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Oral Administration of 17β-Estradiol Over 3 Months Without Progestin Co-Administration Does Not Improve Coronary Flow Reserve in Post-Menopausal Women: A Randomized Placebo-Controlled Cross-Over CMR Study

Juerg Schwitter,[1] Sebastian Kozerke,[2] Jens Bremerich,[3] Christof Baltes,[2] Christine Attenhofer Jost,[1] Martin Birkhäuser,[5] Peter Boesiger,[2] and **Peter Buser[4]**

Clinic of Cardiology, University Hospital Zurich, Switzerland[1]
Institute for Biomedical Engineering, University and ETH Zurich, Switzerland[2]
Division of Radiology, University Hospital Basel, Switzerland[3]
Cardiology, University Hospital Basel, Switzerland[4]
Department of Internal Medicine, University Hospital Berne, Switzerland[5]

ABSTRACT

***Background*: Several large epidemiological outcome studies did not demonstrate a benefit of combined estrogen-progestin replacement treatment (HRT) on cardiovascular events in elderly postmenopausal women. Whether progestin antagonism is responsible for these negative results or the natural estrogen 17ß-estradial (E_2) itself is not effective in the coronary circulation is unknown. *Aim*: To assess the effect of 3 months of E_2 treatment on the coronary circulation, i.e., on coronary flow reserve (CFR), in postmenopausal women without established coronary artery disease (CAD). *Methods*: In a double-blind placebo-controlled cross-over design postmenopausal women (60 $\pm$ 5 years, n $=$ 14) were randomized to either start with placebo or E_2 (Estrofem, Novo Nordisk, Copenhagen, Denmark) 2 mg/d given orally over 3 months and to switch thereafter for another 3 months of therapy. At baseline, a stress echocardiography was performed to exclude CAD. CFR was determined by coronary sinus CMR flow measurements (with motion-adapted gating and interactive acquisition window control; spatial/temporal resolution of 0.8 $\times$ 0.9 mm^2/25–30 ms) which were performed at rest and during vasodilation (dipyridamole 0.56 mg/kg over 4 minutes IV) at baseline, and after 3 and 6 months of therapy, respectively. *Results*: Hemodynamics such as heart rate and systolic and diastolic blood pressure were not different for the control and E_2 group. For CFR and for resting and hyperemic coronary sinus blood flow, no differences between the placebo and E_2 group were found (2-way ANOVA for repeated measurements). Reproducibility of phase-contrast CMR measurements of CFR was $-1.1 \pm 4.9\%$. *Conclusions*: In elderly postmenopausal women without significant CAD, oral administration of E_2 over 3 months without a progestin co-administration does not improve CFR. This finding yields partly explanation for some large epidemiological trials which could not demonstrate a clinical cardiovascular benefit of HRT in elderly women.**

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Correspondence to: J. Schwitter, MD
Cardiology, University Hospital Zurich
Raemistrasse 100, CH-8091 Zurich
Switzerland
tel: (+41) 44/255 3871; fax: (+41) 44/255 4401
email: juerg.schwitter@usz.ch

INTRODUCTION

Several observational studies demonstrated that both estrogen replacement therapy (ERT) and hormone replacement therapy (HRT) (consisting of estrogen plus progestin) reduce the risk of coronary artery disease (CAD) (1–4). A variety of beneficial effects of estrogen on serum lipid levels (5–7), on inflammation (8, 9), arterial compliance and stiffness (10), and vasomotion of the brachial artery (11–14) have been reported. Less data are available for the coronary circulation. The natural 17ß-estradiol (E_2) was shown to induce relaxation of precontracted coronary artery rings and to inhibit calcium influx in isolated cardiac myocytes (15). In a study of postmenopausal women, ethinyl estradiol also acutely attenuated abnormal vasomotor responses of coronary arteries to acetylcholine (16, 17). Despite this experimental and clinical evidence for favorable effects of estrogens on vascular pathophysiology, recent randomized controlled trials showed no benefit in postmenopausal women with CAD (18) or even an increased risk of cardiovascular events in elderly (mean age 63–66 years) women both with established CAD (19) or when predominantly healthy (20), which, however, was not the case in younger women (4, 21) and not in younger women using conjugated equine estrogen (CEE) alone (22, 23).

