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Authors

Hodis, HN
Mack, WJ
Shoupe, D
et al.

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METHODS AND BASELINE DATA FROM A CLINICAL TRIAL DESIGNED TO TEST THE HORMONE TIMING HYPOTHESIS

Short Title: TRIAL TO TEST THE HORMONE TIMING HYPOTHESIS

Howard N. Hodis, MD[1,2,3,4], Wendy J. Mack, PhD[1,3], Donna Shoupe, MD[5], Stanley P. Azen, PhD[3], Frank Z. Stanczyk, PhD[5], Juliana Hwang-Levine, PharmD[4], Matthew J. Budoff, MD[6], Victor W. Henderson, MD, MS[7]

[1]Atherosclerosis Research Unit, Keck School of Medicine, University of Southern California, Los Angeles, CA
[2]Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA
[3]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA
[4]Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA
[5]Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, CA
[6]Los Angeles Biomedical Research Institute at Harbor-University of California at Los Angeles Medical Center, Torrance, CA
[7]Departments of Health Research and Policy (Epidemiology) and Neurology and Neurological Sciences, Stanford University, Stanford, CA

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ELITE Research Group Members

Study Chairman: Howard N. Hodis MD*; Clinical Center Staff: Liny Zurbrugg RN (Clinic Coordinator), Esther Bhimani MA, Martha Charlson RD, Irma Flores MA, Martha Huerta, Thelma LaBree MA, Sonia Lavender MA, Violetta McElreath RN, Janie Teran, Philip Zurbrugg; Ultrasound Image Acquisition and Processing Laboratory: Robert H. Selzer MS* (Director), Yanjie Li MD (Technical Director), Mei Feng MD, Lora Whitfield-Maxwell RN, Ming Yan MD, PhD; Data Coordinating Center: Wendy J. Mack PhD* (Director), Stanley P. Azen PhD*, Farzana Choudhury MS, Carlos Carballo, Laurie Dustin MS, Adrian Herbert, Naoko Kono MPH, George Martinez, Olga Morales; Atherosclerosis Research Unit Core Lipid/Lipoprotein Laboratory: Juliana Hwang-Levine PharmD* (Director), Gail Izumi CLS, Arletta Ramirez CLS, Luci Rodriguez; Gynecology and Mammography: Donna Shoupe MD*, Juan C. Felix MD, Pulin Sheth, Mary Yamashita; USC Endocrinology Laboratory: Carole Spencer; Neurocognition: Victor W. Henderson MD*, Carol A. McCleary, PhD, Jan A. St. John MPH; Kaiser Permanente Medical Center Recruitment Site: Malcolm G. Munro, MD; Cardiac Computed Tomography Core Center: Matthew J. Budoff MD (Director), Lily Honoris MD, Chris Dailing, Sivi Carson; Data Safety Monitoring Board: Leon Speroff MD (Chairman), Robert Knopp MD (deceased), Richard Karas MD, Joan Hilton PhD; Judy Hannah (NIA ex-officio).

*Primary trial investigators

Corresponding author: Howard N. Hodis, MD
Keck School of Medicine
Atherosclerosis Research Unit
2250 Alcazar Street, CSC 132
Los Angeles, CA 90033
Email: athero@usc.edu
Tele: 323-442-1478
Fax: 323-442-2685

PRÉCIS:

ELITE is a timely and unique randomized controlled trial well-positioned to test the hormone therapy “timing hypothesis” in relation to atherosclerosis progression and cognitive change.

ABSTRACT

Objective: To present methods and baseline data from the Early versus Late Intervention Trial with Estradiol (ELITE), the only clinical trial designed to specifically test the timing hypothesis of postmenopausal hormone therapy (HT). The timing hypothesis posits that HT effects depend on the temporal initiation of HT relative to time-since-menopause.

Methods: ELITE is a randomized, double-blind, placebo-controlled trial with a 2x2 factorial design. 643 healthy postmenopausal women without cardiovascular disease were randomized to oral estradiol or placebo for up to 6 years according to number of years-since-menopause, <6 years or ≥10 years. Carotid artery intima-media thickness (CIMT) and cardiac computed tomography were conducted to determine HT effects on subclinical atherosclerosis across strata and longitudinal cognitive assessments to determine HT effects on cognitive aging.

Results: Participants in the early and late postmenopausal strata were well-separated by mean age, 55.4 versus 65.4 years and median time-since-menopause, 3.5 versus 14.3 years, respectively. The expected risk factors were associated with CIMT at baseline in both strata (age, blood pressure and body mass index). Associations with CIMT present in the early but not in the late postmenopausal group that may play a role in responsiveness of atherosclerosis progression according to timing of HT initiation include LDL-C, HDL-C, sex hormone binding globulin and serum total estradiol.

Conclusion: The ELITE randomized controlled trial is timely and unique. Baseline data indicate that ELITE is well-positioned to test the HT timing hypothesis in relation to atherosclerosis progression, coronary artery disease and cognitive change. (NCT00114517; www.clinicaltrials.gov)

INTRODUCTION

The “timing,” “critical window” or “window of opportunity” hypothesis of hormone therapy (HT) was proposed to explain the difference in outcomes between randomized clinical event trials and observational studies of coronary heart disease (1,2), cognition and dementia (3,4). The timing hypothesis posits that HT benefits and risks depend on the temporal initiation of HT relative to time-since-menopause, age or both, which are in turn related to the health of the underlying tissue (i.e., healthy vascular endothelium and extent of atherosclerosis burden) (5,6). The divergent results between the atherosclerosis progression sister trials Estrogen in the Prevention of Atherosclerosis Trial (EPAT) (5) and the Women’s Estrogen Lipid-Lowering Hormone Atherosclerosis Regression Trial (WELL-HART) (6) provided early clinical trial support for this hypothesis. While EPAT demonstrated a reduction of subclinical atherosclerosis progression with HT in postmenopausal women without demonstrable atherosclerosis, WELL-HART demonstrated a null effect in postmenopausal women with documented coronary artery atherosclerosis (5,6). Experimental animal studies (7,8) replicate the divergent effects of HT on atherosclerosis as shown in EPAT and WELL-HART. With completion of EPAT and WELL-HART, a new randomized controlled trial was proposed to the National Institutes of Health (NIH) in early 2002 entitled the Early versus Late Intervention Trial with Estradiol (ELITE).

Data supporting the timing hypothesis has grown considerably over the past decade since initiation of ELITE (1). ELITE remains unique and timely as the only randomized controlled trial specifically designed to test the timing hypothesis of HT in relation to atherosclerosis progression and cognitive change in postmenopausal women. The primary ELITE hypothesis is that postmenopausal HT will reduce the progression of subclinical atherosclerosis, coronary artery disease (CAD) and cognitive change in postmenopausal women without clinical evidence of cardiovascular disease (CVD) when initiated soon after menopause (<6 years) relative to initiation of HT when distant from menopause (≥10 years).

