

## Postmenopausal Therapy Reduces Catalase Activity and Attenuates Cardiovascular Risk

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### Abstract

**Background:** Menopause can lead to alterations in women's health, with changes in the oxidative status of postmenopausal women in whom information regarding the influence of hormone therapy (HT) on antioxidant enzyme activities is limited.

**Objective:** To evaluate the influence of HT on catalase activity; concentrations of lipids and lipoprotein, cholesteryl ester transfer protein, thiobarbituric acid-reactive substances, nitrates, high-sensitivity C-reactive protein and carotid thickness in postmenopausal women.

**Methods:** Ninety-four consecutive women were allocated to one of four groups, without HT and with HT. The latter group was subdivided into women using estrogen and those using estrogen plus progestogen therapy. Plasma biochemical parameters and common carotid intima-media thickness measurements were performed.

**Results:** HT antagonized the decrease in catalase activity after menopause, but had no effect on the levels of cholesteryl ester transfer protein, thiobarbituric acid-reactive substances, lipid peroxide, nitrate, high-sensitivity C-reactive protein, or on the common carotid intima-media thickness. Multivariate analysis showed that estrogen-based HT attenuated the relationship between cardiovascular risk factors and the intima-media thickness of the common carotid.

**Conclusion:** This study indicates that HT in postmenopausal women produces beneficial antioxidant and anti-atherosclerotic effects by ameliorating the plasma lipid and lipoprotein profiles, increasing plasma catalase activity and attenuating the association between cardiovascular risk factors and early atherosclerosis. (Arq Bras Cardiol 2012;99(5):1008-1014)

**Keywords:** Risk factors; cardiovascular diseases; catalase; postmenopause.

### Introduction

The role of oxidative stress in atherosclerosis has received considerable attention, with atherogenesis being triggered by focal inflammation and cellular proliferation<sup>1</sup>. Antioxidant enzymes such as catalase, superoxide dismutase e glutathione peroxidase, protect aerobic cells against oxidative injury caused by reactive oxygen species (ROS) generated during normal cellular metabolism<sup>1</sup>. Oxidative stress resulting from the overproduction of ROS or a deficiency in the enzymatic and non-enzymatic antioxidant systems can lead to a number of pathological conditions and contribute to aging<sup>1</sup>. Menopause can lead to alterations in women's health, with excessive ROS formation and changes in the oxidative status of postmenopausal women<sup>2-5</sup>. However, few studies have examined the influence of hormone therapy (HT) on antioxidant enzyme activity in postmenopausal women and have focused on short-term use of HT.

The cardiovascular effects of HT have been the subject of much debate since the initial findings from the Women's Health Initiative (WHI study) were reported. However, re-analyses of WHI results have suggested that the association between HT use and cardiovascular risk is influenced by several factors, among these, age and time since menopause. Observational and randomized studies have suggested that initiation of hormone replacement therapy (HT) in early postmenopause could be beneficial from a cardiovascular point of view. Conversely, aging, time since menopause and presence of cardiovascular risk factors or cardiovascular disease may decrease its efficacy and increase the risk of cardiovascular events<sup>6</sup>.

In the last ten years, HT for the treatment of menopausal symptoms is a real option that led to recently launched studies on the antioxidant effects of estrogen. In addition to its effects on the post-menopausal symptoms estrogen therapy has been used to treat osteoporosis<sup>7</sup> and many postmenopausal women have been using it.

The intima-media thickness of the common carotid artery (carotid IMT), an appropriate intermediate endpoint for investigating clinically relevant effects on atherogenesis, can be used to assess the early stages of atherosclerosis, since this parameter correlates with risk factors, including age,

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dyslipidemias and oxidative stress<sup>8</sup>. Information regarding the influence of HT on carotid atherosclerosis is limited; whereas experimental and epidemiological studies have reported that HT has a beneficial effect on the progression of carotid IMT in postmenopausal women<sup>9</sup>, others have observed no significant effect<sup>10</sup>. In contrast, it has been reported that while HT may prevent the development of atherosclerotic plaques in postmenopausal women<sup>9</sup>, there is no difference in the carotid IMT between women who had and had not used HT<sup>11</sup>. Lipid and lipoprotein metabolism is markedly altered after menopause<sup>12-14</sup> with a natural reduction in estrogen levels.

