Design Paper

The Estrogen Replacement and Atherosclerosis (ERA) Study: Study Design and Baseline Characteristics of the Cohort

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ABSTRACT: The Estrogen Replacement and Atherosclerosis (ERA) trial is a three-arm, randomized, placebo-controlled, double-blind trial to evaluate the effects of estrogen replacement therapy (0.625 mg/day oral conjugated estrogen) with or without continuous low-dose progestin (2.5 mg oral medroxyprogesterone acetate/day) versus placebo on progression of atherosclerosis. A total of 309 postmenopausal women at five sites underwent baseline coronary angiography and were randomized. Participants will have repeat coronary angiography after an average of 3.25 years of treatment. The primary outcome of interest will be change in minimum diameter of the major epicardial segments, as assessed by quantitative coronary angiography. The primary aim is to test the hypothesis that either form of hormone therapy will slow the progression or induce regression of coronary atherosclerosis compared to placebo. The secondary aims are to assess the effects of the two treatments versus placebo on endothelial function (measured using flow-mediated vasodilator responses), on several presumed mediators of estrogen's effect on atherosclerosis (i.e., plasma lipids and lipoproteins, blood pressure, glucose metabolism, hemostatic factors, and antioxidant activity), on other factors that influence the development of coronary heart disease (i.e., diet, smoking status, exercise, weight, and health-related quality of life issues), and on clinical cardiovascular events.

The ERA trial is the first angiographic endpoint clinical trial to examine the effects of postmenopausal hormone replacement on coronary atherosclerosis in women. It will

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provide an unparalleled opportunity to determine if either regimen of hormone therapy is effective in slowing the progress of angiographically defined coronary atherosclerosis. This study will complement other estrogen replacement trials, such as the PEPI, HERS, and Women's Health Initiative studies, to provide a more comprehensive examination of the effects of estrogen replacement on cardiovascular risk factors, anatomic and functional manifestations of atherosclerosis, and risk for coronary heart disease in postmenopausal women. *Control Clin Trials* 2000;21:257–285

KEY WORDS: *Atherosclerosis, coronary angiography, estrogen replacement therapy, postmenopause, randomized controlled trials, women's health*

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death and disability in women, accounting for 57% of all deaths, far more than breast or other cancers [1]. Because of the increased risk of coronary heart disease (CHD) in women around menopause, it has been speculated that endogenous estrogen has an important cardioprotective effect. This speculation has been supported by observational and case-control studies demonstrating that estrogen replacement therapy in postmenopausal women is associated with lower risk for CHD [2–7]. In epidemiologic meta-analyses [8–10], long-term use of postmenopausal estrogen was associated with a 50% lower risk of CVD. The relationship observed between estrogen replacement and CHD risk is even more striking in older women with coronary artery disease [11, 12].

Despite the observational data suggesting estrogen's cardioprotective effect, its actual benefits and risks have not been fully evaluated. In 1998, the Heart and Estrogen/progestin Replacement Study (HERS) showed no benefit of 4.1 years of estrogen plus progestin therapy for secondary prevention of heart disease in postmenopausal women [13]. Within the overall null effect, there was a reduction in risk after 2 years of treatment unexpectedly offset by an increased risk during the first year. It remains unclear whether this pattern of changing risk over time was simply a chance occurrence combined with no real effect of the regimen, or due to an early adverse and late beneficial effect of the regimen.

A major question concerning the HERS trial results is whether the addition of the progestin medroxyprogesterone acetate (MPA, Provera®) blocked estrogen's cardioprotective effects. Currently, clinicians administer a progestin with estrogen to prevent unopposed estrogen-induced endometrial carcinoma in women with intact uteri. However, MPA and 19-nor testosterone progestins blunt the favorable changes in high-density lipoprotein cholesterol (HDL-C) associated with estrogen use [14, 15]. Furthermore, in nonhuman primates, continuous low-dose MPA substantially reduced estrogen's favorable effect on the progression of coronary atherosclerosis [16]. On the other hand, the attenuation of estrogen-induced increases in HDL-C may be offset by the progestins' protection against estrogen-induced increases in triglycerides and factor VII [17, 18]. Observational studies provide no evidence that combined therapy increases the risk of CHD compared with estrogen alone [19–21]. Use of continuous low-dose progesterone may also improve long-term compliance with estrogen replacement therapy regimens by inducing amenorrhea after 6–12 months of therapy. Thus, the hope has been that the combination of

Table 1 Exclusion Criteria in the ERA Trial

Contraindications for Estrogen Use
- Known or suspected breast or endometrial carcinoma (all potential participants had mammography and endometrial biopsy pre-enrollment)
- History of deep venous thrombosis or pulmonary embolism
- Symtomatic gallstones or elevated liver enzymes (SGOT $> 1.5 \times$ control)
- Fasting plasma triglycerides > 400 mg/dL

Contraindications for Research Angiography
- Recent myocardial infarction (< 4 weeks)
- Renal insufficiency (serum creatinine > 2.0 mg/dL)
- History of life-threatening dye allergy
- $> 70\%$ stenosis of the left main coronary artery
- Uncontrolled hypertension (systolic > 200 mm Hg or diastolic > 105 mm Hg)
- Uncontrolled diabetes (fasting blood glucose $\geqslant 299$ mg/dL)

Factors Not Conducive to Completing the Study
- Planned or prior coronary artery bypass surgery
- Prior mechanical revascularization of the only qualifying ($\geqslant 30\%$ stenosis) lesion
- Inadequate baseline angiogram for quantitative coronary angiography
- Other (non-CHD) diseases likely to be fatal or prevent adequate follow-up (e.g., malignant neoplasms, severe organ failure, alcoholism)
- Participation in other intervention studies
- Plans to leave the area within 3 years

CHD = coronary heart disease; SGOT = serum glutamic oxaloacetic transaminase.

estrogen plus continuous low-dose MPA would retain the cardioprotective effects of estrogen while protecting against endometrial hyperplasia.

More definitive information about the effects of estrogen with and without progestins on anatomic manifestations of atherosclerosis would help in interpreting the HERS results and their relationship to the large body of observational data on estrogen replacement and CHD risk. To clarify these issues, we designed an angiographic endpoint trial comparing unopposed estrogen, or estrogen plus continuous low-dose MPA, versus placebo in women with established coronary atherosclerosis.

Our trial, entitled "Estrogen Replacement and Atherosclerosis" (ERA), will use quantitative coronary angiography and measures of endothelial function to determine the effects of estrogen replacement therapy on the anatomic and functional sequelae of atherosclerosis in coronary and peripheral arteries of postmenopausal women. The ERA trial will complement other estrogen replacement trials, including the Postmenopausal Estrogen/Progestin Intervention (PEPI) study [15], HERS, and the Women's Health Initiative (WHI) [22], to provide a more comprehensive examination of estrogen replacement's effects on cardiovascular risk factors, anatomic and functional manifestations of atherosclerosis, and CHD risk in postmenopausal women.

TRIAL DESIGN AND METHODS

Overview

The ERA trial is a three-arm, randomized, placebo-controlled, double-blind trial to evaluate the effects of estrogen replacement therapy with or without continuous low-dose progestin on angiographic progression or regression of coronary atherosclerosis (Table 1). From January 1996 through December 1997,

a total of 309 postmenopausal women at five sites (Winston-Salem, Charlotte, and Greensboro, NC; Birmingham, AL; and Hartford, CT) underwent baseline coronary angiography documenting significant coronary disease and were subsequently randomized to receive daily (1) 0.625 mg of oral conjugated estrogen, (2) 0.625 mg oral conjugated estrogen plus 2.5 mg oral MPA, or (3) placebo. The women are scheduled to have repeat coronary angiography after an average of 3.25 years of treatment. The primary outcome of interest will be change in minimum diameter of the major epicardial coronary segments measured using quantitative coronary angiography (QCA).