Several mechanisms may explain this discrepancy between these in part negative epidemiological outcome trials in elderly women on one hand and the positive smaller clinical studies, done mostly in younger women, on the other hand. Firstly, coadministration of a progestin to prevent uterine hyperplasia and malignancy could abolish the positive effects of estrogens. Combination of medroxy-progesterone acetate (MPA) with either CEE (24) or E_2 (25) resulted in a reduced flow-mediated vasodilation (FMD) of the brachial artery measured by ultrasound compared to unopposed estrogen treatment. Also, cyclical E_2 combined with norethisterone did not improve FMD (26). Secondly, some procoagulatory effects of estrogens could offset beneficial effects on vascular physiology (27). And the last point, initiation of HRT or ERT in more advanced stages of atherosclerotic disease (28) could prevent hormones from adequate action, since such action may depend on the integrity of the endothelium and estrogen receptors (29), according to the concept that estrogens would primarily prevent development of CAD rather than revert established disease. Besides, CEE or E_2 itself may have limited beneficial long-term effects since many positive experimental and smaller clinical studies assessed acute effects of estrogens (16, 17) rather than long-term outcome. Considering these aspects, the following design was chosen for the current study. Postmenopausal women were randomized blindly to the natural estrogen E_2 or placebo, and no coadministration of progestin was allowed. The treatment period was set to 3 months in order to avoid testing of acute effects, and the study participants must not have obstructive CAD. The aim of this study was to test the hypothesis that E_2 administered orally over 3 months without coadministration of progestins would improve coronary physiology, i.e., coronary flow reserve (CFR) in postmenopausal women with cardiovascular risk factors (RFs) but without established CAD. Due to the restrictive inclusion criteria which would affect the sample size, a cross-over design was applied. Furthermore, to assess the coronary circulation in a longitudinal study, noninvasive phase-contrast cardiovascular magnetic resonance (PC-CMR) flow measurements were performed. The large dimension of the coronary sinus (CS) and the fact that it drains a substantial portion of the left ventricular myocardium are ideal conditions to measure resting and hyperemic flow in this vessel by PC-CMR, and hence, to assess CFR (30–32).

MATERIALS AND METHODS

Study population and design

Fourteen postmenopausal women (mean age 60 ± 5 years, range: 53–67 years) were recruited for this study. They were eligible if they were between 50–70 years of age, had passed natural or surgical menopause for at least 1 year, or had follicle stimulating hormone (FSH) levels > 40 IU/L in case they were hysterectomized. Women with clinically overt CAD and/or with significant stenoses on x-ray coronary angiography and/or a positive stress echocardiography examination (within 6 weeks before study participation) were not eligible as were women with cardiomyopathies or predominant valvular heart disease. Also, calcium antagonist treatment was not allowed (since E_2 may exert calcium antagonistic effects). Further exclusion criteria were HRT or ERT prior to study participation, a history and/or evidence of uterine or breast cancer on gynecological examination (performed within 12 months before study participation), a history of deep vein thrombosis or thromboembolism, and contraindications for CMR examination or dipyridamole administration.

The study protocol was approved by the local Ethics Committees, and all study participants gave written informed consent.

In a double-blind, placebo-controlled, cross-over design the study participants were randomized to either start with placebo or E_2 (Estrofem, Novo Nordisk) 2 mg/d given orally over 3 months and to switch thereafter for another 3 months of therapy (Fig. 1). Randomization was performed by the Institute of Biostatistics, University of Zurich, Switzerland (B. Seifert, PhD). CMR studies were performed at baseline (MR1), and after 3 and 6 months of therapy (MR2 and MR3), respectively.

