Effects of estradiol and norethisterone on lipids, insulin resistance and carotid flow

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Abstract

Objectives: To evaluate the lipid profile, insulin resistance and vasomotricity, and the interaction between these factors, in postmenopausal women receiving hormone therapy.

Methods: A prospective, randomized, double-blind study was carried out in which 77 postmenopausal women received one of the three treatment regimens: (A) 2 mg oral micronized estradiol (E2) (n = 25); (B) 2 mg oral E2 + 1 mg oral norethisterone acetate (NETA) (n = 28); or C) placebo (n = 24), daily for 6 months. Evaluations were carried out at baseline and at the end of treatment on lipid and lipoprotein profiles, homeostasis model assessment of insulin resistance (HOMA-IR) and pulsatility index (PI) of the internal carotid artery by Doppler ultrasonography.

Results: Mean increases of 15.6% and 2.4% and a reduction of 6.4% in high-density lipoprotein (HDL) levels were found for the E2, E2 + NETA and placebo groups, respectively. Reductions of 9.5% and 3.7% and an increase of 12.1% in low-density lipoprotein (LDL), and reductions of 20.0% and 3.8% and an increase of 28.8% in the LDL:HDL ratio were found for the E2, E2 + NETA and placebo groups, respectively (p < 0.001 in all cases). Insulin levels and HOMA-IR decreased 12.8% and 12.3% in the E2 group and increased 12.9% and 16.0% in the E2 + NETA group (p < 0.05), respectively. Carotid PI following treatment was 1.18 ± 0.23, 1.38 ± 0.20 and 1.41 ± 0.21 for the E2, E2 + NETA and placebo groups, respectively (p = 0.0006).

Conclusions: Oral estrogen therapy led to an improvement in lipid profile, insulin resistance and carotid blood flow, which was cancelled when NETA was associated.

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Keywords: Hormone therapy; Estradiol; Norethisterone; Cardiovascular risk; Diabetes; Menopause

1. Introduction

Cardiovascular diseases in general are the principal cause of death in both men and women of the western
world [1]. In addition, this group of diseases incurs serious social and economic consequences, particularly among the surviving victims who may then have to live with sequelae and limitations.

Cardiovascular mortality is low in women of reproductive age; however, the incidence increases rapidly following the onset of menopause. During reproductive years, the incidence is much lower than that of men of similar age [1].

The serum levels of estrogen present in women of reproductive age appear to offer a cardioprotective effect. In addition, it has long been known that deterioration occurs in lipid and lipoprotein profiles following menopause, principally a decrease in the levels of high-density lipoprotein (HDL), and estrogen therapy is known to revert this deterioration [2].

For some time, the role of progestagens in reversing the benefits of estrogen in lipid metabolism has been questioned. It was initially believed that 19-nortestosterone derivatives were more detrimental than 17-hydroxyprogesterone derivatives since the former may offer slight androgenic activity; however, no consensus has yet been established with respect to the alleged poorer profile of the former.

It should be remembered that the risk of cardiovascular disease depends on many other factors, carbohydrate metabolism deserving particular mention. An increase in insulin resistance and deterioration in glucose tolerance is known to occur with advancing age, although the effect of the menopause remains controversial [3]. Moreover, lack of estrogen tends to promote an accumulation of abdominal fat, which is a major cardiovascular risk factor and one of the criteria for the diagnosis of metabolic syndrome [4].

In carbohydrate metabolism, estrogen may have beneficial effects, while the progestagen may be deleterious; however, results may differ depending on the type of progestagen used [5].

Another important aspect with respect to cardiovascular risk is the effect of hormones on arterial flow, estrogen having been shown to reduce flow impedance [6]. Little is known, however, with respect to the beneficial effect of the simultaneous administration of progestagens on arterial blood flow.

In view of the gaps that exist in current knowledge with respect to the effects of postmenopausal hormone therapy (HT) on factors related to cardiovascular risk, particularly carbohydrate metabolism, and the effects of 19-nortestosterone-derived progestagens on these parameters, the present study was developed to evaluate the serum levels of atherogenic markers, insulin resistance and vasomotoricity in postmenopausal women not using HT, in women using estrogen-only therapy and in a group using combined estrogen–progestagen HT.