MATERIALS AND METHODS

Design: ELITE is a single-center, randomized, double-blinded, placebo-controlled, non-invasive serial arterial imaging, prospective trial with a 2x2 factorial design (NCT00114517). Healthy postmenopausal women without clinical evidence of CVD were randomized to active HT or to

placebo according to the number of years-since-menopause, <6 years (early postmenopause) or ≥10 years (late postmenopause). Recruitment was initially based on a 5-year trial (3-year recruitment period and 2 to 5 years of randomized treatment). In the fifth year of randomized follow-up, the trial was extended for an additional 2.5 years of randomized intervention. As part of the ELITE extension, a third cognitive assessment was added and measurements of subclinical coronary artery atherosclerosis were obtained as participants completed the trial.

Treatment: Women with a uterus were randomized to oral micronized 17β-estradiol 1 mg/day with vaginal micronized progesterone gel 4% (45 mg) per day for 10 days each month or to double matched placebos. Women without a uterus were randomized to oral micronized 17β-estradiol 1 mg/day alone or to placebo.

Eligibility criteria: Eligible participants were postmenopausal women without clinical evidence of CVD, with a serum estradiol level <25 pg/ml and cessation of regular menses for a minimum of 6 months who were <6 years or ≥10 years-since-menopause at the time of randomization. Exclusion criteria included: women in whom time-since-menopause could not be determined; fasting plasma triglyceride level >500 mg/dl; diabetes mellitus or fasting serum blood glucose >140 mg/dl; serum creatinine >2.0 mg/dL; uncontrolled hypertension (systolic/diastolic blood pressure >160/110 mmHg); untreated thyroid disease; life threatening disease with prognosis <5 years; history of deep vein thrombosis, pulmonary embolism or breast cancer; current postmenopausal HT.

Randomization: Participants were randomized to active HT or to placebo in strata defined by number of years since menopause, <6 years or ≥10 years. Additional randomization stratification factors included hysterectomy (yes, no) and baseline carotid artery intima-media thickness (CIMT) (<0.75 mm or ≥0.75 mm) (Figure 1). Trial eligibility was assessed at the screening and baseline visits and confirmed at the data coordinating center. Assignment to HT or to placebo in a 1:1 ratio used stratified blocked randomization not identified to study personnel. Randomization lists for each of 8 strata were prepared prior to trial initiation by the trial statistician; blinded study product was prepared based on the stratified randomization lists. Upon determination of trial eligibility and stratum for a given participant, clinic staff pulled the next study product in sequence from the appropriate stratum and recorded the product identification number. The

fidelity of the randomization process was monitored at the data coordination center. Participants, investigators, staff, imaging specialists and data monitors were masked to treatment assignment.

Follow-up: Following randomization, participant evaluations were conducted in a specialized research clinic every month for the first 6 months of the trial and then every other month until trial completion. All baseline evaluations were conducted prior to randomization. Every 6 months, the following fasting blood levels were determined: plasma estradiol and progesterone by extraction and radioimmunoassay (RIA) (5), plasma lipids and lipoproteins (total cholesterol (C), total triglycerides (TG), low-density lipoprotein (LDL)-C, LDL-TG, very low-density lipoprotein (VLDL)-C, VLDL-TG, high-density lipoprotein (HDL)-C and HDL-TG) by preparative ultracentrifugation and enzymatic assays standardized to the CDC using Lipid Research Clinic protocol (5) and hemoglobin A1c by high-pressure liquid chromatography ion exchange chromatography (DCCT approved method) (9). Complete blood count and chemistry panel were completed yearly by Quest Diagnostics.

Following clinical assessment at baseline prior to randomization, blood pressure, pulse rate, weight, waist and hip circumference, and height (only obtained at baseline) were obtained every 6 months; a 12-lead electrocardiogram (ECG) was obtained yearly. Questionnaires covering medical history, angina, claudication, physical activity and smoking and alcohol use were administered every 6 months. Study product compliance, clinical events (MedDra-coded) and non-study medication and nutritional supplement use were ascertained at every clinic visit. Participants completed 3-day dietary booklets prior to the baseline visit and prior to each subsequent clinic visit.

A reproductive history was obtained at baseline and the Center for Epidemiologic Studies Depression scale and the Women’s Health Questionnaire (10) covering physical and emotional health were administered at baseline prior to randomization and every 6 months. Participants completed flushing, vaginal bleeding and cramping and breast pain diaries that were collected at baseline and at each clinic visit. Baseline, annually and as indicated, mammography, gynecological examinations including a Pap smear, transvaginal uterine ultrasound and endometrial biopsy (if indicated) were performed.

Primary endpoint: All women meeting trial inclusion criteria underwent 2 baseline B-mode carotid artery ultrasound examinations to acquire standardized images for CIMT measurements. The first CIMT was assessed at the screening visit and was used to determine randomization stratum. The second CIMT was conducted at the baseline visit prior to randomization in the women meeting the final trial inclusion criteria. The time interval between the first and second CIMT was 2-4 weeks. The primary trial endpoint is the rate of change of the right distal common carotid artery (CCA) far wall intima-media thickness (IMT) in computer image processed B-mode ultrasonograms over the 2 baseline examinations and serial examinations completed every 6 months over trial follow-up (5). High resolution Bmode ultrasound imaging and measurement of far wall CIMT was conducted using standardized procedures and technology specifically developed for longitudinal measurements of atherosclerosis change (Patents 2005, 2006, 2011). Details of the ultrasound acquisition and CIMT measurement procedures are described elsewhere (5,11-13) and in the supplemental material.

Secondary endpoints: The secondary endpoints include measurement of cognition and coronary artery atherosclerosis. Cognitive function was determined at a baseline cognitive examination before randomization and at 2 follow-up examinations at 2.5 years post-randomization and at completion of randomized treatment. A comprehensive neuropsychological battery was used to assess a broad spectrum of cognitive skills, emphasizing standardized tests sensitive to age-associated change in middle-aged and older adults. The primary cognitive outcome will be change in a composite measure of verbal memory calculated as an average of standardized scores from four neuropsychological tests weighted by the inverse inter-test correlation matrix. Secondary cognitive endpoints will include changes in global cognition (based on 14 neuropsychological tests), changes in executive function and changes in individual neuropsychological tests, as well as the proportion of women with large memory change scores that may represent increased risk for Alzheimer's disease. Details of the neuropsychological tests, procedures and statistical analyses are described elsewhere (14,15).

Subclinical coronary artery atherosclerosis was assessed with cardiac computed tomography (CT) scanning for coronary artery calcium (CAC) and cardiac computed tomography angiography (CCTA) for measurement of coronary artery stenoses in a single session using a dedicated cardiac GE 64 slice multi-detector computed tomography (64 MDCT) scanner as participants completed randomized treatment. The CT end points will represent a comprehensive

assessment of coronary artery atherosclerosis across all of the coronary artery segments of total CAC, total stenosis score, total plaque score and composition of coronary atherosclerotic plaque. Details of the CAC and CCTA methods are described elsewhere (16,17) and in the supplemental material.

