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SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 953–959

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Estrogen treatment improves arterial distensibility, fibrinolysis, and metabolic profile in postmenopausal women with type 2 diabetes mellitus

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Received 6 October 2004; accepted 23 March 2006

Abstract

The effects of isolated estrogen therapy on the hemostatic system and arterial distensibility were determined in postmenopausal females with type 2 diabetes mellitus. This was a prospective nonrandomized study of 19 subjects (age, 56.2 ± 4.7 years; body mass index, 27.8 ± 2.4 kg/m^2 [mean ± SD]). Inclusion was done after 2 months of glycemic and blood pressure control. The study consisted of 4 months of placebo treatment immediately followed by an equal period of oral conjugated equine estrogens (CEE) 0.625 mg/d. Measures included anthropometrics, a metabolic profile (oral glucose tolerance test and fasting glycated hemoglobin, total cholesterol and fractions, and triglyceride levels), and coagulation and fibrinolytic factors at the end of the placebo period and after 4 months of oral CEE. Conjugated equine estrogen therapy decreased plasminogen activator inhibitor 1 (placebo × CEE: 16.33 ± 9.11 × 13.08 ± 8.87 UI/mL, $P < .03$) and increased factor VIII activity (134.11% ± 46.18% × 145.33% ± 42.04%, $P < .04$). An increase in high-density lipoprotein cholesterol levels (placebo × CEE: 42.47 ± 6.80 × 53.32 ± 11.89 mg/dL, $P < .01$), and a decrease in glycated hemoglobin (8.45% ± 1.30% vs 7.58% ± 1.06%, $P < .02$) and in fasting glucose levels (121.51 ± 21.05 x111.21 ± 20.74 mg/dL, $P = .02$) followed CEE therapy. Pulse wave velocity and augmentation index were performed by applanation tonometry and were obtained at the end of the placebo period (placebo), again after an intravenous load of 1.25 mg of CEE (short-term), and after 4 months of oral CEE (long-term). A significant decrease in central (carotid-femoral) pulse wave velocity was seen both after short- and long-term CEE (placebo vs short-term vs long-term: 9.36 ± 2.58 vs 8.26 ± 2.20 vs 7.98 ± 1.90 m/s, respectively [analysis of variance, $P < .03$]; placebo vs short-term, $P < .05$; placebo vs long-term, $P < .01$), whereas augmentation index decreased only after long-term CEE (placebo vs short-term vs long-term: 39.14% ± 6.94% vs 37.48% ± 8.67% vs 34.3.3% ± 8.11% [analysis of variance, $P < .05$], respectively; placebo vs long-term, $P < .05$). Long-term administration of CEE leads to an improvement in fibrinolysis and arterial distensibility, associated with an increase of the intrinsic coagulation pathway in postmenopausal women with type 2 diabetes mellitus.

1. Introduction

Type 2 diabetes mellitus (DM) is a strong risk factor for cardiovascular disease (CVD), and this risk may be higher for postmenopausal women [1]. The mechanisms involved in this process include obesity, disturbances in carbohydrate and lipid metabolism [1,2], hypertension [1], altered hemostasis [2,3], endothelial dysfunction [4], and increased arterial stiffness [5].

Hyperglycemia and hyperinsulinemia also reduce fibrinolysis by stimulating plasminogen activator inhibitor 1 (PAI-1) synthesis and inhibiting the tissue plasminogen activator factor in endothelial and smooth muscle cells [3]. Type 2 DM reduces the main inhibitors of coagulation (antithrombin III and C-reactive protein) and favors an increase of clot formation by activating thrombin and coagulation factors (fibrinogen, factors VII, VIII, and X) [2].

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doi:10.1016/j.metabol.2006.03.003

In addition, the loss of the elastic fibers in blood vessels with aging changes their mechanical properties, especially in the large arteries, increasing their stiffness and the cardiovascular risk (CVD) [5-7]. Diabetic arteries appear to age faster and earlier when compared with nondiabetic ones [5,8].