In this study, we investigated the serum catalase activity; serum concentrations of lipids and lipoprotein, cholesteryl ester transfer protein, thiobarbituric acid-reactive substances (TBARS), nitrate and hs-CRP plasma levels in postmenopausal women with or without HT, in order to determine whether postmenopausal HT influences the levels of oxidative stress (a proinflammatory marker). The thickness of the common carotid intima-media was used as an early marker of atherosclerosis.

## Methods

### Subjects

Ninety four postmenopausal women (POMW) mean age: 59 years, were assigned to one of two groups: those without HT (WHT, n = 63) and those with HT (WHT, n = 31); the women in the latter group were further subdivided into those using estrogen therapy (ET, conjugated equine estrogen, 0.625 mg/d; n = 20) and those using estrogen plus progestogen therapy (EPT, methoxyprogesterone acetate, 2.5-5 mg/d; n = 11). They were sequentially recruited at the University Clinic during a one-year period, according to the criteria presented below.

The POMW were clinically evaluated at the Dyslipidemia and Menopause Outpatient Clinics of the School of Medical Sciences of UNICAMP. The presence of menopause was defined among women  $\geq 40$  years old with at least one year of natural menopause or surgical bilateral oophorectomy, in accordance with the criteria of the North American Menopause Society<sup>14</sup>. The exclusion criteria for participation in the study were: severe dyslipidemia (HDL cholesterol  $\leq 40$  mg/L, LDL cholesterol  $\geq 190$  mg/L and triglycerides  $\geq 400$  mg/L), smoking, alcoholism, severe systemic disease including nephropathy, endocrinopathy and pneumopathy.

The criterium for body mass index (BMI)  $\leq 27$  kg/m<sup>2</sup><sup>13</sup> was reached in half of the women studied.

Vasomotor symptoms (hot flashes and night sweats) were the main indication for the use of systemic HT<sup>14</sup>. All POMW had been on HT with continuous oral estrogen (0.625 mg/d) or continuous estrogen (0.625 mg/d) and methoxyprogesterone acetate (2.5 or 5 mg/d) for at least one year (mean of five years). All of the subjects gave written informed consent to participate in the study.

This investigation was approved by the institutional Research Ethics Committee (protocol no. 314/2004).

### Samples

Blood samples were collected from patients by venipuncture into ethylenediamine tetraacetic acid (EDTA) after a 12 h fast. Plasma was obtained by centrifugation at 1000 g for 10 min at 4°C.

### General Analytical Methods

Total cholesterol and triglycerides were determined by an enzymatic-colorimetric method (Hitachi 917, Roche, Mannheim, Germany). Plasma LDL and HDL cholesterol were analyzed in sample supernatants by a homogeneous direct enzymatic-colorimetric method after precipitation of apoB 100-containing lipoproteins. Apolipoproteins (AI and B 100) were determined by nephelometry.

Nitrate, the stable metabolite of nitric oxide, was measured with Greiss reagent in a commercial nitrate/nitrite assay kit (Cayman Chemical Co, Ann Arbor, Michigan, USA)<sup>15</sup>. Catalase activity, thiobarbituric acid-reactive substances (TBARS) and lipoperoxidases (LPO) concentrations were assayed in plasma by ELISA and colorimetric methods (Cayman Chemical Co, Ann Arbor, Michigan, USA)<sup>16,17</sup>. The concentration of high-sensitive CRP was determined with a Behring latex-enhanced CRP assay and a Behring nephelometer analyzer system (Dade Behring, Tokyo, Japan).

The intima-media thickness of the common carotid artery was measured with an HDI 1500 ultrasound system (ATL Ultrasound, Botheli, WA, USA) fitted with a 7-12 MHz color Doppler probe. The carotid IMT was calculated as the mean of five measurements in the far wall of the left and right common carotid arteries, according to a standardized method<sup>9</sup>. Individual results (expressed in mm) were the average of the left and right carotid IMT.

