PROTOCOL

**WOMEN'S ANGIOGRAPHIC VITAMIN AND ESTROGEN
(WAVE)**

June 14, 1997

CONFIDENTIAL

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1. OBJECTIVE

The WAVE trial is a factorial randomized double masked controlled clinical trial. The trial will randomize women to active or placebo hormone replacement therapy (estrogen and, when appropriate, estrogen with progesterone), and to active or placebo vitamin combination (1000mg/day of vitamin C and 800 IU/day of vitamin E). For each patient, the minimum lesion diameter (MLD), i.e., the minimum lumen diameter in a lesion, will be measured for all segments containing a lesion either at baseline or at follow up. The change in MLD between baseline and follow-up will then be computed for all such segments. The primary outcome for each patient is the time adjusted average of the changes in MLDs from baseline to follow- up.

The primary null hypotheses of the trial are:

a) The average change in MLD of women receiving hormone replacement therapy will not differ significantly from that of women receiving placebo.

b) The average change in MLD of women receiving antioxidant therapy will not differ significantly from that of women receiving placebo.

2. BACKGROUND

2.1 INTRODUCTION

Cardiovascular disease is the leading cause of death among women in the United States; more women die from cardiovascular disease than from all other causes combined (1). Coronary disease in women differs from men in several important aspects: lipid profiles and their associated cardiovascular risk in women differ from those in men (2); cigarette smoking may convey even greater risk in white women than in white men; increased risk of cardiovascular disease after menopause and the potential role of estrogen replacement therapy in reducing this risk is specific to women. Although traditional cardiovascular risk factors are generally predictive in women as well as men, the percent of women included in risk factor modification studies has been substantially less than men.

The Multiple Risk Factor Intervention Trial, which assessed the value of modifying hypercholesterolemia, hypertension and cigarette smoking on cardiovascular death, recruited only men (5). Large, masked, lipid-lowering drug trials such as the Lipid Research Clinics Coronary Primary Prevention Trial (6), the WHO cooperative trial (7), the Helsinki Heart Study (8) the Coronary Drug Project (9) and the West of Scotland Primary Prevention trial, included only men. Insufficient numbers of women have been enrolled in angiographic trials to determine whether they derive the same benefit from cholesterol-lowering as do men (Table 1).

In addition to gender differences in cardiovascular risk, substantial differences exist within the population of women with coronary disease. African American women from 35 to 74 years have a higher likelihood of coronary death than white women (1). Risk factor profiles also differ; African American women were more likely to have hypertension and diabetes, whereas white women had higher lipid levels and were more likely to smoke cigarettes in the Charleston Heart Study (3). Other studies have reported

a variety of similarities and differences in cardiovascular risk profiles in African American and white women.

Coronary disease is *the* most important health problem in postmenopausal women in the United States. Coronary disease in women differs from that in men, and women have been systematically excluded or under-represented in clinical trials in cardiology. Coronary disease is an even greater health problem among African American women than among white women. These considerations provide a compelling rationale for an angiographic trial in women which includes a significant proportion of ethnic minorities.

Table 1. Men and Women in Coronary Angiographic Trials
of Cholesterol Lowering or Risk Reduction

	Men	Women
Cholesterol Lowering		
NHLBI Type II (10)	94	22
CLAS (11)	162	0
FATS (12)	120	0
SCOR (13)	31	41
STARS (14)	74	0
MARS (15)	226	20
CCAIT (16)	245	54
HARP (17)	70	9
POSCH (18)	760	78
PLAC-1 (19)	326	82
MAAS (20)	336	45
REGRESS (21)	890	0
Post-CABG (140)	1243	108
CIS (22)	254	0
LARS (23)	22	15
Sub Total	4853	474
Risk Reduction		
Lifestyle (24)	36	5
Heidelberg (25)	92	0
SCRIP (26)	207	39
Sub Total	335	44
Total	5788	518

2.2 ESTROGEN AND CARDIOVASCULAR PROTECTIONEstrogen and Cardiovascular Protection

The Framingham Study identified a striking increase in coronary heart disease among post- compared to pre-menopausal women (4). In contrast, U.S. national health statistics have not shown a change in the rate of death from cardiovascular disease following menopause (1), suggesting that menopause per se does not increase cardiovascular risk. Data from the Nurses' Health Study indicated that, whereas natural menopause did not increase the risk of coronary heart disease, bilateral oophorectomy did increase cardiovascular risk (27). Although the relationship between menopause and development of cardiovascular disease remains to be established, the relationship between hormone replacement therapy and cardiovascular disease has been the focus of clinical investigation for the past 25 years.

In 1965 the first major trial of coronary pharmacologic intervention, the Coronary Drug Project, randomized over 8000 men with prior myocardial infarction to five drugs, including oral conjugated estrogen, 2.5 or 5 mg daily or placebo; an increase in the risk of non-fatal myocardial infarction and thromboembolism was detected among recipients of the higher estrogen dose (28). Lung cancer was subsequently reported to be more common in those receiving the lower estrogen dose than in placebo recipients (29). Although these adverse outcomes may have resulted from the very high doses rather than a gender specific toxicity, enthusiasm for exogenous estrogen therapy in men has subsequently waned. Since 1970, a number of large cohort and case-control studies (30 - 48) and one randomized trial (49) have examined estrogen's role in prevention of cardiovascular disease among postmenopausal women. Meta-analyses of these studies have reported the relative risk for coronary heart disease in women who have ever used estrogen compared to those who never used estrogen as 0.65 (50), 0.55 (51), and 0.58 (52).

Among women with angiographically-confirmed coronary artery disease, the protective benefit of estrogen appeared even more striking with relative risks of 0.37-0.5 in women who ever used compared to those who never took estrogen (53, 54, 55). In one

such recent study, women with critical coronary disease were less likely to have ever used estrogen than were women with non-critical coronary disease (OR 0.35). Of the 1178 women with ≥70% reduction in luminal diameter of one or more coronary arteries, only 60% of women who had never used estrogen (n=1108) were alive after ten years, compared with 97% (p=0.007) of women who had used estrogen (n=70). Among 644 women with less severe coronary stenoses, ten-year survival among nonusers of estrogen was 85% compared with 96% survival among estrogen users (p=0.27) (42). Although these studies suggest a possible protective role for hormone replacement, the shortcomings of observational studies underscores the importance of a randomized, placebo-controlled trial to determine the risk vs. benefit of hormone replacement in postmenopausal women.

Resistance to estrogen replacement among postmenopausal women and their physicians is attributable to the increased risk of endometrial hyperplasia and cancer (56, 57) and possible augmented risk of breast cancer associated with estrogen use. Some epidemiologic studies have identified an association between estrogen replacement therapy and breast cancer, whereas others have not (58, 59, 60).

Concern about endometrial cancer, which is clearly associated with estrogen use, has led to the widespread practice of administering progestins in conjunction with estrogen to limit this risk (61). Progestins attenuate the favorable effect of estrogens on blood lipids (62); it is not known whether the cardiovascular protective benefits of estrogen are similarly attenuated. Evidence from female Cynomolgus monkeys suggests that treatment with estrogen plus a progestin is as effective as estrogen alone for prevention of low density lipoprotein accumulation in coronary arteries (63, 64). Epidemiologic comparison of estrogen alone to estrogen plus progestin includes a prospective Scandinavian cohort study which reported a relative risk of MI of 0.69 among women taking estrogen alone compared with non-users and a relative risk of 0.53 in women taking estrogen plus progestin (65). The sole randomized, placebo-controlled trial in 168 women observed a 70% reduction in the risk of myocardial infarction during 10 year follow up in women taking conjugated estrogen (2.5 mg/day) plus

medroxyprogesterone acetate (10 mg/day) for seven days per month (44). This conclusion was drawn, however, from only four myocardial infarctions.

Several current studies will remedy, at least in part, our dearth of knowledge regarding the clinical role of hormone replacement therapy for cardiovascular prophylaxis. The Heart & Estrogen/Progestin Replacement Study, a placebo-controlled, randomized study, will determine the impact of continuous low dose estrogen plus progestin on clinical endpoints in women with angiographically-documented coronary disease. The Women's Health Initiative includes a randomized comparison of hormone replacement therapy for primary cardiovascular protection. The WAVE trial proposed in this protocol will complement these two studies which have clinical endpoints.

2.3 ESTROGEN – MECHANISMS OF ACTION

The mechanisms underlying estrogen's putative protection remain a topic of debate, as the proposed benefit does not appear to be solely, or even predominantly attributable to favorable effects on plasma lipids (66). Other proposed mechanisms include antioxidant effects, modulation of vasoreactivity (67, 68), the coagulation system (69) and growth factors (70).

2.3.1 Lipid Effects

Estrogen has a number of effects on plasma lipids in women including increasing high density lipoprotein (HDL), apoA-1 and very low density lipoprotein (VLDL)-triglycerides, and reducing low density lipoprotein (LDL) and Lp(a) (57, 71, 72).

In women, low HDL cholesterol is an even more striking predictor of coronary heart disease than in men (73). The rise in plasma HDL observed in women taking estrogen is primarily due to an increase in particles containing only apoA-I, as opposed to those containing apoA-I and apoA-II. ApoA-I only particles stimulate cholesterol efflux from peripheral cells and are inversely correlated with coronary disease in men (74).

Increased plasma triglyceride concentrations predict coronary disease in women (75); the relative risk is increased by concomitant low HDL cholesterol and/or elevated plasma apoB. The high triglyceride/low HDL/high apoB phenotype often reflects an atherogenic dyslipidemia such as hyperapoB, the atherogenic lipoprotein phenotype associated with LDL subclass pattern B, familial combined dyslipidemia, syndrome X or familial dyslipidemic hypertension. The increase in VLDL triglycerides appears to result from an increase in the ostensibly less atherogenic large VLDL compared to more atherogenic small VLDL species. This may explain the apparent dichotomy between estrogen's apparent anti-atherogenic effect and the increase it induces in triglyceride concentrations.

Lp(a) predicts cardiovascular risk in women as well as in men (76). Individuals with elevated plasma Lp(a) have impaired coronary vasomotor responses to acetylcholine, which may contribute to the increased coronary risk associated with Lp(a) (77). Estrogen reduces plasma Lp(a) concentrations, although this effect also appears to be attenuated by progestins (78). The extent to which progestins attenuate estrogen's effects on HDL and Lp(a) parallels the androgenic potency of the progestogen (79).