On the other hand, the increase in arterial stiffness after menopause in healthy and nonhealthy women [8] can be improved by unopposed estrogen therapy (ET) and combined estrogens-plus-progestins therapy [8-10]. The vascular cells express estrogen receptors that respond to estrogen exposure by nongenomic and genomic mechanisms. The former occurs very fast, without gene modification, in minutes after estrogen exposure by endothelium-dependent and endothelium-independent effects related to its effects on ion channels (potassium channels overture) and nitric oxide (NO) release. Longer-term effects of estrogen include increase of genetic expression of crucial vasodilation enzymes (prostacyclin and NO synthase), lower expression of vascular genes products such as preproendothelin, down-regulation of tissue angiotensin-converting enzyme and angiotensin II type 1receptors, decrease of PAI-1 synthesis, and modulation of the adhesion molecules [10].

Increase of the coagulation and impaired fibrinolysis occurs after the onset of menopause [10] and can be partially reversed by ET or estrogens-and-progestins therapy because they have a favorable impact on fibrinolysis [10,11]. However, those treatments may activate coagulation [12,13].

Postmenopausal women with type 2 DM can benefit from unopposed or combined therapy with conjugated equine estrogens (CEE) in terms of metabolic profile (reduction of total cholesterol and increase in high-density lipoprotein cholesterol [HDL-C] levels, improvement in glycated hemoglobin) and fat distribution [14], but the effects of ET on the large conduit arteries and on the hemostatic system in this population are still controversial [14-17] and have not been fully studied. Differences among studies in ET or estrogens-plus-progestins therapy prescriptions, including administration route (oral or transdermic), doses of estrogen, presence or absence of associated progestin (that can abolish some estrogen effects), and therapy duration, account for some of the controversy.

Therefore, we aimed to study the short- and long-term effects of oral ET in type 2 diabetic postmenopausal women on metabolic and hemostatic parameters and arterial distensibility, the last one through noninvasive methods: applanation tonometry and pulse wave velocity (PWV).

2. Materials and methods

2.1. Subjects

Subjects were recruited from the Diabetes Outpatient Clinic of the Hospital das Clinicas of the University of Sao Paulo Medical School (Brazil). The hospital's research ethics committee approved the study, and all of the subjects gave their written informed consent to participate in it, having been considered of minimal risk by the ethics committee.

The study patients (n = 14) were all women with type 2 DM who had been postmenopausal for 2 to 10 years and had no hormone therapy or lipid-lowering drugs for at least 4 months before the study. Treatment regimens included diet and sulfonylureas. Ten subjects were hypertensive, taking captopril (n = 5), captopril plus low-dose hydrochlorothiazide (n = 3), or only hydrochlorothiazide (n = 1). Baseline characteristics are given in Table 1.

The diagnosis of postmenopausal status was made based on amenorrhea and hormonal measurements (serum follicle-stimulating hormone [FSH] >30 UI/L and plasma estradiol <10 pg/mL). The diagnosis of diabetes was defined according to the American Diabetes Association [18] criteria.

Exclusion criteria included any severe concomitant illness, usual contraindication to ET (antecedents of CVD or previous thromboembolism), smoking, dyslipidemia (total cholesterol and triglyceride levels >250 mg/dL), and diabetic proliferative retinopathy and nephropathy (serum creatinine >1.6 mg/dL or uncontrolled hypertension-blood pressure >170 × 110 mm Hg). Metabolic control was defined as fasting glycemia of less than 140 mg/dL and glycated hemoglobin up to 1.5% above the reference range (reference range, 4.7%-8.5%).

On inclusion, all patients performed a mammogram and a transvaginal ultrasound with endometrium thickness measurement. The 4-month period of treatment with CEE was considered safe in terms of endometrial hyperplasia [19]. At the end of the study all patients with an intact uterus (n = 9) had another transvaginal ultrasound and received 5 mg/d of oral medroxyprogesterone for 15 days to prevent endometrial hyperplasia.

2.2. Study design

This was a placebo-controlled study in postmenopausal women with type 2 DM. After 2 months of glycemic and blood pressure stabilization, a 4-month placebo period was immediately succeeded by the same duration of oral CEE (0.625 mg/d) treatment. An oral glucose tolerance test (OGTT; 75 g glucose) with glucose and insulin determinations every 30 minutes for 2 hours was performed at the end of each phase (placebo and CEE). Total cholesterol and fractions, triglycerides, glycated hemoglobin blood levels, and hemostatic parameters were determined at the end of both periods.

