The effects of hormone replacement therapy combined with vitamins C and E on antioxidants levels and lipid profiles in postmenopausal women with Type 2 diabetes☆

Mustafa Nazırog˘lua,*, Mehmet Şimşekb, Halil Şimşekc, Nurettin Aydilekc, Zeynep Özcanb, Remzi Atılganc

aDepartment of Physiology, Veterinary Faculty, Fırat University, TR-23119 Elazıg˘, Turkey
bDepartment of Obst. and Gynaecol., Medical Faculty, Fırat University, TR-23119 Elazıg˘, Turkey

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Abstract

Background: Recent studies have demonstrated that oxidative modification of low-density lipoprotein (LDL) involving the formation of lipid peroxides (MDA), exerts several biological effects that may contribute to the onset and progression of cardiovascular diseases in postmenopausal women with Type 2 diabetes (DPMW). Therefore, the aim of our study was to evaluate the effect of hormone replacement therapy (HRT), vitamin C and E (VCE) treatments on lipid profiles, glucose and MDA levels as well as antioxidant vitamins and enzymes in plasma and red blood cells (RBC) in diabetic or non-diabetic postmenopausal women (PMW).

Methods: Oral HRT and VCE supplementation for 6 weeks were compared with HRT treatment in 40 non-diabetic PMW and 40 DPMW.

Results: In the 40 postmenopausal women (PMW) and 20 postmenopausal women with DPMW who received oral HRT and 20 DPMW who received HRT plus VCE, there was a significant fall in MDA, total cholesterol, LDL-cholesterol and triglyceride values. Glycated haemoglobin (HbA1c) in the DPMW was significantly improved with oral HRT and VCE although no significant change in white blood cell counts, vitamin A and HDL values occurred. Additionally, a fall in plasma glucose, HbA1c and platelet values also occurred in the PMW and DPMW groups by oral HRT and VCE treatments. There was a significant increase in plasma vitamin E and beta-carotene concentrations, catalase, glutathione peroxidase and reduced glutathione in RBC and plasma in DPMW by treatments with HRT and/or VCE.

Conclusions: Daily VCE and HRT administrations both in PMW and DPMW seem to produce significant improvement in antioxidants concentrations, and the metabolic control of lipids and glucose. The HRT and VCE supplementations may strengthen the antioxidant defense system due to reducing blood glucose and lipid metabolites, and they may play a role in preventing cardiovascular diseases in postmenopausal women with Type 2 diabetes.

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Keywords: Oxidative stress; Menopause; Glycated hemoglobin; Lipid peroxide; Vitamins C and E

Abbreviations: DPMW, postmenopausal women with Type 2 diabetes; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HRT, hormone replacement therapy; LDL, low-density lipoprotein; MDA, malondialdehyde; PMW, postmenopausal women; RBC, red blood cells; ROS, reactive oxygen species; SOD, superoxide dismutase; VCE, vitamins C and E; VLDL, very low-density lipoprotein.

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* Corresponding author. Institut für Physiologie, Klinikum der RWTH, Pauwelsstr. 30, D-52057 Aachen, Germany. Tel.: +49-241-80-88-809; fax: +49-241-80-82-434.
E-mail address: mnazirogla@physiology.rwth-aachen.de (M. Nazırog˘lu).
1. Introduction