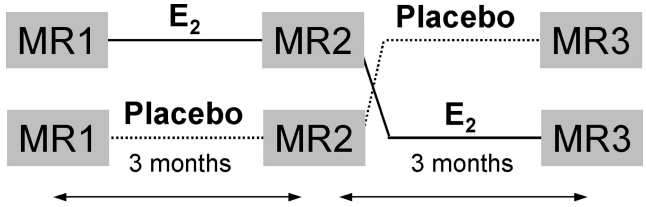

Figure 1. All women eligible for study participation were randomly assigned to a treatment/placebo arm in a double-blind fasion and switch after 3 months to the alternative study arm. Schematic of the double-blind placebo-controlled cross-over study design. E_2: 17ß-estradiol.

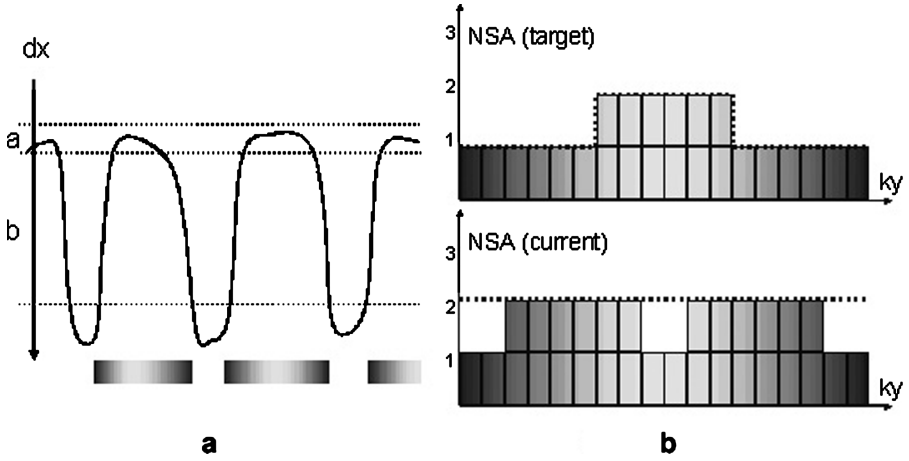

Figure 2. a) Motion Adapted Gating (MAG) controls phase-encoding according to the actual displacement (dx) of the diaphragm. Displacements within the 5 mm window (a) led to proposals for profile $k_y = 0$. Within a 12 mm window (b) MAG was performed. b) A target function defines the minimum number of signal averages (NSA [target]) required per profile k_y. In case of short-term drifts profiles other than the required can be averaged until the target function is fulfilled (NSA [current]).

MR imaging

Each subject was imaged in supine position on a 1.5 T system (Philips Medical Systems, Best, The Netherlands) using a 5 element cardiac phased-array coil. After measurement of left ventricular volumes and function by short-axis cine images, localizers were acquired depicting the CS in-plane. The site of flow measurements was then defined by an imaging plane transecting the center of both, the left atrium and the aortic root, thereby cutting the CS perpendicularly (31). To allow for velocity mapping with sufficient spatial and temporal resolutions, free-breathing acquisitions employing a modified navigator-based motion-adapted gating (33) and tracking (34) approach was implemented. A cubic weighting function was used to control phase-encoding according to the actual displacement of the diaphragm (Fig. 2a). Small displacements of less than 5 mm led to the acquisition of central k-space profiles according to the target function explained below, whereas larger displacements triggered encoding of higher spatial frequencies. Only diaphragm displacements within a 17 mm constant gating window were marked valid. Displacements beyond the 17 mm window led to rejection of the acquired data. Accepted data could potentially be averaged in the central 45% of k-space according to the target function shown in Figure 2b, thereby improving the signal-to-noise ratio in the final images while efficiently using data in case of high gating efficiencies for the given scan time of approximately 4 min. For all respiratory positions inside the gating window of 17 mm, tracking was performed with a correction factor of 0.8, i.e., a displacement of the diaphragm of 10 mm was scaled to 8 mm at the level of the coronary sinus cross-section (35). Since breathing patterns can exhibit temporary changes and sudden drifts in particular during stress exams in some subjects, the operator was allowed to adapt the gating window level interactively during the scan, thereby preventing varying gating efficiencies.