The arterial compliance study was performed in 14 patients 3 times: twice on the last day of the placebo

Table 1
Clinical characteristics of the 19 patients

Parameters	Mean ± SD (range)
Age (y)	56.2 ± 4.7 (48-62)
BMI (kg/m^2)	27.8 ± 2.4 (24.4-32.8)
Menopause (y)	5.3 ± 1.7 (2-10)
Known duration of diabetes (y)	4.7 ± 3.7 (2-16)
Duration of hypertension (y; n = 9)	13.1 ± 8.3 (2-20)
Waist-to-hip ratio	0.89 ± 0.04 (0.84-0.93)
Race	12 White/7 nonwhite

Table 2
Effects of oral CEE therapy on clinical, metabolic, and hormonal parameters (N = 19)

Parameters (mean ± SD)	Placebo	CEE
Weight (kg)	69.2 ± 8.0	69.2 ± 7.7
Waist-to-hip ratio	0.89 ± 0.01	0.88 ± 0.01
Systolic blood pressure (mm Hg)	128.6 ± 14.3	132.5 ± 16.1
Diastolic blood pressure (mm Hg)	79.1 ± 6.5	79.7 ± 9.0
Fasting glycemia (mg/dL)	121.5 ± 21.1	111.2 ± 20.7*
Fasting insulin (μUi/mL)	12.1 ± 9.2	11.2 ± 6.5
Glycated hemoglobin (%)	8.5 ± 1.3	7.6 ± 1.1**
AUC glucose (mg dL^{-1} min^{-1})	12336 ± 4519	12496 ± 3786
AUC insulin (μUI mL^{-1} min^{-1})	3050 ± 2950	3014 ± 2262
Total cholesterol (mg/dL)	201.6 ± 42.5	200.3 ± 11.9
HDL-C (mg/dL)	42.5 ± 6.8	53.3 ± 11.9**
LDL-C (mg/dL)	127.0 ± 37.94	119.5 ± 32.60
Triglycerides (mg/dL)	143.5 ± 57.6	137.2 ± 62.7
FSH (mUI/mL)	62.5 ± 18.0	47.1 ± 19.3**
Estradiol (pg/mL)	11.6 ± 2.4	57.3 ± 20.5**

The placebo period was 4 months, which was immediately followed by 4 months of oral CEE. AUC indicates area under the OGTT curve.
* $P < .03$.
** $P < .002$.

period, which included a basal one (placebo) and a second, 20 minutes after 1.25 mg of CEE intravenously (short-term). The third measurement was taken after 4 months of oral CEE 0.625 mg /d (long-term).

Compliance with ET was confirmed by tablet counting and FSH/estradiol levels on inclusion, at 40 days, and at the end of the study (120 days). Before the inclusion, and for the preceding 2 months, patients were advised to eat an isoenergetic diet according to the American Diabetes Association recommendation [20] and were kept under adequate glucose and blood pressure control. After this stabilization period on the protocol, subjects were asked to maintain their usual dietary intake and physical activity level. A medical interview was done every 3 weeks for blood glucose, blood pressure determinations, as well as the anthropometric measurements: weight, height, body mass index (BMI; kg/m^2), waist-to-hip ratio, waist circumference, and hip circumference [21] were done before and after CEE treatment.

2.3. Laboratory methods

Blood samples were collected at the end of the placebo and active drug (CEE, 0.625 mg/d) between 08:00 and 09:00 AM, after a 12-hour overnight fast and resting in the supine position for 40 minutes. In the day before the test, patients were advised to avoid caffeine and consume low-fat food. Blood was withdrawn from an antecubital vein into tubes containing 3.8% sodium citrate, in a 9:1 proportion (blood and citrate) with minimal stasis, and the first sample was used only for an analysis excluding hemostatic factors.

2.4. Hemostatic analysis

Plasma was separated within 30 minutes of the collection by centrifugation at 2500*g* for 10 minutes at 4°C, and the plasma aliquots were then frozen and stored at −70°C for subsequent simultaneous analysis in duplicate of all the samples, thus avoiding interassay variations. All determinations were done at the Fundação Pró-Sangue-Hemocentro Sao Paulo (Brazil). Fibrinogen was measured by the Clauss [22] clotting technique (Fibriquick Assay, Sigma); the intra-assay coefficient of variation (CV) was of 8%. Determination of the activity of the coagulation factors (V, VII, VIII) was performed by colorimetric method using plasma deficient in the respective factors (factor-deficient plasma, Sigma, St Louis, MO; CV of 4%) and antithrombin III activity by chromogenic assay (antithrombin III Accucolor, Sigma Diagnostics); the CV was 4.4%. Two markers of the plasma fibrinolytic activity were measured (PAI-1 antigen and activity) with monoclonal antibodies. The quantitative determination of PA-1 antigen was performed by a procedure described by Ranby et al [23] (Imulyse PAI-1, Umea, Sweden) and PAI-1 activity by an immunoactive quantitative assay [24] (Chromolize PAI-1, Biopool, Umea, Sweden), the intra-assay CV being 5% and 3.7%, respectively.