2. Methods

For this prospective, randomized, double-blind, placebo-controlled study, initial sample size was calculated at 16 women for each group, in order to have a power of 80% to detect a difference of 0.15 in pulsatility index of carotid artery (type I and II error rates: 5% and 20%, respectively, and S.D. of 0.24). This sample size was also calculated as being able to detect a difference of 7.5 mg/dL in high-density lipoprotein with a power of 90%, considering a S.D. of 10.0 (type I and II error rates: 5% and 10%, respectively).

Inclusion criteria comprised: age 40–60 years; postmenopausal; last menstrual period at least 1 year previously; not having been in use of postmenopausal hormone therapy for at least 3 months prior to inclusion in the study. The exclusion criteria adopted were non-spontaneous menopause; practice of any physical exercise other than walking; endometrial thickness >5 mm as measured by transvaginal ultrasonography; presence of obstructive lesion with carotid vascular flow restriction detected by Dopplerfluxometry; abnormal mammography or cervical cytology; fasting glucose >99 mg/dL; medical history of cardiovascular disease, venous or arterial thromboembolism, carotid occlusive disease, diabetes mellitus, acute or chronic liver disease, alcohol consumption; use of any of the following medications: vitamin supplements (B6, B12 or folic acid), androgens, raloxifene, tamoxifen, statins, barbiturates, hydantoin, carbamazepine, phenylbutazone, meprobamate and rifampicin.

All participants were from Jundiaí, a city of metropolitan São Paulo. They signed an informed consent form prior to inclusion in the study. The study was conducted at Gynecologic Clinic of Jundiaí School of Medicine. The study protocol and the consent form were approved by the Institution’s Internal Review Board.

The medical history of all participants was registered at admission and complete general physical
and gynecological examinations were carried out during which height, weight and blood pressure were recorded. Transvaginal ultrasonography, mammography and cervical cytology were performed whenever examinations carried out within the 6-month period preceding enrollment to the trial were not available. The women were randomly divided into three treatment groups, treatment lasting for 6 months in all groups:

- **Group A**: treated with 2 mg oral micronized estradiol daily (E2).
- **Group B**: treated with a combination of 2 mg micronized estradiol and 1 mg norethisterone/day orally (E2 + NETA).
- **Group C**: received oral placebo.

Prior to and following treatment, the participants were submitted to blood sampling for biochemical measurements, always performed in the morning following 12 h of fasting. Doppler fluxometry of the carotid artery was also carried out at baseline and at the end of the treatment period. Plasma insulin levels were measured using an immunulite radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Total cholesterol (TC), high-density lipoprotein and triglyceride (TG) levels were measured using an enzymatic colorimetric method (Diasys, Brazil). Low-density lipoprotein (LDL) levels were calculated using Friedewald’s formula [7].

Insulin resistance was measured indirectly using a homeostasis model assessment (HOMA-IR) [8,9] based on fasting plasma insulin and glucose concentrations. Doppler fluxometry evaluations of the carotid artery were performed by the same examiner at baseline and following treatment. A Phillips SD800 ultrasound scanner with a 7.5-MHz transducer and color flow mapping (duplex scan) was used. The examinations were performed following 4 h of fasting and at least 1 h of rest in a room in which the temperature was maintained constant, light was low and noise was minimal. The common right and left carotid arteries were examined with the patient in the dorsal decubitus position with her neck stretched and supported on a pillow. Pulsatility index (PI) was calculated from the spectral analysis of the waves most representative of the speed of flow, and was obtained using the following formula in which A is the maximum systolic velocity, B is the diastolic velocity and C is the mean velocity of the entire cardiac cycle:

\[
\text{PI} = \frac{A - B}{C}
\]

The PI used for these comparisons is the mean PI of the right and left carotids.

2.1. Statistical analysis

One-way analysis of variance (ANOVA) was used for comparisons between groups with respect to their clinical characteristics: age, BMI, time since menopause, number of smokers and arterial blood pressure. The same statistical method was used to compare biochemical parameters, HOMA-IR index and PI. Means and 95% confidence intervals (95% CI) of the individual percentage variations in biochemical parameters, HOMA-IR and PI were also calculated, and the means of the groups were compared using ANOVA.