### Statistical Analysis

Results are expressed as means  $\pm$  SD. Statistical analyses were carried out using ANCOVA with rank transformation, adjusted for age and BMI, or the Mann-Whitney test. Spearman's test was used to examine the correlation between variables in the groups. Multiple linear regression analysis with stepwise criteria for the selection of variables was used to assess the influence of lipoproteins, apolipoproteins, cholesteryl ester transfer protein (CETP), catalase, nitrate, TBARS, LPO and hs-CRP serum concentrations on carotid IMT. The results were expressed as coefficients of determination ( $R^2$ ) that represented the percentage of variation in the dependent variable that was explained by the independent variables. A value of  $p < 0.05$  (two-tailed test) indicated significance. All data and statistical analyses were performed with the SAS statistical package (SAS Institute Inc, Cary, NC, USA).

## Results

Table 1 summarizes the clinical and biochemical characteristics of the subjects. There was no difference in the mean age or blood pressure of the WHT and WHT groups, but a significant difference was observed in the BMI (29 kg/m<sup>2</sup> and 26 kg/m<sup>2</sup>, respectively;  $p = 0.021$ ) and waist circumference (89 cm and 83 cm, respectively;  $p = 0.007$ )

**Table 1 - Anthropometric and radiological parameters of postmenopausal women with (wht) and without (wtht) hormone replacement therapy (ht)**

Group/ Parameter	Age (years)	BMI (kg/m <sup>2</sup> )	SBP (mmHg)	DBP (mmHg)	WC (cm)	IMT(mm)
POMW	58±8 (94)	28±5 (94)	120±1.0(94)	80±0.9 (94)	87 ± 13 (92)	0.88±0.2 (84)
WHT	59±7(63)	29.2 <sup>1</sup> ±5(63)	120±1.1(63)	80.1±0.1(63)	89 <sup>3</sup> ±14(63)	0.89±0.19(55)
WHT	56±8(31)	26.4 <sup>1,2</sup> ±4(31)	120±0.9(31)	79±0.8(31)	83 <sup>3</sup> ±10(31)	0.85±0.14(29)
ET	56±9(20)	25.7 <sup>2</sup> ±4(20)	120±0.5(20)	79±0.7(20)	80 <sup>4</sup> ±9(20)	0.83±0.14(20)
EPT	57±7(11)	28.0±4(11)	120±1.4(11)	80±0.9(11)	88±9(11)	0.91±0.14(9)

The values are the mean±SD of the number of subjects indicated in parentheses, POMW: postmenopausal women; WHT: without HT; WHT: with HT; ET: estrogen therapy; EPT: estrogen plus progesterone therapy; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; IMT: carotid intima-media thickness; WC: waist circumference; <sup>1</sup>p(29.2x26.4)=0.021; <sup>2</sup>p(26.4x25.7); <sup>3</sup>p(89x83) =0.007; <sup>4</sup>p=0.0184 (Mann-Whitney test between 2 groups and Kruskal-Wallis test between 3 groups).

of the two groups. The mean carotid IMT ranged from 0.83 mm to 0.91 mm, with no difference between the two postmenopausal groups.

The mean serum total cholesterol concentration in POMW exceeded the maximum recommended value of 200 mg/dL (Table 2). Higher LDLchol and NHDLchol (non HDL cholesterol) levels were observed in WHT versus WHT postmenopausal women (p = 0.013 and 0.048 for LDL and NHDLchol, respectively) (Table 2), whereas lipoprotein (a) remained unchanged. Increased HDLchol was observed in postmenopausal women with estrogen therapy, compared to those with estrogen plus progesterone

therapy (p = 0.041). Insulin levels were identical in all groups (not shown). The Castelli II index (LDLchol/HDLchol) decreased in HT with estrogen. CETP activity was similar in all groups.

Table 3 shows that, with the exception of catalase, which increased by approximately 42% in the WHT and EPT groups, there were no significant differences in the levels of the other biomarkers of oxidative stress and inflammation between the groups.