2.3.2 Antioxidant Effects

Vascular endothelial injury has long been proposed as the starting point in formation of atherosclerotic plaque (80). The earliest lesion of atherosclerosis, the fatty streak, is characterized by foam cells laden with cholesterol esters accumulating beneath the endothelium. The monocytes that become foam cells adhere to and penetrate damaged endothelium (81, 82, 83). Oxidized, but not native, LDL is a potent chemoattractant for circulating monocytes, contributes to cell injury, stimulates transformation of monocytes to foam cells and is taken up by monocytes (84). Evidence for the role of oxidized low density lipoprotein in human atherogenesis includes demonstration of elevated lipid-peroxide levels in patients with cardiovascular disease (85, 86) and identification of oxidatively modified LDL in aortic lesions (87, 88). Seventeen-beta estradiol inhibits LDL oxidation and cholesterol ester formation in cultured macrophages (89) and protects

cultured endothelial cells from the cytotoxicity of oxidized LDL (90). Estrogen also increases hepatic 7-alpha-hydroxylase mRNA and activity in primates (91, 92).

Although oxidized LDL has received the lion's share of recent scientific attention, HDL oxidation now also appears to play an important role in atherogenesis. Native HDL protects LDL from oxidation (93), impedes monocyte chemotaxis (94) and mediates reverse cholesterol transport in which cholesterol is removed from the vessel wall and returned to the liver (95). Estrogen protects HDL to a much greater extent than LDL. Progesterone, testosterone and androstenedione do not appear to affect lipoprotein oxidation. Banka estimates that in the presence of HDL, LDL oxidation in culture is reduced 25%; in the presence of HDL+ estrogen, LDL oxidation is reduced by 50%.

2.3.3 Vasoreactivity

Estrogen modulates vascular reactivity in ovariectomized Cynomolgus monkeys (96) and in women (97, 98). Proposed mediators of estrogen's effect on vascular tone include nitric oxide (99, 100) and cyclic GMP (101). Both acute and chronic estrogen appear effective; co-administration of progestin has yielded conflicting results. The causal link between abnormal coronary vasoreactivity and atherosclerosis remains to be established.

2.3.4 Coagulation System

Elevated plasma fibrinogen and factor VII levels are associated with cardiovascular disease (102). In the Atherosclerosis Risk in Communities Study, women taking estrogen, with or without progestin, had lower fibrinogen levels than non-users. Women taking estrogen had higher factor VII levels, than non-users, but this elevation was attenuated in women taking estrogen + progestin (103). Reports of estrogen's impact on other components of the coagulation system have been inconsistent, possibly because of interactions between hormone replacement and other thrombotic stimuli such as smoking, and to estrogen's effect on triglycerides, which affect expression, availability and activity of a number of clotting factors.

2.4 ANTIOXIDANTS AND CARDIOVASCULAR DISEASEAntioxidants and Cardiovascular Disease

Antioxidants inhibit production of oxidized LDL (104) and, thus, are candidate anti-atherosclerotic agents. In mammals, tocopherols are secreted by the liver as constituents of VLDL (105, 106), subsequently appearing in low density and high density lipoproteins as a consequence of VLDL catabolism (107). In general, plasma vitamin E levels increase with total lipid content and about half the vitamin E is associated with LDL (108). Each LDL particle contains about 6 vitamin E molecules and smaller amounts of beta-carotene, lycopenes, retinyl esters and about 1000 molecules of polyunsaturated fatty acid (109, 110). Vitamin C in the surrounding medium initially provides concentration-dependent protection against oxidation, regenerating reduced tocopherols (111). Following ascorbate depletion, tocopherols are lost, then lycopenes, retinyl esters and carotenes are depleted at lower rates (112-116). Once the antioxidants are depleted, unsaturated bonds of C18:2 and C20:4 are peroxidized, leading to formation of lipid peroxides, malondialde-hyde and lipid-soluble aldehydes.

Several lines of evidence support a protective role for vitamin E against oxidative damage. Vitamin E attenuates endothelial damage in vitro following exposure to neutrophil-generated hydrogen peroxide (117), and inhibits production and release of partially reduced oxygen from activated macrophages (118, 119). Vitamin E deficiency enhances the susceptibility of mammalian heart membranes to oxidative damage during subsequent in vitro exposure to free radicals (120, 121). In humans, vitamins E and C, individually and in combination, reduce lipid peroxidation (122, 123, 124).

Demonstration of anti-atherosclerotic benefit has been more difficult than demonstration of effects on lipid oxidation. Early, small studies suggested vitamin E was effective in treatment of symptomatic peripheral vascular disease (125, 126, 127). A combination of selenium and vitamin E appeared to prevent coronary atherosclerosis in rabbits fed a high fat diet (128), but this finding has not been reproduced in man.

Several European epidemiologic studies have suggested a relationship between dietary consumption of antioxidants and coronary artery disease. In one analysis, blood levels of vitamin E in men from 16 different study populations were inversely correlated with mortality from ischemic heart disease. Stepwise regression analysis indicated that mortality was predictable to 62% by lipid-standardized vitamin E and to 83% when vitamin A was included (129). In another European study, dietary intake of beta-carotene and vitamins C and E was assessed in Scottish men and women with coronary disease and in controls without coronary disease. Men without heart disease were more likely to have high dietary intake of beta-carotene and vitamins C and E; no such significant relationship was apparent in women (130).

Two large prospective cohort studies in the United States have evaluated the relationship between antioxidant consumption and coronary disease. The Nurses' Health Study reported a reduced risk of coronary heart disease in women with comparatively high consumption of vitamin E and beta-carotene (131, 132). Vitamin C consumption did not appear to confer protection. Antioxidant vitamins appeared to provide secondary cardiovascular protection among women with prior coronary disease as well (133). The Health Professionals Follow up Study, which included only men, also reported reduced frequency of coronary disease among individuals with higher vitamin E consumption. Carotene intake was inversely associated with coronary disease among smokers (134); vitamin C was not associated with reduced risk of coronary disease. A smaller cohort study of elderly Massachusetts residents (135) identified a 45% reduction in the risk of cardiovascular death among subjects with high beta-carotene intake (95% confidence interval 0.34-0.77, p=0.004).

One large randomized trial of antioxidant supplementation for coronary prevention has been completed. The antioxidant arm of the Physician's Health Study, a randomized trial of beta carotene (50 mg qod) in men, recently reported no difference between treatment groups in the incidence of lung cancer, all cancer or cardiovascular death (136). Trials of antioxidants in women are ongoing. The Women's Health Study (135) (WHS) is assessing beta-carotene and vitamin E as well as aspirin for primary cardiovascular and cancer prophylaxis. The Women's Antioxidant and Cardiovascular

Study (WACS) is also in progress, assessing vitamin E, vitamin C and beta carotene for secondary prevention in 8000 female health professionals.

A widespread perception that antioxidant vitamins were safe was recently challenged in a report from the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, which reported findings among 29,133 male Finnish smokers followed for 5-8 years (137). Subjects were randomized to Alpha-tocopherol, 50 mg/day, beta-carotene, 20 mg/day, or placebo in a factorial design. Excess lung cancer was observed among beta-carotene recipients, an 18% difference by the end of the study (95% confidence interval, 3-36%, p=0.01). This group also had slightly more prostate and stomach cancer, more death from ischemic heart disease and stroke. Total mortality was higher among beta-carotene recipients (95% confidence interval 1-16%, p=0.02). The enhanced risk of lung cancer was subsequently confirmed in the beta Carotene and Retinol Efficacy Trial (CARET) (138) which randomized 18,314 smokers, former smokers and asbestos workers to beta carotene (30 mg) + vitamin A (25,000 IU) or placebo. After mean follow up of 4 years, the relative risk of lung cancer in active drug recipients was 1.28 (95% CI 1.04-1.57, p=0.02); relative risk of cardiovascular death was 1.26 (0.99-1.61). These two studies underscore the importance of controlled clinical trials, such as WAVE, to evaluate the efficacy and safety of other antioxidant interventions.

2.5 *WHY AN ANGIOGRAPHIC TRIAL* **Why an Angiographic Trial?**

Regression studies with control groups ranging in size from 13 to 333 subjects have demonstrated atherosclerotic progression rates of 24-92% during follow up periods of 1-10 years (10-21, 139). Mean age in these studies was about 50 years, quite young compared to what might be expected in a group of postmenopausal women. Participants in past studies had an array of coronary risk factors and documented coronary disease, as will subjects in WAVE. Previous studies (159-161) demonstrate the relationship of angiographic assessed progression of atherosclerosis with the risk of future clinical events. Concordance of angiographic trials and those with clinical endpoints is supported

by studies demonstrating efficacy of lipid-lowering drugs for cardiovascular prevention. The results of WAVE will be analyzed in relation to those of ongoing hormone replacement and antioxidant trials with clinical endpoints.

3. THE STUDY COHORT

3.1 PATIENT ELIGIBILITY

The eligibility and exclusion criteria are as broad as possible to increase the generalizability of the results while maintaining patients' safety and the scientific integrity of the trial. There is no upper or lower age limit for participants, however, participants must meet all inclusion criteria and have none of the exclusion criteria. All potential participants with any single exclusion criteria will be excluded. However, such patients may be reconsidered for eligibility at a later time (for example, if a patient had an MI less than four weeks prior to randomization, they may be reconsidered at a later time). However, all of the trial's inclusion and exclusion criteria must be reevaluated when they are reconsidered.

3.2 INCLUSION CRITERIA

To be eligible for randomization, women must:

1. Be postmenopausal by one of the following criteria:

    a. Have had a bilateral oophorectomy at any age,

    b. Be $\geq$ 55 years of age (or have an FSH $\geq$ 40, if under age 55)

    c. < age 55 and FSH $\geq$ 40

    Women under 45 years of age without a bilateral oophorectomy, or FSH $\geq$ 40 are excluded.

2. Have a WAVE protocol angiogram performed within four months of randomization, while hemodynamically stable, demonstrating at least one vessel segment with a 15-75 percent stenosis. The qualifying artery may not have been intervened (dilated, atherectomized or stented). If the angiogram was performed within 2 weeks of a myocardial infarction, the qualifying segment may not be the infarct segment.

3. Provide signed informed consent.

3.3 EXCLUSION CRITERIA

1. Concurrent hormone replacement therapy (HRT) which will not be terminated. If on HRT for at least three months when screened, a washout period of 3 months is advisable. Women using estrogen vaginal cream are eligible if they use it no more than 25 percent of the time.

2. Concurrent vitamin supplements exceeding the RDA for vitamins C (> 60mg/day) and/or E (> 30 IU/day), which the patient is unwilling to stop. Women taking vitamins will be offered the alternative of stopping and taking Centrum Silver, provided by the WAVE trial.

3. History of breast cancer or mammogram suggestive of cancer without a subsequent negative biopsy.

4. History of endometrial carcinoma without subsequent hysterectomy. However, a woman with stage I endometrial cancer treated at least five years prior to randomization is eligible.

5. Any abnormal uterine bleeding or any endometrial hyperplasia at baseline. Women may become eligible after evaluation and treatment.