ERA's primary aim is to test the hypothesis that unopposed estrogen and estrogen plus MPA will slow the progression or induce regression of coronary atherosclerosis versus placebo based on QCA measurements. Secondary aims are to assess the effects of both treatments versus placebo on endothelium-dependent vasodilator capacity, and on mediators of estrogen's effect on atherosclerosis (i.e., plasma lipids and lipoproteins, blood pressure, glucose metabolism, hemostatic factors, and antioxidant activity). Other CHD-related factors (i.e., diet, smoking status, exercise, weight, and health-related quality of life issues) will also be measured and considered as potentially important confounders of health-related quality of life and on clinical cardiovascular events.

Eligibility Criteria

The eligibility criteria were designed to include as many women as possible who might potentially benefit from estrogen replacement therapy, while excluding any for whom participation might be unnecessarily dangerous or who were unlikely to complete the study. Women under the age of 80 years were eligible if they had coronary artery disease according to a baseline angiogram and were postmenopausal. We defined as "postmenopausal" women aged 55 years or older without natural menses for at least 5 years, or who met one of the following criteria: (1) no natural menses for at least 1 year and serum follicle-stimulating hormone (FSH) level $>$ 40 MIU/mL; (2) documented oophorectomy; or (3) reported bilateral oophorectomy, FSH $>$ 40 MIU/mL, and estradiol $<$ 25 pg/mL.

Coronary artery disease was defined as at least one stenosis of 30% in any single coronary artery. Angiographic eligibility was determined based on a clinically indicated coronary angiogram obtained less than 4 months before randomization or a de novo research angiogram obtained in women likely to have coronary disease and who met the other eligibility criteria.

Exclusion Criteria

Exclusion criteria are listed in Table 1. Women also were excluded if they did not successfully complete the placebo run-in phase of the study, a 4-week period before randomization when potential participants were required to take daily placebos and keep a diary documenting medication consumption. (This diary was also used to record vaginal bleeding after randomization.) Subjects who returned to the clinic after 4 weeks and were $>$ 80% compliant with the placebo medications based on pill count and diary were scheduled for the baseline angiogram (if needed). Women on estrogen at the initial screening

were eligible for ERA if they were willing and able to stop taking estrogen during the run-in phase and willing to be randomized to active treatment or placebo during the follow-up period. We anticipated that < 50% of postmenopausal women with heart disease would be current users of estrogen, based on our experience in screening for HERS [23]. Women who had percutaneous transluminal coronary angioplasty (PTCA) during their baseline angiogram remained eligible if at least one coronary segment with 30% stenosis or more was not crossed by the PTCA equipment. Women who underwent clinically indicated baseline coronary angiography less than 4 weeks after a myocardial infarction also were eligible.

The sample size and power for ERA were based on using two sample *t* tests to compare pre- and postdifferences in mean minimum diameter between arms. Two comparisons will be made: placebo to estrogen, and placebo to combination therapy, each at the 0.025 level. ERA plans to randomize 300 women, and has 80% power to detect a 0.0542 mm difference between arms in 39-month change in mean minimum diameter, assuming a pooled standard deviation of 0.1145 mm and 15% dropout. Because the primary analysis will actually be done by analysis of covariance with adjustment for baseline and other covariates, the power should be in excess of 80%.

Recruitment

A detailed description of the recruitment activities for the ERA trial is the subject of a separate report. In summary, three strategies were used: community-wide media-based announcements; targeting enriched cohorts identified through hospital records, prospective hospital admissions, and screening logs from other studies; and strategies focused on minority recruitment. We identified 73,327 eligible women from these sources. The best yield was from self-referrals (2.9%), followed by local hospital patient lists (1.2%), Health Care Financing Administration lists (1.02%), and screening logs from other studies (0.65%). Yields at the five clinic sites averaged 1.09% overall. Recruitment lasted for 24 months. The average age of the participants was 65.7 years, and 81.9% were Caucasian (Table 2). Baseline physical characteristics of the cohort are listed in Table 3, and cardiovascular medication use at baseline is listed in Table 4.

Randomization Design

Women returned for a randomization visit after completion of the screening visits, baseline angiogram, endometrial evaluation, mammogram, and placebo run-in. Prestratification was employed for randomization to avoid the possibility of substantial imbalances across treatment groups for lipid-lowering therapy at baseline and hospital where the angiogram was performed (total of ten strata). The eligible women were assigned to treatment groups using a permuted block randomization based on pretrial values of these variables. Adjustment for pretreatment values of the stratification factors will be made in the analysis of group differences; therefore, the analysis will match the design and the estimated variance will not be positively biased [24].

Table 2 Baseline Demographic Characteristics of Subjects in the ERA Trial

	Group 1 (n = 103)	Group 2 (n = 102)	Group 3 (n = 100)	Overall (n = 305)
Mean age (yr)[a]	65.5	65.5	66.2	65.7
Mean time since menopause (yr)[a]	22.1	23.8	23.1	23.0
Ethnicity (%)				
White	77.7	74.5	75.0	75.7
Black	14.6	13.7	14.0	14.1
Native American	4.9	5.9	6.0	5.6
Hispanic	0.0	0.0	1.0	0.3
No data	2.9	5.9	4.0	4.3
Smoking status (%)				
Never	35.0	40.2	30.0	35.1
Former	48.5	38.2	52.0	46.2
Current	16.5	21.6	18.0	18.7
Percent with history of[a]				
Angioplasty	49.0	41.9	51.0	47.2
Diabetes	28.8	29.5	25.0	27.8
Heart attack	41.3	55.2	48.0	48.2
Hypertension	72.1	68.6	60.0	67.0
Hysterectomy	61.5	65.7	56.0	61.2
Ovariectomy[b]	29.8	44.8	23.0	32.7
Taking lipid med.	32.7	30.5	30.0	31.1

[a] Group sizes for these data: group 1, n = 104; group 2, n = 105; group 3, n = 100; overall, n = 309.

[b] p = 0.02 between groups. All other p-values not significant.

The treatments were randomly assigned to participants within each stratum using a computerized randomization system. A randomization list (a sequence of treatment assignments of about 20% more than the expected enrollment in a given stratum) was generated using random blocks (4, 8, 12) such that, at

Table 3 Baseline Physical Characteristics of Subjects in the ERA Trial

	Group 1 (n = 103)	Group 2 (n = 102)	Group 3 (n = 100)	Overall (n = 305)
Waist circumference (cm)	94.1	91.7	93.6	93.1
Hip circumference (cm)	108.4	107.9	107.2	107.8
Waist/hip ratio	0.9	0.9	0.9	0.9
Subscapular skinfold (mm)	24.6	23.9	23.2	23.9
Tricep skinfold (mm)	26.0	26.1	25.9	26.0
Systolic BP (mm Hg)	136.2	134.6	131.5	134.1
Diastolic BP (mm Hg)	74.1	74.5	73.4	74.0
Ejection fraction (%)	60.0	58.7	59.8	59.5
Percent with EF $\leq$ 40%	5.8	3.8	3.0	4.2
Baseline angiographic data across groups[a]				
Percent Stenosis				
Mean	66.26			
Minimum	0			
Maximum	100			

[a] Data available for 307 subjects.

For all comparisons between groups, p-values not significant.

BP = blood pressure; EF = ejection fraction.

Table 4 Baseline Concomitant Medications for Subjects in the ERA Trial[a]

Percent Taking	Group 1 (n = 104)	Group 2 (n = 105)	Group 3 (n = 100)	Overall (n = 309)
ACE inhibitor	26.9	16.2	23.0	22.0
Aspirin	73.1	69.5	67.0	69.9
Beta blocker	46.2	46.7	39.0	44.0
Calcium blocker	50.0	54.3	61.0	55.0
Diuretic	31.7	30.5	29.0	30.4
Insulin	11.5	10.5	13.0	11.7
Nitrates	38.5	29.5	50.0	39.2
Oral hypoglycemic	13.5	18.1	16.0	15.9
Other BP meds[b]	6.7	7.6	0	4.9

[a] Each subject counted once per reported medication.

