of arteriolar tone. There are many reports about the effect of estrogen treatment on aorta, carotid, uterine, ovarian and peripheral arteries using this technique. These studies reveal controversial results about the vasodilating action of estrogen. We have undertaken this trial to examine the short term effect of oral HT on internal carotid and renal arteries' PIs.

## Materials and Methods

Fifty postmenopausal women who applied to the Menopause Outpatient Clinic of 2<sup>nd</sup> Gynecology and Obstetrics Clinic at Şişli Etfal Training and Research Hospital, İstanbul, participated in the study. Women with climacteric complaints, at least 12 months postmenopausal, with plasma FSH >40 IU/L and estradiol <20 pg/ml, who had not taken exogenous estrogen recruited into the study. Prior to treatment, family and personal histories were taken. All went through physical examination and had transvaginal pelvic ultrasound scan. Baseline mammographies, cervicovaginal PAP smears, bone densitometries, hormonal and biochemical tests were all performed for all women. Contraindications included a history of cancer of endometrium, liver or breast, surgical menopause and having a risk for cardiovascular disease. Informed consent was taken from all subjects for this study that was approved by the institutional review board.

Daily continuous combined 2 mg estradiol hemihidrate and 1 mg norethisterone acetate (NETA) were given to all patients for 6 months. Before the start of the study and after 6 months of therapy, blood flow was measured by color flow pulsed Doppler ultrasonography. All examinations were performed in a similar manner by using modern equipment (Toshiba SS A-270 A, Tokyo, Japan) by the same specialist. Internal carotid and renal artery PIs were assessed in 32 of the participants who completed the trial.

Renal artery Doppler examinations were performed with 3.75 mHz phased-array transducer. Spectral tracings were obtained from the renal arteries at the location of the renal hilum. The sweep time was set to the highest possible value (100 mm/sec) with the lowest spectral filter. The gain was set such that background echoes were barely visible. The Doppler gate width was kept small, and the angle of insonation was maintained at or lower than 60. Patients were examined in the lateral decubitus position.



**Figure 1.** Pulsatility indices of internal carotid and renal arteries before and after treatment.

\* RICA=right internal carotid artery; LICA=left internal carotid artery; RRA=right renal artery; LRA=left renal artery.

**Table 1.** Demographic characteristics of the patients

	Before treatment	After treatment	T value	P value
Age (years)	62 (50-72)			
Time since menopause (years)	4 (1-8)			
Weight (kg)	71.73 ± 18.24	71.54 ± 18.15	0.68	0.501
Systolic arterial pressure (mmHg)	115.31 ± 14.36	114.06 ± 12.14	0.72	0.474
Diastolic arterial pressure (mmHg)	71.87 ± 9.98	72.50 ± 9.50	-0.39	0.701

**Table 2.** Pulsatility indices of internal carotid and renal arteries before and after treatment

	Initial PI	Final PI	T value	P value
Right internal carotid artery	0.87±0.17	0.89±0.13	-0.65	0.518
Left internal carotid artery	0.84±0.12	0.86±0.11	-0.92	0.363
Right renal artery	0.98±0.17	1.01±0.14	-0.97	0.341
Left renal artery	0.98±0.16	0.98±0.15	-0.09	0.928

Bilateral carotid arterial Doppler examination was performed with the same machine by using a linear-array transducer with frequency of 7 MHz. Spectral Doppler waveform measurements were obtained at the location just beyond the bulb widening in the internal carotid artery. A single measurement was recorded at this location. In all cases, the measured angle of insonation was kept below 60. The peak systolic and end-diastolic velocities were determined from velocity waveforms by using electronic calipers during the examination and PI were calculated.

The results were analysed by t test for paired samples. Before undertaking the study, we didn't make any attempt to estimate the sample size required to show a significant difference in the PI because normal ranges for PI of these arteries in postmenopausal women are not readily available. This is a pilot study; nonsignificant results would lead to a larger trial.

**Results**

The demographic characteristics of the patients are summarised in Table 1.

The mean age of the postmenopausal women was 62 years. The mean duration of menopause was 4 years. There were no significant differences before and after a 6-month treatment with regard to weight, systolic and diastolic arterial pressures ( $P>0.05$ ).

The internal carotid and renal arteries PIs at the start and after treatment were shown in Table 2 and represented by Figure 1.

The confidence interval (CI) for treatment difference of right and left carotid arteries were respectively as follows:  $P=0.518$ , 95% CI -0.073–0.038 and  $P=0.363$ , 95% CI -0.072–0.027. The confidence interval of right and left renal arteries were respectively  $P=0.341$ , 95% CI -0.086–0.31 and  $P=0.928$ , 95% CI -0.050–0.046. There were no statistically significant differences between PIs before and after a 6-month treatment in both arteries ( $P>0.05$ ).