6. Abnormal Pap smear. Women with atypical squamous cells of uncertain significance should be evaluated per standard of care, and are eligible. Women with dysplasia of grade CIN-I or greater are ineligible until treated.

7. Uncontrolled diabetes mellitus or hypertension.

8. A myocardial infarction less than 4 weeks prior to randomization.

9. Planned or prior coronary artery bypass grafting.

10. Most recently documented fasting triglycerides >500 mg/dL, $\leq$ four months prior to randomization.

11. Creatinine>2.0 mg/dL.

12. Symptomatic gallstones.

13. NYHA class IV congestive heart failure or known ejection fraction less than 25%.

14. History of hemorrhagic stroke or bleeding diathesis.

15. History of pulmonary embolism, or idiopathic deep venous thrombosis (DVT).

16. History of osteoporosis, unless it is being treated with non-hormonal therapy.

17. Anticipated survival less than three years.

18. Concurrent participation in another masked clinical trial. Participation in an interventional device trial or short term post-PTCA anti-thrombotic trial is permitted so long as follow-up angiography is not part of that trial. If angiography is required, that study's exit angiogram can serve for evaluation as the qualifying angiogram for WAVE.

19. Angiogram not meeting protocol criteria, as judged by the WAVE Central Angiography Laboratory.

20. Unlikely to adhere to the protocol in the opinion of the investigator.

4. TRIAL INTERVENTIONS

Each woman randomized into the WAVE trial will receive either ether active or placebo hormone replacement therapy (HRT) and either active or placebo antioxidant supplement.

4.1 SELECTION OF HORMONE REPLACEMENT THERAPY

The selection of the HRT for the WAVE trial was guided by the following considerations: the medications selected should be well studied; approved for use in the United States; the safety concerns and side effects should be well known and it must be possible to address them as either an exclusion criteria or through careful post-randomization monitoring. There are currently limited data available comparing the cardiovascular effects of the various commercially available estrogen preparations. In the United States, the most commonly prescribed estrogen preparation is conjugated equine estrogen (CEE). CEE is currently being studied in the Women's Health Initiative (WHI), the Heart and Estrogen Progestin Replacement Study (HERS) and Estrogen Replacement and Atherosclerosis (ERA) trials. Using this preparation in the WAVE trial will allow for comparisons among some of the current major cardiovascular trials in women using HRT. Therefore the WAVE trial will use CEE (0.625 mg daily) as the estrogen component of its HRT.

Recent data in the Postmeopausal Estrogen/Progestin Interventions trial (PEPI) indicate that the use of unopposed estrogen in women with a uterus is associated with significant compliance problems and increased prevalence of side effects (152). However, in PEPI, the combination of CEE with a progestin significantly reduced the risk of endometrial hyperplasia. Therefore, in the WAVE trial women without a uterus will receive an unopposed CEE (Premarin), while women with a uterus will receive Prempro, a combination of a CEE and continuous progestin, 2.5 mg medroxyprogesterone acetate

(MPA). Premarin, Prempro and their respective placebos, will be supplied by Wyeth-Ayerst. Each will be provided as a single capsule containing either active or placebo medications.

Several considerations led to the selection of the progestin. Concern has been raised that the addition of a progestin may diminish the putative cardiovascular benefits of estrogen replacement therapy. Progestins alone tend to raise LDL and lower HDL cholesterol levels. In the PEPI trial, all hormone regimens lowered LDL cholesterol levels, but medroxyprogesterone acetate (MPA) significantly attenuated the estrogen related increase in HDL cholesterol levels. Oral micronized progesterone in doses of 1 and 2 mg appeared to have the least detrimental effect on plasma lipoprotein levels. However there is little safety information on the long-term use of continuous micronized progesterone. The Nurses Health Study data suggests that the addition of progestin does not appear to attenuate the cardioprotective effects of post menopausal estrogen use. The choice of MPA will also allow for it to be combined in a single capsule with the CEE selected. By reducing the number of daily capsules that each woman with a uterus will have to take, compliance in the HRT arm of the trial should be increased.

4.2 SELECTION OF ANTIOXIDANTS

A dose-ranging study to assess protection of LDL from oxidation suggested that the minimum dose of alpha tocopherol needed was 400 IU per day with further prolongation of the lag phase of LDL oxidation at 800 and 1200 IU (141). There was wide inter-individual variation at identical vitamin E doses. The correlations between plasma LDL, alpha-tocopherol and oxidation kinetics were approximately 0.5-0.6 for most parameters. A subgroup analysis from the Cholesterol Lowering Atherosclerosis Study relating angiographic progression of coronary lesions to in-trial vitamin E intake, suggested that intakes above 100 IU of vitamin E among the male study subjects were protective (142).

Vitamin C is a water-soluble essential vitamin with antioxidant properties and possible beneficial effects on coagulation and fibrinolytic factors (143). It spares vitamin E and may thus enhance the effects of vitamin E. Epidemiologic data are mixed, but some studies have reported a lower incidence of stroke (144) and a lower cardiovascular mortality with increased vitamin C intake (145, 146). The effect of Vitamin C on progression of coronary disease was not apparent in the study of Hodis (142).

Vitamin E is generally well tolerated, but prolongation of prothrombin time among vitamin K deficient patients has been documented and, in rare cases, it may result in clinical bleeding (147). A more recent study using 800 or 1200 IU in patients concurrently prescribed warfarin did not demonstrate any significant effects on coagulation parameters (148). Vitamin C also appears to be quite safe even at large doses (150). Although earlier studies suggested that high vitamin C intakes may predispose to nephrolithiasis by increasing oxalate concentration in the urine more recent studies suggest that the increased urine oxalate content is a measurement artifact (151). Consistent with this observation, an increase in renal stone formation was not apparent in the Health Professionals' Study at doses of vitamin C of 1500 mg and higher when compared to intakes of less than 250 mg (149).

Women will be randomized to an active anti-oxidant therapy consisting of Vitamins E and C or their matching placebos. The preparations will be provided as 400 IU capsules of vitamin E and 500 mg tablets of vitamin C, BID for a total daily dose of 800 IU vitamin E and 1000 mg vitamin C. The active and placebo vitamins will be supplied by BASF/Knoll.

5. MASKING

To promote uniform follow-up of all trial participants, all randomized women and clinical center personnel responsible for randomization and follow-up will, to the extent possible, remain masked to treatment assignments until the end of the trial. This will increase the probability that all women will receive similar concomitant treatments, testing and evaluation during the conduct of the trial. It is likely however that due to the occurrence of side effects, some women and/or clinical personnel will assume knowledge of one of their treatment assignments. Women will be instructed to not discuss gynecological issues with WAVE cardiology staff. Rather, each center will have a study gynecologist, or designated personnel to whom women can address concerns about side effects.

Each woman with a uterus will be asked to fill out a bleeding diary for the first six months following randomization. The diary will be returned to the gynecology staff. After review of the entries at the first six month visit, a decision by the OB/GYN physician will be made as to whether or not the diary may be discontinued.

Personnel at the central angiographic laboratory and the central biochemistry laboratory, will also be masked to treatment assignments. This will permit unbiased assessments of the trial's primary and secondary outcomes.

6. INFORMED CONSENT

Each Principal Investigator will be required to submit the WAVE protocol to their local IRB for approval. As part of this process, an appropriate informed consent form will be developed at each site. A copy of the IRB approval letter and a copy of the consent form approved by the local IRB will be forwarded to the NHLBI project office. No randomizations will be permitted prior to the receipt of these documents. The consent form will, at a minimum, meet the guidelines from the Code of Federal Regulations, part 46 – Protection of Human Subjects. A sample informed consent form is included in Appendix A. The SCC will send reminders for annual renewals of each center's IRB approval and will request that copies be sent to the NHLBI project office.

7. STUDY VISITS

7.1 SCREENING

It is anticipated that on average each of the five WAVE clinics will randomize 90 women. To accomplish this goal each coordinator will monitor the daily catheterization laboratory logs and other potential sources, to identify likely candidates for the trial. The coordinators will be free to design an identification program that is efficient for their local conditions. However, it is anticipated that the initial identification of potential WAVE participants will be by: reviewing logs of diagnostic catheterizations; pre- and post-intervention catheterizations; reviewing existing databases to identify women from other research programs. A goal of the WAVE trial is to have an appropriately ethnically diverse study cohort. To accomplish this, each site will develop a plan to recruit minority women. This will include educating cardiology fellows about the trial, recruiting nurse practitioners from clinics that serve minority populations, reviewing records from ongoing studies that are recruiting minority women, interacting with community leaders to provide information about the trial and development of recruitment and retention aids that are culturally sensitive. Prior to randomization, the clinics will submit their proposed screening procedures to the Study Coordinating Center (SCC), which will produce a summary of all the programs to be distributed to all five sites. Prior to randomizing any patient, a local investigator will contact the patient's physician. It is crucial to obtain the commitment and support of the patient's physician in assisting and maintaining compliance to the protocol for the duration of the trial.

The procedures to be followed and the data to be collected at the screening and follow-up visits are outlined below.

7.2 SCREENING VISIT

The study will be described to the potential participant and if she agrees to participate, an informed consent form should be reviewed and signed.

If creatinine and fasting triglycerides have been assessed within the past twelve months, no blood collection is necessary at this visit. If they are not available, the patient will be asked to fast for at least twelve hours prior to coming to clinic and a blood sample will be taken. This should be sent to the local laboratory for analysis of creatinine, triglycerides, FSH and INR, as necessary.

A general physical (including a mammogram, if none has been done in the last 12 months, a PAP smear and a pelvic examination) is to be completed. If a mammogram has been done within the past twelve months, the coordinator will obtain a medical release from the patient and obtain a copy of the results. For women participating in the Brachial Artery Reactivity Study (see appendix D) and the Effects of Hormone Replacement Therapy on Electrocardiographic and Scintigraphic Measures of Myocardial Ischemia Study (see appendix E) appropriate assessments will be performed. However, a woman who is otherwise eligible may refuse either or both of these studies and still be randomized into the WAVE trial.

Comment [TBC1]:

7.3 RANDOMIZATION

Women who have met all inclusion criteria and have none of the exclusion criteria, and who have signed the informed consent, will be scheduled for a randomization visit. Prior to the randomization visit all women who did not have a blood collection at the screening visit will be advised to fast for at least twelve hours prior to the visit. A blood sample will be collected for central analysis and a local sample for assessment of INR if needed. Data to be collected are indicated in Table 2.

Randomization to one of the four possible study groups (active HRT and placebo antioxidants, active HRT and active antioxidants, placebo HRT and active antioxidants, placebo HRT and placebo antioxidants) will be by a telephone call to the Study Coordinating Center. After confirmation of the patient's eligibility the SCC will issue a

unique patient identifier and medication codes that the clinical center coordinator will use to obtain the woman's study medications. The unique patient identifier will be used on all study forms, laboratory samples, angiograms and any other necessary study documents. Each woman will have a one in four chance of being assigned to each of the four study groups. Randomization will be stratified by clinical center (n=5) and by whether a hysterectomy has been performed. Each woman will be given a seven month supply of her study medications, a medication instruction sheet, a bleeding calendar for those women with a uterus, a bottle of Centrum Silver vitamins (if she requests it), and an appointment for the one month visit.