[b] For these data, $p = 0.011$ between groups. For all other comparisons between groups, p-values not significant.

ACE = angiotensin-converting enzyme; BP = blood pressure.

any point on the list, the maximum discrepancy in the size of the treatment groups is a fixed, small number for each stratum. The computer program was used to: (1) determine eligibility of the patient; (2) if eligible, access the randomization list and assign the first unassigned treatment for the patient; (3) record all the randomization information; and (4) print out a session log.

The data necessary for randomization were entered into the computer under a forms management program and were checked for validity as entered. Any keyed data out of range or otherwise determined to be invalid were rekeyed immediately. After all necessary information was keyed, the data were displayed for verification and correction. Only after the entered data were verified and the eligibility criteria met was a treatment assignment displayed. The patient was then registered in a write-protected file. A manual system backup, and a computer printout of the planned assignments including treatment identification numbers, were available for use by a designated member of the data management staff in case of a computer failure.

Screening and Follow-up Visits

The participants are seen in the General Clinical Research Center (GCRC) outpatient unit in Winston-Salem or the satellite clinics in Greensboro, Charlotte, Hartford, or Birmingham for all screening and follow-up visits except the gynecology examinations, mammograms, and angiograms. The clinic schedule is arranged for patient safety and comfort as well as efficiency of personnel and space resources.

Women who complied with the placebo run-in (i.e., took $\geq 80\%$ of expected number of pills based on pill count) and met the other eligibility criteria had blood drawn for serum electrolytes, hemoglobin, hematocrit, platelet count, and prothrombin time; had a 12-lead electrocardiogram (EKG) recorded; and were scheduled for a baseline angiogram (if needed). If the angiographic criteria were also met, they then returned for a clinic visit to be randomized into the trial. The order of data collection was designed to delay the angiogram until it was certain that a woman otherwise qualified. If a woman was excluded

because of an abnormal laboratory finding, her local physician was notified promptly. The local physician was also notified of the patient's participation and received a copy of all prescreening laboratory results. At that time, we requested the patient's and local physician's cooperation in refraining from use of postmenopausal hormone replacement to reduce drop-ins. For women with a clinically indicated coronary angiogram as their baseline angiogram who were otherwise eligible, the order of prescreening events was necessarily reversed. The two outpatient screening visits, placebo run-in, cervicovaginal cytology screening, mammography, and randomization visit were completed within 4 months of the baseline angiogram. Subjects were scheduled to return after 3 months, 6 months, and then every 6 months thereafter. Participants will also return to the gynecology clinic for annual cervicovaginal cytology screening and mammograms. Closeout angiograms will occur during a 9-month period, for a mean follow-up of 3.25 years (range 2.6–3.9 years).

At each follow-up visit, the clinic staff will document the number of pills dispensed and returned and all concomitant prescription medications. Compliance will be measured as percent of expected pills taken based on pill count. Women will be classified as compliant if they take $\geqslant$ 80% of expected pills during the study. Both cardiology and gynecology sites are monitored periodically to review procedures, check data collection forms for completeness, and answer questions about site-specific issues that may arise. In addition, the data management staff continually monitors visit and form completion and submits queries to the clinics to resolve confusing or missing data.

Baseline and Follow-up Gynecology Evaluations

Each patient who completed the initial screening underwent a baseline gynecologic examination (including the breast, abdomen, and pelvis) to ensure that she was free of malignant and premalignant disease. The pelvic examination included a Pap smear for cervical cytology, an endometrial aspiration, and a bimanual and rectovaginal examination. If an endometrial aspiration could not be performed, a transvaginal ultrasound with measurement of the endometrial thickness was done. We anticipated that approximately 10–15% of the eligible participants would have cervical stenosis that would preclude endometrial sampling and therefore require transvaginal ultrasound. In accordance with current American College of Obstetrics and Gynecology guidelines [25], all women in the unopposed estrogen arm (approximately 33% of the participants) should undergo annual endometrial aspiration for detection of endometrial hyperplasias. To maintain the blind, all subjects, including those assigned to continuous combined therapy, will undergo this procedure annually. Mammography was also performed, if possible, on the day of the baseline gynecologic examination, but at any rate before the second screening visit and annually thereafter. Adverse events relating to abnormalities of the breast, hormone replacement therapy, or the genitourinary tract in general will be treated according to established guidelines.

Baseline and Follow-up Coronary Angiography

High-quality coronary angiograms acquired in an identical standardized fashion at both baseline and after an average of 3.25 years of follow-up are

required for ERA. After an overnight fast, women scheduled for a de novo baseline angiogram were admitted to the day-hospital unit at one of the five hospitals approximately 2 weeks after their last clinic appointment. After examination, an intravenous drip line was started, and the EKG and laboratory results from the clinic visit were reviewed by a research nurse and the angiography personnel. On arrival at the catheterization laboratory, subjects received 0.4 mg nitroglycerin sublingually unless their systolic blood pressure was < 100 mm Hg. A minimum of three pairs of standardized biplane views of the left coronary artery and one pair of standardized biplane views of the right coronary artery were obtained using 6F catheters and non-ionic contrast material. Additional views were also obtained as needed for optimal display of the entire coronary tree according to the discretion of the angiographer. Columniation was maximized and image intensifier-to-patient distance minimized while still showing the areas of interest. A research nurse recorded the preoperative medication, image geometry (camera angles, table height, subject-to-intensifier distance), and catheter used for each injection. During the follow-up angiogram, all aspects of the baseline procedure will be duplicated. When possible, the same catheterization laboratory will be used for the baseline and follow-up studies. The angiographer will review the baseline angiogram before performing the follow-up study. If a woman requires a clinically indicated angiogram before the end of the trial, every effort will be made to assure that the angiogram is done in accordance with the ERA protocol and all pertinent information recorded. Clinically indicated coronary angiograms obtained within 6 months of the scheduled closeout angiogram will be treated as the final angiogram. When possible, pre-intervention (percutaneous transluminal coronary intervention or coronary artery bypass grafting) angiograms that occur during the follow-up period will also be sent to the QCA Core Laboratory for evaluation of the involved segments before the intervention. Thus, the length of follow-up will be a segment-specific variable that may vary within the same subject.

Core Laboratory Review of Baseline Angiograms

Because the primary outcome measure will require high-quality coronary angiography at baseline and follow-up, it would be very costly to randomize women whose baseline angiograms were unusable. Thus, we screened all baseline angiograms and their associated documentation to confirm the presence of a qualifying (30% or greater) lesion. The prerandomization angiogram review was performed at the Wake Forest Cardiology Image Analysis Laboratory within 48 working hours of receipt so as not to delay enrollment of eligible women. A similar strategy was successfully used in previous clinical trials in which our laboratory participated. A summary of the QCA review of the worst lesion and baseline data from the catheterization reports is found in Table 5.

Maintaining the blind for angiography personnel is critical for the integrity of the results. QCA operators will be blind both to treatment assignment and to temporal sequence of the films. It is equally important that the final quantitative results are assigned correctly to the baseline or follow-up film time period. Therefore, at the central ERA site, a staff member not involved with angiography will generate a set of participant-specific label pairs with "A" or "B"

Table 5 Baseline Extent and Severity of Angiographically Defined Disease in the ERA Trial

	Mean ± SD	Range
Qualifying lesion severity		
Minimum diameter (mm)	1.57 ± 0.53	0–3.06
Diameter stenosis (%)	47.3 ± 13.6	30–100
Location of qualifying lesion: *n* (%)		
Left main	3 (1)	
Left anterior descending	108 (35)	
Circumflex	71 (23)	
Right coronary	127 (41)	
No. of vessels ⩾ 50% stenosis	*n* (%)	
Left main	3 (1)	
0		92 (30)
1		98 (31)
2		77 (25)
3		39 (13)

SD = standard deviation.