Scan parameters for phase-contrast velocity mapping were as follows: measured spatial resolution: $0.8 \times 0.9\ mm^2$, slice thickness: 5 mm, temporal resolution: 25–30 ms depending on heart rate, velocity encoding (venc): 60 cm/s/π, T_E: 7.8 ms. The two velocity encoding segments were balanced and measured consecutively in the same heart phase. Scan durations were 4:28 min and 4:05 min on average assuming heart rates of 65 beats/min and 80 beats/min and gating efficiencies of 85% and 77% during the resting and stress states, respectively.

Statistics

Values are given as mean ± SD. The assessment of treatment effects on CFR, resting, and hyperemic CS flow as well as on blood pressure and heart rate involved a repeated measures ANOVA (2 within factors: treatment groups: E_2 vs placebo and time points: baseline vs follow-up), followed by paired t tests and Bonferroni's correction ($p < 0.05/4$ is significant because of 4 comparisons; StatView v5.0.1, SAS Institute Inc., USA). Baseline measurements included all MR1 measurements and follow-up measurements included the MR2 and MR3 measurements for each treatment group (i.e., MR2 and MR3 for E_2 group vs MR2 and MR3 for the placebo group).

Reproducibility for CFR, resting, and hyperemic CS flow measurements was calculated from baseline and follow-up studies under the placebo regimen (all MR1 studies vs MR2 and MR3 studies of the placebo group). Reproducibility is given as the mean ± SD of paired measurements ($= SD_{Diff}$) and corresponding 95% confidence intervals (36).

For an assumed difference in CFR between E_2 and placebo of approximately 10% (37) with an interstudy reproducibility of 4.9% ($= SD_{Diff}$) by PC-MR, the study yields a power of 90% to detect differences at a p value of 0.01 in a sample size of 15 subjects (38).

Table 1. Characteristics of postmenopausal women

n	14
Age (years)	58 ± 5
Height (cm)	161 ± 6
Weight (kg)	67 ± 9
Body mass index (kg/m^2)	26 ± 3.7
Estradiol (menopause <100 pmol/L)	48 ± 13
Risk factors:	2.0 ± 0.9
Arterial hypertension	8 (57)
Hypercholesterolemia	4 (29)
Diabetes mellitus	2 (14)
Smoking	1 (7)
Positive family history	1 (7)
Medication:	
Beta-blockers	6
ACEI	4
Statins	4
Insulin	2
History of hysterectomy	5
Stress-Echocardiography	11
X-ray coronary angiography	3

Data are mean ± SD or number of patients (%). ACEI = angiotensin converting enzyme inhibitors.

RESULTS

Demographics of the study population are given in Table 1. Two patients had to be excluded from the study due to headache over several days (n = 1) and vomiting after vasodilation at MR1 (n = 1) resulting in 12 patients included in the analysis. In the 12 women, 1 CMR study (MR 3) was not evaluable due to caffeine intake at the day of the CMR study, in 2 patients 1 CMR study was technically inadequate. An example of CMR images for MR1, MR2, and MR3 is given in Figure 3, together with corresponding resting and hyperemic CS flow curves in Figure 4. Medication included antihypertensive treatment (in 8 patients), statins (in 4), and antidiabetic treatment (in 2), which were all continued during the entire study. Hemodynamics such as heart rate, systolic, and diastolic blood pressures were not different for the control and E_2 group as shown in Figures 5 and 6, respectively. No differences between the placebo and E_2 group were found for hyperemic CS blood flow ($p = 0.60$; p value for the interaction between treatment groups and time-points, repeated measures ANOVA), neither for resting CS blood flow ($p = 0.51$; Fig. 7), nor for CFR ($p = 0.16$; Fig. 8).

Reproducibility for CFR measurements with PC-MR expressed as mean difference ± SD of paired measurements determined in the placebo group with a mean CFR of 2.55 ± 0.33 was −0.021 ± 0.13 (−1.1 ± 4.9%).