Strong correlations were observed between three risk factors for atherosclerosis (age, hs-CRP and waist circumference) and carotid IMT in the WHT group, but

**Table 2 - Lipid, lipoproteins and cholesteryl ester transfer protein levels in postmenopausal women with (wht) and without (wtht) hormone replacement therapy (ht)**

Parameter/Group	POMW	WHT	WHT	ET	EPT
Cholesterol (mg/dL)	224±39 (94)	229±40 (63)	215±36 (31)	215±37 (20)	213±36 (11)
HDLchol (mg/dL)	61±14 (94)	60±12 (63)	65±17 (31)	69±17 <sup>3</sup> (20)	57±14 <sup>3</sup> (11)
LDLchol (mg/dL)	133±36 (94)	140±34 <sup>1,4</sup> (63)	118±37 <sup>1</sup> (31)	115±40 <sup>4</sup> (20)	126±29 (11)
NHDLchol (mg/dL)	164±40 (94)	170±40 <sup>2</sup> (63)	151±40 <sup>2</sup> (31)	148±40 (20)	157±41 (11)
Triglycerides (mg/dL)	153±89 (94)	147±66 (63)	165±224 (31)	158±88 (20)	178±176 (11)
LDLchol/HDLchol	2.4±0.7 (94)	2.41±0.7 <sup>5,6</sup> (63)	1.93±0.8 <sup>5</sup> (31)	1.76±0.8 <sup>5</sup> (20)	2.27±0.8 (11)
APO A1 (mg/dL)	172±26 (94)	167±24 (63)	181±30 (31)	188±33 (20)	167±17 (11)
APO B100 (mg/dL)	120±27(94)	123±28 (63)	115±25 (31)	113±23 (20)	116±29 (11)
Lp(a) (mg/dL)	29±30 (94)	28±29 (63)	32±30 (31)	24±27(20)	44±35 (11)
CETP (% CE transfer)	32 ± 12 (93)	32 ± 11 (61)	32 ± 13 (31)	31 ± 14 (20)	34 ± 11 (11)

The values are the mean±SD of the number of subjects indicated in parentheses. ), POMW: postmenopausal women; WHT: without HT; WHT: with HT; ET: estrogen therapy; EPT: estrogen plus progesterone therapy; APO A1: apolipoprotein A1; APO B: apolipoprotein B; CETP: cholesterol ester transfer protein; HDLchol: high-density cholesterol lipoprotein; LDLchol: low-density cholesterol lipoprotein; LDLchol/HDLchol: Castelli II; Lp(a): lipoprotein (a); and NHDLchol: no high-density cholesterol lipoprotein <sup>1</sup>p= 0.013; <sup>2</sup>p= 0.048; <sup>3</sup>p=0.041; <sup>4</sup>p=0.034; <sup>5</sup>p=0.017 and <sup>6</sup>p=0.013. (Mann-Whitney test between 2 groups and Kruskal-Wallis test between 3 groups.

**Table 3 - Oxidative and inflammatory biomarkers in postmenopausal women with (wht) and without (wht) hormone replacement therapy (ht)**

Parameter/Group	Catalase (nmol/min/ml)	Nitrate (µM)	TBARS (nmol/tmp/gprot)	Lipid peroxides (umoles/L)	hs-CRP (mg/L)
WHT	22±24 <sup>1</sup> (62)	8.6±6(62)	2.8±1.1(55)	4.3±1.0(29)	0.5±0.8(61)
WHT	38±30 <sup>1</sup> (30)	9±7(31)	2.4±0.9(26)	5.3±4.8(24)	0.4±0.4(29)
ET	39±31(20)	8.2±5(20)	2.4±0.8(17)	5.8±5.7(17)	0.5±0.4(19)
EPT	37±30 <sup>2</sup> (10)	10±11(11)	2.4±1.1(9)	4.2±0.9(7)	0.4±0.4(10)

The values are the mean±SD of the number of subjects indicated in parentheses; WHT: without HT; WHT: with HT; ET: estrogen therapy; EPT: estrogen plus progestogen therapy; hs-CRP: high-sensitivity C-reactive protein; nitrate: nitrate/nitrites; and TBARS: thiobarbituric acid-reactive substances. <sup>1</sup>p (22x38)= 0.015; and <sup>2</sup>p (22x37)= 0.029. (Mann-Whitney Test between 2 groups and Kruskal-Wallis test between 3 groups.