2.5. Biochemical analysis

Total cholesterol, HDL-C, triglyceride, and glucose levels were determined by conventional methods. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula. Glycated hemoglobin was determined by ion exchange chromatography (Labtest, Sao Paulo, Brazil) [25]. Insulin was analyzed using a competitive radioimmunoassay from Linco Research (St Charles, MO). This method is based on a double-bounded antibody (polyethyineleglycol [PEG]) technique.

2.6. Measurements

2.6.1. Arterial distensibility study

Arterial distensibility was evaluated at sites with different anatomical and physiologic properties: elastic (carotid and femoral) and muscular (dorsalis pedis) vessels. Two indexes of arterial distensibility described elsewhere [26,27] were used to evaluate the effect of CEE on the arterial tree through noninvasive methods: carotid applanation tonometry (augmentation index [AI]) and PWV, the latter at 2 different sites, along the central (carotid-femoral [CF]) and peripheral (femora-pedialis [FP]) arteries. Patients fasted 12 hours before the study and were free of all medications for at least 14 hours (48 hours for diuretics) to avoid any possible

Table 3
Effect of oral CEE therapy on the hemostatic factors

Parameters (mean ± SD)	Placebo	CEE
Factor VII (%) activity	148.1 ± 56.4	129.9 ± 51.7
Factor VIII (%) activity	134.1 ± 46.2	145.33 ± 42.0*
Antithrombin III (%) activity	98.4 ± 22.8	93.05 ± 13.0
Fibrinogen (mg/dL)	327.5 ± 89.7	309.1 ± 69.5
PAI-1 antigen (ng/mL)	20.99 ± 13.8	16.52 ± 12.5
PAI-1 activity (UI/mL)	16.33 ± 9.1	13.08 ± 8.9**

The placebo period was 4 months, which was immediately followed by 4 months of oral CEE.
* $P < .04$
** $P < .03$.

Table 4
Influence of short-term CEE and long-term oral CEE on hemodynamic determinations (n = 14)

Parameters (mean ± SD)	Basal	Short-term	Long-term
Heart rate (beats per minute)	66.1 ± 11.2	64.2 ± 15.2	66.1 ± 11.5
Mean blood pressure (mm Hg)	104.0 ± 14.1	103.4 ± 15.3	101.4 ± 13.5
AI (%)	39.1 ± 6.9	37.5 ± 8.7	34.3 ± 8.1*
Central PWV (m/s)	9.4 ± 2.6	8.3 ± 2.2*	7.9 ± 1.9**
Peripheral PWV (m/s)	12.1 ± 2.6	11.3 ± 3.7	12.3 ± 2.9

Basal represents a period at the end of the placebo phase; short-term, at the end of the placebo phase, 20 minutes after a short-term intravenous load of 1.25 mg of CEE; and long-term, 4 months of oral CEE therapy 0.625 mg/d. Data were examined using ANOVA with Tukey post hoc analysis. AI (ANOVA, $P < .05$): basal vs long-term ($P < .05$) and basal × short-term and short-term × long-term (not significant). Central PWV (CF) (ANOVA, $P < .03$): basal × short-term ($P < .05$), basal × long-term ($P < .01$), and short-term × long-term (not significant). Peripheral PWV (FP): not significant.
* $P < .05$.
** $P < .01$.

immediate interference from the drugs in the study. All subjects were studied by a single investigator (AN) in a quiet room with constant light and room temperature between 21°C and 22°C. Patients rested in a supine position for 20 minutes before any arterial compliance measurement was taken. Blood pressure was measured through an automatic device (Dynamapp, Miami, FL) every 5 minutes throughout the evaluation. The coefficient of variation for the same data was less than 10% in all tests.