TABLE 2

WAVE Summary	Screening	Randomization	IM	3M	6M	12M	18M	24M	30M	36M	Exit
Consent	X										
Creat, trig, FSH (if needed)	X										
INR	Obtain at either visit		X								
Study Bloods	Obtain at either visit			X			X			X	
Ht/wt, BP, waist/hip	Obtain at either visit					X		X		X	
Randomization form		X									
Breast, pelvic, Pap, mamm, breast self-exam teaching	X					X		X		X	
Sign medical release	X					X		X		X	
Brachial reactivity	Obtain between consent and initiation of study medication			X							
Dipyridamole SPECT/ETT				X						X	
ECG		X				X				X	
Patient tracking form		X		Update as needed							
Bleeding calendar		X	X	X	X	prn	prn				
Dispense study meds		X			X	X	X	X	X		
Adherence assessment					X	X	X	X	X	X	X
Quality of Life		X		X			X		X		
Follow-up visit*					X	X	X	X	X	X	X
Angiogram	X										X
Physical exam		X				X		X		X	

*Includes: side effects, concurrent medications, internal medical history and adherence

7.4 SCHEDULED FOLLOW-UP

Following randomization all WAVE trial participants will be scheduled for visits at 1, 3, 6, 12, 18, 24, 30 and 36 months. In addition a woman who was randomized during the early months of recruitment, may require a 42 and possibly a 48 month visit. The actual number of visits will depend on the date of randomization and the occurrence of interim fatal or non-fatal events. The requirements of each visit are summarized in Table 2.

7.4.1 One Month Visit

The one month visit serves to reinforce the relationship between each randomized woman and the local WAVE clinical center staff, including her gynecological contact. Any initial side effects and management options will be discussed and agreed upon. No measure of adherence will be obtained at this visit. For women on Coumadin, an INR will be assessed locally and the three month follow-up visit will be scheduled.

7.4.2 Three Month Visit

The patient will be asked to fast for at least twelve hours before the visit and a study blood sample will be collected for central analysis. A designated individual will discuss any symptoms or side effects identified by the patient. For those women participating in either the brachial reactivity or SPECT/ETT study, the appropriate assessments will be performed and the six month visit scheduled. No measure of adherence will be collected.

7.4.3 Semi-Annual Visits (at 6, 18, 30 and, if necessary 42 months)

The participant will be asked to bring in all unused study medication and all other medications she has taken since the last study visit. For the 18 month visit, the participant will be asked to fast 12 hours before visit. During each semi-annual visit a designated individual will

review and discuss any symptoms potentially attributable to either study medication. The coordinator or his/her designee will:

1. Document any clinical outcomes that have occurred since the last visit and record current medications on a follow-up form;

2. Record study medication capsule/tablet count on a follow-up form; dispense a new supply of study medication and provide a new bleeding calendar, if appropriate;

3. At the 18 month visit only, draw serum for fasting total cholesterol, HDL cholesterol and triglycerides assays;

4. Schedule the next appointment and remind the participant to call with questions.

7.4.4 Annual Visits (at 12, 24, 36 months)

The participant will be asked to bring in all unused study medication and all other medications she has taken since the last study visit. At the 36 month visit, the participant will be asked to fast 12 hours before coming to the visit. During each annual visit, a designated individual will review and discuss any symptoms potentially attributable to either study medication. The coordinator or his/her designee will:

1. At the 36 month visit, draw fasting study bloods for processing and shipping to the central biochemistry laboratory. Feed participant if desired. At the 36 month visit (42 month for some women), obtain pre-angiogram bloods, if necessary;

2. Document any clinical outcomes that have occurred since the last visit and record current medications on a follow-up form;

3. Record study medication capsule/tablet count on follow-up form; dispense a new supply of study medication;

4. At the 12 and 36 month visits obtain an ECG;

5. Perform a breast examination, provide breast self-exam teaching, perform pelvic examination, obtain a Pap smear (if uterus present and none done within past 12 months) and complete the physical exam form;

6. Ask participant to sign a medical release. Make arrangements to obtain any mammogram report done within the past 12 months, or schedule a mammogram;

7. At the 36 month visit (42 month for some women), schedule the exit angiogram and the visit.

8. At the 36 month visit, perform appropriate assessments for patients in the Effects of Hormone Replacement Therapy on Electrocardiographic and Scintigraphic Measures of Myocardial Ischemia Study.

Comment [TBC2]:

7.4.5 Exit Visit

This visit will take place after the final angiogram of the patient. During the visit, the coordinator or his/her designee will:

1. Discuss results of angiogram.

2. Reinforce participant's valued contribution to the study. Discuss treatment options until the entire cohort is unmasked at the end of the trial.

3. Discuss the procedure for informing the participant and her designated physician of the trial's results.

7.4.6 Additional Visits

Additional visits will be scheduled as required by the patient's health. These visits are not considered study visits and this protocol does not mandate particular procedures or data collection for such visits.

8. MONITORING OF ADHERENCE AND SAFETY

8.1 MONITORING ADHERENCE

Starting at the 6 month visit, and at each subsequent study visit, a measure of adherence to the assigned treatment regimens will be collected. Women will be asked to return all unused study medications at each visit. The study coordinator will ask each woman if there have been any problems adhering to the medication schedule. The coordinator will count the number of returned capsules/tablets. This will be used to estimate adherence to the protocol. To discourage out-of-trial use of vitamins and improve protocol adherence, patients will be provided with a multivitamin (Centrum Silver), with a minimal antioxidant vitamin content (60 mg C, 45 IU E, 6000 IU VitA-carotene), supplied to the trial by BASF/Knoll.

In addition to the capsule/tablet counts, plasma collected at baseline and 36 month visits will be analyzed for vitamins C and E. This will allow an additional measure to correlate with the capsule/tablet count data.

8.2 MONITORING PATIENT SAFETY

The NHLBI will appoint an independent Data and Safety Monitoring Board (see Section 12.2.1) to monitor all aspects of the WAVE trial. The SCC will prepare reports summarizing all aspects of the WAVE trial, including: screening, randomization, baseline, follow-up, adherence, side effects, and outcomes. In addition, specific requests may be made for additional reports by the DSMB. The DSMB will convene approximately every six months, either by telephone conference call or in person.

8.3 MONITORING POTENTIAL ANTIOXIDANT THERAPY SIDE EFFECTS

At high doses, vitamin E may result in a bleeding diathesis through its interaction with vitamin K dependent clotting factors and its antiplatelet effects. To monitor the former, all women will have a prothrombin time (INR) assessment prior to randomization and at the one month visit. If the patient is on an anticoagulant, the INR results will be sent to her private physician so that the anticoagulant dose can be appropriately adjusted to maintain the desired INR range. If anticoagulant therapy is initiated after randomization, this procedure will also be followed.

There are no specific side effects of vitamin C requiring laboratory tests for monitoring.

Data will be collected to estimate and compare the frequency of: hemorrhagic stroke; episodes of major (requiring a transfusion) and minor bleeding of other sites; renal stones; episodes of nausea or gastrointestinal upset.

If a patient cannot tolerate full doses of the study antioxidant medications due to nausea or other gastrointestinal side effects, dosage will be transiently reduced to 50 percent of the planned daily dose. Once the side effects have resolved, an attempt should be made to resume full doses of the medications. If this is not tolerated, patients will be maintained at 50 percent of the planned daily dose for the remainder of the trial.

Vitamin E/placebo therapy will be stopped after the occurrence of a hemorrhagic stroke. In the case of a major bleed at another site vitamin E/placebo therapy may be stopped temporarily or permanently at the discretion of the local WAVE investigator. If other predisposing factors (e.g., peptic ulcer, adjustment of Coumadin dosing, etc.) develop, modification or temporary discontinuation of vitamin E/placebo should be considered.

8.4 MONITORING POTENTIAL HORMONE REPLACEMENT THERAPY SIDE EFFECTS

8.4.1 Symptomatic Management

Women will be instructed on palliative management of symptoms such as breast tenderness or bloating. The management of these side effects includes dietary changes, use of a mild diuretic for fluid retention, vaginal lubricants and reassurance. If these symptoms continue and are not tolerated, the dose of study hormone drug will be reduced in frequency to 5 days per week or less and then slowly increased to attempt to achieve the protocol dosing regimen.

8.4.2 Vaginal Bleeding

If bleeding occurs during the first six months post randomization, the patient will be managed by the following algorithm: a) If no endometrial aspiration or biopsy has been performed within the last twelve months, endometrial aspiration will be recommended for any bleeding. If the patient refuses and bleeding has stopped, she will continue on study drug and will be reassessed at the 6 month visit.   If she has had further bleeding, endometrial aspiration will again be recommended. If the patient still refuses, consider vaginal ultrasound to measure endometrial thickness. If this is ≥ 5 mm, the patient should proceed to endometrial aspiration and the study drug be discontinued if she refuses. If the endometrial thickness is < 5 mm, she may continue on study drug (consider getting the patient's signature on a form describing that ultrasound is less sensitive for determining endometrial pathology than aspiration); b) If endometrial aspiration has been performed within the past twelve months, and was benign, no further assessment is warranted for one year post aspiration. However, if bleeding is heavy (soaking 1 or more pads per 24 hours) during the first six months however, endometrial aspiration will be recommended even if performed within the past twelve months.

If endometrial biopsy has been performed, the following algorithm will be followed based on the results of the biopsy:

1. Normal atrophy, secretory or proliferative continue randomized therapy. If insufficient tissue is available, a second biopsy should be obtained and a vaginal ultrasound performed.

2. Simple or complex hyperplasia. Stop randomized hormone therapy, and treat with medroxyprogesterone 10-20 mg qd for 2-3 months followed by repeat biopsy. If the repeat biopsy is negative, study drug may be restarted and the patient should be scheduled for follow-up biopsy in one year. If the woman starts to bleed once the study drug is restarted, consider adding medroxyprogesterone 2.5 mg daily to the study drug for 3 to 4 months. The additional medroxyprogesterone should then be stopped and the presence of bleeding reassessed on study drug alone. If the biopsy shows hyperplasia, the study hormone replacement therapy should be permanently discontinued and the patient referred to a gynecologist for further management.

3. Atypia or cancer. Stop study hormone replacement therapy and refer the patient to her private gynecologist or internist.