Sample size: The sample size was based on a projected mean treatment group difference in the CIMT change rate among women <6 and ≥ 10 years since menopause (placebo-treated rate minus estradiol-treated rate) of 0.0144 and 0.0021 mm/year, and standard deviation (SD) of CIMT change rate of 0.02 mm/year. Sample size based on CIMT progression required 83 participants in each cell (126 participants per cell including an anticipated 25% dropout) to detect treatment-by-time-since-menopause interaction in the 2x2 factorial design with 80% power, testing at a two-sided alpha of 0.05. This sample size was also projected to yield a power of 96% to detect a significant treatment group difference in the CIMT progression rates in the early postmenopausal group.

Statistical analysis:

Baseline comparisons will be made between treatment arms (within time-since-menopause strata) with respect to demographic, medical history, baseline risk factors (blood pressure, smoking, lipids), previous hormone use, estradiol and progesterone levels and baseline CIMT. Analytical methods will include two-sample t-tests (or a non-parametric analog) for continuous variables and chi-square procedures for categorical variables. All treatment group comparisons on baseline variables will be performed for the entire randomized study sample and for the group of subjects with repeat (post-randomization) carotid ultrasounds.

Evaluation of potential biases introduced by differential dropout (comparing subjects with and without post-randomization ultrasound by treatment group) will be determined. Analytic methods will utilize standard univariate procedures for the total sample and by time-since-menopause strata to compare these subject groups on baseline demographics and baseline levels of laboratory and lifestyle variables.

On-trial levels of laboratory variables (plasma lipids, lipoproteins, glucose, hemoglobin A1c, and estradiol and progesterone levels) will be compared between treatment groups. Analytic

methods will include marginal models with generalized estimating equations. Independent factors will be treatment group, time-since-menopause and randomization stratification factors; the baseline levels of the on-trial dependent variables will be included as a covariate. The primary tests of interest are the overall treatment group comparisons. Differences in the randomized treatment effect by menopause strata will also be tested with addition of a product interaction term. Statistical evaluations will report the overall test for treatment group difference, the overall test for differences between the time-since-menopause groups as well as the interaction p-value. Treatment group comparisons will be performed for the group of subjects with any post-randomization carotid ultrasound and for the adherent participants (adherent analysis) defined as ≥80% compliance with estrogen therapy.

The primary endpoint will be the per-subject rate of change in the right distal CCA far wall IMT. Statistical analyses will use mixed effects models; treatment group and time-since-menopause group will be included as indicator variables, with the randomization stratification variables included as covariates. Random effects will be specified for the subject-specific intercept (baseline CIMT) and slope (CIMT rate of change). The overall treatment group difference in the mean CIMT rate will be tested with a 2-way treatment group by time-on-study interaction. A 2-way time-since-menopause group-by-time on-study interaction term will test whether the mean CIMT progression rate differs among the 2 menopause groups. The hypothesis that the treatment group effects on the CIMT rate differs by time-since-menopause will be tested with a time-since-menopause-by-treatment-by-time on-study interaction term. Treatment group comparisons will be performed for the group of subjects with any post-randomization carotid ultrasound and for the adherent participants (≥80% compliance with estrogen therapy).

CAC and CCTA outcomes obtained at the end-of-study will be compared between treatment groups: 1) primary CCTA measures – total stenosis score and total plaque score; and, 2) secondary CCTA measures -- number of coronary artery segments with calcified, non-calcified and mixed plaque. CAC measures will include presence of any CAC (dichotomous variable) and CAC score. General linear models (GLM) will be used, specifying these CAC and CCTA measures as dependent variables. The 2 randomization stratification variables (type of menopause and dichotomous baseline CIMT) will be included as covariates. To account for the fact that the end-of-study visit will differ across subjects, indicator variables for the study visit at which the CAC and CCTA measures were obtained will also be included as covariates. The

primary independent variables of interest will be indicator variables for each of the study design factors, testing for differences in the end-of-study CAC and CCTA measure by treatment group and menopause stratum. A treatment-by-menopause stratum interaction will test whether the treatment group differences differ by time-since-menopause. Treatment group comparisons will be performed for all participants who have the CAC and/or CCTA end points and for the estradiol adherent participants.

Safety analysis and evaluation of adverse events will be performed on all randomized subjects using exact methods, comparing events among the four study groups [early postmenopausal-estradiol treated; early postmenopausal-placebo treated; late postmenopausal-estradiol treated; late postmenopausal-placebo treated]) since the primary question is whether any given group shows an elevated risk for adverse events. The following major clinical events will be evaluated and compared: 1) cardiovascular events, including fatal/nonfatal myocardial infarction (MI), silent MI and sudden death, hospitalization for unstable angina and coronary revascularization procedures (coronary artery bypass grafting, percutaneous transluminal coronary angioplasty); 2) stroke; 3) venous thromboembolism (deep vein thrombosis and pulmonary embolism); 4) cancer (breast, uterine, ovarian, gastrointestinal, lung); 5) bone fractures; and, 6) all-cause mortality and noncoronary mortality.

ELITE was approved by the Institutional Review Board of the University of Southern California. All participants provided written informed consent before trial-related procedures were conducted. Participant safety and trial conduct were overseen by an External Data Safety Monitoring Board appointed by the National Institute on Aging, National Institutes of Health with expertise in women's health, menopausal health, hormone replacement therapy, cardiovascular disease, clinical trials and biostatistics.

RESULTS

Recruitment and Screening: Recruitment for ELITE commenced in May 2005. The first participant was randomized on July 13, 2005; the last participant was randomized on September 30, 2008. A total of 643 participants were randomized. The original sample size estimates required randomization of 504 participants. Since the response and recruitment to ELITE was large, it was decided to randomize a higher number of participants to the trial, in particular

women in the late postmenopausal group since previous HT use was high in this group, and to compensate for any higher anticipated dropout rate in this older group.

To obtain the 643 participants, 2166 candidates were telephone prescreened with a series of questions from a standard interview form. Of these 2166 telephone prescreened candidates, 1271 were ineligible. The remaining 895 candidates were further screened during a clinic visit from which the 643 participants were randomized. In total, 1523 (70%) candidates were ineligible or refused enrollment. The primary reasons for ineligibility or refusal included: current HT (171 women), 6-9 years postmenopausal (142 women), clinical signs or symptoms of CVD (81 women), diabetic or serum glucose ≥140 mg/dl (21 women), history of breast cancer (37 women), and not postmenopausal by trial criteria (23 women).

Baseline Results: Table 1 contrasts demographic factors and the results of the baseline examinations between the <6 years-since-menopause and ≥10 years-since-menopause strata. In the early postmenopausal stratum, participant mean age was 55.4 years and the median time-from-menopause was 3.5 years. In the late postmenopausal stratum, participant mean age was 65.4 years and the median time-from-menopause was 14.3 years. Overall, 32% of the women were from a minority racial or ethnic group and 99% of the participants had a high school or greater education. Smoking history was similar between strata and only 3.4% of the participants were active smokers. As expected, hot flashes were more common in the early postmenopausal group relative to the late postmenopausal group. A major difference between strata was that more women in the late postmenopausal group previously used HT and were currently using antihypertensive and lipid-lowering medications than the women in the early postmenopausal group. More women in the late postmenopausal group underwent surgical menopause than the women in the early postmenopausal group (16.1% versus 3.3%, respectively).