Postmenopausal women with Type 2 diabetes (DPMW) have a high prevalence of cardiovascular risk factors, thus morbidity and mortality from cardiovascular disease (CVD) is increased [1]. In non-diabetic women CVD increases at menopause [2] when lipids and glucose concentrations in plasma, all recognized CVD risk factors, increase [3,4]. Moreover, the diabetic state is characterized by an increased rate of lipoprotein oxidation, which may play a role in the development of arteriosclerosis [5]. Diabetes can affect lipoprotein metabolism in many ways. The diabetic’s lipoprotein profile is characterized by changes in lipoprotein concentrations and composition [6]. Diabetic patients show increases in triglycerides and decreases in high-density lipoprotein (HDL) cholesterol levels [3]. In fact, diabetes itself is well established as a high-risk factor for CVD. Oral estrogen’s beneficial effects include reducing total cholesterol and low-density lipoprotein (LDL) cholesterol, through enhanced LDL receptor binding and clearance [1,7]. Additionally, by reducing hepatic lipase activity, this hormone treatment promotes the formation of larger and less atherogenic LDL cholesterol particles [1,7]. Oral estrogen may also inhibit LDL oxidation due to its inherent antioxidant property [1,3,4]. Thus, diabetic women, who have a 2-4-fold higher risk for CVD than in non-diabetic women [1,3], could benefit possibly from the use of hormone replacement therapy (HRT) with a reduced risk of CVD and arteriosclerosis [12,13]. Previous studies in diabetic patients and animals have reported that VCE supplementation improves beta-cell function and tissues insulin resistance, increases plasma insulin and can lower blood glucose and glycated hemoglobin levels [2,14]. VCE supplementation can decrease or inhibit lipid peroxidation and overcomes abnormalities in endogenous antioxidant defense system in diabetic patients [12,13] and animals [7–9]. The enzymatic protection against superoxide radicals, which are formed in the metabolism of many toxic agents, is provided by superoxide dismutase (SOD). It converts superoxide radicals into hydrogen peroxide [9,10]. The further degradation of hydrogen peroxide into water and oxygen can be catalyzed by catalase. In addition, selenium-dependent glutathione peroxidase (GSH-Px) plays an important role in the degradation of hydrogen peroxide and organic hydro peroxides [7–9]. Reduced form of glutathione, GSH, serves as the hydrogen donor for both the se-dependent and se-independent GSH-Px [11].

To our knowledge, there are few papers on malondialdehyde (MDA) and antioxidant levels in the blood of DPMW. In addition, because the incidence of diabetes is higher in men than in women until women reach menopause [1,2], a protective role of HRT and VCE against the development of DPMW is an area of great research interest. Therefore, the present study was aimed at investigating the possible changes plasma lipid and glucose levels, plasma antioxidant vitamin concentrations, plasma and red blood cell (RBC) enzyme activities in PMW and DPMW before and after oral HRT and VCE treatments. A combination of VCE supplementation was chosen since vitamin C, as well as being a free radical scavenger, also transforms vitamin E to its active form [12].
2. Subjects and methods

2.1. Chemicals

All chemicals were obtained from Sigma (St. Louis, MO) and all organic solvents from Merck Chemical (Germany) except VCE and HRT. The oral form of vitamin C (Radoxan, ascorbic acid) and E (Ephynal, dl-α-tocopheryl acetate) was obtained from F. Hoffman La Roche (Istanbul, Turkey). HRT (premelle, 0.625-mg oestradiol and 5-mg medroxyprogesterone) pills were obtained from Wyeth (Istanbul, Turkey). All reagents were analytical grade. All reagents except the phosphate buffers were prepared daily and stored at +4 °C. The reagents were equilibrated at room temperature for half an hour before use when the analysis was initiated or reagents containers were refilled. Phosphate buffers are stable at +4 °C for 1 month.

2.2. Subjects

Ethics Committee of the Medical Faculty approved the study plan by protocol no. 2002-13. All subjects volunteered for the trial and they gave written consent. Postmenopausal women with or without Type 2 diabetes were recruited from outpatient clinics within the Firat Medical Center of Firat University. The study was performed in 40 PMW and 40 DPMW (aged 45 to 65 years; mean age 51 years). Type 2 diabetes was defined as diabetes diagnosed after the age of 45 years. Postmenopausal was defined as the cessation of menses for greater than a year in the presence of climacteric symptoms (hot flushes, night sweats, genital atrophy), or biochemically, follicular stimulating hormone >25 IU, with serum oestradiol <0.40 pmol/l. PMW or DPMW taking insulin or lipid lowering therapy or antioxidant vitamins within the last 6 months or HRT within the last 3 months were excluded. Moreover, patients with liver or thyroid diseases were excluded. None were consuming alcohol.