8.4.3 Management of Abnormal Mammograms

Annual mammograms are required in this patient group. If the mammogram is abnormal and further evaluation can be performed within 30 days, the subject can continue on study HRT. If evaluation takes longer, the study HRT will be discontinued and restarted when evaluation proves negative for carcinoma. If the mammogram is abnormal but not suggestive of cancer, the patient will be continued on study HRT and the appropriate follow up mammograms obtained.

8.4.4 Decision to Stop Study Medication

The site study gynecologist, in consultation with the patient's physician will make decisions on whether to stop study medication because of a gynecological adverse event or an increased risk of deep venous thrombosis (i.e., need for prolonged bed rest due to a fracture,

prolonged illness). Unmasking the patient's treatment assignment is usually not necessary and should only be considered as a last resort.

9. QUALITY OF LIFE ASSESSMENT

9.1 Objective

The purpose of the quality of life assessment in the WAVE clinical trial is to monitor the qualitative consequences of hormone replacement therapy and/or antioxidant treatment in a group of postmenopausal women who have angiographically established coronary artery disease. The quality of life outcomes are not expected to be related to the primary outcome (change in MLD). They may, however, be related to mortality rate, and potential confounders such as treatment noncompliance, incidence of intercurrent catheterizations, and side effects of treatment.

9.2 Instruments

The following psychometrically established instruments will be administered to all patients at randomization and at the 18 and 30 month visits:

1. The Medical Outcome Study Short Form (MOS-36) (153)
2. The Center for Epidemiological Studies - Depression Scale (CES-D) (154)
3. The Seattle Angina Questionnaire (SAQ) (155)
4. The Duke Activity Status Index (DASI) (156)
5. The MOS - Sleep Questionnaire (157)

In addition, the SAQ and DASI, will also be administered at 3 months for those patients in the Effects of Hormone Replacement Therapy on Electrocardiographic and Scintigraphic Measures of Myocardial Ischemia Ancillary Study.

Comment [TBC3]:

9.3 Response Shift Analysis

A potential difficulty in analyzing quality of life outcomes is that the basic metric a patient uses to make quality of life judgments may be affected by time, by the treatment or by adverse events: a patient's concept of what constitutes a "good" quality of life may evolve in the course of the trial. For example, a patient answering questions about how much her health limits

her activities may have very different expectations at baseline and at 30 months. She may describe her activities as very limited at baseline but, having adapted her daily routines and lowered her expectations over time, may report only minor limitations at 30 months even if, objectively, her status has changed little.

Because the patients' responses to the quality of life questions are subjective, it is important to determine whether the criteria they use to make quality of life judgments remain approximately constant over the course of the trial. If they do not, simple comparisons between the baseline and the follow-up responses will be misleading. The following procedure will be used to assess the extent of the change over time in the basic metrics used by the patients to make quality of life judgments:

- at the 30 month visit, after the patient completes the MOS-36 form, she will be asked to fill it out again from the point of view of how she felt at the time of the baseline visit. For patients whose criteria have remained fairly stable, this retrospective assessment should agree fairly well with the responses on the MOS-36 completed at baseline. If there have been significant changes in the criteria, however, the answers on the retrospective versions of the MOS-36 would be expected to differ significantly from those on the baseline versions.

- The difference between the baseline and the 30 month responses will be assessed using a separate statistical test for each question on the MOS-36. The test will be performed on the entire cohort and then in each of the following subgroups: the patients in each treatment group, the patients who had adverse events, and the patients who did not have adverse events. Adjustments will be made for multiple comparisons.

This approach, which is commonly used in educational research, is called a response-shift analysis (158). If significant differences between the baseline and retrospective answers are found within particular quality of life domains, all further analyses for those domains on the MOS-36 will compare the 30 month results to the baseline and to the retrospective analyses. Otherwise, only comparisons between the 30 month and baseline responses will be reported.

10. STATISTICAL DESIGN AND ANALYSES

10.1 OVERVIEW OF STUDY DESIGN

The WAVE trial is a randomized double masked placebo controlled factorial clinical trial. The trial will randomize women to active or placebo HRT (estrogen or, when appropriate, estrogen with progesterone), and to active or placebo vitamin combination (1000mg/day of vitamin C and 800 IU/day of vitamin E). The trial will be conducted at five participating clinical centers and will randomize a total of 450 postmenopausal women with angiographically documented coronary artery disease. The primary objective of the WAVE trial is to determine whether hormone replacement or antioxidant therapy favorably affect coronary atherogenesis during approximately a three year study interval. Response to the treatments will be assessed by the mean, for each woman, of the changes in minimum lesion diameter (MLD), of study defined lesions, as estimated at baseline and at the exit angiogram, between the randomized treatment groups.   Two null hypotheses will be tested in this trial:

1. There is no difference in average change in mean lesion diameter per year
    between women who receive hormone replacement therapy and women who receive placebo.

2. There is no difference in average change in mean lesion diameter per year
    between women who receive antioxidants and women who receive placebo.

Although both hypotheses will be tested, no adjustment will be made for multiple comparisons because the trial is powered to test both hypotheses at the 0.05 level.

10.2 PRIMARY ENDPOINT

10.2.1 Definition of the Primary Endpoint

The primary endpoint will be evaluated in all segments containing 15 - 75% lesions noted on the entry angiogram or any new lesions $\geq$ 15% observed on a follow-up angiogram

within the study duration. These segments are referred to as the study endpoint segments. The change in MLD of each segment is divided by the time between the entry arteriogram and the final angiogram within the prespecified study follow-up-period. An exception will be made for intercurrent angiograms and revascularization procedures: these procedures will provide the concluding measurements for those segments that were intervened. For such segments, the change in MLD will be divided by the time between the baseline angiogram and the pre-procedure intercurrent angiogram. The study goal is to obtain a protocol angiogram within the last six months of the scheduled end of the trial. With recruitment anticipated to occur over a 12-15 month period, this implies an anticipated average follow-up of approximately 40.5 months. If the patient has had a coronary angiogram for clinical indications within six months of the planned study termination, the results from that arteriogram can be used in lieu of an exit angiogram. If the angiogram has occurred more than six months before the end of the trial an additional angiogram will be requested.

Patients eligible for entry into the study will have one or more segments containing 15 - 75% stenosis which have not been influenced by prior catheter interventions. These are referred to as qualifying segments and lesions. Segments identified on the entry as containing a 15 - 75% stenosis or on the concluding arteriogram(s) as containing any lesion ≥15% are considered endpoint segments. This definition automatically includes those segments which originally qualified the patient for study entry. Segments included in this analysis must be in the main coronary vessels or their major branches and have a reference diameter >1.5 mm. Segments or lesions progressing to 99 - 100% stenosis are automatically assigned a MLD of 0.0 mm. Non-visualized segments distal to total occlusions are considered lost to follow-up.

The 15 - 75% lesion determination is based on the mean of the most anatomically relevant proximal and distal reference margins that can be ascertained. These reference margins may extend proximally or distally beyond the margins of the segment, if the lesion/disease is extensive. Segments may range in length from 3 to 25mm.

Intercurrent angiograms are those performed for clinical, rather than protocol indications after the entry angiogram, but prior to the scheduled exit angiogram. Intercurrent angiograms performed immediately prior to any catheter based interventions will be assessed by the Central Angiographic Laboratory to identify whether either the original qualifying segments or new segments have been subject to manipulation that would alter their subsequent natural history. Intervened segments which contain a new stenosis (i.e. ≥15%) are included as endpoint segments. However, their exposure times are adjusted in accordance with the interval between the initial angiogram and the intercurrent angiogram.

10.2.2 Primary Analysis

The change in MLD will be computed for each of the patient's study endpoint segments **.** The follow up angiogram is not necessarily the same for all the segments in a patient: for segments that were revascularized prior to the concluding angiogram, the MLD will be read from the angiogram before the revascularization. For segments that were not revascularized before the concluding angiogram, the MLD will be read from the concluding angiogram. Each of the changes in MLD will then be divided by the time between the entry angiogram and the follow up angiogram. For patients who have revascularization procedures prior to the concluding angiogram, the changes in MLD of various segments may thus be divided by different quantities. These changes in MLD, divided by the appropriate quantities are then averaged for the patient. This average is the primary endpoint for the patient. A segment with a 1 mm change at the time of an intercurrent angiogram at 6 months will therefore have more weight in the average than another segment with a 1 mm change after 3 years. With the time adjustement described above, the primary endpoint is basically a measure of the average rate of change in MLD in diseased segments.

The primary endpoint in the treated and control groups will be compared using a nonparametric rank test based on the Van der Waerden scores. This test, which is similar to a Wilcoxon test, was selected for two reasons. First, for the sample sizes contemplated in this study, it has essentially the same power as the usual t-test under the assumption of normality and

better power than the t-test if the data are markedly non-normal. Second, it readily accommodates patients who had an MI or died by assigning them the worst rank. It also has better power than the Wilcoxon test under the assumption of normality. The primary analysis for the study will be conducted using the "intention to treat" paradigm, with all patients retained in their original randomization group, regardless of their level of compliance with their assigned treatments.

10.3 Secondary Analyses

10.3.1 By Patient Analyses

a) *By patient, change in the mean (rather than minimum) lesion diameter of all study endpoint segments*. The mean segment diameter extends over a segment length that is identical on the entry and exit angiogram.

b) *By patient, change in the mean diameter of non-disease containing segments* (i.e. segments that do not contain a lesion $\geq$ 15% on either the entry or exit angiogram). The mean segment diameter extends over a segment length that is identical on the entry and exit angiogram. Segments included in this analysis must be in the main coronary vessels or their major branches and at least 1.5 mm in diameter.

c) *By patient, analysis based on categorizing patients as exhibiting overall progression, regression, mixed change or no change*. To classify patients, the MLD of each study endpoint segment will be categorized as decreasing by > 0.4 mm (progression), increasing (regression) by > 0.4 mm or neither (no change). A patient will be categorized as exhibiting
   - overall progression if they have at least one segment progression without any segment regression,
   - overall regression if they have at least one segment regression without any segment progression,
   - mixed change if they have both progressing and regression segments, and
   - no change if none of their segments have progressed or regressed.

The analysis will be repeated using the mean (instead of the minimum) lesion diameter of study endpoint segments, and then for the mean lumen diameter of non-diseased segments

10.3.2 By Segment Analyses

(This provides the opportunity to include lesion, segment and vessel characteristics unique to each lesion and segment in multivariate analyses).

a) *By segment, change in MLD of all study endpoint segments.* The change is adjusted for the actual time interval between the entry arteriogram and the follow-up arteriogram(s).

b) *By segment, change in the mean (rather than minimum) lesion diameter of all endpoint segments.* The mean segment diameter extends over a segment length that is identical on the entry and exit angiogram.

c) *By segment, change in the mean diameters of all non-disease containing segments* (no lesion $\geq$ 15% on either the entry or exit angiogram). The mean segment diameter extends over a segment length that is identical on the entry and concluding angiogram. Segments included in this analysis must be in the main coronary vessels or their major branches and at least 1.5 mm in diameter.

d) *By segment, change in the mean diameters of quantitated segments.* This includes both study endpoint segments and quantitated segments with no lesion $\geq$ 15%. The segments included in this analysis must be in the main coronary vessels or their major branches, and must be at least 1.5 mm in diameter. The mean segment diameter extends over a segment length that is identical on the entry and concluding angiogram.