DISCUSSION

The current study results indicate that a 3 month treatment period of postmenopausal women with E_2 does improve neither maximum myocardial perfusion nor CFR. In the current study population of postmenopausal women without established CAD, CFR ranged from 2.35 to 2.96. In a study using PET imaging similar CFR, values of 2.05 to 2.51 were obtained in postmenopausal women with RFs (37). In this study by Campisi et al , CFR and dipyridamole-induced hyperemic myocardial blood flow were 22% and 11% higher during HRT than without therapy, respectively, although these differences did not reach statistical significance. However, in their study both, ERT and HRT were mixed in the active treatment group, which could explain a lack of CFR improvement due to progestin antagonism. In the current study with unopposed E_2 treatment, no difference was found between the E_2 and the placebo group for both CFR and dipyridamole-induced hyperemic myocardial blood flow. These data indicate that CFR and hyperemic myocardial blood flow do not increase with either E_2 alone or with combined estrogen/progestin treatment. Assuming that CFR and maximum myocardial blood flow are reflecting coronary vascular health status, the current findings are in line with large clinical prospective trials which demonstrated in elderly postmenopausal and predominantly healthy women no beneficial effect of HRT (20) and ERT (23) on cardiovascular event rates.

When comparing the current study results on coronary circulation obtained in women without CAD with those of other vascular territories such as the brachial or carotid arteries in

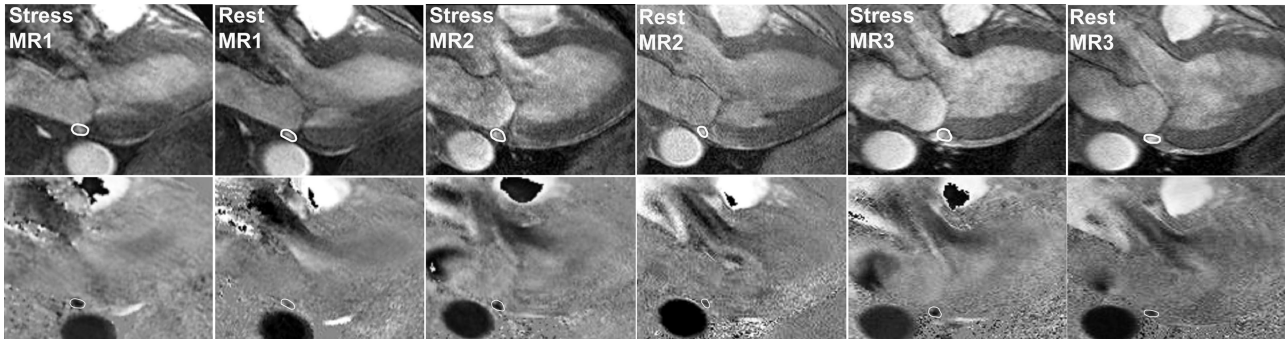

Figure 3. Example of magnitude and phase contrast MR images (top and bottom row, respectively) for a subject randomized for placebo during the first 3 months, followed by 17ß-estradiol treatment during the following 3 months. In the magnitude images (top row), the coronary sinus was delineated by a region-of-interest (thick line), which was copied to the phase contrast, i.e., flow images (bottom row, thin line).