Table 2 contrasts clinical, laboratory and CIMT variables between years-since-menopause strata. The average CIMT was greater in the women in the late postmenopausal stratum than the women in the early postmenopausal stratum, 0.787 versus 0.748 mm, respectively. The average baseline systolic blood pressure was slightly greater and the average diastolic blood pressure was slightly lower in the women in the late postmenopausal stratum relative to the women in the early postmenopausal stratum. These average blood pressure differences between the women in the 2 strata likely reflect the greater baseline use of anti-hypertension medications by the women in the

late postmenopausal stratum relative to the women in the early postmenopausal stratum, 28.8% versus 18.5%, respectively (Table 1). The slightly lower average baseline fasting LDL-C and higher HDL-C levels in the women in the late postmenopausal stratum relative to the women in the early postmenopausal stratum, as well as the equivalence in baseline fasting plasma cholesterol and triglycerides between strata (Table 2) is likely a reflection of the greater baseline use of lipid-lowering medications (the majority (96%) of which were from the statin class) by the women in the late postmenopausal stratum relative to the women in the early postmenopausal stratum, 23.7% versus 14.8%, respectively (Table 1). Although the hemoglobin A1c value was slightly lower among the women randomized to the early postmenopausal stratum relative to the women randomized to the late postmenopausal stratum, the fasting serum glucose level and body mass index were equivalent between strata (Table 2).

Table 3 shows the age- and race-adjusted linear associations of CIMT with baseline characteristics according to postmenopausal strata. The expected risk factors of blood pressure and body mass index (BMI) were positively associated with CIMT in both strata. LDL-C, triglycerides, HDL-C (inverse) and sex hormone binding globulin (SHBG) (inverse) were associated with CIMT in the early postmenopausal stratum but not in the late postmenopausal stratum, although the interaction between strata for these variables was not statistically significant. Pulse rate and the serum levels of total estradiol differed across strata (interaction p-value = 0.02). Serum total estradiol was negatively associated with CIMT ($p = 0.09$) among the women randomized to the early postmenopausal stratum but not among the women randomized to the late postmenopausal stratum. For pulse, a higher rate was positively associated with CIMT in the early postmenopausal stratum but not in the late postmenopausal stratum.

DISCUSSION

Results of the recruitment, screening and randomization phase of ELITE indicate that the design and methods produced excellent baseline circumstances to conduct a randomized controlled trial for the test of the timing hypothesis of HT on vascular disease and cognition. By design, there is excellent separation without overlap in the time from menopause between the group of women <6 years-since-menopause (median, 3.5 years) and ≥10 years-since-menopause (median, 14.3 years). This outcome provides an optimal setting in which we will be able to determine the effects of HT on atherosclerosis progression, CAD and cognitive change in strata of women with

characteristics similar to those described in key studies. The early postmenopausal group is comparable to postmenopausal women initiating HT in clinical practice near the time of menopause and as represented in most observational studies (18,19) and in the Danish Osteoporosis Prevention Study (DOPS) randomized trial (20). The late postmenopausal group is more comparable to women in the Women's Health Initiative (WHI), in which participants were on average 64 years old and 12 years-since-menopause when randomized; a profile not representative of women who typically initiate HT (1). Baseline CVD risk factors across postmenopausal strata such as smoking, BMI, blood pressure, lipids, glucose and hemoglobin A1c are similar while the baseline CIMT value is lower in the early postmenopausal group relative to the late postmenopausal group. Baseline usage of lipid-lowering and anti-hypertensive medications was greater in the late postmenopausal group than in the early postmenopausal group.

Although traditional and expected risk factor associations with CIMT (age, blood pressure and BMI) are present at baseline in both the <6 and ≥10 years-since-menopause strata, several important associations evident in the early but not in the late postmenopausal group may have relevance to the timing hypothesis and that may play a role in the responsiveness of atherosclerosis progression to HT. Of these differences, perhaps the most important are the significant positive LDL-C and inverse HDL-C and SHBG associations with baseline CIMT in the early postmenopausal group. We have previously shown that the LDL-C lowering and HDL-C raising effects of HT account for 30% of the effect of randomized HT in reducing atherosclerosis progression (21). The highly significant inverse association of SHBG with CIMT in the early postmenopausal group may be of particular importance as we have previously shown that the HT-related reduction in atherosclerosis progression occurs in women with an increase in SHBG in response to HT (22). The significant interaction across postmenopausal strata for total serum estradiol levels and CIMT appears consistent with the timing hypothesis. Although not statistically significant, the inverse association of total serum estradiol levels (in the relatively restricted range of postmenopausal levels) with CIMT in the early postmenopausal group suggests that raising estradiol levels may decrease progression of atherosclerosis in this group of women. In the late postmenopausal group, there is no association of total serum estradiol levels with CIMT suggesting that raising estradiol levels may have no effect on atherosclerosis progression in this group of women. Whether these differential risk factor associations with CIMT at baseline according to strata play a role in determining whether HT reduces

atherosclerosis progression in women who are <6 years-since-menopause relative to women who are ≥10 years-since-menopause remains to be determined. Although the strata are sufficiently separated according to time-since-menopause, certain baseline factors could obscure our results and the timing hypothesis for this trial. One possible variable is age since age and time-since-menopause tend to co-vary and it has not been determined which variable may be a stronger factor in determining the potential responsiveness of atherosclerosis progression to HT. Although there is a baseline mean 10-year difference in age between postmenopausal strata, there is a large overlap in age across strata.

With the beneficial effects of HT in reducing the progression of atherosclerosis in EPAT reported in 2001, we hypothesized that intervention early in the atherosclerosis process at the start of menopause may be the key to cardiovascular disease prevention with HT (5). This led to the hypothesis that HT may be more effective as primary prevention when initiated in the early stages of atherosclerosis rather than as secondary prevention when atherosclerosis is established (6). In other words, HT may be effective in the prevention but not in the treatment of vascular disease (6). With publication of EPAT's sister study WELL-HART, where the effect of HT on established atherosclerosis was null, we concluded that the difference in outcomes between the two trials (EPAT and WELL-HART) may have resulted from the timing of the intervention relative to the stage of atherosclerosis as measured by the different imaging methods used in these trials (6) (carotid artery wall thickness in EPAT as a measure of early, asymptomatic, subclinical atherosclerosis and quantitative coronary angiography in WELL-HART used to evaluate late-stage, symptomatic atherosclerosis) (5,6). In support of these human trial findings is an accumulating literature from animal studies showing that estrogen has little effect in reversing atherosclerosis once it is established, whereas it significantly reduces the extent of atherosclerosis if therapy is initiated at an early stage of atherosclerosis development (7,8).