(randomly allocated to baseline and follow-up for each subject). This staff person then will remove the film leader with patient and date identifiers and replace it with a label whose identical mate will be affixed to the leader (Figure 1). The labeled films will then be sent in unmarked canisters to the QCA Core Laboratory at Wake Forest for analysis. Similarly, all laboratory analyses have no identifying information as to patient or treatment assignment.

Primary Outcome: QCA Methods

Analysis of the cineangiograms is complex and can be influenced by many factors beyond the actual edge detection algorithm. Strict adherence to the following protocol will assure uniform and objective measurements to determine eligibility in the ERA trial and for the primary and secondary outcomes.

Sampling Strategy and Frame Selection

Past experience suggests that analysis of standardized proximal segments of the coronary vasculature yields the best results (Figure 2). Segments 1–10 are conventional coronary anatomic definitions. We also include up to four additional segments at the discretion of the QCA staff for any other segment that may fall outside the definitions of segments 1–10, such as the left main, a second large diagonal, or perhaps a second or third large obtuse marginal vessel. Whenever an additional vessel segment is identified it must be analyzed in both films, and all ten (or up to 14) segments will be analyzed regardless of the presence or absence of a qualifying lesion.

We employ a three-step process for frame selection before QCA analyses. First, the projection that best displays the vessel segment of interest in both films is selected. The projection should be as close as possible to perpendicular to the long axis of the vessel segment of interest, and free of significant overlapping of other vessels. Second, in each film the cardiac cycle is selected during

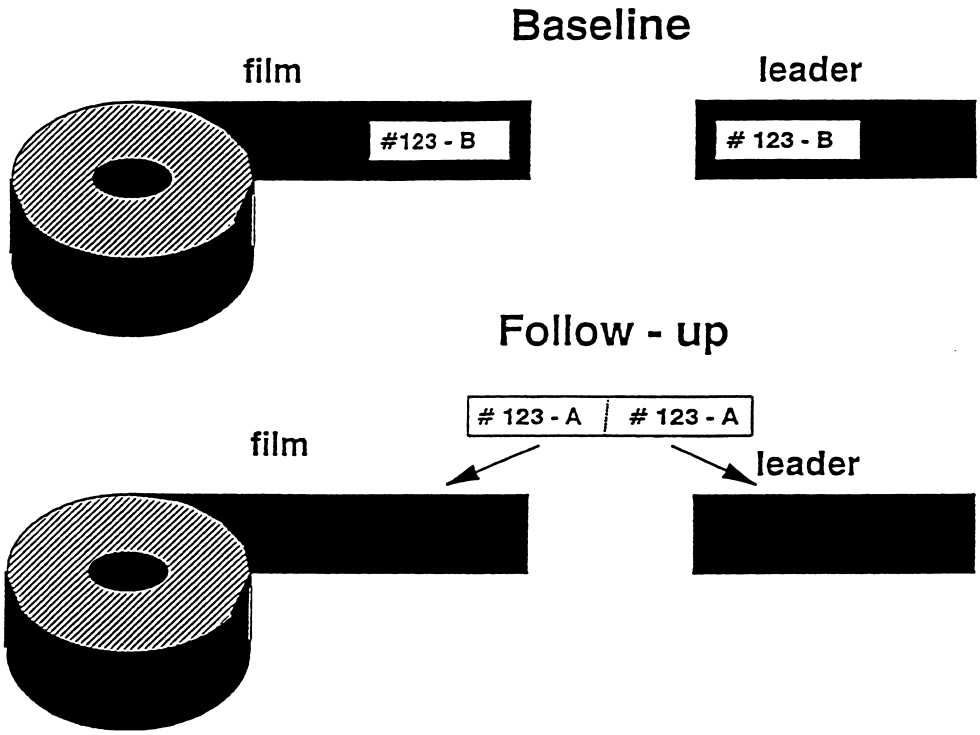

Figure 1 Schematic illustration for labeling scheme to maintain the blind on coronary catheterization film canisters.

which maximum opacification occurs. Finally, the exact frame with the clearest edges as close to end diastole as possible is selected. When practicable, a projection, cycle, and frame will be used in which both the catheter's distal portion and the vessel segment of interest are in the same frame.

QCA Instrumentation and Analysis

The QCA equipment used for ERA includes a pair of Sony 3500 cine/video projectors generating upscanned highline rate video images, a frame grabber (Epix) installed in a 66MHz 486 PC, and QCA-Plus software. The QCA software has been extensively tested and validated both in our laboratory [26, 27] and elsewhere [28], and versions of the software have been used in previous angiographic trials [29, 30]. One particularly helpful feature of the software is the ability to compare films side-by-side. QCA-Plus also has an extensive, automated database that generates SAS files for subsequent data analysis.

Each selected frame will be optically magnified (approximately 3×), digitized, and stored on a dedicated personal computer along with the ERA identification number, vessel segment, and frame number in the QCA-Plus database. Automated edge-detection software is applied to the region of the image in question to define the boundaries of the vessel. The algorithm automatically determines the minimum diameter, average diameter, percent diameter stenosis

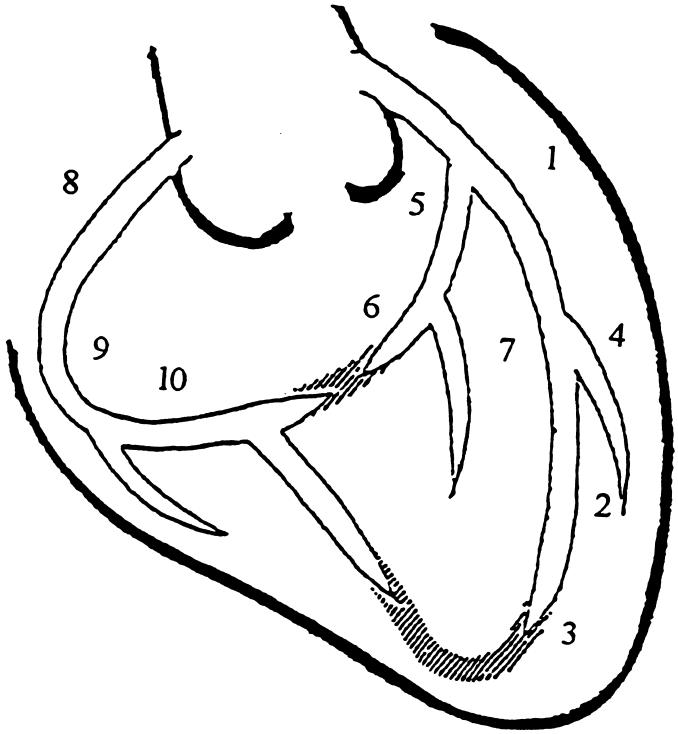

Figure 2 Anatomic locations of coronary segments used for quantitative coronary angiography in the ERA trial. 1 = proximal left anterior descending (LAD) artery, from origin of the LAD to the first major diagonal; 2 = mid LAD, from first major diagonal to distal third of the LAD; 3 = distal LAD, from the second major diagonal to the apex; 4 = LAD diagonal, the major diagonal branch; 5 = proximal circumflex, from origin of the circumflex to the first major obtuse marginal; 6 = distal circumflex, from first major obtuse marginal to the distal circumflex *or* second obtuse marginal if the distal circumflex is a trivial vessel; 7 = obtuse marginal, first major obtuse marginal or intermedius branch; 8 = proximal right coronary artery (RCA), proximal third of the RCA up to the first major right ventricular freewall branch if present (not shown); 9 = mid RCA, middle third of the RCA from first major right ventricular freewall branch (if present) to the acute marginal branch or the crux of the heart; 10 = distal RCA, from the acute marginal or crux of the heart to the posterior descending.

(based on an automated estimate of mean proximal vessel diameter), proximal and distal diameter segment length, and resistance.