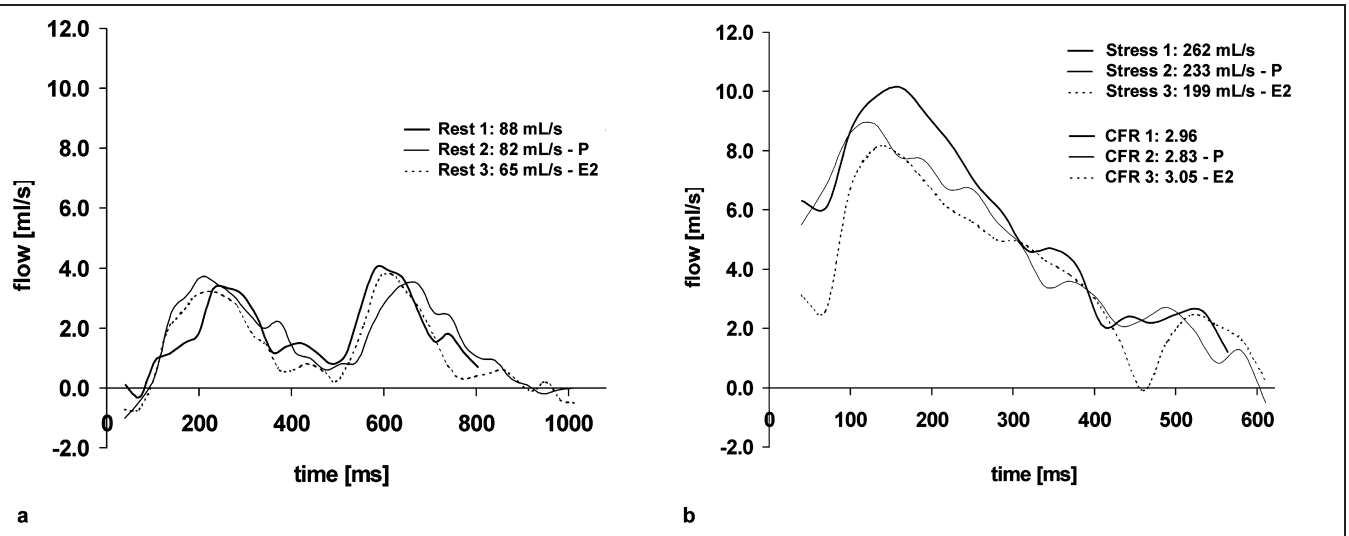

Figure 4. Example of resting and stress coronary sinus flow curves derived from the image set shown in Figure 3. P = placebo, E2 = 17ß-estradiol, Rest 1–3, Stress 1–3, and CFR 1–3 indicate baseline, first follow-up, and second follow-up CMR study, respectively.

postmenopausal women without CAD, similar findings were reported. In a large prospective study testing FMD of the brachial artery as a marker of vascular health, 253 postmenopausal women were randomized to either HRT or no therapy and were followed for a mean of 2.9 years (26). After elimination of 153 patients from analysis due to drop outs, in the 100 remaining patients, no difference in FMD was found between HRT and the placebo group. Similarly, in another large study 321 postmenopausal women were recruited for measurements of carotid artery distensibility and were randomized to low and high dose HRT or no therapy. Drop out was 46% and in the remaining population, no effect of HRT on distensibility was found (39). Finally, in a randomized, double-blind, placebo-controlled trial unopposed E_2 reduced progression of intima-media-thickness measured by sonography in the carotid arteries in postmenopausal women compared with placebo but did not influence progression in those women with controlled lipid levels (40).

In postmenopausal women with established CAD neither HRT nor ERT had a beneficial effect on the coronary macrocirculation, i.e., progression of coronary artery stenoses was not altered (41). However, there were positive effects of E_2 on coronary circulatory function in postmenopausal women with established CAD by attenuating acetylcholine-induced coronary artery constriction (17). Similarly, in the forearm microcirculation of postmenopausal women blood flow at baseline (i.e., prior to E_2 treatment) of 10.2 mL/min/100 g (during acetylcholine infusion) increased to 17.9 mL/min/100 g after 12 weeks of E_2 treatment (42). In this study, also sodium nitroprusside-augmented microcirculatory flow of 10.3 mL/min/100 g further increased to 17.9 mL/min after 12 weeks of E_2 treatment. In an CMR study in healthy premenopausal women, the forearm macrocirculation also exhibited increased FMD during midcycle compared with the menstrual phase with high and low levels of E_2, respectively (43).

These findings illustrate that E_2 mechanisms of action may differ in different vascular beds, such as in coronary microcirculation (37 and present study), coronary macrocirculation (17, 41), brachial macrocirculation (26, 43), forearm microcirculation (42), and carotid artery macrocirculation (40). Also the status of the endothelium, i.e., preclinical disease (37, 40 and the present study) vs established atherosclerotic disease (17, 41) may influence the effects of E_2 treatment on the vascular parameters measured. This could be one reason among many others for the different effects of E_2 in different vascular territories since atherosclerosis is known to progress differently in various vascular regions. It is noteworthy that large randomized clinical trials were negative when performed in women with proven CAD with respect to clinical outcome (18) as well as morphologic