Since 2001, there has been a large accumulation of data from randomized trials strongly supporting the timing hypothesis (Table 4) suggesting that women respond differentially to HT according to timing of HT initiation relative to age and/or time-since-menopause (1). Meta-analyses of the cumulated data show that the effect of HT on CHD and total mortality is null when HT is initiated in women >60 years of age and/or >10 years-since-menopause whereas, in women who initiate HT when <60 years of age and/or <10 years-since-menopause, there is a 32% reduction in CHD and 39% reduction in total mortality relative to placebo (23-26). The

magnitude of CHD and total mortality reduction for women <60 years of age and/or <10 years-since-menopause when randomized to HT is similar to observational studies of populations of women who initiated HT at or near the time of menopause (18,19).

In a more recent randomized trial with a PROBE (prospectively, randomized, open with blinded endpoint evaluation) design, the DOPS provides data for long-term use of oral estradiol alone and estradiol with sequential norethisterone acetate when initiated in healthy young perimenopausal and early postmenopausal women (20). DOPS included 1,006 postmenopausal women who were on average 50 years old and 7 months postmenopausal. The composite primary trial end point of mortality, MI or heart failure was significantly reduced by 52% (HR, 0.48; 95% CI, 0.27-0.89) and by 39% (HR, 0.61; 95% CI, 0.39-0.94) after 10 years of randomized treatment and 16 years of total follow-up (10 years of randomized treatment and 6 years of post-intervention follow-up), respectively. Total mortality was reduced by 43% (HR, 0.57; 95% CI, 0.30-1.08) after 10 years of randomized treatment and by 34% (HR, 0.66; 95% CI, 0.41-1.08) after 16 years of total follow-up. Remarkably similar to the DOPS results are the 11-year WHI trial follow-up (7 years of randomized treatment and 4 years of post-intervention follow-up) data. Women who were 50-59 years of age when randomized to conjugated equine estrogen (CEE) relative to placebo had significant reductions in CHD by 41% (HR, 0.59; 95% CI, 0.38-0.90), total myocardial infarction (MI) by 46% (HR, 0.54; 95% CI, 0.34-0.86) and total mortality by 37% (HR, 0.73; 95% CI, 0.53-1.00) (27). These treatment benefits significantly differed from women aged 60-69 and 70-79 years (p-value for interaction, p=0.05, p=0.007 and p=0.04, respectively), indicating that the CEE effect on these outcomes differed by age of HT initiation (27).

Other evidence that HT initiation in young postmenopausal women in close proximity to menopause may reduce CHD derives from 1,064 women who participated in the WHI CAC sub-study in which 50-59 year old women who were randomized to CEE had significantly less CAC at year 7 of the trial compared with women randomized to placebo (28). There was no older age group in this sub-study to evaluate whether HT influenced CAC when initiated in women >60 years old. Results of ELITE will provide the necessary data to determine the effect of HT on CAC in younger postmenopausal women with HT initiation in close proximity to menopause relative to older postmenopausal women with HT initiation distant from menopause. In addition, for the first time, the effects of HT on CAD will be determined with CCTA in an asymptomatic

population of women under the same circumstances in ELITE. The Kronos Early Estrogen Prevention Study (KEEPS) will provide data for low-dose HT on CIMT and CAC in women randomized within 3 years of menopause.

In addition to mammalian hormones, other agents that bind to the estrogen receptor exert similar CHD beneficial effects in young postmenopausal women as HT. Among women <60 years of age when randomized to raloxifene, a selective estrogen receptor modulator, CHD was significantly reduced by 41% (HR, 0.59; 95% CI, 0.41-0.83) relative to placebo (29). Women older than 60 years of age showed no benefit from raloxifene. In the Women's Isoflavone Soy Health (WISH) study, a randomized controlled trial in which the effects of high-dose isoflavone soy protein supplementation (plant estrogens that preferentially bind to estrogen receptor-beta) on the progression of subclinical atherosclerosis were determined, women who were randomized within 5 years of menopause to isoflavone soy protein supplementation had a significant reduction in progression of subclinical atherosclerosis relative to placebo whereas isoflavone supplementation among women more than 5 years beyond menopause when randomized had no significant effect (30).

The totality of randomized trial data consistently shows that HT reduces the incidence of CHD and total mortality in young postmenopausal women (<60 years old) who initiate HT in close proximity to menopause (<10 years-since-menopause) (Table 4) (1). These findings parallel the consistent reduction in CHD and total mortality reported from observational studies where the majority of women initiated HT within 6 years of menopause (18,19). Over the past 5 decades, 40-50 observational studies have consistently shown that HT is associated with a 30-50% reduction in CHD and 20-60% reduction in total mortality in postmenopausal women (18,19). Although the cumulated literature is consistent and intriguing, no clinical trial has specifically tested the timing hypothesis. ELITE is both timely and unique. Over the past decade, the timing hypothesis has matured into a well-supported hypothesis awaiting study. As a result of randomization and as baseline data indicate, ELITE is well-positioned to test the timing hypothesis of HT in relation to atherosclerosis progression, CAD and cognitive change.

Table 1. Demographic characteristics at baseline

Characteristic	<6 Years-Since-Menopause (n=271)	≥10 Years-Since-Menopause (n=372)	P-value[1]
Mean age, years	55.4 (4.1)[2]	65.4 (6.0)	<0.001
Race or ethnicity			0.03
White, non-Hispanic	174 (64.2%)	266 (71.5%)	
Black, non-Hispanic	24 (8.9%)	36 (9.7%)	
Hispanic	41 (15.1%)	49 (13.2%)	
Asian	32 (11.8%)	21 (5.7%)	
Education			0.05
College graduate	195 (72.0%)	233 (62.6%)	
High school or some college	75 (27.7%)	137 (36.8%)	
Less than high school	1 (0.4%)	2 (0.5%)	
Current smoker	11 (4.1%)	11 (3.0%)	0.45
Median time since menopause, years	3.5 (1.9,5.0)[3]	14.3 (11.4,18.6)	<0.001
Type of menopause			<0.001
Natural	262 (96.7%)	312 (83.9%)	
Surgical	9 (3.3%)	60 (16.1%)	
Hot flashes, any within previous month[4]	173/243 (71.2%)	157/324 (48.5%)	<0.001
Past use of hormone therapy, yes	138 (50.9%)	321 (86.3%)	<0.001
Hypertension medications	50 (18.5%)	107 (28.8%)	0.003
Lipid-lowering medications	40 (14.8%)	88 (23.7%)	0.005

[1]Statistical tests utilized the student two-sample t-test for continuous variables, chi-square test for discrete variables and the Wilcoxon rank sum test for median time-since-menopause.
[2]Mean (SD)
[3]Medan (interquartile range)
[4]By flushing diaries

Table 2. Clinical and laboratory characteristics at baseline[1]