Data Management

The measured data (including minimum diameter, average diameter, percent diameter stenosis, proximal and distal diameters, segment length, and resistance) will be recorded on the QCA worksheet, and an image file containing the vessel segment, identified boundaries, and luminal dimensions will be permanently stored in the database. A hard copy of the image and results will

also be a permanent part of the study records. A similar file and hard copy will be made of any separate frames and subsequent analysis required for catheter images. In addition, the technician using the standardized scheme printed at the top of every form will make a manual sketch of the coronary tree. Any missing data will be accompanied by a comment explaining the reason for missing data by the QCA technician and approved by the image processing laboratory director. Every worksheet will be reviewed by the principal investigator before acceptance.

All the QCA data will be downloaded as SAS data files from the QCA-Plus database. The data files will be subjected to automated range and consistency checks. Any ambiguous value will be returned to the QCA technician for clarification.

Despite rigorous attention to complete QCA evaluation of the coronary tree, there will always be some unevaluable vessel segments. If the data for a given segment are not available because of unavoidable overlapping of vessels or because the segment is anatomically absent, these data will be treated as true missing data, as will segments distal to a total occlusion. However, the actual totally occluded segment will be treated separately (see below).

Data Imputation and Other QCA Data Issues

Statistical approaches to missing data define two distinct cases. If the reason a value is missing is not related to the progression of atherosclerosis, it will be considered missing at random; otherwise, it will be considered not missing at random. For values that are missing at random, the likelihood techniques proposed for the analysis are valid, and no special adjustment is needed.

Vessel segments not missing at random may occur in two ways: (1) complete occlusion of the proximal portion of an artery will make distal segments unmeasurable, or (2) measurements of segments of recanalized arteries will not reflect the disease process. In the first case, although the missingness for a particular segment is not directly related to the stenosis in that segment, it is caused by a large amount of stenosis in a proximal site. Because stenosis within a patient is more homogeneous than between patients, this value is not missing at random. In the second case, the physical alteration of a segment causes its subsequent measurement to be unreflective of the natural progression of atherosclerosis. Furthermore, it may cause measurements of distal segments to be unrepresentative of the disease process as well.

To avoid bias in the treatment of measurements not missing at random, several analyses will be performed in sequence. For segments that have recanalized, analyses will be run with and without these segments deleted. If there is no difference, the segments in question will be excluded from the final analysis. If there is a difference in the analyses, a large value for the stenosis will be imputed using the data of similar patients. Segments that are missing due to a proximal stenosis will be handled in three ways: (1) minimum diameter will be set to zero, (2) minimum diameter will be set to its baseline value in the same segment, and (3) the segment will be removed from the analysis. If there are no differences between results from these three approaches, the segments will be deleted.

Other Secondary Outcome Measures

Endothelium-Dependent Vasodilator Capacity

Estrogen significantly improves endothelium-dependent vasodilator capacity [31, 32]. However, there are few data on the relationship between endothelial function and progression of coronary atherosclerosis. To study this, ERA participants will undergo endothelial function testing before closeout, while still receiving study medication. Two-dimensional ultrasound is used to measure flow-mediated endothelial-dependent vasodilator responses in the brachial artery [33]. This will permit a comparison of vasodilator responses among women randomly assigned to receive long-term unopposed estrogen or estrogen plus progestin versus placebo.

The methodology for brachial artery ultrasound examinations and subsequent data analysis are described in detail elsewhere [27]. Briefly, subjects arrive at the clinic in a fasting state and lie supine on a table in a quiet, temperature-controlled room. Electrocardiogram electrodes and a pediatric blood pressure cuff are placed and the subject is allowed to rest for a brief period. Once the subject is comfortable and her blood pressure stable, images of the brachial artery are obtained using an Acuson (Mountain View, CA) Aspen device with a 7.5 or 10 MHz linear array transducer. When the near and far arterial walls are simultaneously visualized, the sonographer begins recording baseline images of the brachial artery for 1 minute. The automatic cuff inflator then is activated to inflate a blood pressure cuff placed on the proximal forearm to 50 mm Hg above the subject's resting systolic pressure. The cuff remains inflated for 4 minutes while imaging continues. The cuff is then deflated and imaging continues for another 2 minutes.

Digitizing the frames of interest, identifying vessel diameter using set anatomic boundaries, and calculating relevant parameters are all performed using software developed in the Wake Forest University Image Processing Laboratory and designed to minimize the amount of editing required. Digital images are acquired in a gated fashion on the R-wave, stored in a DICOM format, and then downloaded via an Ethernet port to a server in the laboratory for later analysis. Data from the baseline period before cuff inflation are compared with data from the baseline period after cuff release. Time from cuff release to maximum diameter and the area between the diameter curve and each of the baseline diameters are also automatically determined. The laboratory database contains: patient, sonographer, and reader identification; diameter profile; quality-control measures; and all of the numerical data. Intra- and inter-reader variability is low, and within-subject reproducibility is good (data not shown).

Clinical Cardiovascular Events

At each clinic visit, participants will be questioned about interval hospitalizations. The hospital discharge summary will be obtained for each hospitalization. A preliminary cardiovascular event form will be filled out for suspected primary nonfatal cardiac arrests, acute myocardial infarctions, congestive heart failure, unstable angina resulting in hospitalization, coronary artery bypass grafts, coronary angioplasty, nonfatal strokes, transient ischemic attacks, or other vascular surgery or angioplasty. A similar form will also be completed if the ERA

clinic is informed of a participant's death. Collection of the required information to document the event (including discharge diagnosis, EKGs, cardiac enzymes, procedure notes, and, in the event of out-of-hospital death, a standardized interview with the woman's physician, family members, and witnesses) will begin as soon as the preliminary event form is filled out. The required documentation, designed to make the most accurate determination possible regarding the cause of death or the nature of the morbid event, will be compiled within 6 weeks and then forwarded to an independent, experienced adjudicator (Dr. Bruce Psaty), whose classification rules for clinical cardiovascular events are nearly identical to those in HERS [23].

Plasma Lipids, Carbohydrate Metabolism, Blood Pressure Regulation, Hemostasis, Antioxidant Activity, and Estrogen Levels

Estrogen-associated changes in plasma lipids, glucose and insulin levels, elements of the renin/angiotensin system, hemostasis factors, and antioxidant activity have been cited as potential intermediate steps in the causal pathway between estrogen and cardiovascular risk reduction. Other plasma factors such as Apo-E isoforms may not be influenced by hormone replacement but still may confound estrogen's effects on atherosclerosis. Table 6 shows values for all blood measures obtained at baseline. The lipid profile and estrogen levels will be measured annually. All other factors will be measured once at baseline and once at the end of the trial (except Apo-E isoforms, which are measured at baseline only). Because of special handling requirements, the renin/angiotensin and plasma antioxidant capacity measures were only measured in women at Wake Forest. We will obtain and store sufficient plasma to measure all of the identified factors along with additional plasma, serum, and DNA for other variables yet to be identified.

Physical Measurements

Physical measurement will include measures of body fat distribution based on skin-fold thicknesses and waist-to-hip ratios, body mass index based on height and weight, and systolic and diastolic blood pressure based on resting supine measurements. Baseline physical characteristics of the cohort are listed in Table 7.