2.3. Groups and HRT and VCE supplemenation

The PMW and DPMW were divided into three groups as follows: Oral HRT (0.625-mg oestradiol and 5-mg medroxyprogesterone) in first group was given to 40 PMW without diabetes for 6 weeks. Oral HRT in second group was given for 6 weeks to 20 DPMW. HRT and 1 g of vitamin C and 600 mg of vitamin E in the third group were given orally for 6 weeks with breakfast to 20 DPMW. Fasting blood samples from the four groups were collected from each woman before starting therapy and after 6 weeks of HRT and/or VCE therapy.

2.4. Blood collection and preparation of blood samples

Venous blood (5 ml) was taken from the antecubital vein, using a monovette system of blood collection, into anticoagulated tubes containing sodium EDTA, protected against light after an overnight fast with all morning medication omitted. One milliliter of anticoagulated blood was used for hematological and HbA1c analysis. The remaining anticoagulated blood was separated into plasma and RBC by centrifugation at 1500 × g for 10 min at +4 °C. RBC samples were washed three times in cold isotonic saline (0.9%, v/w), then haemolyzed with a ninefold volume of phosphate buffer (pH 7.4). Two milliliters of plasma was used for the detection of glucose, triglyceride and lipid profile. Haemolyzed RBC and plasma samples were stored at −30 °C. Haemolyzed RBC and plasma were stored for <3 months pending on measurement of enzymatic activity. The remaining haemolyzed and plasma was used for immediate MDA and vitamin assay. Blood hematological and plasma biochemical parameters were measured within 6 h following blood taking.

2.5. Lipid peroxidation (MDA) assay

Lipid peroxidation (as MDA) levels in plasma and haemolized RBC were measured with the thiobarbituric-acid reaction by the method of Placer et al. [16] as described in previous studies [8,9]. The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of malondialdehyde equivalents generated by acid catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane. The values of MDA were expressed as nmol/ml for plasma or nmol/mg protein for RBC. Every sample was assayed in duplicate, and the assay coefficients of variation for MDA were less than 3%.
2.6. GSH-Px, catalase and GSH assay

The methods of Goth [17,18] were used for the determination of catalase activities both in haemolyzed RBC and plasma. The yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against blank using a spectrophotometer (Shimadzu 2R/UV, Kyoto, Japan). The GSH content in RBC and plasma was measured at 412 nm using the method of Sedlak and Lindsay [19] as described own studies [8–10]. The samples were precipitated with 50% trichloracetic acid and then centrifuged at 1000 \( \times \) g for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris–EDTA buffer (0.2 mol/l; pH 8.9) and 0.1 ml of 0.01 mol/l 5,5\(^{-}\)-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min, and then read at 412 nm on the spectrophotometer. GSH-Px activities in RBC and plasma were measured at 37 \( ^\circ \)C and 412 nm according to Lawrence and Burk [20].

2.7. Protein determination

The protein content in the plasma and haemolyzed RBC was measured by method of Lowry et al. [21] with bovine serum albumin as the standard.

2.8. Plasma vitamins A and E and beta-carotene analyses

Vitamins A (retinol) and E (alpha-tocopherol) were determined on frozen plasma samples by a modification of the method described by method of Desai [22] as described in previous studies [8,9]. One hundred microliters of plasma was saponified by the addition of 0.3-ml 60% (w/v in water) KOH and 2 ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70 \( ^\circ \)C for 30 min. Twenty-microliter portions of the methanol extracts were chromatographed on high-performance liquid chromatography. Fluorimetric detection of vitamin A used excitation and emission wavelengths of 330 and 480 nm, respectively. The relevant wavelengths for alpha-tocopherol detection were 292 and 330 nm. Calibration was performed using standard solutions of all-trans retinol and alphatocopherol in methanol.

The levels of beta-carotene in plasma samples were determined according to method of Suzuki and Katoh [23]. Two milliliters of hexane was mixed with 0.5-ml plasma and absorbance was measured at 453 nm in the spectrophotometer.