Variable	<6 Years-Since-Menopause (n=271)	≥10 Years-Since-Menopause (n=372)	P-value
Carotid artery intima-media thickness, mm	0.748 (0.095)	0.787 (0.109)	<0.001
Body mass index, kg/m²	27.2 (5.4)	27.4 (5.4)	0.67
Pulse rate, beats/min	65.5 (5.1)	66.1 (5.2)	0.14
Systolic blood pressure, mmHg	116.8 (12.9)	118.7 (11.9)	0.06
Diastolic blood pressure, mmHg	76.1 (7.1)	74.3 (7.0)	0.001
Serum lipids			
LDL cholesterol, mg/dl	139.4 (32.1)	134.3 (30.8)	0.04
HDL cholesterol, mg/dl	64.4 (16.0)	67.1 (18.6)	0.06
Total cholesterol, mg/dl	224.8 (33.9)	223.0 (33.4)	0.49
Triglycerides, mg/dl	105.1 (53.7)	108.0 (55.7)	0.51
Glucose, mg/dl	95.5 (10.4)	94.5 (10.2)	0.23
Hemoglobin A1c, % (n=269,369)	5.5 (0.5)	5.7 (0.4)	0.002
Sex hormone binding globulin, nmol/L (median, interquartile range) (n=268,370)	46.1 (32.5,61.1)	51.1 (37.7,66.9)	0.01
Total estradiol, pg/ml (median, interquartile range) (n=269,370)	8.0 (5.8,11.4)	7.7 (5.8,10.5)	0.17

[1]All variables are summarized as mean (standard deviation) unless otherwise stated. Comparison of means between postmenopausal strata utilized the student two-sample t-test; comparison of medians utilized the Wilcoxon rank sum test
LDL=low-density lipoprotein
HDL=high-density lipoprotein

Characteristic	<6 Years-Since-Menopause (n=271)		≥10 Years-Since-Menopause (n=372)		P-value for interaction
	Beta (SE)	p-value	Beta (SE)	p-value	
Education					
College graduate	Ref		Ref		0.75
High school or some college	-0.003 (0.012)	0.79	-0.007 (0.011)	0.55	
Less than high school	-0.011 (0.089)	0.90	0.080 (0.075)	0.29	
Current smoker	-0.007 (0.027)	0.79	0.003 (0.032)	0.94	0.80
Type of menopause					
Natural	Ref		Ref		0.06
Surgical	0.073 (0.030)	0.02	-0.011 (0.015)	0.47	
Hot flashes, any within previous month[2]	0.0006 (0.013)	0.96	-0.002 (0.012)	0.88	0.89
Past use of hormone therapy, yes	-0.007 (0.011)	0.51	-0.026 (0.016)	0.09	0.25
Hypertension medications	0.005 (0.014)	0.73	0.004 (0.012)	0.77	0.88
Lipid-lowering medications	0.017 (0.015)	0.27	0.0003 (0.013)	0.98	0.38
Body mass index, kg/m^2	0.002 (0.001)	0.02	0.003 (0.001)	0.007	0.40
Pulse rate, beats/min	0.003 (0.001)	0.002	-0.0003 (0.001)	0.78	0.02
Systolic blood pressure, mmHg	0.002 (0.0004)	<.0001	0.002 (0.0005)	<.0001	0.72
Diastolic blood pressure, mmHg	0.002 (0.0008)	0.02	0.002 (0.0008)	0.05	0.82
Serum lipids					
LDL cholesterol, mg/dl	0.0004 (0.0002)	0.02	0.0003 (0.0002)	0.13	0.76
HDL cholesterol, mg/dl	-0.001 (0.0003)	0.005	-0.0004 (0.0003)	0.20	0.33
Total cholesterol, mg/dl	0.0002 (0.0002)	0.11	0.0001 (0.0002)	0.50	0.59
Triglycerides, mg/dl[3]	0.066 (0.028)	0.02	0.0009 (0.030)	0.98	0.12

Glucose, mg/dl	0.0006 (0.0005)	0.24	0.0007 (0.0005)	0.17	0.92
Hemoglobin A1c, % (n=269,369)	0.006 (0.011)	0.58	-0.010 (0.014)	0.44	0.40
Sex hormone binding globulin, nmol/L (n=268,370)[3]	-0.082 (0.027)	0.002	-0.010 (0.028)	0.71	0.13
Total estradiol, pg/ml (n=269,372)[3]	-0.032 (0.019)	0.09	0.037 (0.026)	0.16	0.02

Table 3. Baseline associations with carotid artery intima-media thickness[1]

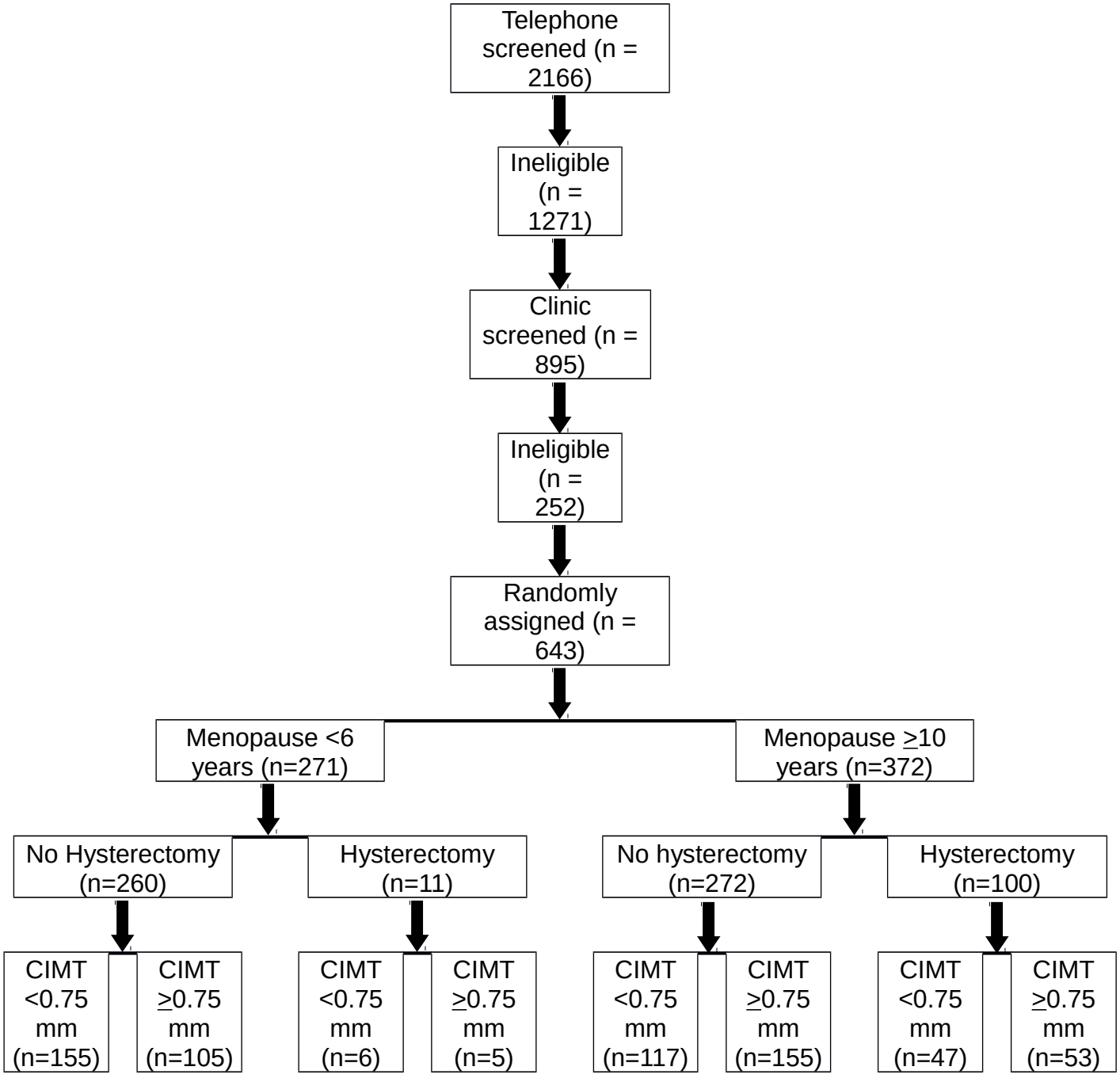
[1]Multiple linear regression analyses were conducted for each variable, adjusting for age and race.
[2]By flushing diaries
[3]Log transformed