Physical Activity and Smoking Status

Both daily physical activity and current smoking status may influence outcomes in the trial. Randomization should address the potential effects of these components on outcomes. However, given the importance of each of these behaviors to recurrent disease, we will characterize the subjects' physical activity and smoking status at both baseline and follow-up. Smoking status (current and prior) will be assessed with standard instruments employed in other surveys, including the WHI [22]. Physical activity will be assessed with the Yale Physical Activity Survey [34]. This is a brief interviewer-administered questionnaire that requires approximately 20 minutes to complete. The instrument was

Table 6 Selected Baseline Laboratory Values for All Subjects in the ERA Trial (mg/dl) (mean ± SD)

Lipids	
Total cholesterol	216.2 ± 42.88
LDL-C (estimated)	135.2 ± 38.13
Beta quant LDL-C	137.1 ± 42.09
HDL-C	44.1 ± 11.95
HDL-C subfraction 2	4.8 ± 4.31
HDL-C subfraction 3	39.4 ± 10.98
Triglycerides	193.0 ± 108.0
Lipoprotein(a)	41.0 ± 38.39
Apolipoprotein A	135.7 ± 23.35
Apolipoprotein B	116.4 ± 25.46
Apolipoprotein E phenotypes	*n* (%)
2/2	1 (1.0)
3/2	47 (15.6)
3/3	159 (52.8)
4/2	7 (2.3)
4/3	73 (24.3)
4/4	12 (3.9)
Missing	8 (2.6)
Carbohydrate Metabolism	
Glucose	
Fasting	106.0 ± 29.30
1 hour	183.5 ± 60.24
2 hour	152.9 ± 65.01
Insulin	
Fasting	14.2 ± 10.63
1 hour	183.5 ± 60.24
2 hour	60.9 ± 33.35
Glycated hemoglobin[a]	11.4 ± 2.85
Antioxidant Activity[b]	
Azo lag (min)	76.2 ± 13.43
Azo rate (pm/min)	157.9 ± 30.39
Cu lag (min)	55.4 ± 17.32
Cu rate (pmole/min)	2070 ± 448.4
Hemostasis	
Fibrinogen	420.38 ± 96.54
Factor VII	136.00 ± 56.00
PAI-1[b]	360.46 ± 454.41
Blood Pressure Regulation[b]	
Renin	1.78 ± 4.76
Angiotensin II, (1–7)	36.01 ± 23.46
Estrogen (s)	
Estradiol	12.72 ± 7.46
Estrone	16.54 ± 8.27
Sex hormone-binding globulin	31.50 ± 17.84

[a] Only done in diabetic subjects ($n = 86$).

[b] Only done in subjects at Wake Forest ($n = 102$).

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PAI-1 = plasminogen activator inhibitor-1.

Table 7 Physical Characteristics of All ERA Subjects at Baseline (n = 309; mean ± SD)

Systolic blood pressure (mm Hg)	133.9 ± 17.48
Diastolic blood pressure (mm Hg)	74.0 ± 8.93
Waist circumference (cm)	92.9 ± 15.28
Hip circumference (cm)	108.0 ± 14.13
Waist/hip ratio	0.861 ± 0.093
Subscapular skinfold (mm)	23.8 ± 11.37
Tricep skinfold (mm)	25.9 ± 10.60

SD = standard deviation.

developed and validated in a population of older adult men and women (mean age 71) to be specifically sensitive to activity levels among an older population.

Dietary Data Collection

To characterize the baseline dietary intake of ERA participants, as well as monitor their intake during the study, we will use the semiquantitative Food Frequency Questionnaire (FFQ) originally developed by Willett et al. [35], with slight modifications from the Atherosclerosis Risk in Communities (ARIC) study. The FFQ assesses usual dietary habits, defined as the average intake over the last year. The interviewer-administered version took about 15 minutes in ARIC (subjects aged 50+). In addition, the interviewer need not be a trained dietitian, and completing the FFQ is fairly easy both for the interviewer and the participant. Because the beneficial effects of antioxidants on cardiovascular disease are linked to supplemental intakes [36, 37], we will also capture vitamin and supplement use with the ARIC vitamin survey form. The Willett questionnaire has been validated in women.

Health-Related Quality of Life

Health-related quality of life (HRQL) is generally accepted as an appropriate measure of treatment efficacy in most clinical research, augmenting the more traditional measures of morbidity and mortality [38, 39]. The measures selected for use in the ERA trial include the Medical Outcomes Study (MOS) 36-item health survey (MOS SF-36) [40], the MOS social support survey form [41], the Center for Epidemiologic Studies-Depression (CES-D) short form [42], and an adaptation of the Social Integration Index [43]. These measures should have sufficient sensitivity to detect an HRQL-related effect of treatment, yet are simple and short enough to guard against excessive participant burden. Details on baseline HRQL measures in the ERA cohort will be described in another paper.

Data Analysis

The primary trial endpoint is change in minimum coronary lesion diameter as measured by QCA. The analysis will be an intention-to-treat analysis among those women with both baseline and follow-up angiographic data. Inability to

collect the outcome of interest (angiographic follow-up data) due to death or losses due to follow-up is an unavoidable element of QCA trials. Based on previous studies, we anticipate loss due to death at 1%/year and an overall angiographic follow-up rate of 85%. The effects of hormone replacement therapy will be determined using one-way analysis of covariance (ANCOVA). Analysis of group differences will adjust for the prerandomization levels used in the blocked randomization to provide the correct variance estimates for the randomization design [24]. In addition, the analyses will adjust for prerandomization values of conventional cardiovascular risk factors significantly associated with minimal diameter (e.g., lipids) after adjusting for other terms in the model. This corrects for chance imbalances in the prognostic factors between the groups and reduces the variance of the estimate of group differences, and thus increases the power of the comparisons. While lumen diameter is the unit of measurement, group comparisons will be based on patients as the unit of analysis, as patients are randomized to treatment groups. The analysis of lumen diameter will account for intrasubject correlations by fitting an unstructured covariance matrix. If this is not possible due to numerical instability, the best fitting covariance structure that allows for estimated variance to vary across segments will be used. All tests of hypotheses and reported *p*-values will be two-sided.

Factors measured several times (e.g., plasma lipids, blood pressure, glucose metabolism, total antioxidant activity, dietary intake, HRQL) will be analyzed across time and compared between groups using repeated measures analysis of variance, adjusting for the prerandomized values as covariates. Follow-up angiographic measurements will be modeled using a mixed model analysis of variance with the baseline measurement, clinic, and use of lipid-lowering drugs at entry as covariates. Variables identifying study arm, segment location in the coronary tree, and patient identity as a random effect will be included.

Regression diagnostics, residual plots, and exploratory analyses will be performed to find appropriate transformations of the variables for multivariable regression analyses. Order of priority in choosing a transformation will be to satisfy the (1) linearity assumption, (2) homogeneity assumption, and (3) normality assumption. Simple associations between variables will be estimated by Spearman's nonparametric rank correlation coefficient. Associations between continuous measures will be explored using bivariate plots and spline-fitting algorithms. Categorization or linear regression between (or possible transformations of) the covariates will be used if deemed appropriate from the exploratory results. Test of hypotheses concerning differences in the relationship between two continuous measures between treatment groups will be tested by the interaction term(s) in regression models.

Missing Data

Statistical approaches to missing data depend on classification of the probabilistic mechanism governing the missingness. If the probability that a response is missing depends on covariate information but not on the response's value, the data are said to be missing at random. If the probability that a response is missing depends on the response's value and possibly also on covariate information, the data are neither missing at random nor observed at random.

In our analysis, if the reason a value is missing is not related to the level of atherosclerosis, it will be considered missing at random. In this case, the likelihood techniques proposed for the analysis are valid; no special adjustment is needed.

If the probability of observing a missing value is related to the level of atherosclerosis, we will perform sensitivity analyses to assess the impact of missing values for inferences on our primary hypotheses. Segment measurements missing due to the level of atherosclerosis may occur in two ways: (1) complete occlusion of the proximal portion of an artery will make distal segments unmeasurable, (2) measurements on recanalized arteries will not reflect the disease process. In the first case, although the missingness for a particular segment is not directly related to the stenosis in that segment, it is caused by a large amount of stenosis in a proximal site. As stenosis within a patient is more homogeneous than between patients, this value is not missing at random. In the second case, the physical alteration of a segment causes its subsequent measurement to be unreflective of the natural progression of atherosclerosis, especially when the intervention was prompted by disease symptoms. It may also cause measurements of distal segments to be unrepresentative of the disease process.

Segments that are completely stenosed will be included in the analysis. Values missing due to a proximal occlusion will be treated several ways and each resulting dataset analyzed separately. Imputation strategies will include: (1) setting the measurement to zero, and (2) setting the measurement to its baseline value in the same segment. In these imputed datasets, an indicator variable for imputed values will be included in the analysis. An analysis removing the segment from the analysis will also be done. If inferences for the primary hypotheses do not differ in these three analyses, the segments will be deleted. If the handling of these missing values affects the inferences, they will be imputed using that value among measured segments from the same women representing the most progression.