2.9. Biochemical and hematological parameters analyses

Triglycerides, glucose, total cholesterol, HDL and LDL cholesterol values in plasma were determined using routine kits in an autoanalyzer (Olympus AU 600, Tokyo, Japan). Very low-density lipoprotein (VLDL) cholesterol value was calculated by the formula: VLDL cholesterol equals total cholesterol minus (LDL plus HDL cholesterol). White blood cells (WBC) and platelets counts were determined with an automated blood counter (Beckman Coulter, Miami, USA). Blood HbA1c was also measured by routine kit (Alfabiotech, Milano, Italy) using the autoanalyzer. To exclude hepatic, renal and thyroid and liver dysfunction, electrolytes and creatinine, thyroid function tests in plasma were performed.

2.10. Statistical analyses

All results are expressed as means ± S.D. To determine the effect of treatment, data were analyzed using one-way ANOVA repeated measures. \( P \) values of less than 0.05 were regarded as significant. Significant values were assessed with LSD test. Data were analyzed using SPSS statistical program (version 10.0 software, SPSS Chicago, IL, USA).

3. Results

3.1. MDA levels and antioxidant vitamins and enzymes concentrations

The changes in vitamins A and E and beta-carotene in plasma are shown in Table 1. There were no statistical differences in the vitamin concentrations between baseline and HRT supplemented PMW groups. However, there was significant decrease in the DPMW in the plasma vitamin E (\( p < 0.01 \)) and beta-carotene (\( p < 0.05 \)) concentrations. The current study results showed that plasma beta-carotene and vitamin E levels in the DPMW were significantly (\( p < 0.01 \) and \( p < 0.001 \)) increased after 6 weeks of
oral HRT and vitamin C, E (VCE) supplementation in the PMW and DPMW groups were not associated with any changes in plasma vitamin A level.

The changes in MDA and GSH levels, catalase and GSH-Px activities in plasma and RBC are shown in Tables 1 and 2. When compared to PMW, MDA

Table 1
The effects of oral HRT and vitamin C, E (VCE) supplementation on lipid peroxidation (MDA), antioxidant vitamin, reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase levels in the plasma of postmenopausal diabetic or non-diabetic women

<table>
<thead>
<tr>
<th>Study group</th>
<th>Menopause</th>
<th>Menopause and diabetes</th>
<th>Menopause and diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>HRT 6-week</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>n = 40</td>
<td>n = 40</td>
<td>n = 20</td>
</tr>
<tr>
<td>GSH-Px (IU/g prot.)</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.2</td>
<td>0.7 ± 0.3a</td>
</tr>
<tr>
<td>GSH (μmol/ml)</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1a</td>
</tr>
<tr>
<td>Catalase (kU/g prot.)</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.8</td>
<td>1.0 ± 0.8a</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.2 ± 0.2</td>
<td>0.9 ± 0.3d</td>
<td>1.4 ± 0.4a</td>
</tr>
<tr>
<td>Vitamin A (μmol/l)</td>
<td>1.7 ± 0.6</td>
<td>2.0 ± 0.6</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Beta-carotene (μmol/l)</td>
<td>1.7 ± 0.8</td>
<td>2.1 ± 0.2</td>
<td>1.2 ± 0.6a</td>
</tr>
<tr>
<td>Vitamin E (μmol/l)</td>
<td>12.2 ± 4.2</td>
<td>12.8 ± 2.6</td>
<td>7.2 ± 3.6b</td>
</tr>
<tr>
<td>Vitamin E/LDL-chol.</td>
<td>3.1 ± 1.3</td>
<td>3.7 ± 1.1d</td>
<td>1.6 ± 0.7b</td>
</tr>
<tr>
<td>Beta-carotene/LDL-chol.</td>
<td>0.5 ± 0.2</td>
<td>0.7 ± 0.1d</td>
<td>0.3 ± 0.1a</td>
</tr>
</tbody>
</table>

(mean ± S.D.).