Student’s t-test for paired data was used for intra-group comparisons between baseline and post-treatment values of biochemical parameters, HOMA-IR and PI. Student’s t-test was also used for comparisons at the end of treatment between the two groups, whenever necessary.

Multivariate analyses using HDL, HOMA-IR and PI as dependent variables and type of treatment (only estrogen, estrogen + progestin, or placebo) as independent variable were made using ANOVA. Bonferroni’s correction for multiple comparisons was applied.

Statistical calculations were made with Addinsoft XLSTAT™ 2007 and Microsoft Excel™ 2007. Where appropriate, the data are presented as mean ± S.D. Statistical significance was established at 5%.

3. Results

Of the 89 women included in the study, 12 failed to complete the trial, 3 in Group A, 2 in Group B and 7 in Group C. In Group A, the discontinuations were due to mastalgia (1) and irregular vaginal bleeding (1), while one patient was lost-to-follow-up. In Group B, the two discontinuations were due to irregular vaginal bleeding that was considered unacceptable by the patients. Of the seven discontinuations in Group C, three abandoned the study because their hot flushes became worse, one because of mastalgia, one due to
Table 1
Mean values and S.D.s of the clinical characteristics of the placebo, estradiol (E2) and estradiol/norethisterone acetate (E2 + NETA) groups prior to initiation of treatment

<table>
<thead>
<tr>
<th></th>
<th>E2 (n = 25)</th>
<th>E2 ± NETA (n = 28)</th>
<th>Placebo (n = 24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.6 ± 3.4</td>
<td>52.1 ± 3.7</td>
<td>52.5 ± 4.8</td>
<td>0.7132</td>
</tr>
<tr>
<td>TSM (years)</td>
<td>3.72 ± 3.1</td>
<td>3.89 ± 2.8</td>
<td>4.04 ± 3.0</td>
<td>0.9295</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 4.9</td>
<td>27.0 ± 4.6</td>
<td>26.2 ± 4.3</td>
<td>0.7897</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.2 ± 11.6</td>
<td>129.6 ± 16.4</td>
<td>129.6 ± 14.9</td>
<td>0.4622</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.6 ± 10.4</td>
<td>85.0 ± 10.4</td>
<td>82.9 ± 10.4</td>
<td>0.7600</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>0.7111</td>
</tr>
</tbody>
</table>

TSM: time since menopause; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PI: pulsatility index of the carotid artery.

carotid obstruction (violation of the exclusion criteria), one due to myocardial infarction and one due to lost-to-follow-up.

Therefore, a total of 77 patients concluded the study, 25 in Group A, 28 in Group B and 24 in Group C. The age of patients varied from 45 to 61 years (mean 52.1 ± 4.0 years). Time since menopause ranged from 1 to 14 years with a mean of 3.9 ± 2.9 years and median of 3. Smoking was reported by 15 women (19.5%). The demographical and clinical characteristics at baseline are shown in Table 1 and no statistically significant differences were found between the three groups.

Table 2 shows the means and S.D.s of the biochemical parameters evaluated in each group at baseline and following treatment, and the intra-group and inter-group comparisons.

There was a statistically significant increase in HDL only in Group A. Comparison between the three groups showed that these differences were statistically significant at the end of the treatment. Likewise, LDL decreased significantly in Group A, while showing a trend towards decreasing less and increasing in Groups B and C, respectively. There was a statistically significant difference between the groups at the end of treatment. There was a statistically significant decrease in the LDL:HDL ratio in the group treated with estradiol (A) and a statistically significant increase in the group that received placebo (C). In addition, there were statistically significant differences between the three groups at the end of treatment. No differences were found among the groups in triglyceride levels at baseline and at the end of study.

During the multivariate analysis, we found a statistically significant direct correlation between E2 treatment and HDL with a correlation coefficient of +0.541. The Bonferroni’s test showed a p-value of <0.0001 for the difference of HDL levels between ‘with’ and ‘without’ E2, with higher levels of HDL with estradiol treatment, while NETA led to decreased levels of HDL, and the coefficient was −0.278 (p = 0.023).