**Discussion**

Numerous evidence have suggested a physiological action of sex steroids upon the cardiovascular system and coherence of epidemiological studies have raised the possibility of a positive action of estrogens in preventing cardiovascular disease, especially through atheroma inhibition and other vascular wall-related mechanisms. One of the best researches about this effect of estrogens is Nurses' Health Study. Women on HT had lower mortality rates and this beneficial effect, most relevant in high risk group for coronary artery disease disappeared on long terms (16). Cardioprotective effects of HT in postmenopausal women have been suggested by observational data, but not proved in randomised trials. For example, although the methodology is debatable, Women's Health Initiative has shown increased incidence of thromboembolic events and has questioned the use of HT for primary prevention.

There are few studies about secondary prevention of estrogens. Even though they seem to support the positive role of estrogens, the Heart and Estrogen/Progestin Replacement Study failed to demonstrate the positive actions suggested by epidemiological studies and the efficacy of estradiol in the treatment of postmenopausal women with cardiovascular disease has been questioned (13,17).

In this study based on the hypothesis that postmenopausal hormone might protect against cardiovascular disease, possibly by arterial vasodilatation and reduced blood pressure, we tested the short term effects of estradiol hemihydrate and NETA. Using Doppler ultrasonography, we investigated the internal carotid artery because it is one of the major arteries likely to represent the general vasculature and renal artery supplying one of the most important organs in the regulation of hemodynamic changes, both of which are easily accessible. To our knowledge, this is the first report evaluating about renal arterial flow by doppler ultrasonography during hormone therapy. Arterial pulsatility index seems

to increase after menopause, probably revealing reduced compliance and increased peripheral resistance within aged vessels (18). After a 6-month of treatment, we didn't find any significant changes in PIs of both arteries. Likewise, Darj et al. couldn't find significant decreases in PIs of common, external and internal carotid arteries after 6 months of HT (19). Similarly, Pan et al. stated that vascular impedance was not influenced significantly in the common and internal carotid arteries after 6 months of HT (20). On the other hand, De Leo V et al. showed improvement in internal carotid PI after a 3-month treatment (21). Penotti et al. reported rapid reductions during a 6-month period of HT of PIs in internal carotid and middle cerebral arteries (22). Jackson et al. also found a decrease in carotid artery PI after 20-24 weeks of HT (18). Both oral and transdermal hormone replacement therapy were reported to reduce impedance to flow in the internal carotid artery after a six-month treatment (23). In a study evaluating long term effects of oral sequential combined HT, carotid PI decreased significantly and this decrease, already detectable during the second month, continued up to 12 months. Also, addition of NETA did not counteract drops in carotid PI (24). In Penotti's study, cyclical medroxyprogesterone acetate did not modify the positive effect of estrogen on the reactivity of the blood vessels (22). In our study, we also used NETA. The effect of progesterones, replaced after menopause to prevent endometrial disease, on cardiovascular disease remains controversial, as some, but not all, negate the vasodilatory effects of estrogens (25,26). NETA has been shown *in vitro* to possess vasodilatory properties (15). However, in our study, we didn't find any effect of NETA. In a study by Naessen et al. long term estrogen therapy was associated with minor reduction in common carotid artery, less in external carotid and negligible in internal carotid artery; therefore, they concluded that effects on carotid vascular resistance did not seem to be a major mechanism in the long-term protective effect of estrogen therapy on cardiovascular risk (27).

The effect of combined estrogen-progesterone HT on blood pressure has been studied in many researches (25). The reported blood pressure changes during HT range from neutral to a moderate blood pressure lowering effect. These variations may be due to many different types and dosages of hormones (26). In our study, there were no significant differences in blood pressure before and after treatment.

At present, it is not possible to make an evidence based clinical decision regarding the key question about the real actions of estradiol in the prevention of cardiovascular disease in postmenopausal women. Many reports reveal positive effect of hormone therapy on cardiovascular performance, specifically on left ventricular function parameters and on aortic elasticity properties (28,29). It is claimed that even though women have greater arterial stenosis compared to men, they have less plaque and that the plaque area is a stronger predictor of outcomes than is stenosis (10).

The discrepancy between the trials trying to quantify the effects of HT may be explained by insufficient adjustment for lifestyle factors in observational studies and insufficient consideration of methodological flaws in experimental studies, both of which lead to an overestimation of the protective effects of hormone replacement. Hormone replacement may affect the pathophysiology of cardiovascular disease in several ways, both directly and indirectly, both negative and positive. Progress in this field requires well-designed, large scale, prospective, randomized clinical studies and the isolation of the positive effects.

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