Table 2
The effects of oral HRT and vitamin C, E (VCE) supplementation on the levels of lipid peroxidation (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase in the RBC of postmenopausal diabetic or non-diabetic women

<table>
<thead>
<tr>
<th>Study group</th>
<th>Menopause</th>
<th>Menopause and diabetes</th>
<th>Menopause and diabetes</th>
</tr>
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<tr>
<td></td>
<td>Baseline</td>
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</tr>
<tr>
<td></td>
<td>n = 40</td>
<td>n = 40</td>
<td>n = 20</td>
</tr>
<tr>
<td>GSH-Px (IU/g prot.)</td>
<td>19.5 ± 5.3</td>
<td>24.5 ± 5.4d</td>
<td>18.1 ± 6.2a</td>
</tr>
<tr>
<td>GSH (μmol/g prot.)</td>
<td>40.0 ± 13.2</td>
<td>41.3 ± 18.8</td>
<td>39.0 ± 18.4</td>
</tr>
<tr>
<td>Catalase (kU/g prot.)</td>
<td>38.3 ± 10.3</td>
<td>47.6 ± 12.1</td>
<td>33.5 ± 9.1a</td>
</tr>
<tr>
<td>MDA (nmol/mg prot.)</td>
<td>4.3 ± 1.2</td>
<td>3.6 ± 1.1d</td>
<td>4.2 ± 1.3</td>
</tr>
</tbody>
</table>

(mean ± S.D.).

Table 1

 oral HRT and vitamin C, E (VCE) supplementation in the PMW and DPMW groups were not associated with any changes in plasma vitamin A level.

The changes in MDA and GSH levels, catalase and GSH-Px activities in plasma and RBC are shown in Tables 1 and 2. When compared to PMW, MDA
levels in plasma and RBC were significantly \((P<0.05\) and \(P<0.01\)) higher in DPMW. On the other hand, 6 weeks of oral HRT and VCE supplementations in DPMW was associated with a fall in MDA level of both plasma and RBC \((P<0.01)\). Catalase and GSH-Px activities and GSH levels in plasma and RBC samples were not statistically change by the oral HRT supplementation in the PMW. However, catalase and GSH-Px activities and GSH levels in plasma and RBC increased significantly \((p<0.05\) and \(p<0.01)\) in DPMW after 6 weeks of oral HRT and VCE supplementation.

### 3.2. Levels of triglycerides and lipid profile

The changes in triglycerides, total cholesterol and lipoprotein cholesterol in plasma are shown in Table 3. Triglycerides, total cholesterol, LDL and VLDL cholesterol values were significantly \((P<0.05)\) higher in the DPMW baseline group than in the PMW baseline group. However, 6 weeks of HRT and VCE treatments was associated with the fall in the triglyceride, total cholesterol, LDL and VLDL cholesterol values in three groups. Oral HRT and VCE supplementation in all groups were not associated with any change in the HDL cholesterol value.

### 3.3. Changes in biochemical and hematological parameters

Fasting plasma glucose, HbA1c, platelet and WBC values in control, patient and treatment groups are shown in Table 3. Plasma glucose, values were significantly \((P<0.001)\) higher in DPMW baseline group than in the PMW baseline. However, a fall in HbA1c, plasma glucose and platelet values also occurred after 6 weeks of oral HRT and VCE treatment. HbA1c in postmenopausal women with diabetes significantly \((P<0.05)\) improved with oral HRT and VCE although no significant change in WBC counts.

### 4. Discussion

Postmenopausal hormone reduction is important risk factor for CVD and arteriosclerosis both in PMW and DPMW. Many studies have demonstrated that the HRT can improve the lipid profile and fasting glucose