10.3.3 Additional Analyses

a) *Study of lesions >75%:* change in the, by patient, average of the MLD of all segments containing >75% stenosis on the entry angiogram. The change is adjusted for the actual time interval between the entry arteriogram and the follow-up arteriogram. Many of these segments may become subject to catheter based or surgical interventions during the course of the study.

b) *Study of post PTCA restenosis:* change in the MLD of any stenosis having catheter interventions from the immediate post PTCA procedure to the exit angiogram. All lesions that were treated within 6 weeks prior to or during the entry angiographic procedure are included in this analysis. However, there must be at least a 6-month, in study, interval between the interventional procedure and the follow-up angiogram which may be either a protocol mandated, concluding angiogram or a clinically indicated intercurrent angiogram. The primary analysis for this study will be based on the one lesion per patient that shows the greatest change from initial eligibility to follow-up. A second analysis will consider each PTCA treated lesion as an individual statistical entity. Both of these analyses will include the type of catheter-based intervention along with multiple other standard angiographic lesion characteristics as covariates.

10.4 SAMPLE SIZE CALCULATIONS

The sample size calculations are based on a change in mean MLD using a t-test. The antioxidant and HRT components are treated as separate trials (no interaction is assumed). No adjustment for multiple comparisons will be made.

The type 1 error rate a was 0.05 (2-tailed). Although a rank test will be used, given the sample sizes contemplated in the study, the power of the rank test is essentially the same as that of the t-test under the assumption of normality. The power calculations are based on the effect size, defined as the expected difference in outcome between treatment and control divided by the

standard deviation of the outcome (the outcome here is the change in mean MLD between baseline and follow-up). The effect sizes for four published studies are summarized below.

	STARS	FATS	SCRIP	MARS
	0.86	0.51	0.29	0.14

The weighted average of these effect sizes, weighing by the number of patients in each study is 0.334. With this effect size and a power of 0.90, the required total sample size is N=380. Assuming a dropout rate of 15%, 450 patients are needed. If a 20% dropout rate is assumed, the power of the test for various effect sizes is summarized in the table below:

	N=450	N=425	N=400	N=375	N=350
ES=0.30	0.81	0.79	0.77	0.74	0.71
ES=0.35	0.91	0.90	0.88	0.86	0.83
ES=0.40	0.97	0.96	0.95	0.93	0.92

Based on these considerations, 400 patients is considered the minimum acceptable sample size for the study. The target sample size for the study is 450 patients.

10.5 INTERIM ANALYSES

During the trial, the external Data and Safety Monitoring Board (DSMB) will meet periodically to review trial results. Since the primary outcome of most patients is not expected to be available until the last year of the study, interim reports to the DSMB will focus on any safety

data or secondary outcomes available before the end of the study. As a result, it will not be

necessary to adjust the significance level of the primary analysis for multiple looks.

11. PUBLICATION POLICY

11.1 INTRODUCTION

The Women's Angiographic Vitamin and Estrogen (WAVE) trial is an important scientific investigation that will require major resources and an important commitment of time and effort from the investigators and the participants. The investigators have the responsibility and the right to communicate the results of the WAVE trial in a timely and accurate manner to the scientific community, and through them to the general public.

It is important that the WAVE investigators have equal opportunity to participate in the formulation of the study, the analyses of data, the writing of manuscripts and the presentation of results. The NHLBI Project Office, the Study Coordinating Center (SCC) and the Core Laboratories shall have equal status with the clinical center investigators in participating in all of these activities. With the approval of the principal investigators, associate investigators at the clinical sites (and the SCC and Core Laboratories) are encouraged to participate in these processes.

11.2 PRESERVATION OF THE INTEGRITY OF THE TRIAL

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual center will not report the data collected from its center alone. All presentations and publications using WAVE trial data must protect the main objectives of the trial. Data that could be perceived as threatening the masking will not be presented prior to release of the primary WAVE outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be given by the WAVE Steering Committee. WAVE results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about WAVE that are shared with national media should be approved by the Steering Committee before release.

11.3 REVIEW PROCESS AND PUBLICATION SUBCOMMITTEE

The purpose of the review process is to ensure that the WAVE results are presented in an accurate and timely fashion, and in a manner that adheres to the highest scientific standards. The review process and the Publications Subcommittee should encourage participation of all investigators and facilitate the publication of as much worthy information as possible from the trial.

The primary WAVE papers are defined as a main Design and Methods paper and papers that present the key endpoint data by treatment group. The number and general content of the primary papers will be planned by the Publication Subcommittee and approved by the Steering Committee. For each paper thus identified, a committee of volunteers from among the centers will be charged by the Publications Subcommittee to produce a report within a stated time limit. The draft manuscript will be circulated to the Project Office, the SCC, the Core Laboratories and the clinical center Principal Investigators for comment. The revised paper will be approved by the Steering Committee, upon recommendation of the Publications Subcommittee. Comments on a draft manuscript must be returned to the writing group within 3 weeks, and the Publication Subcommittee must either approve or suggest further revisions to a revised manuscript within three weeks. Upon approval of the Publications Subcommittee, the Steering Committee will be polled within three weeks as to whether or not they approve the paper.

11.4 SECONDARY PAPERS AND ANCILLARY STUDIES

Any investigator may propose a secondary manuscript or an ancillary study to the Publications Subcommittee. The proposal must contain enough information to judge the scientific validity of the proposal, must clearly define any additional procedures that WAVE participants will undergo, and must define the costs of the proposal and how they will be covered. An ancillary study must not begin until it has been approved by the Publications Subcommittee. The Publications Subcommittee will assess the scientific validity of the proposal and the degree to which it may impact on the primary goals of the study. An ancillary study or

secondary manuscript will not be approved if it threatens the integrity of the main WAVE Trial. The Publications Subcommittee may recommend changes to a proposal, either to improve it or to make it more feasible within the context of the main trial. Proposals will be circulated to all sites and investigators from all sites will be welcome to participate. The originator of the proposal will be responsible for allocating and for coordinating the work involved, for keeping the Publications Subcommittee abreast of the progress of the project, and for completing the project within the predefined period.

Disputes within an approved project will be settled by the Publications Subcommittee. The Steering Committee will approve or reject all proposals for secondary manuscripts and ancillary studies, upon recommendation of the Publications Subcommittee. Secondary manuscripts and manuscripts resulting from ancillary studies will follow the approval process outlined above for primary papers.

11.5 ABSTRACTS AND ORAL PRESENTATIONS

Abstracts must be submitted to the Publications Subcommittee at least two weeks before the submission deadline. An abstract may not be submitted for presentation until approval of the Publications Subcommittee is obtained. Abstracts that contain primary WAVE results will be treated like primary WAVE papers and, as described above, they will be planned by the Publications Subcommittee, circulated to all centers and approved by the Steering Committee before submission.

11.6 COMPOSITION AND ROLE OF THE PUBLICATION SUBCOMMITTEE

The Publication Subcommittee will consist of the principal investigators from each clinical site and the SCC or their designees, the Project Officer or his designee and a representative of each of the Core Laboratories. The Steering Committee may at any time modify the composition of the Publication Subcommittee.

The Publications Subcommittee shall:

1. Plan the number and content of the primary WAVE papers and abstracts, assign a committee of volunteers to each paper and monitor the progress of each writing group to ensure that the WAVE results are communicated in a timely manner;

2. Review all WAVE papers and abstracts to ensure their scientific validity and accuracy; recommend improvements to the writing groups and approval or rejection to the Steering Committee;

3. Evaluate proposals for secondary manuscripts and ancillary studies to ensure their scientific integrity and their feasibility within the main WAVE Trial, without compromising the primary aims of the trial;

4. Arbitrate disputes among or within working groups as to authorship, manuscript content or other issues;

5. Encourage participation by all investigators in the design, analysis and writing or primary and secondary papers.

11.7 AUTHORSHIP GUIDELINES

The main WAVE paper will be authored by the "WAVE Trial Investigators" if this approach is acceptable to the journal editors, and all WAVE Trial personnel will be listed in the Appendix. Whether other primary WAVE manuscripts, such as the Methods paper, are also handled in this manner will be determined by the Publications Subcommittee.

Authorship of secondary papers and ancillary studies will be arranged by the investigator who makes the proposal, in consultation with the other members of the working group. The

authorship guidelines of the target journal should be followed. Authorship disputes will be arbitrated by the Publication Subcommittee.

12. STUDY ORGANIZATION

12.1 INTRODUCTION

The WAVE trial study organization includes the following components: the NHLBI, the Data and Safety Monitoring Board, the Steering Committee and its subcommittees, the Executive Committee, the Study Coordinating Center, the Clinical Centers, Central Angiographic Laboratory, Drug Distribution Center and the Central Biochemistry Laboratory. In the following section each component and the interrelationship among these components will be described. The organization structure that is depicted in Figure 1 was developed to facilitate the conduct of the study by ensuring careful and uniform adherence to the Protocol and the Manual of Operations.

12.2 STRUCTURE

12.2.1 NHLBI

The Director of the National Heart, Lung and Blood Institute (NHLBI) is responsible for the use of Institute funds and the management of Institute programs. He has ultimate responsibility for the conduct of the WAVE trial and serves as the final decision-maker for all major decisions affecting the trial. The Institute Director appoints the Chair and members of the Data and Safety Monitoring Board (DSMB). The Project Officer of the NHLBI represents the Director of the NHLBI and is responsible for ensuring that the scientific and technical objectives of the study are consistent with the mission and responsibilities of the NHLBI. The Project Officer, or his designate, is a member of the study's Executive and Steering Committees and a voting member of each of the subcommittees of the Steering Committee.

12.2.2 Data and Safety Monitoring Board

The Data and Safety Monitoring Board is appointed by the Director of the NHLBI. The members, who are not otherwise affiliated with the study, include experts in cardiology, angiography, gynecology, use of antioxidant therapy, biostatistics, bioethics and other relevant disciplines. The DSMB reviews the Protocol for the main study, any proposed modifications to the Protocol, and the protocols for all proposed ancillary studies.