Table 4. Coronary heart disease and total mortality in women initiating hormone or selective estrogen receptor modifier (SERM) therapy before age 60 years and/or within 10 years-since-menopause

Studies	Age, years	Time-Since-Menopause	Hormone Therapy	Coronary Heart Disease % Reduction (Risk Ratio; 95% Confidence Interval)	Total Mortality % Reduction (Risk Ratio; 95% Confidence Interval)
DOPS, 10 year[20]	50	7 mo	E2 alone and E2 + NETA sequential	↓ **52%** (0.48; 0.27-0.89)	↓ **43%** (0.57; 0.30-1.08)
DOPS, 16 year[20]				↓ **39%** (0.61; 0.39-0.94)	↓ **34%** (0.66; 0.41-1.08)
WHI-E, 11 year[27]	<60		CEE alone	↓ **41%** (0.59; 0.38-0.90)	↓ **27%** (0.73; 0.53-1.00)
WHI-E[31]		<10 yrs	CEE alone	↓ **52%** (0.48; 0.20-1.17)	↓ **35%** (0.65; 0.33-1.29)
WHI-E+P[31]		<10 yrs	CEE+MPA continuous combined	↓ **12%** (0.88; 0.54-1.43)	↓ **19%** (0.81; 0.52-1.24)
WHI-E/E+P[31]		<10 yrs	CEE alone and CEE+MPA continuous combined	↓ **24%** (0.76; 0.50-1.16)	↓ **24%** (0.76; 0.53-1.09)
WHI-E[31]	<60		CEE alone	↓ **37%** (0.63; 0.36-1.09)	↓ **29%** (0.71; 0.46-1.11)

WHI-E+P[31]	<60		CEE+MPA continuous combined	↑ **29%** (1.29; 0.79-2.12)	↓ **31%** (0.69; 0.44-1.07)
WHI-E/E+P[31]	<60		CEE alone and CEE+MPA continuous combined	↓ **7%** (0.93; 0.65-1.33)	↓ **30%** (0.70; 0.51-0.96)
Meta-analysis[23]	<60	<10 yrs		↓ **32%** (0.68; 0.48-0.96)	
Meta-analysis[24]	54				↓ **39%** (0.61; 0.39-0.95)
Bayesian meta-analysis[25]	55				↓ **27%** (0.73; 0.52-0.96)
RUTH[29]	<60		Raloxifene	↓ **41%** (0.59; 0.41-0.83)	
Observational studies[18,19]	30-55	<5 yrs		↓ **30-50%**	↓ **20-60%**

DOPS = Danish Osteoporosis Prevention Study
WHI-E = Women's Health Initiative-estrogen alone trial
WHI-E+P = Women's Health Initiative-estrogen + progestin continuous combined trial
WHI-E/E+P = WHI-E and WHI-E+P trials combined into a single analysis
RUTH = Raloxifene Use for the Heart trial
E2 = estradiol
NETA = Norethisterone acetate
CEE = Conjugated equine estrogens
MPA = Medroxyprogesterone acetate


Telephone screened (n = 2166)
Ineligible (n = 1271)
Clinic screened (n = 895)
Ineligible (n = 252)
Randomly assigned (n = 643)
Menopause <6 years (n=271)
Menopause ≥10 years (n=372)
No Hysterectomy (n=260)
Hysterectomy (n=11)
No hysterectomy (n=272)
Hysterectomy (n=100)
CIMT <0.75 mm (n=155)
CIMT ≥0.75 mm (n=105)
CIMT <0.75 mm (n=6)
CIMT ≥0.75 mm (n=5)
CIMT <0.75 mm (n=117)
CIMT ≥0.75 mm (n=155)
CIMT <0.75 mm (n=47)
CIMT ≥0.75 mm (n=53)

Legend

Figure 1

CONSORT flowchart for the Early versus Late Intervention Trial with Estradiol summarizing the recruitment and randomization process.

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SUPPLEMENTAL MATERIAL

Primary endpoint: Acquisition of ultrasound images and measurement of carotid artery intima-media thickness (CIMT) were conducted with standardized procedures and technology specifically developed for longitudinal measurements of atherosclerosis[1-10] (Patents 2005, 2006, 2011). In brief, high resolution Bmode ultrasound carotid artery images were acquired with a Siemens Acuson CV70 (Mountain View, California) ultrasound imaging system using a linear array 7.5 MHz transducer. Single lead electrocardiogram (ECG) and ultrasound images were simultaneously recorded. The jugular vein and carotid artery were imaged transversely and longitudinally with the former vessel stacked above the latter. All images contained internal anatomical landmarks for reproducing probe angulation. The baseline image for each individual was used as an online guide for follow-up examinations on a split-screen system designed for repeat image acquisition for longitudinal studies. For each individual, depth of field, gain, ultrasound input power, dynamic range, monitor intensity setting and all other instrumentation settings used at baseline examination were maintained for all follow-up examinations. This establishes standardization for instrument setup encompassing the full dynamic range of the ultrasound echo across all examinations within the same participant. These standardized procedures result in reproducible imaging and processing of the same portion of the arterial wall at each examination necessary for accurately tracking atherosclerosis change[1-8]. Arterial dimensions including the far wall CIMT was measured at sub-pixel resolution using automated computerized edge detection software[7,8] (Patents 2005, 2006, 2011). CIMT was determined as the average of 70 to 100 measurements between the intima-lumen and media-adventitia interfaces along a 1 cm length just proximal to the carotid artery bulb at the same point of the cardiac cycle. This method standardizes the location and the distance over which CIMT is measured, ensuring comparability within and across participants[7,8]. This CIMT method is correlated with the change in coronary artery disease assessed by quantitative coronary angiography[9] and is predictive of clinical coronary events[10]. The coefficient of variation of repeated CIMT measurements is typically <3% and often approaches 1%. Other ultrasound imaging end points that will be available for further analysis include arterial stiffness, plaque quantification and analysis and qualitative assessment of the arterial wall and plaques (e.g., gray-scale median).