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### Table 3

The effects of oral HRT and vitamin C and E (VCE) on selected cardiovascular risk factors in the blood of postmenopausal diabetic or non-diabetic women (mean ± S.D.)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Menopause</th>
<th>Menopause and diabetes</th>
<th>Menopause and diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n=40)</td>
<td>HRT 6-week (n=40)</td>
<td>Baseline (n=20)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.9 ± 0.8</td>
<td>4.4 ± 0.6\textsuperscript{d}</td>
<td>13.3 ± 2.9\textsuperscript{c}</td>
</tr>
<tr>
<td>T. Cholesterol (mmol/l)</td>
<td>5.8 ± 0.6</td>
<td>5.0 ± 0.2\textsuperscript{d}</td>
<td>6.0 ± 0.5\textsuperscript{a}</td>
</tr>
<tr>
<td>HDL-Chol. (mmol/l)</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>LDL-Chol. (mmol/l)</td>
<td>3.4 ± 0.5</td>
<td>3.0 ± 0.2\textsuperscript{d}</td>
<td>3.9 ± 0.4\textsuperscript{a}</td>
</tr>
<tr>
<td>VLDL-Chol. (mmol/l)</td>
<td>1.2 ± 0.3</td>
<td>0.8 ± 0.2\textsuperscript{d}</td>
<td>1.5 ± 0.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.5 ± 0.7</td>
<td>2.0 ± 0.3\textsuperscript{d}</td>
<td>2.7 ± 0.7\textsuperscript{a}</td>
</tr>
<tr>
<td>Platelet count ((\times 10^{9}/l))</td>
<td>313.1 ± 33.5</td>
<td>288.9 ± 90.6\textsuperscript{d}</td>
<td>308.0 ± 77.9</td>
</tr>
<tr>
<td>WBC count ((\times 10^{9}/l))</td>
<td>6.8 ± 1.6</td>
<td>7.7 ± 1.7</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>–</td>
<td>10.4 ± 2.9</td>
<td>7.0 ± 1.4\textsuperscript{e}</td>
</tr>
</tbody>
</table>

(mean ± S.D.).

\(\textsuperscript{a}p<0.05.\)

\(\textsuperscript{b}p<0.01.\)

\(\textsuperscript{c}p<0.001\) vs group baseline of menopause + HRT.

\(\textsuperscript{d}p<0.05.\)

\(\textsuperscript{e}p<0.01.\)

\(\textsuperscript{f}p<0.001\) vs group menopause + HRT.

\(\textsuperscript{g}p<0.05.\)

\(\textsuperscript{h}p<0.01.\)

\(\textsuperscript{i}p<0.001\) vs group baseline each group.

\(\textsuperscript{j}p<0.05.\)

\(\textsuperscript{k}p<0.01.\)

\(\textsuperscript{l}p<0.001\) vs group menopause + HRT.
levels in postmenopausal women with or without diabetes thus reducing the coronary risk [1]. Sex steroids can also exert beneficial effect on CVD because oestrogens have an antioxidant activity on LDL [7]. The current report shows that excess ROS apparently contributes to the etiology of diabetic complications in DPMW because diabetes induced an increase in the plasma and RBC lipid peroxide levels in DPMW. However, HRT and/or VCE treatments in DPMW have also the potential to prevent increasing lipid peroxide as well as the decrease in the investigated antioxidant vitamins and enzymes. In our study a significant reduction in total cholesterol, triglycerides, fasting glucose, HbA1c, LDL and VLDL cholesterol plasma levels was also observed after HRT and VCE therapies, but no change was found in HDL-cholesterol plasma levels.

Type 2 diabetes mellitus is a syndrome characterized by insulin resistance, derangement in carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia. In both insulin-dependent and non-insulin-dependent diabetes, oxidative stress was shown to be increased [1,2,8]. It is accepted that both correlate to oxidative stress the imbalance between the generation of oxygen derived radicals and the organism’s antioxidant potential. It has been shown in various studies that diabetes mellitus is associated with increased formation of free radicals, and with heavy oxidative stress [8–12]. As a result of an increase in the formation of free radicals in diabetes, the balance normally present in cells between radical formation and protection against them is disturbed [8,11]. This will lead to oxidative damage of cell components, e.g. proteins, lipids, and nucleic acids [11].

Our findings show that MDA levels in plasma and RBC both in MPW and DPMW were higher than baseline group while it decreased significantly after HRT and VCE treatment in these groups (Tables 1 and 2). As regards changes in plasma and RBC levels of MDA both in MPW and DPMW treated with and/or VCE, there are few data in literature. Clemente et al. [24,25] have reported that serum levels of lipid peroxides in postmenopausal women decreased after treatment with HRT. Tranquilli et al. [26] found that reduction of MDA in platelet membranes from menopausal women menopausal women is due to HRT treatment.