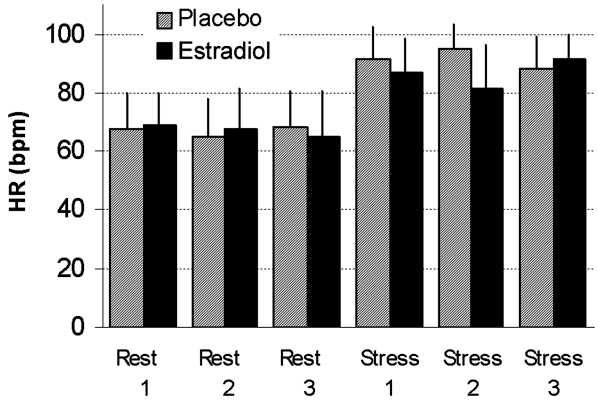

Figure 5. Bar graph demonstrating heart rates (HR in beat per minute) during resting and stress condition for the placebo and 17ß-estradiol group. No significant differences occurred. Bpm = beats per minute.

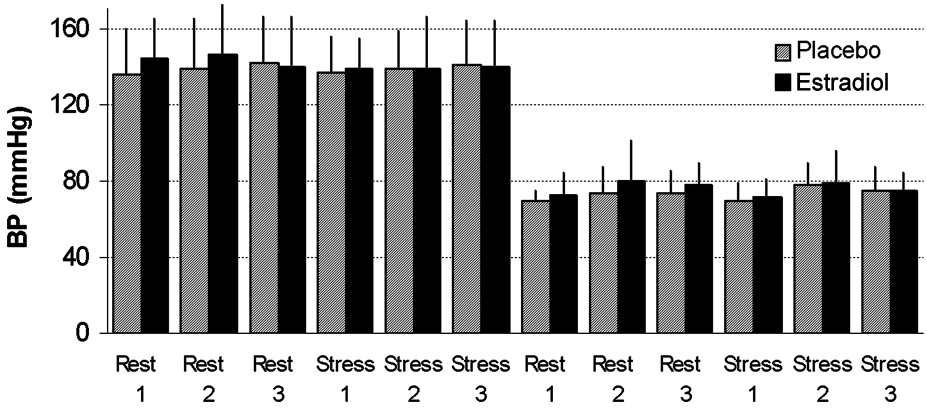

Figure 6. Bar graph demonstrating systolic and diastolic blood pressure (BP) during resting and stress condition for the placebo and 17ß-estradiol group. No significant differences occurred.

progression of CAD (19, 41). In the present study, participants had preclinical CAD only and, thus, a lack of an E_2 effect on CFR does not directly explain the negative clinical outcome of the large clinical trials mentioned above. While estrogen receptors are diminished in atherosclerotic lesions in women (29), the estrogens themselves may show different affinities or different conformations of the ligand-receptor complex yielding different effects in different tissues. The field of selective estrogen receptor modulators (SERM) opens the opportunity to design specific compounds which exert beneficial effects in the cardiovascular system, while being inactive in other tissues, such as breast or endometrium. Raloxifene, a SERM approved for osteoporosis prevention and treatment, was shown to restore endothelial nitric oxide release in ovariectomized female rats (44) and in a subpopulation of women at increased cardiovascular risk in the MORE trial (Multiple Outcomes of Raloxifene Evaluation), fewer cardiovascular events occurred with raloxifene versus placebo (45). In extended follow up, this advantage was no longer present, which was discussed in light of selection bias, but could also be a consequence of changes in receptor density with progressive disease (46).

In addition to these pathophysiological considerations based on prospective, randomized, double-blind, and placebo-controlled trials (23, 40), some other positive studies (11, 13, 24, 47–51) had some major limitations in study design. Several of these positive studies had no placebo control group (13, 24, 48–50), were observational in nature (51), or were small in sample size (24), which makes correction for underlying RFs profiles problematic (unless a cross-over design is applied). In the present study, limitations with regard to study design were minimized, and as a result, it could be demonstrated that unopposed E_2 administered orally over 3 months had no beneficial effect on the myocardial microcirculation in postmenopausal women without CAD.