The variations in glucose and insulin were not statistically significant in any of the groups; however, insulin resistance decreased in Group A and this decrease was marginally significant. The HOMA-IR index following treatment was 2.38 ± 1.30, 3.65 ± 2.86 and 2.54 ± 1.01 for Groups A–C, respectively, and the inter-group differences were statistically significant (p = 0.04). However, the intra-group variations failed to reach statistical significance.

There was no correlation between E2 treatment and HOMA-IR at multivariate analysis, but, NETA treatment influenced negatively this parameter, with coefficient of +0.305, with statistically significant differences between ‘with’ and ‘without’ NETA (p = 0.021).

There were statistically significant differences in the PI of the carotid artery between the three groups at the end of the treatment period, the lowest value being found in the group treated with estradiol alone (1.18 ± 0.23, 1.38 ± 0.20 and 1.41 ± 0.21 for Groups A–C, respectively) (p = 0.0006). The decrease in PI in Group A was marginally significant (p = 0.0511).

In the multivariate analysis, there was a significant inverse correlation between E2 therapy and IP, with coefficient of −0.454, and a direct correlation between NETA use and IP with coefficient of +0.405. The differences between ‘with’ and ‘without’ the treatments were statistically significant (Bonferroni’s test, p = 0.0004 for E2 and p = 0.001 for NETA).

Analysis was also carried out on mean individual variations, and a reduction in total cholesterol of 3.4% was found in Group A (E2 alone), a decrease of 1.5%
Table 2
Means and S.D.s of the initial and final levels of biochemical parameters and HOMA-IR in the estradiol (E2), estradiol/norethisterone acetate (E2 + NETA) and placebo groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial (median ± d.p.)</th>
<th>Final (median ± d.p.)</th>
<th>Intra-group comparison (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>222.0 ± 44.1</td>
<td>211.4 ± 40.8</td>
<td>0.1537</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>219.7 ± 31.6</td>
<td>215.8 ± 32.0</td>
<td>0.2305</td>
</tr>
<tr>
<td>Placebo</td>
<td>214.5 ± 41.8</td>
<td>226.8 ± 47.5</td>
<td>0.1514</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.7931</td>
<td>0.3915</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>50.4 ± 11.7</td>
<td>56.8 ± 10.6</td>
<td>0.0008</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>49.2 ± 11.2</td>
<td>50.1 ± 11.5</td>
<td>0.4679</td>
</tr>
<tr>
<td>Placebo</td>
<td>47.8 ± 10.8</td>
<td>43.0 ± 9.6</td>
<td>0.0733</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.7206</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>149.7 ± 38.9</td>
<td>134.0 ± 38.3</td>
<td>0.0062</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>147.8 ± 28.3</td>
<td>141.4 ± 28.3</td>
<td>0.0614</td>
</tr>
<tr>
<td>Placebo</td>
<td>146.3 ± 35.7</td>
<td>160.1 ± 38.9</td>
<td>0.0533</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.9418</td>
<td>0.0326</td>
<td></td>
</tr>
<tr>
<td>LDL/HDL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>3.06 ± 1.03</td>
<td>2.40 ± 0.88</td>
<td>0.0002</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>3.11 ± 1.05</td>
<td>2.95 ± 1.00</td>
<td>0.1688</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.14 ± 1.01</td>
<td>3.85 ± 1.22</td>
<td>0.0079</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.9637</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>109.6 ± 54.7</td>
<td>103.4 ± 82.8</td>
<td>0.5675</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>113.6 ± 50.8</td>
<td>122.0 ± 34.6</td>
<td>0.3692</td>
</tr>
<tr>
<td>Placebo</td>
<td>102.3 ± 44.8</td>
<td>118.3 ± 54.9</td>
<td>0.0850</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.7203</td>
<td>0.5031</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>82.8 ± 8.3</td>
<td>82.8 ± 7.7</td>
<td>1.000</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>84.4 ± 6.8</td>
<td>86.0 ± 7.6</td>
<td>0.1118</td>
</tr>
<tr>
<td>Placebo</td>
<td>85.2 ± 7.4</td>
<td>82.7 ± 10.2</td>
<td>0.2856</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.5469</td>
<td>0.2767</td>
<td></td>
</tr>
<tr>
<td>Insulin (mcU/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>14.5 ± 7.4</td>
<td>11.6 ± 6.3</td>
<td>0.0591</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>15.2 ± 10.7</td>
<td>17.0 ± 13.1</td>
<td>0.1568</td>
</tr>
<tr>
<td>Placebo</td>
<td>12.6 ± 7.5</td>
<td>12.8 ± 6.0</td>
<td>0.7705</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.5469</td>
<td>0.0928</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>2.99 ± 1.62</td>
<td>2.38 ± 1.30</td>
<td>0.0567</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>3.21 ± 2.43</td>
<td>3.65 ± 2.86</td>
<td>0.1420</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.67 ± 1.72</td>
<td>2.54 ± 1.01</td>
<td>0.5416</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.6198</td>
<td>0.0406</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>1.37 ± 0.32</td>
<td>1.18 ± 0.23</td>
<td>0.0511</td>
</tr>
<tr>
<td>E2 + NETA</td>
<td>1.40 ± 0.30</td>
<td>1.38 ± 0.20</td>
<td>0.6404</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.40 ± 0.34</td>
<td>1.41 ± 0.21</td>
<td>0.8394</td>
</tr>
<tr>
<td>Inter-group comparison (p)</td>
<td>0.9332</td>
<td>0.0006</td>
<td></td>
</tr>
</tbody>
</table>