It is important to note that in the studies conducted by Wen et al. [5] and Bureau et al. [6], alpha-tocopherol and beta-carotene levels in serum and RBC also were not modified in PMW by HRT supplementation. Clemente et al. [25] reported that the HRT did not modify significantly alpha-tocopherol and beta-carotene serum levels in postmenopausal women, while alpha toc/LDL and beta-car/LDL ratios significantly increased after HRT therapy. In agreement with the findings of Feingold et al. [27], Sargeant et al. [28], Clemente et al. [25], Bureau et al. [6], the HRT did not modify significantly alpha-tocopherol and beta-carotene plasma levels in the PMW, while alpha toc/LDL-cholesterol and beta-car/LDL-cholesterol ratios significantly increased after HRT therapy. Our finding differs from the effect of hormone therapy in pre-menopausal women [4] who showed a decrease in vitamin E levels using combined oral contraceptives.

Increased generation of ROS in studied samples of PMW and DPMW may also be related to changes in activities of enzymatic antioxidants. In this study, decreased activities of key antioxidants, GSH and GSH-Px, were found in the plasma and RBC of DPMW. However, an increase in the level of MDA in the blood of DPMW was prevented by HRT and VCE treatments. Reports of other authors confirm this phenomenon. Yadav et al. [29] demonstrated a significant diminution of GSH-Px activity in the kidney of rats with STZ-induced diabetes. Çay et al. [8] and Naziroğlu and Çay [9] found decreased GSH-Px activity in the lens, liver and muscle, plasma and erythrocyte of rats with 24-day diabetes. Damasceno et al. [30] demonstrated reduced GSH-Px and GSH activities in the liver and kidneys of rats with 12-week diabetes. The increased level of glycation in the plasma and RBC cells was accompanied by a reduced activity of the enzyme. This relation corresponds to results of this study pointing to a reduction of GSH-Px activity accompanied by significant hyperglycemia and increased levels of glycated proteins, which suggest that the decreases in GSH-Px and GSH levels may be conditioned by its enhanced glycation.

Improved fasting glucose levels after VCE administration, with a consequently greater inhibition of MDA, might explain the significant decline in plasma triglycerides, total cholesterol, LDL and VLDL found
in postmenopausal diabetic women of this study. As far as a HRT plus VCE-induced decline in plasma total cholesterol, LDL and VLDL cholesterol is concerned, one can hypothesize that the antioxidant power of VCE itself could preserve LDL-cholesterol from the peroxidation phenomenon [10,11]. Furthermore, VCE have a direct effect on plasma cholesterol levels because they contribute to inhibiting cholesterol biosynthesis [7]. A hepatic overproduction of VLDL and decreased activity of lipoprotein lipase might be further attributable to the enhanced plasma triglycerides levels in the study.

HbA1c and plasma glucose levels in DPMW were improved with oral HRT and, similarly, Cefalu [1] has also found that HRT and VCE have either improved glycemic control or had no adverse effect on glycemic control in diabetic women. In addition, Shoff et al. [14] also reported that HRT improves insulin sensitivity in women with impaired glucose tolerance, as shown by decreased concentrations without a change in insulin levels following a glucose tolerance test. In a study with PMW, current users of HRT also had significantly lower levels of HbA1c and plasma glucose compared with non-users [28]. Also, Paolisso et al. [31] suggested that chronic vitamin E administration reduced plasma glucose. In the same reports as well as in others, HbA1c and fasting plasma glucose in diabetic rats and women have been reduced by VCE supplementation [8,14,15,32]. These reductions in plasma glucose and HbA1c were associated with improved glucose homeostasis [1].

It is concluded that both enzymatic and lipophilic antioxidants in the plasma and RBC were decreased in DPMW when compared to the PMW. These alterations may contribute to a decreased cellular scavenging capacity, and thus, an increase of oxidative stress as shown by the increase in lipid peroxidation. However, oral VCE supplementation in DPMW seems to produce significant improvement on antioxidants concentrations. We observed that HRT and VCE treatments affect antioxidants status and glucose metabolic control, and lipid profile in the DPMW. Therefore, VCE supplementation in postmenopausal diabetics could reduce the imbalance between uncontrolled ROS generation and scavenging enzyme activities, and thereby potentially serve as a simple and useful prophylactic factor.

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