2.6.1.1. Carotid-femoral and femora-pedialis PWV. Flow waves were recorded from the right common carotid, right femoral, and right pedialis arteries with the use of nondirectional transcutaneous Doppler flow probes (model 810-A, 10 MHz, Parks Medical Electronics, Aloha, OR), as previously described [27]. The PWV is determined by the quotient of a distance and time measure.

For the calculation of CF-PWV, the distance measure is the distance between the midpoint of the manubrium (taken as a locator of the aortic arch) and the femoral pulse transducer, minus the distance between the manubrium and the carotid pulse transducer. For calculation of the femoral-pedialis PWV (FP- PWV), the distance measured is the distance between the femoral and pedialis pulses.

The time measured is the interval from the QRS complex onset to the foot of the femoral pulse wave, minus the time from the QRS complex onset to the foot of the carotid pulse recording (for the carotid PWV), and the interval from the QRS complex onset to the foot of the *pedialis* pulse wave minus the time from the QRS complex onset to the foot of the femoral pulse recording (for FP-PWV) [7].

2.6.2. Augmentation index

Carotid arterial pressure waveforms were obtained from the right common carotid artery by applanation tonometry with the use of a pencil-sized probe over the pulsation of the artery, as previously described [26]. The AI was used to quantify the augmentation of systolic pressure in central arteries due to early return of wave reflections [7,26]. The early return of wave reflections causes an inflection in the ascending portion of the pressure wave of central arteries. We constructed a computer algorithm using the derivatives of the pressure wave to determine the timing and amplitude of the foot, inflection, and peak of the pressure wave contour. More than 10 sinus beats were averaged, to calculate AI, with the peak of the R wave from the simultaneously recorded electrocardiogram being used as a timing marker. The AI was defined for each averaged waveform as the height from the inflection point to the peak of the pressure waveform, divided by the total height from foot to the peak, and expressed as a percentage. The use of AI derived from noninvasively recorded pressure waveforms of the carotid artery as an index of the contribution of wave reflections to late systolic pressure augmentation in central arteries has been previously validated [7,26].

2.7. Statistical analysis

Logarithmic transformation was performed on the variables of arterial distensibility and hemostatic parameters. Short- and long-term effects of ET on the arterial distensibility (PWV and AI) were examined by repeated-measures analysis of variance (ANOVA), using the Tukey test for post hoc analysis. Paired Student *t* test was used to study the reaming data. Pearson test for correlation was performed between parameters of arterial distensibility and hemostatic and metabolic factors, and other variables (age, known time of diabetes, BMI, and menopause duration). Values are presented as mean ± SD.

3. Results

3.1. Vascular, hemostatic, and metabolic results

Four months of ET resulted in significant reduction in glycated hemoglobin levels and an increase in HDL-C

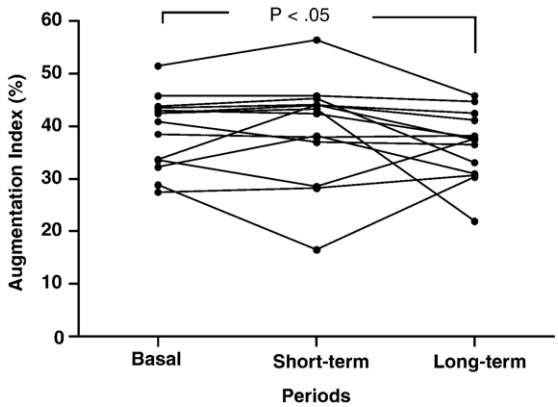

Fig. 1. Influence of short-term CEE and long-term oral CEE on the AI (n = 14). Basal represents a period at the end of the placebo phase; short-term, at the end of the placebo phase, 20 minutes after a short-term intravenous load of 1.25 mg of CEE; and long-term, 4 months of oral CEE therapy 0.625 mg/d. Analysis of variance with Tukey post hoc analysis, $P < .05$; basal × long-term, $P < .05$; basal × short-term and short-term × long-term, not significant.