For segments that have been recanalized, if a pre-procedure measurement from an interim angiogram is available, it will be imputed and an indicator for this condition will be included in the analysis. Otherwise, these missing values will be imputed several ways and each resulting dataset analyzed separately. Imputation strategies will include: (1) setting the measurement to zero, and (2) setting the measurement to its baseline value in the same segment. In these imputed datasets, an indicator variable for imputed values will be included in the analysis. An analysis removing the segment from the analysis will also be done. If inferences for the primary hypotheses do not differ in these three analyses, the segments will be deleted. If the handling of these missing values affects the inferences, they will be imputed using that value among measured segments from the same women representing the most progression.

Data Management

The data management responsibilities of this study include data collection, secure data storage, data retrieval, and preparation for data analysis. Much of the data management tasks are handled by the computer-based system installed

in the clinical centers and maintained by the Data Management Core at Wake Forest. In addition, each clinic site stores one hard copy of all questionnaires, mammogram results, Pap smear and endometrial biopsy reports, EKGs, and clinical records until the end of the trial. All copies are stored in a locked room and access is restricted to study personnel. Confidentiality of data is scrupulously maintained. Identification numbers that uniquely identify subjects are used. In addition, study medication is stored in a locked cabinet in the clinics.

Other Design Features

Plans for Retention and Enhancement of Compliance

The results of a trial can be affected by subjects dropping out of the study or by noncompliance with the intervention, leading to underreporting of possible therapeutic as well as toxic effects. We planned several steps to minimize dropouts and maximize compliance. These include a simple study regimen, placebo run-in, careful attention to informed consent, frequent use of reminder phone calls and postcards, providing transportation or travel reimbursement when needed, annual social events, a quarterly newsletter, ERA brochures and wallet cards, and careful monitoring of retention during the trial. These methods have been successfully employed to achieve excellent retention rates in several recent clinical trials at our institution.

Hypercholesterolemia in ERA Participants

Perhaps the most difficult question concerning risk-factor control for the ERA trial was whether to treat elevated low-density lipoprotein cholesterol (LDL-C) or low HDL-C. The importance of this question is accentuated by the National Cholesterol Education Program (NCEP) Adult Treatment Panel guidelines [44] for management of elevated LDL-C in patients with coronary artery disease. However, after careful consideration, and consultation from many experts (including two members of the NCEP panel), we decided not to include a formal lipid-lowering intervention in the ERA trial participants with elevated LDL-C. There are three reasons for this decision: (1) Most of the data used to support the NCEP guidelines were derived from studies of middle-aged men, and their applicability to postmenopausal women is unclear. In fact, data from the Lipid Research Clinics suggest that LDL-C shows no association with CVD risk in postmenopausal women [4]. (2) LDL-C may be in the causal chain between estrogen use and prevention of atherosclerosis; thus, a lipid-lowering intervention could bias a hormone replacement treatment effect and/or obscure differences between the opposed and unopposed regimens. (3) Less than half of all postmenopausal women with elevated cholesterol in our geographic area were being treated with drug therapy when ERA began. (4) Furthermore, HDL-C (and triglycerides in an opposite fashion) may also be in the causal chain, and almost no women in our geographic area take HDL-C–raising medications despite a diagnosis of heart disease.

Thus, women in ERA were informed of their lipid status, instructed about a prudent diet, and referred to their local physician for questions concerning their risk factor status. Similarly, all participants were advised of the benefits

of smoking cessation and regular physical activity (under the supervision of their personal physician). Patients were also advised of elevated fasting blood glucose levels or blood pressure, and were encouraged to seek follow-up from their personal physician. Uncontrolled diabetes or hypertension were exclusion criteria for the study, and prompted direct contact with the participant's physician if detected during the screening or follow-up.

Maintaining the Blind for Participants and Clinic Staff

Estrogen as an intervention may unblind participants because of its characteristic side effects. The clinic staff may also be unblinded through discussions with participants about these adverse effects, including vaginal bleeding, breast tenderness, bloating, ankle edema, and headaches. All questions and management of all adverse effects related to these signs and symptoms are directed to the gynecology physician and nurse, who are completely separate from the other ERA clinic staff. All participants received laminated cards with separate phone numbers to call for gynecologic questions, symptoms, or adverse events (the same strategy used in HERS and WHI). All ERA participants will have annual pelvic examinations whether or not they have a uterus, and all women with a uterus will have annual endometrial evaluations regardless of treatment assignment.

Unblinding Procedures

Unblinding is kept to an absolute minimum. However, it may occasionally be necessary to unblind some of the investigative team responsible for directing the subject's subsequent management. Precautions are taken to minimize the impact of such unblinding on staff responsible for data collection, data analysis, and formulating study conclusions. Unblinding could be necessary for management of symptoms or disease, evaluation of serious adverse experiences, evaluation of unexpected event rates, and analysis of results (scheduled unblinding).

The gynecologist at each site can determine the study drug assigned to any patient if such information is required for further management of that participant with respect to vaginal bleeding. If unblinding is necessary to make the appropriate decisions about management, the gynecologist is required to contact a designated member of the data management staff during regular working hours to obtain the treatment assignment. Whenever unblinding is required for management of serious adverse effects, an adverse effects reporting form is generated by the gynecologist.

The second situation in which unblinding would be necessary is if the Data Safety and Monitoring Board (DSMB) detects an alarming number of events during their routine monitoring of adverse events and clinical events. The DSMB can unblind the data in two stages. If a higher than expected adverse event rate is observed, at their request, information concerning the event rate stratified by treatment group will be provided. However, this stratification will not indicate the exact treatment assignment, but instead simply divide the adverse events into three nonspecified treatment assignments. If there is a significantly higher rate of adverse events in one of the three treatment groups

then, at the request of the DSMB, fully unblinded data will be made available to them.

Scheduled unblinding at the end of the study will occur only after all of the primary outcomes have been determined and the final data set is cleaned and prepared for analysis. At the end of the study, the effectiveness of the blind will be tested among the participants and the clinic staff as an element in the final clinic visit.

Monitoring for and Management of Adverse Effects/Toxicity

Participant safety is a major concern for the ERA investigators. Major adverse effects should be minimized by our excluding during the screening phase any women with medical conditions in which estrogen or estrogen plus progestin are contraindicated (i.e., history or evidence of breast or endometrial carcinoma, cholecystitis, and thromboembolism) or in whom angiography would be unnecessarily dangerous. Subsequently, participants will be carefully monitored for the development of any adverse effects potentially related to the various hormone regimens. Minor adverse effects such as water retention, bloating, breast tenderness, weight gain, headaches, or mood swings will be monitored at each clinic visit with a self-administered questionnaire. Screening for the development of asymptomatic endometrial of breast pathology will be performed on an annual basis. Vaginal bleeding and other gynecologic adverse events will be reported directly to the study gynecologist.

Major adverse effects possibly related to the study include: death; any hospitalization for a life-threatening event; diagnosis of endometrial, breast, or any other malignancy; diagnosis of symptomatic gallstones or thromboembolic events (deep venous thrombosis or pulmonary embolism); or any abnormal physical finding or laboratory value considered clinically significant by an ERA investigator. Women and their families are instructed to inform the ERA staff of any of the above potential adverse effects immediately. For any major adverse experience reported during the study, the nature, duration, intensity, and any remedial action taken will be recorded. In general, women who experience one of the nonfatal serious adverse effects will discontinue study medications, but continue to be followed. Women with an intercurrent illness for whom estrogen might be inappropriate may have their study medications discontinued temporarily (without unblinding) by the clinic gynecologist.