**Table 4 - Correlation coefficients for carotid INTIMA-MEDIA THICKNESS versus metabolic or anthropometric variables in postmenopausal women without hormone replacement therapy (wht) and those on estrogen plus progestogen therapy (ept)**

WHT (n=55)	Age	WC	hs-CRP
Right IMT (mm)	0.386 (0.004)	0.316 (0.018)	0.430 (0.043)
Left IMT (mm)	0.400 (0.003)	0.0186 (0.316)	0.306 (0.026)
Mean IMT (mm)	0.414 (0.002)	0.296 (0.028)	0.308 (0.025)
EPT (n=9)	SBP	LDLchol	-
Right IMT (mm)	-	0.826 (0.011)	-
Left IMT (mm)	0.734 (0.025)	0.766 (0.027)	-
Mean IMT (mm)	0.686 (0.041)	0.826 (0.002)	-

The corresponding p values are indicated in parentheses. EPT: estrogen plus progestogen therapy; hs-CRP: high-sensitivity C-reactive protein; IMT: carotid intima-media thickness; LDLchol: low-density lipoprotein cholesterol; SBP: systolic blood pressure; and WC: waist circumference.

these were abolished by HT in the EPT group (Table 4). However, the EPT group still retained important correlations between carotid IMT and LDLchol and systolic blood pressure, two well-known markers for atherosclerosis.

Regression analysis showed that, in WHT women, carotid IMT was positively correlated with age and nitrate and hs-CRP levels, but negatively correlated with Lp (a) levels (Table 5). In WHT women, carotid IMT was positively correlated with age and waist circumference, whereas in ET women, the carotid IMT was negatively correlated with age and lipoprotein levels. The latter finding suggests a protective action of estrogen.

## Discussion

Oxidative stress has a central role in lipid peroxidation and inflammation associated with atherosclerosis, with inflammation being particularly important in mediating all stages of this disease<sup>18</sup>. However, the usefulness of antioxidant measures to treat patients with atherosclerosis has not been conclusively proven.

HT has cardioprotective actions that may be associated with changes in circulating lipoprotein levels<sup>19</sup>. As shown here, women with HT had significantly lower plasma levels of LDLchol (p = 0.013) and NLDLchol (p = 0.048), and a lower LDLchol/HDLchol ratio (p = 0.001), although ET women had higher HDLchol than EPT women (p = 0.041).

Changes in the plasma lipoprotein profile, mediated by the transfer of neutral lipids, such as cholesteryl ester and triglyceride, are the best known function of CETP<sup>20</sup>. Experimental and epidemiological studies indicate that CETP may play an important role in the development of atherosclerosis<sup>20-21</sup>, although the precise effects of CETP on atherogenesis are still controversial. In humans, an increased incidence of coronary heart disease has been associated with a deficiency<sup>22</sup> or increase<sup>23</sup> in CETP. Although CETP is a potential therapeutic target<sup>24</sup>, the usefulness of interventions involving this protein will depend on clarification of its role in atherogenesis. As shown here, CETP was unaffected by HT, in agreement with a previous study in postmenopausal women<sup>5</sup>.

Free radical-induced lipid peroxidation has been proposed as an etiological factor in ageing after menopause and in various age-related diseases, including atherosclerosis. In the present study, there were no differences in the serum LPO and TBARS levels (indicators of lipid peroxidation and free-radical production, respectively) between the WHT and WHT groups. These data differ from previous findings<sup>25</sup> and from the significantly higher TBARS levels in postmenopausal women under HT<sup>26</sup>. The discrepancy between our study and the already-mentioned report may be related to differences in the mean ages of the groups that were studied, namely, 59 and 56 years in our WHT and WHT women, respectively, compared to 47 and 52 years for WHT and WHT women, respectively, in Kesim et al's study<sup>26</sup>.