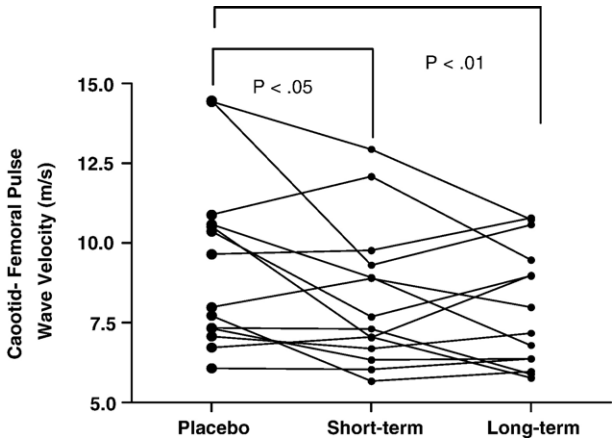

Fig. 2. Influence of short-term CEE and long-term oral CEE on the central PWV (CF, n = 14). Basal represents a period at the end of the placebo phase; short-term, at the end of the placebo phase, 20 minutes after a short-term intravenous load of 1.25 mg of CEE; and long-term, 4 months of oral CEE therapy 0.625 mg/d. Analysis of variance with Tukey post hoc analysis, $P < .03$; basal × short-term, $P < .05$; basal × long-term, $P < .01$; short-term × long-term, not significant.

levels. Weight; waist-to-hip ratio; fasting glucose; insulin, total cholesterol/LDL-C/very low-density lipoprotein cholesterol, and triglyceride levels; and glucose and insulin areas under the OGTT curve were unchanged after 4 months of CEE (Table 2).

There was a decrease in PAI-1 and an increase in factor VIII activities after CEE therapy. No significant change was observed in the other hemostatic parameters analyzed (Table 3).

Long-term oral CEE therapy decreased the CF-PWV (ANOVA, $P < .03$) and AI in relation to the placebo period (ANOVA , $P < .05$) (Table 4, Figs. 1 and 2). In addition, a significant decrease in CF-PWV was seen after the immediate test (20 minutes after 1.25 mg of intravenous CEE) (Table 4 and Fig. 2). The FP-PWV remained stable. There was no deterioration in systolic or diastolic blood pressure control during the experiment. Responders on the immediate test (30% for the AI and 50% for CF-PWV, respectively) also did it in the long-term one (50% for AI and 70% for CF-PWV) (Figs. 1 and 2). Pearson test showed a negative correlation between chronic glucose area under the curve and long-term CF-PWV ($r = -0.56$ $P < .04$). No significant correlation was seen between the parameters of arterial distensibility and the other variables (data not shown).

4. Discussion

Several effects of ET and estrogens-plus-progestins therapy are well known in nondiabetic subjects: reduction of total cholesterol, increase in HDL-C levels [10,28-30], reduction of fasting glycemia [30], improvement of vascular [8,9] or endothelial function [10], and fibrinolysis [11]. Estrogen therapy was also shown to potentiate endothelium-dependent and endothelium-independent vasodilatation in nondiabetic and diabetic subjects [10,17].

This is the first study to assess the immediate (non-genomic) and long-term effects (genomic) of CEE in women with type 2 DM on central and peripheral arterial distensibility. Both the elastic and muscular vessels were analyzed by measurements of PWV. Our results indicate that 4 months of oral CEE therapy improved the arterial distensibility, the metabolic profile (indicated by lower levels of fasting glycemia and glycated hemoglobin and higher HDL-C levels), and fibrinolysis, accompanied by a slight deterioration of the coagulation factors analyzed.

No change in insulin sensitivity was observed by us (glucose and insulin areas under the OGTT curve did not change) or others [14,15]. The improvement in glucose metabolism seems to be related to the stimulation of liver fatty acid metabolism, suppression of hepatic glucose production [30], and decrease in cytokine production [31] induced by CEE therapy.

The lower PAI-1 activity verified after 4 months of CEE suggests an enhancement of fibrinolytic potential and, possibly, an improvement in endothelial dysfunction, because PAI-1 is the most important inhibitor of fibrinolysis [3,11]. This result is in accordance with other studies [11,15,16]. High concentrations of CEE in the portal circulation can inhibit the synthesis of PAI-1 in the liver or increase its clearance [11]. Indirectly, an improvement on glycemic control and lipid profile could lower PAI-1 levels by interacting with endothelial cells [3].

The increase of factor VIII's activity suggests the estrogen induction of a partially procoagulant state and a deleterious effect, favoring thrombosis that possibly contributed to secondary fibrinolysis activation. Estrogen therapy and estrogens-plus-progestins therapy have shown controversial effects on factor VIII in nondiabetic women, either increasing [13] or not changing it [32]. The other clotting factors analyzed did not change.