Data Safety and Monitoring Board

The independent DSMB meets twice yearly (once in Winston-Salem and once by conference call) to review all potential side effects and advise as necessary with respect to participant safety. Surveillance of adverse effects and cardiovascular events will be modeled after guidelines established by PEPI, HERS, and the Cardiovascular Health Study (CHS). The nature, duration, and intensity of any adverse experiences will be recorded at the scheduled clinic visits. All adverse experiences will be reported to the DSMB. Meetings can be called as needed at the discretion of the chair of the DSMB (Dr. Alan Guerci). The DSMB members and other leaders of the ERA study are listed in Appendix A.

Quality Control Procedures and Measurements

An ERA operations manual was distributed to all clinic sites, investigators, and core laboratories in 1995. A revised and updated version was distributed in early 1998. Twice-yearly monitoring of each clinic site is conducted by one of us (Dr. Sharp), and gynecology staff are evaluated separately at similar intervals. Adherence to the operations manual will be assured by periodic assessment and retraining. Monthly conference calls keep all sites aware of procedures to be followed and address any questions that may arise. North Carolina staff meet monthly in Winston-Salem to address any outstanding issues and plan for upcoming portions of the trial. A 2-day training session focused on closeout procedures was held in 1998 for all staff. See the following section for more details. A complete list of ERA clinic investigators and staff is found in Appendix B.

Closeout

A final closeout clinic visit will be scheduled 2 weeks after the follow-up angiogram. At this time, participants will be asked to return all remaining study medications. In addition, a questionnaire about blinding will be administered. Investigators and staff will express their appreciation for the participants' long-term investment. We will also unblind women at this time. When unblinding is done, each participant's personal physician also will be informed whether she was taking an active drug or a placebo. Furthermore, continuation of study medications (or, among those taking placebo, initiation of therapy) will be suggested if indicated by the study results. The participant's trial experience will be summarized and forwarded to her personal physician (and cardiologist, if applicable).

DISCUSSION

The ERA trial is the first angiographic endpoint trial to examine the effects of postmenopausal hormone replacement on progression or regression of coronary atherosclerosis in women. By randomizing women to unopposed estrogen, estrogen plus MPA, or placebo, it will provide an unparalleled opportunity to determine if either regimen is effective in slowing the progress of angiographically defined coronary atherosclerosis.

Quantitative measurement of coronary luminal narrowing using arteriograms has provided a meaningful outcome measure for several trials of interventions designed to inhibit progression or initiate regression of coronary atherosclerosis using lipid-lowering drugs, ileal bypass surgery, or lifestyle modification [45–50]. Despite the relatively small (albeit statistically significant) changes in lumen diameter in the treatment groups in many of these studies, patients assigned to active therapy experienced significant clinical benefits. These included improved myocardial perfusion by positron emission tomographic scanning [51], a reduction in fatal and nonfatal myocardial infarctions [47], and fewer episodes of progressive angina requiring angioplasty or bypass surgery [46]. More recently, data from Waters et al. [52] clearly demonstrate a strong correlation between actual changes in lumen dimensions (not just treatment assignment) and subsequent cardiovascular morbidity and mortality.

Table 8 Comparison of Selected Characteristics: ERA and HERS Cohorts at Baseline (mean ± SD)

Criteria	HERS	ERA
Sample size	2763	309
Mean age (yr)	66.7 ± 5.3	65.9 ± 7.1
Nonwhite *n* (%)	312 (11.3)	56 (18.1)
Smoking status		
Current *n* (%)	360 (13.0)	57 (18.6)
Former *n* (%)	1352 (49.0)	141 (46.1)
Never *n* (%)	1051 (38.0)	108 (35.3)
Hypertension		
History *n* (%)	1619 (58.6)	207 (67.0)
SBP (mm Hg)	135.0 ± 21.0	133.9 ± 17.5
DBP (mm Hg)	73.1 ± 10.5	74.0 ± 8.9
Diabetes *n* (%)[a]	634 (22.9)	86 (27.8)
BMI (kg/m^2)	28.6 ± 5.3	30.1 ± 8.4
Lipids (mmol/L)		
TC	5.92 ± 1.10	5.58 ± 1.10
LDL-C	3.75 ± 1.10	3.49 ± 0.98
HDL-C	1.30 ± 0.53	1.14 ± 0.31
TG	1.87 ± 0.53	2.41 ± 1.31

[a] Self-report.

BMI = body mass index; DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SD = standard deviation; TC = total cholesterol; TG = triglycerides.

Similar data have been published from the placebo group in the Program on the Surgical Control of Hyperlipidemias (POSCH) [53]. In the aggregate, these studies make a strong case for use of QCA endpoints as a surrogate measure for the real endpoint of interest—clinical events [54].

The ERA trial will also provide valuable information about hormone therapy's effects on endothelial function and its relation to progression of coronary disease in women. Estrogen's documented favorable effects on nitric oxide–mediated endothelium-dependent vasodilation are thought to be a key mechanism by which estrogen may favorably influence vascular health [26, 55–60]. But considerable uncertainty remains about MPA's effect on this mechanism [26, 61, 62]. The ERA trial will provide the first direct comparison of the effects of long-term unopposed estrogen and estrogen plus MPA on endothelial function in women. Endothelial function is also being used as a surrogate endpoint in clinical trials of angiotensin-converting enzyme inhibition [63] and lipid-lowering therapy. However, the degree to which endothelial function is correlated with actual progression of coronary disease remains to be seen. This question will also be examined in the ERA trial.

The similar baseline characteristics in the ERA and HERS cohorts (Table 8) may predict how the angiographic, endothelial, and blood factor changes in ERA could relate to the clinical events observed in HERS. Whether estrogen plus progestin causes an early increase in CHD risk, as suggested in HERS, will not be easily evaluated in the ERA trial because of the few anticipated clinical events. Nevertheless, the stored serum, plasma, and DNA in the ERA trial provide an unprecedented opportunity to examine the effects of estrogen

and estrogen plus MPA on markers of inflammation and propensity for thrombosis that were not possible from the HERS trial. Measurements of change in Factor V, von Willebrand's factor, fibrinogen, Lp(a), and PAI-1 have already begun. Plans are underway to add measures of C-reactive protein, interleukin-1α, interleukin-6, transforming growth factor-β, and soluble adhesion molecules to more fully characterize the effects of the ERA regimens on possible factors in the early increase in CHD risk seen in HERS.

Three other National Institutes of Health-funded angiographic endpoint trials of hormone replacement are currently underway. The Women's Estrogen/Progestin Lipid Lowering Atherosclerosis Regression Trial (WELL-HART) is examining the effects of estradiol administered with or without cyclic MPA. In the Women's Angiographic Vitamin and Estrogen (WAVE) trial, women will receive estrogen plus MPA and/or supplemental vitamins C and E, or a placebo. The Estrogen and Graft Atherosclerosis Regression (EAGAR) trial is studying the effects of estrogen plus MPA on atherosclerosis progression in women with bypass grafts. Together with ERA, these trials will provide substantial new data on the effects of estrogen replacement therapy on coronary atherosclerosis in women and complement the large existing database on prospective, quantitatively evaluated coronary disease progression already available in men.

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APPENDIX A: COMMITTEE MEMBERS FOR THE ERA TRIAL

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Herrington DM, Reboussin DM, Klein KP, et al. entitled "The Estrogen Replacement and Atherosclerosis (ERA) Study: Study Design and Baseline Characteristics of the Cohort" (Controlled Clinical Trials, 2000;21:257–285).

In the article listed above, please note that on page 259, under "Trial Design and Methods" section, "Overview" paragraph, fourth line: the years should be 1995 and 1996, respectively.

Further, on page 282, reference 26 should be: Williams JK, Anthony MS, Honoré EK, et al. Regression of atherosclerosis in female monkeys. Arterioscler Thromb Vasc Biol 1995;15:827–836. Also on page 282, reference 27 should be: Honoré EK, Williams JK, Washburn SA, Herrington DM. The effects of disease severity and sex on coronary endothelium-dependent vasomotor function in an atherosclerotic primate model. Coronary Artery Dis 1996;7:579–585.

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