NB: TC, total cholesterol; TG, triglycerides; PI, pulsatility index of the carotid artery. The statistical significant p-values are in bold.

* Student’s t-test.

b ANOVA.

in Group B (E2 + NETA) and an increase of 7.4% in Group C (placebo). Likewise, HDL increased 15.6% in Group A and 2.4% in Group B, while decreasing 6.4% in Group C. LDL decreased 9.5% in Group A and 3.7% in Group B, but increased 12.1% in Group C, resulting in a reduction of 20.0% in the LDL:HDL ratio in Group A and 3.8% in Group B, but an increase of 28.8% in Group C. All the variations in the lipid profile, except triglycerides, were statistically significant and are shown in Fig. 1.

The differences between the groups in the mean variations of glucose, insulin, HOMA-IR and PI failed to reach statistical significance in the global analysis (Fig. 2). However, there was a statistically signifi-
Fig. 1. Means of the individual percentage variations in the parameters of the lipid profile between baseline measurements and those found at the end of the treatment period in the E2, E2 + NETA and placebo groups. Inter-group differences were statistically significant for all parameters evaluated, except for triglycerides. (Bars represent parameter averages; vertical lines represent 95% confidence intervals. Numbers near bars are the average values.)

4. Discussion

The present study was developed to investigate the effects of estrogen and estrogen + progestagen HT not only on lipid profile but principally on insulin resistance, which is strongly correlated with cardiovascular risk, and also on carotid flow, comparing continuous hormone therapy with estrogen alone and estrogen in combination with a progestagen with a placebo group.

The present study found statistically significant inter-group differences in all the parameters in the lipid profile following treatment, except in triglycerides. There was an increase in HDL and a decrease in LDL in the group treated with E2. LDL decreased in the estrogen–progestagen group and increased in the placebo group; however these differences were only marginally significant. The LDL:HDL ratio decreased significantly in the E2 group and increased significantly in the placebo group. However, the improvement in the lipid profile achieved by estradiol was practically annulled by the addition of norethisterone in Group B in which there was merely a trend towards improvement. This finding was confirmed by the multivariated analysis which showed a direct correlation between estrogen treatment and HDL levels, and an inverse effect of NETA.