The members of the DSMB will monitor the progress of recruitment, compliance, data quality, clinical endpoint data, adverse drug responses and side effects. Prior to the end of the trial, summary endpoint data presented by randomization group is confidential and shared only with the DSMB. The DSMB will review reports approximately twice a year, either in person or by conference call, and make its recommendations concerning the conduct of the WAVE trial directly to the Director of the NHLBI. The Principal Investigator and Co-Investigators of the Study Coordinating Center, the NHLBI Project Officer and other designated Institute staff and the chairman of the Steering Committee are ex-officio members of the DSMB.

During the active recruitment phase, the DSMB will monitor the progress of recruitment and the random allocation of participants to treatment and control groups and may recommend modifications in recruitment goals and procedures as needed. At any time, the DSMB may recommend discontinuation of the study or one of its treatment arms on any of the following grounds:

A. An adverse effect attributable to either of the study treatments that is sufficient to override any potential benefit and preclude its further use in post-menopausal women;

B. compelling evidence from this or another study of a significant beneficial effect of either of the study treatments, such that its continued denial to the control group is ethically untenable;

C. a very low probability of successfully addressing the study hypotheses within a feasible time frame, because of inadequate recruitment, compliance, etc.

The Director, NHLBI will make the final decision on whether or not to continue the study.

12.2.3 Steering Committee

The Steering Committee is the representative body of study participants. The voting members of the Steering Committee include the Principal Investigator of each of the five clinical centers, the NHLBI project officer, and the Principal Investigator of the Study Coordinating Center. The Steering Committee provides overall scientific direction for the study through consideration of recommendations from the subcommittees. The business of the Steering Committee will be conducted in accordance with customary parliamentary procedures. The Steering Committee will meet at least once each year to review the progress of the study and to monitor non-endpoint data. The Steering Committee will not have access to endpoint data and treatment group differences until the trial is completed. The Steering Committee will establish subcommittees to support its objectives. The subcommittees currently include: Publications, Interventions, Quality of Life, End Points, Recruitment and Adherence, Laboratory, Conflict of Interest. The members of the subcommittees are appointed from among the investigators at the clinical centers, SCC, and NHLBI. The chairman of each subcommittee will be either a Principal or Co-Investigator from one of the clinical centers or the SCC.

All subcommittees have specific responsibilities outlined below and may assume other responsibilities as requested by the Steering or Executive Committees.

A. Publications Subcommittee: decides on the timing and content of publications and presentations. They will review all study manuscripts prior to submission. They will review all research requests for use of study patients or any study data. They will forward their recommendation for each proposal to the Steering Committee.

B. Interventions Subcommittee: Selected the HRT and antioxidant vitamins, specified the dosage and the management of side effects.

C. Quality of Life Subcommittee: Selected the Quality of Life instruments to be used in the Quality of Life component of the WAVE trial.

D. End Points Subcommittee: Will develop definitions and algorithms for angiographic and clinical endpoints. Will implement a mechanism for the timely, masked classification of clinical events by study cardiologists. Members will include the principal investigator of the Central Angiography Laboratory, a cardiologist from the SCC and investigators from the Clinical Centers.

E. Recruitment and Adherence Subcommittee: Will oversee progress of recruitment and of minority recruitment initiatives, facilitate exchange of information regarding recruitment and adherence among centers, and advise the Steering Committee as needed on the maintenance and enhancement of recruitment and adherence rates.

F. Laboratory Subcommittee: Will advise the Steering Committee on appropriate laboratory methods for study assays to achieve study objectives and will assist the SCC in monitoring the performance of the Central Biochemistry Laboratory.

G. Conflict of Interest Subcommittee: Will draft guidelines regarding outside activities of study investigators that represent potential conflicts of interest. The SCC will collect annual disclosure statements from investigators regarding relevant activities and distribute them to the subcommittee for annual review and evaluation.

12.2.4 Study Coordinating Center

The Study Coordinating Center (SCC) provides overall coordination for all aspects of the study. The SCC investigators participate in all aspects of the design and implementation of the WAVE trial. The Principal Investigator is a voting member of the Steering Committee. The

Principal Investigator and Co-Investigators provide scientific and technical service to the Steering Committee and each of its subcommittees. The SCC has the responsibility for implementing the systems necessary for randomization, drug distribution, data collection, editing, management and statistical analyses. It is responsible for providing appropriate and timely data reports to the DSMB, the Steering Committee and its subcommittees, and the Executive Committee. The SCC will oversee all aspects of the clinical centers' performance, train and certify the centers' staff, and oversee the subcontracts for the Central Biochemistry and the Core Angiographic laboratories. The SCC will also oversee and participate in the following activities:

1. Preparation of the Protocol, forms, and Manual of Operations.

2. Development of the experimental design and statistical analysis for the study.

3. Reproduction and distribution of data collection forms. The SCC will work with the clinical investigators as needed to pre-test forms and procedures.

4. Solicitation, awarding, and oversight of subcontracts for central angiographic and biochemistry laboratories. Oversight of the interagency agreement with the Drug Distribution Center

5. Coordination of training of Clinical Center personnel to standardize all study procedures and data collection.

6. Monitoring Clinical Center and central angiographic and biochemistry laboratory performance and data quality. Provision of summary performance reports to the Steering Committee at their meetings.

7. Provision of detailed and up-to-date semi-annual statistical reports of study progress to the Data and Safety Monitoring Board (see above).

8. Logistical support (as needed) and minutes for study meetings.

9. Coordination and supervision of endpoint verification activities.

10. Preparation of study manuscripts in collaboration with Clinical Center investigators.

12.2.5 Clinical Centers

The five participating clinical centers are staffed by a Study Coordinator and a Principal Investigator. The Principal Investigator works with the other members of the Steering Committee to conduct the study in accordance with the Protocol and Manual of Operations. The clinical center is expected to perform the following functions:

A. Screen, obtain informed consent and randomize acceptable numbers of eligible patients.

B. Obtain entry and exit angiograms suitable for quantitative analysis.

C. Dispense study medication in the manner outlined in the WAVE Protocol

D. Maintain contact with patients

E. Schedule and perform follow-up visits, submit data collection forms to the SCC, and respond to data queries in an appropriate manner, as outlined in the Protocol and the trial Manual of Operations.

F. Obtain Protocol-required validating information on endpoints.

G. Attend all study meetings.

12.2.6 Pharmaceutical Companies

Two pharmaceutical companies will provide the drugs and their matching placebos to the study: Wyeth-Ayerst will provide Premarin, Prempro and MPA, and BASF/Knoll will provide vitamins C and E. The pharmaceutical companies will provide the study medication to the Drug Distribution Center (DDC). They will have no access to study data prior to the publication of the trial results.

12.2.7 Drug Distribution Center

The Drug Distribution Center (DDC) will receive the study medication from the pharmaceutical companies. They will receive, store, bottle, label and distribute the drugs to the Clinical Centers. A representative of the DDC should attend all WAVE trial Steering Committee meetings.

12.2.8 Central Biochemistry Laboratory

The Central Biochemistry Laboratory (CBL) will coordinate the collection, local processing, and shipment of baseline and follow-up blood specimens from each collaborating clinic. The CBL will serve as the long term storage repository of these specimens. They will perform assays as required by the Steering Committee. A member of the CBL should attend all Steering Committee meetings.

12.2.9 Central Angiographic Laboratory.

An independent central angiographic laboratory (CAL) will provide detailed acquisition guidelines with standardized film sequence, angulations, calibration, and vasodilator use. The CAL will also provide a detailed description of the angiographic methodology, a protocol for acquisition of the baseline and follow-up angiograms, a method to monitor local acquisitions, the

angiographic system used for the study, and provide documentation of their quality assurance standards. All baseline cineangiograms will be semiquantitatively reviewed at the CAL within one week of the baseline procedure to document angiographic quality and patient eligibility for study entry. CAL will perform formal masked qualitative morphologic and quantitative analysis using a validated, automated edge-detection algorithm on all baseline and follow-up angiograms at the completion of the follow-up examination. Based on pre-determined criteria, these readings will be used for the assessment of disease progression or regression. A member of the CAL should attend all Steering Committee meetings.

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14. APPENDIX A - SAMPLE INFORMED CONSENT FORM

INFORMED CONSENT FORM - RESEARCH STUDY

Women's Angiographic Vitamin & Estrogen Trial (WAVE)

Principal Investigator -
Telephone -

Introduction
You are being asked to take part in a research study. Before you decide to take part, you need to understand the risks and benefits so that you can make an informed decision. This is known as informed consent. This consent form provides information about the research study which has been explained to you. Once you understand the study and the activities it requires, you will be asked to sign this form if you want to take part. You are free to choose if you take part.

Purpose
The purpose of the WAVE research study is to find out if two treatments, hormone replacement therapy and antioxidant vitamins, reduce the risk of narrowing or closing off in the blood vessels supplying the heart. This research study is being carried out at 5 centers around the country; a total of about 450 women will participate. Participation will last about 3-4 years.

Description of Procedures
Dr. _____ and his/her staff are inviting postmenopausal women (those who have gone through the change of life) to participate in this study if they either have had recent heart catheterization or are scheduled for upcoming heart catheterization. If you agree, you will have your heart catheterization as scheduled. Careful records will be kept of the camera views used and several extra views, taking a few seconds each, may be filmed to be sure that pictures of any narrowings in your blood vessels are clear. If you have any narrowings which meet study requirements (15-75% narrowed), you may be eligible to join the study. If not, you will be so informed by a study staff member.

Ultrasound pictures of the blood vessels in your heart may be recorded during your heart catheterization by placing a small ultrasound device in your blood vessels through the same tubes used for the rest of the heart catheterization. (You cannot feel the ultrasound device.) You may join the research study without having the ultrasound pictures. You will be notified if these ultrasound pictures are planned.

If your heart catheterization results are suitable, you will be scheduled for a screening visit to ensure that you can safely join the study. At that visit you will be asked about your medical

background, have a brief physical examination including breast and pelvic exams (including Pap smear if you have not had one within the past year), and give a blood sample (_____ tablespoons).You will need to have a mammogram if you have not had one within the past year. You will not be charged for the examinations or tests.

You may also be asked to have two additional tests, a dipyridamole SPECT and a brachial reactivity study. The dipyridamole SPECT is a common type of stress test in which a medication called dipyridamole and a small amount of radioactivity are given to you through a vein. A special camera takes pictures of the radioactivity to measure the blood flow to your heart. The brachial reactivity study measures the ability of the artery in your arm to dilate in response to nitroglycerin and after temporarily blocking blood flow in your arm. A blood pressure cuff will be placed around your arm and ultrasound pictures of your artery recorded. You may join the WAVE research study without having the dipyridamole SPECT or the brachial reactivity study.