**Table 5 - Multiple linear regression coefficients for carotid intima-media thickness in postmenopausal women with (wht) and without (wtht) hormone replacement therapy (ht)**

Group	Independent variables	Parameters	p	Cumulative R <sup>2</sup>
WTHT (n=20)				<b>0.8788</b>
	Age	0.0022	<b>&lt;0.001</b>	
	Lp(a)	-0.0014	<b>0.035</b>	
	Nitrates	0.0095	<b>0.003</b>	
	hs-CRP	0.2298	<b>0.000</b>	
WHT (n=13)				<b>0.3810</b>
	Age	0.0043	0.416	
	WC	0.0118	<b>0.048</b>	
ET (n=9)				<b>0.8748</b>
	Age	-0.1120	<b>0.026</b>	
	LPO	-0.1724	<b>0.041</b>	

hs-CRP: high sensitivity C-reactive protein, Lp(a): lipoprotein (a), LPO: lipoperoxides and WC: waist circumference, WTHT: without HT, WHT: with HT, ET: estrogen therapy.

The nitric oxide synthase expressed within the vascular wall is a target of estrogen action and produces a number of beneficial effects on vascular biology. As a woman ages, NOS becomes increasingly higher and starts to produce superoxide, a dangerous reactive oxygen species. It is the biochemical environment around NOS that will determine whether estrogen produces a beneficial nitric oxide or deleterious (superoxide) product, and can account for this dual and opposite nature of estrogen pharmacology<sup>27</sup>.

The lack of difference in the serum nitrate/nitrite levels of WTHT and WHT women agreed with data reported by kesim et al.<sup>26</sup>, but differed from those of Bednarek-Tupikowska et al.<sup>25</sup> and Salhotra et al.<sup>28</sup>, who reported significantly higher nitrite levels in postmenopausal women under HT.

Age-dependent deficiency of estradiol and antioxidant glutathione in blood of postmenopausal women causes compensatory activity of catalase, which is not always enough to counteract oxidative stress.

Several reports have suggested a connection between estrogen exposure and catalase activity. Catalase is a primary antioxidant defense component that catalyzes the decomposition of hydrogen peroxide to water<sup>29-30</sup>. HT antagonized the decrease in catalase activity normally associated with menopause (catalase activity: 22% in WTHT women, compared to 37% in ET women;  $p = 0.029$ ).

These findings differed from those of other studies that reported no difference in catalase activities of WTHT and WHT women<sup>27</sup> or higher levels in postmenopausal women<sup>31-36</sup>.

Treatment of normal human breast epithelial cells in culture with estradiol was shown to decrease cellular catalase activity<sup>37</sup>. Further, treatment of breast cancer cell lines with estradiol resulted in decreased catalase activity in the estrogen receptor (ER)-positive but not the ER-negative cell lines<sup>37</sup>.

The discrepancy between our study and other reports may be related to the small number of patients in each group studied. The magnitude of the decrease in catalase activity may depend on other factors such as race, diet and the individual expressing forms of the catalase polymorphism, although our study did not permit detailed subgroup analysis by diet status, race or catalase polymorphism<sup>38</sup>. Escalante Gómez and Quesada Mora<sup>39</sup> studied the effect on DNA oxidation as a possible explanation for the aging process itself and the effects that HT has on them, and found no statistical difference for protein oxidation and catalase activity among the groups. They concluded that HT decreases oxidative damage to both DNA and lipids in postmenopausal women, in corroboration to our findings<sup>39</sup>.

In addition, comprehensive information regarding potential confounding factors were available, and the HT-use information was collected using a detailed questionnaire, which, although subject to recall bias, referred to behavior in the past.

HT did not alter the carotid IMT in WHT women, or in the subgroups ET and EPT. However, mean carotid IMT was positively correlated with age, waist circumference and hs-CRP levels; this correlation disappeared with HT. In addition, multiple regression analysis showed negative relationships for age and LPO after estrogen HT, indicating a protective effect of hormonal treatment. This small study indicates the need for more extensive studies to investigate the differential roles of whether postmenopausal HT influences the levels of oxidative stress.

## Conclusions

This study indicates that HT in postmenopausal women produces beneficial antioxidant and anti-atherosclerotic effects by ameliorating the plasma lipid and lipoprotein profiles, increasing

plasma catalase activity and attenuating the association between cardiovascular risk factors and early atherosclerosis.

### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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