Secondary vascular endpoints: Subclinical coronary artery atherosclerosis was assessed with cardiac computed tomography (CT) scanning for coronary artery calcium (CAC) and cardiac computed tomography angiography (CCTA) for measurement of coronary artery stenoses. In brief, using a dedicated cardiac GE 64 slice multi-detector computed tomography (64 MDCT) scanner, participants underwent sequential non-contrast CAC followed by contrast CCTA scans in a single session. A Field of View (FOV) of 25 cm was adjusted to include the heart from below the carina to just below the diaphragm. A non-contrast CT CAC scan was performed using prospective ECG gating at 70-80% of the R-R interval according to standardized procedures[11] with the following CT imaging parameters: tube voltage = 120 killivolts (kV); tube current = 150 milliamperes (mA); gantry rotation speed = 0.35 seconds; slice thickness = 2.5 mm; rows = 64; range = 128-160 mm. CCTA was performed using prospective ECG gating at 70-80% of the R-R interval with the following CT parameters[12]: tube voltage = 100 kV for subjects who weigh <85 kg and 120 kV for subjects >85 kg, tube current = 300-600 mA (based on body habitus), gantry rotation speed = 0.35 seconds, slice thickness = 0.5-0.625 mm, rows = 64, range = 128-160 mm. CCTA was performed during a 5 ml/sec intravenous iodinated contrast infusion. Participants were given sublingual nitroglycerin 1 minute prior to initiation of CCTA to improve epicardial vasodilation and a β-blocker to maintain heart rate between 50 to 70 beats per minute, if required.

CAC was calculated from the non-contrast CT images using standard methods as previously described[11]. CAC was defined as a plaque of at least 3 contiguous pixels (area 1.02 mm^2) with a density of >130 HU. The lesion score was calculated by multiplying the lesion area by a density factor derived from the maximal HU within this area[13]. A total CAC score was determined by summing the individual lesion scores from each of 4 anatomic sites (right coronary, left main, left anterior descending, left circumflex).

Thin CT sections (0.5-0.625 mm) were transferred to a dedicated workstation (GE Advantage Workstation 4.4, GE Healthcare, Milwaukee, Wisconsin) for CCTA image analysis. Curved maximum intensity projection (MIP) was performed for each coronary artery segment at the end-diastolic frame or the frame with the least motion artifact. Based on the curved MIP, areas of abnormalities were identified and the points of minimum luminal diameter within the abnormal

areas determined. Multi-planar reformatting was also used to generate cross-sectional images of coronary segments using semi-automated software (GE Advantage Workstation 4.4, GE Healthcare, Milwaukee, Wisconsin). This yields a vessel centerline using the full-width-half-maximum standard method to delineate the contrast-filled vessel as well as automatic maximum and minimum diameters at any cross-section along the vessel centerline. A cross-sectional 5 mm MIP image was reconstructed and semi-automatic software used to outline the intimal surface providing cross-sectional vessel area. In cases where coronary segments were normal, the most proximal cross-sectional image was used for analysis. CT values were measured in the most visible images such as axial source or multi-planar images of the long axis at each site of the coronary arteries. All data sets were evaluated based on the modified 15-segment model of the American Heart Association[14]. The right coronary artery was defined to include segments 1-4, the left main artery and the left anterior descending artery to include segments 5-10, and the left circumflex artery to include segments 11-15. The intermediate artery was designated as segment 16 if present. Side branches were used as anatomical landmarks to define coronary segments.

The degree of coronary artery stenosis was assessed by using axial images, multi-planar reconstructions and curved MIPs to assess the degree of luminal narrowing in all assessable coronary segments. Segments with stenosis 1-25% diameter narrowing were defined as having minimal stenosis, 26%-50% diameter narrowing was defined as mild stenosis, 51-75% diameter narrowing was defined as moderate stenosis and those with >75% diameter narrowing was defined as severe stenosis. Percent diameter stenosis and not area stenosis was determined as the spatial resolution of CCTA cannot achieve the precision of quantitative coronary angiography. The most narrowed diameter in each segment was determined even if the plaque was eccentric. A segment stenosis score was generated based on the degree of underlying stenotic disease in each segment (0=no plaque, 1=1-25% stenosis, 2=26-50% stenosis, 3=51-75% stenosis, 4=>75% stenosis). The extent scores of all 15 individual segments were summed to yield a Total Stenosis Score (TSS) ranging from 0 to 60. In addition, each coronary territory (right coronary artery, left main, left anterior descending and left circumflex artery) was also scored according to presence of most significant lesion.

Plaque quantification was determined by manually tracing the plaque area per slice in all affected coronary segments. The plaque area of each coronary plaque visualized in at least 2 adjacent slices (reconstructed slice thickness 0.6 mm) was determined on all affected slices. The total plaque per segment was summed. A semi-quantitative plaque score previously utilized will be applied in each subject[15]. Each plaque was multiplied by 1 for small plaque, 2 for medium plaque and 3 for large plaque. A small plaque was defined as <1 mm in diameter perpendicular to the artery, medium as 1-2 mm in diameter and large as >2 mm. Total Plaque Score (TPS) was determined by summing the number of evaluable coronary segments with individual plaque scores (maximum plaque score = 45 [score of 3 for all 15 segments]). Because of difficulty making measurements from vessels <1.5 mm in diameter, we used both a 'raw' score (total of 45) and a percent plaque score that normalizes the plaque burden if the total number of measurable segments varies from subject to subject. The Percent Plaque Score (%PS) was calculated as follows: (number of segments with plaque / total number of assessable segments) x 100.

Composition of coronary atherosclerotic plaque was evaluated from both axial source images and multi-planar reconstruction images of the long axis at each site of the coronary arteries. Each coronary segment was classified as either normal (no plaque), containing non-calcified plaque, containing mixed plaque with either predominantly non-calcified plaque (<50% of plaque area occupied by calcium) or containing calcified plaque (>50% of plaque area occupied by calcium)[16]. Calcified atherosclerotic plaque was defined as any discernible structure that: 1) had a CT density greater than the contrast-enhanced lumen; 2) was clearly assignable to the coronary artery wall; and, 3) was identified in at least 2 independent planes[17]. Non-calcified atherosclerotic plaque was defined as any discernible structure that: 1) had a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue; 2) was clearly assignable to the coronary artery wall; and, 3) was identified in at least 2 independent planes[17].

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