These effects of sex steroids have long been known [1,2,10] and, only to cite some of the most known reports, are in agreement with the results of the WHI study, which reported reductions in LDL of 12.7% both with estrogen therapy and estrogen–progestagen therapy, and an increase of 14.0% in HDL with estrogen therapy and 7.3% with combined therapy. There was no reduction in total cholesterol in the estrogen group; however, total cholesterol decreased 5.4% in the com-
bined therapy group, which is explained by the fact that there was a greater increase in HDL in the estrogen group [11,20]. This combination of results would lead to a greater reduction in the LDL:HDL ratio in the treatment with estrogen alone compared to combined HT, which is compatible with the findings of the present study despite the differences in the progestagens used. Also, a recent meta-analysis evaluated 107 studies and concluded that a mean reduction of 17.4% occurred in the LDL:HDL ratio with oral HT [12] which is compatible with our findings.

A dose–response effects of both estrogen and progestin probably exists, and Lobo et al. have showed that low doses of medroxyprogesterone acetate and conjugated estrogen induced favorable changes in lipids, lipoproteins, but a careful analysis of their results reveals that the effect of only estrogen on lipid profile is some better than that of combination HT [22].

We found a reduction in the pulsatility index of the carotid artery only in the estradiol group, which had the lowest index value compared to the other groups at the end of hormone treatment. This results in an improvement in blood flow in this group. This finding is in agreement with other studies that have shown an improvement in Doppler flow both in central and in peripheral territory [6,13,14]. Estradiol promotes this favorable alteration by means of direct arterial vasodilatation, but principally mediated by nitric oxide [15] and there is an evidence that estradiol may lead to an increase in the levels of this substance [16]. This vasodilatory effect also appears to be dependent on the time between the onset of menopause and the beginning of HT, since a study has been carried out that shows vasodilatation following estrogen therapy alone in a group of younger postmenopausal women, although the increase in nitrites and nitrates occurred both in the younger and older women [14]. The probable explanation is that the arterial walls of older women, those who have been postmenopausal for longer, are already more rigid and consequently less responsive to the increase in nitrites and nitrates.

Although the present study failed to detect any improvement in arterial flow with combined therapy, De Leo et al. reported an improvement in this flow even with combined estrogen–progestagen therapy [17]. One of the possible explanations for this may be, once
again, the type of progestagen, since these investiga-
tors used cyproterone acetate, a pregnane-derivative, 
by obtaining results that were repeated in another study
[18]. In support of this explanation, another study with 
norethisterone also failed to find any improvement in 
pulsatility index, in agreement with the findings of our
study [19].

One Brazilian study found no difference between 
estriadiol and estradiol plus norethisterone acetate 
effects on pulse-wave velocity and arterial stiffness
[23], but, they have studied hypertensive post-
menopausal women, and, again, a possible explanation 
is that arterial walls of hypertensive women were 
already rigid.

The effect of the association estradiol plus norethis-
teron on carotid and uterine vascular resistance was 
also evaluated by another Brazilian investigators and 
they concluded that this oral continuous HT was effec-
tive in reducing impedance to flow in uterine, but not 
in carotid circulation [24]. The conclusion towards 
carotid territory is in agreement to ours, because we 
only found reduction in pulsatility index with only 
estriadiol therapy. Their results also suggest that the 
effects of HT vary by vascular site.

Our multivariate analysis reinforced the beneficial 
effect of estrogen treatment in carotid artery flow and 
the opposite effect of NETA, at least in our population 
within first years after menopause.

With respect to insulin levels, statistical signifi-
cance was found only in the comparison of mean 
variations between the group treated with E2 ver-
sus the E2 + NETA and placebo groups. The lowest 
absolute value of the HOMA-IR index was found in the 
estriadiol group and the difference between the 
groups was statistically significant. There was also a 
significant difference between the mean reduction of 
12.3% in HOMA-IR in the E2 group and the mean 
increase of 16.0% in the E2 + NETA group. Although 
the mean increase in HOMA-IR in the placebo group 
was even greater, this difference was not statistically 
different from that of the E2 group, except marginally. 
This is explained by the wider spectrum of vari-
ation in this parameter in the placebo group, reflected in the larger confidence interval, which may be due to 
the lower number of participants who concluded the 
study in this group in which there was a greater 
incidence of drop-outs as a result of the menopausal 
symptoms.

That is also a limitation of our study, because some evaluated parameters, principally carbohydrate 
metabolism ones, had a greater variability, and a larger size of population could helped us in this aspect. A high 
drop-out rate in placebo group caused by menopause 
symptoms is another problem with this kind of study.