Limitations of the study

Although the study was performed in a placebo-controlled, randomized, double-blind fashion, there still remain some

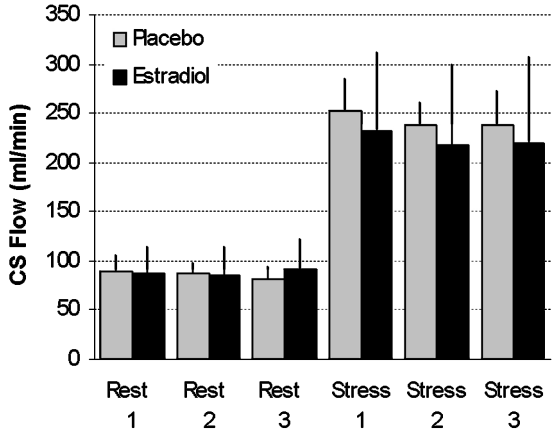

Figure 7. Bar graph demonstrating coronary sinus (CS) blood flow during resting and stress condition for the placebo and 17ß-estradiol group. No significant differences occurred.

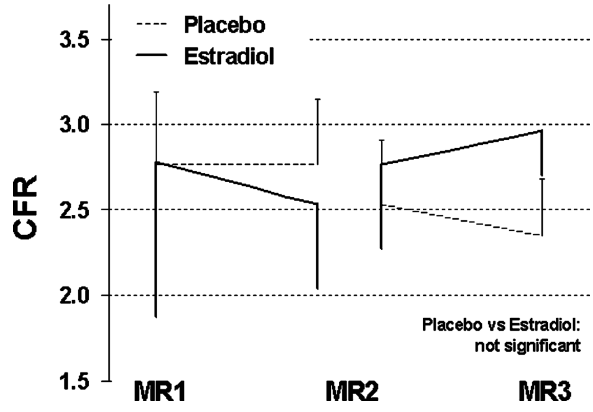

Figure 8. Mean coronary flow reserve (CFR) for all time points. 17ß-estradiol treatment did not improve CFR in this cross-over design.

limitations. Particularly, the treatment period of 3 months might have been too short for E_2 treatment to fully develop the beneficial changes in vascular physiology and/or morphology. However, if the treatment periods would have been considerably longer, this could have led to a selection bias toward women with high acceptance of hormone treatment (subjecting the study to a limitation similar to observational studies) and maybe a higher risk of side effects of E_2. A second limitation is the age of the patients; as it has been suggested by a large prospective, randomized, double-blind and placebo-controlled clinical trial (21, 23, 52), women with a distance less than 10 years from menopause may behave differently than women with a more distant menopause ("window of opportunity").

Dipyridamole increases myocardial blood flow by acting on smooth muscle cells. Increased flow then induces endothelium-mediated vasodilation by increased endothelial shear stress. Thus, dipyridamole indirectly stimulates endothelial-dependent flow increase, and accordingly, an impairment of dipyridamole-induced hyperemic flow has been shown in the presence of RFs (37). Therefore, it appears justified to use this pharmacological agent to test whether E_2 could revert or mitigate some adverse effects of RFs on endothelial function.

Finally, neither serum lipids nor inflammatory markers were measured in this study to focus on the single question: whether E_2 can improve coronary vascular function without addressing potential mechanisms of E_2 action. The treatment regimens between women were somewhat heterogeneous. The cross-over design, however, should mostly correct for this limitation (no treatment was changed during the placebo and treatment phase in any patient besides the study drug).

Despite the relatively small sample size in this study, the nonsignificant p value (> 0.05) for differences in CFR indicates that E_2 treatment affects the CFR by less than $\pm 8\%$ (at a power of 80%), i.e., the effect of E_2, if at all present, is unlikely to be of clinical relevance.

CONCLUSIONS

In elderly postmenopausal women without significant CAD, oral administration of E_2 over 3 months without a progestin coadministration does not improve CFR. This finding yields partly explanation for some large epidemiological trials which could not demonstrate a clinical cardiovascular benefit of HRT in elderly women.

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