The progression of elastic arterial stiffness that occurs with aging is accelerated in hypertension, altered renal function, diabetes, and dyslipidemia [4-7], contributing to increased CVD [4-7,26,27]. Postmenopausal women with type 2 DM experience more structural and functional vessel changes compared with nondiabetic matched females [5,8,17]. A reduced bioavailability of NO present in the endothelium in type 2 DM [4,7] seems to be the common end point of the classic metabolic syndrome (dyslipidemia, hypertension, hyperglycemia, and hyperinsulinemia) and nonclassic syndromes (chronic low-grade inflammation, endothelial dysfunction, increased oxidative stress, advanced glycation end products and activation of the polyol pathway). All these changes can damage the vessel wall and favor a stiffer vasculature [4,7].

The beneficial effects of ET and estrogens-plus-progestins therapy on arterial distensibility were found in healthy [8-10] and diabetic women [17] with few exceptions [14].

The decrease in CF-PVW after short-term intravenous physiologic estrogen points to a direct effect on central arterial distensibility because there was insufficient time for

activation of nuclear receptors. At the caveolae of endothelial cells, estrogens increase NO production by stimulating NO synthase, which triggers smooth muscle cell guanylate cyclase, inducing calcium-activated potassium channels [10], thus relaxing smooth muscle and promoting vasodilatation. This is to our knowledge the first description of a short-term effect of CEE in the arterial stiffness in diabetic women. Long-term CEE therapy seems to have a greater impact on central arterial distensibility as expressed by the greater decrease in PWV CF and AI. All the responders on the immediate test did it so in the long-term one (Figs. 1 and 2), favoring an estrogen-dependent effect.

Effects of long-term ET on arterial compliance depend on direct and long-term action on vessel wall, with emphasis at the functional level: down-regulation of angiotensin I receptor, increase vasodilatatory product expression (bradykinin), and gene expression of prostacyclin and NO synthase [18-20], Furthermore, the increase in NO production may inhibit monocyte adherence, thus preventing earlier stage of atherogenesis. In addition, the vasculoprotective effects of estrogens promote endothelial cell growth and vessel repair, halt the proliferation of vascular smooth cells [10,33], decrease intimal thickness [8,10], and prevent qualitative changes in the collagen fibers [33], although its effect on elastic fibers remains controversial. Hyperglycemia may lead to impaired nicotinamide adenine dinucleotide phosphate (NADPH) supply, activation of the diacyl-glycerol–protein kinase C pathway, and increase in angiotensin levels with induction of PAI-1 expression. Thus, indirectly, an improvement in the metabolic status seen in our experiment could have played a role in the improvement of the central arterial distensibility in postmenopausal subjects with type 2 DM in this and several other studies [14,15,17,31]. Corroborating, a mild negative correlation between glucose area under the curve and CF-PWV measurement after long-term ET ($r = -0.56$, $P < .04$) suggests a possible role of estrogen-induced glycemic control for the long-term response.

The decrease in PWV and AI was not reflected in blood pressure levels, as already reported by others, suggesting a greater effect of CEE on the stiffness of large arteries than on peripheral resistance [7].

Many factors could have contributed to the favorable results observed by us. Among them, glycemic and blood pressure control before and during the protocol and exclusion of severe dyslipidemia, hypertension, smoking, and known cardiovascular disease. Moreover, when compared with population studies like the Heart and Estrogen/Progestin and Women's Health Initiative [34,35], which found no benefit on cardiovascular risk, our patients were younger, leaner, and have fewer years since menopause. The data presented in our study are consistent with the emerging idea that estrogen treatment, when initiated to newly menopausal women, may be beneficial [33,36-39].

In conclusion, CEE therapy in postmenopausal women with type 2 DM was associated with a reduction in arterial stiffness and an improvement in lipid metabolism, glycemic control, and fibrinolysis, with a slight deterioration of coagulation, without increase in weight or blood pressure [40,41]. The overall long-term effects of this medication in postmenopausal women with type 2 DM require further study.

Acknowledgments

This study was supported in part by the FAPESP and CAPES institutions. The authors thank Whyeth-Ayerst for donation of medication.

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