The fall in insulin following treatment with estra-
diol is in accordance with data already reported in the 
scientific literature, including the reported findings of 
the WHI study [11,20]. It should be emphasized that in 
this latter study, a reduction occurred both in patients 
using estrogen therapy alone and in those using com-
bined HT, although in the latter group, the reduction in 
insulin was only marginally significant [11]. It is impor-
tant to remember that the progestagen used in the WHI 
study was medroxyprogesterone acetate, whereas in the 
present study norethisterone acetate was used, the 
former being a pregnane derivative, while the latter is a 
nortestosterone derivative. In addition, the difference in 
the size of the populations studied should be taken into 
consideration. Another relevant aspect is that progesta-
gens derived from 19-nortestosterone, particularly first 
generation progestagens including norethisterone, may 
be associated with a reduction in glucose tolerance 
and an increase in insulin levels [5] which is compat-
ible with our findings. On the other hand, there have 
been concerns for quite some time regarding carbohy-
drate metabolism with the use of medroxyprogesterone 
acetate, principally in parenteral contraceptive prepa-
rations [21] and, in the case of the WHI study, this 
substance was used orally in doses much lower than 
those used for contraception. Also, Lobo et al., studying 
749 postmenopausal women receiving several dosage 
regimens of conjugated estrogens and the same pro-
gestin of WHI, reported only minimal changes in 
carbohydrate metabolism [22].

A recent meta-analysis concluded that post-
menopausal HT results in a mean reduction of 12.9% in 
HOMA-IR, principally with respect to the oral route of 
administration, a finding compatible with our findings 
for estrogen therapy alone [12]. Nevertheless, that pub-
lication failed to discriminate between estrogen therapy alone and estrogen–progestagen therapy, as was done in the present study. The probable explanation may be 
the fact that more studies have been carried out using 
estrogen therapy alone, principally the older studies, 
and that the number of studies carried out on the effects 
of HT on the HOMA-IR index is relatively small. The
fact that oral estrogen has a greater impact on the reduction in insulin resistance may be due to the mechanism of the first pass through the liver, principally if it is remembered that the pancreatic venous return has been shown to occur mainly by the portal system; however, a direct effect on the pancreas may also be present, and the exact mechanisms of this action are still unknown [25]. Moreover, there have been reports in the scientific literature of a reduction in sensitivity to insulin with combined HT [26] and also an improvement with estrogen therapy alone [27,28], and these data are in agreement with the findings of the present study.

It is noteworthy that a recent study showed not only different effects but even contradictory effects with different progestagens and concluded that there is a beneficial effect of norethisterone on carbohydrate metabolism, whereas medroxyprogesterone does not confer this effect [29]. In opposite way, however, other study found that conjugated estrogen plus medroxyprogesterone acetate reduced HOMA-IR [30]. As norethisterone has a more androgenic profile, it is to be expected a more deleterious action in carbohydrate metabolism, notwithstanding, norethisterone has no glucocorticoid effect, distinctly of medroxyprogesterone which may present this effect [31]. Nevertheless, it should be emphasized that our data showed a deleterious effect of norethisterone on the benefits of estradiol in insulin resistance.

These contradictions in the literature clearly show that the effects of HT on carbohydrate metabolism are less intense than the effects of this therapy on lipid metabolism; however, their evaluation is more difficult and requires larger sample sizes. Therefore, important variables not yet detected may be creating confounding effects. In conclusion, the use of oral estradiol alone by postmenopausal women leads to a significant improvement in lipid profile, in the carotid pulsatility index as result of arterial vasodilatation, and in insulin resistance evaluated by the HOMA-IR index. The addition of norethisterone, a synthetic progestagen derived from nortestosterone, cancelled out all the benefits conferred by the estrogen. In view of the many variables involved in carbohydrate metabolism, it is possible that new studies designed to detect and isolate confounding factors, may determine the existence of a dependent relationship between the parameters evaluated here. Moreover, different progestagens may exert different impacts on lipid and carbohydrate metabolism and on estrogen-mediated vasodilatation.

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References