If the results are satisfactory and you wish to join the study, you will be be enrolled and given study medication. The hormone replacement will be either Premarin 0.625 mg each day (for women with a hysterectomy) or Premarin 0.625 mg + medroxyprogesterone 2.5 mg each day (for women with a uterus). The antioxidants will be vitamins C (500 mg) + vitamin E (400 IU), both taken twice daily. These drugs are approved for sale in the United States; they are not experimental. You will take one tablet of hormone study medication and two capsules of antioxidant study medication in the morning and two capsules of antioxidant study medication in the evening for the duration of the study; the doses of study medication can be adjusted if you should develop a side effect. Whether you are given active medication or inactive medication (placebo) will be determined by chance. You will have an equal chance of receiving each of the following drug combinations.

      Active hormone replacement + active antioxidant vitamins
      Active hormone replacement + inactive antioxidant vitamins
      Inactive hormone replacement + active antioxidant vitamins
      Inactive hormone replacement + inactive antioxidant vitamins

Neither you nor the research staff will know which treatment you are receiving. You will be asked not to take other hormone replacement therapy, vitamin C or vitamin E while participating in the study. If you wish, you will be given a multivitamin tablet (Centrum) to take daily.

You will be asked to return for a clinic visit one month and 3 months later to be sure you are not having any problems with the study medication. Every 6 months you will be asked to return to clinic to be given a new supply of study medication and to tell the study staff about any changes in your health. Annual mammograms, breast and pelvic exams will be performed, and a blood sample collected at 3, 18, 24 and 36 months. The dipyridamole SPECT may be repeated at 3 months and 3 years; the brachial reactivity study may be repeated at 3 months.

At the end of 3 years you will have a heart catheterization for comparison with your original study. This catheterization may be performed for research purposes, not necessarily because of medical need. If ultrasound pictures of your heart arteries were recorded during your entry heart catheterization, they may be repeated.

Benefits and Risks

Possible benefits to you include a 3 out of 4 chance that you will be receiving active hormone replacement and/or antioxidant vitamins for free. You will receive free physical examinations, mammograms and Pap smears for the study. Benefit to others includes contributing to the understanding of ways to prevent heart disease in women. Although heart disease is the leading cause of death in women, they have often been excluded from past studies of heart disease.

Risks associated with coronary angiography - Some additional views may be filmed during your angiogram, increasing the amount of radiation you receive. For example, instead of having 6 views of your coronaries filmed, you may have 8 or 9. You will be having a second angiogram performed as part of the study, with associated risks which include bleeding (1%, that is, 1 out of 100) , stroke (0.1%, that is, 1 out of 1000) and death (0.05%, that is, 1 out of 2000) . The amount of radiation during heart catheterization is about 20 rads, which is about 67 times the amount of natural environmental radiation the average person receives in the United States each year.

If you have ultrasound pictures taken of your heart arteries, there is a small risk (0.3%, that is 3 out of 1000) that a coronary artery may be damaged by the ultrasound device.

Adverse effects of the study drugs - Estrogen (Premarin) may cause bloating, nausea, vomiting, abdominal cramps, rash, headache, dizziness, depression, breast tenderness and enlargement, and changes in thyroid activity. Vaginal spotting or bleeding is common, occurring in as many as 1/3 of women, but usually resolves within several months. In some women it may persist. For your safety, if you have persistent or severe bleeding you may be asked to have an endometrial aspiration, in which a few cells from the lining of the uterus are sucked into the thin tube (about the width of a pencil lead) during pelvic exam. Endometrial aspiration may cause abdominal cramps. Fibroids (non-cancerous tumors of the uterus may increase in size while taking estrogen. Estrogen use has been associated with a 2-fold increased risk of gallstones. Among 50-62 year old women not taking estrogen, 10-15% are thought to have gall bladder disease, thus the expected frequency among estrogen users would be 20-30%. Estrogen, when given alone, is also associated with increased of endometrial cancer (cancer of the lining of the uterus). Medroxyprogesterone is given with estrogen in this study to protect against development of endometrial cancer. Prolonged use of estrogen may be associated with a slightly increased risk of breast cancer. This is the reason for the annual mammograms and breast exams performed as part of this study. Women with known breast cancer or endometrial cancer may not participate in this study.

Medroxyprogesterone may cause rash, vaginal bleeding, depression or nausea. Hormone replacement has been associated with a 3-fold increased risk of blood clots in the legs and lungs. If you have had blood clots in your legs within the past 10 years or have ever had blood clots in your lungs, you should not participate in this research study.

Vitamins C and E are thought to be safe in the doses used in this study.

Mammography, blood drawing and endometrial aspiration may be associated with temporary discomfort. Breast compression is required during mammography, which may be uncomfortable. Blood drawing involves a needle stick and may result in bruising at the site. If you have the dipyridamole SPECT pictures, the amount of radiation is about 0.4 rad, equivalent to 1.3 times the amount of natural environmental radiation the average person receives in the United States each year. There is no risk with the brachial reactivity study, although the blood pressure cuff may be uncomfortable while inflated.

Alternate Treatments
An alternative to joining this study is to take hormone replacement and/or antioxidant vitamins prescribed by your physician.

Costs

Participation will cost only your time and travel. Transportation or parking expenses associated with study visits may be reimbursed. Study medication will be provided to you without charge. If your heart catheterizations are done because you and your physician believe them to be important to your health care, they will be billed under normal financial arrangements. If, on the other hand, one or both of your heart catheterizations are being done only for research purposes, the research study will pay for that catheterization. Any charges associated with mammograms, Pap smears and endometrial biopsies will be paid by the study. You will not be paid for your participation.

Taking part in this research study is unlikely to result in injury or harm to you. If you require immediate medical care, you should go to an emergency room. Otherwise the doctor in charge of this study will take care of you or help you get the care you need. You will be sent a bill for whatever medical care you receive. All or part of your bill may be paid by your health insurance. The National Heart, Lung and Blood Institute will not pay for the care. You should not expect anyone to pay you for pain, worry, lost income, or non-medical care costs that may occur from taking part in the study. This position does not prevent you from pursuing whatever appeals may be available under the law.

Confidentiality

The results of this research study will be given to the Study Coordinating Center for this research study at the George Washington University, and to the National Heart, Lung and Blood Institute. Your medical and research records may be also be provided to the Food and Drug Administration and the United States Department of Health and Human Services. Except for these entities, medical and research study records will be kept confidential unless you authorize their release, or the records are required by law (i.e., court subpoena). You will not be identified by name in any reports or publications of this study.

Right to Withdraw

You may decide to stop this study at any time. Your care and relations with the doctors and nurses working on this research study will not be changed in any way if you decide not to participate in the study or to stop the study.

Voluntary Consent

Your participation in this research study is voluntary. If you have any questions about the study, you should contact Dr._____. Also, if you have any questions about your rights as a participant in this study, please call_____, who is your representative and is not affiliated with this research study.

Signatures

By signing this consent form you are agreeing that you understand this consent form, have had an opportunity to ask questions, have had your questions answered to your satisfaction, and agree to take part. You will be given a copy of this consent form.

_____   _____   _____

Signature of participant   Print name   Date

_____   _____   _____
Signature of person obtaining consent   Print name   Date

_____   _____   _____
Signature of principal investigator or designee   Print name   Date

15. APPENDIX B - ORGANIZATIONAL FLOW CHART

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                          ┌──────────────┐
          ┌─────────┐     │  Data and    │
          │         │     │   Safety     │
          │  NHLBI  │─────│  Monitoring  │
          │         │     │    Board     │
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│ Study           │ │Hartford│ │Duke  │ │Medlantic│ │Univ. of│ │Johns   │
│ Coordinating    │ │Hospital│ │Univ. │ │Research │ │Alabama │ │Hopkins │
│ Center          │ │        │ │      │ │Institute│ │        │ │Univ    │
│ George          │ └───────┘ └──────┘ └─────────┘ └────────┘ └────────┘
│ Washington      │
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│ Core    │ │ Drug       │ │ Core       │
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│Laboratory│ │ Center     │ │ Laboratory │
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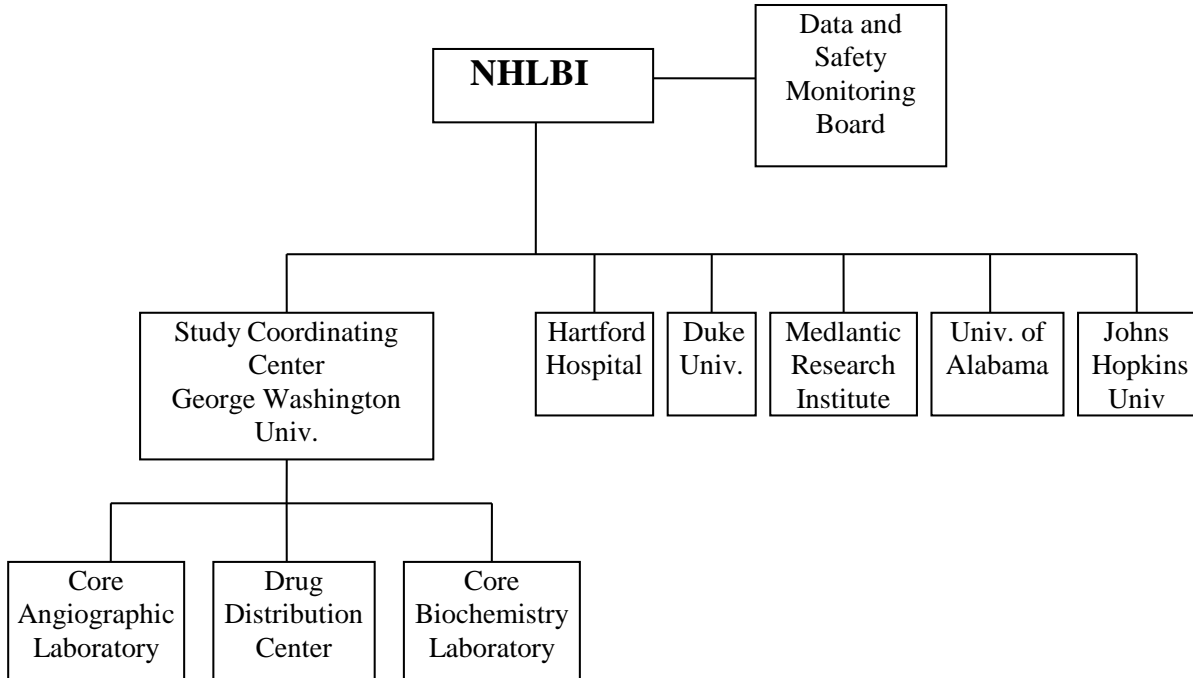
16. APPENDIX C - A STATEMENT BY THE WAVE INVESTIGATORS ON CONFLICT OF INTEREST

The Women's Angiographic Vitamin and Estrogen (WAVE) Trial is a multicenter factorial study which is designed to evaluate the effect of either hormone replacement or the use of an antioxidant supplement on the progression (or regression) of arteriosclerosis in the coronary arteries. Women will be randomized to receive either, neither or both of the active therapies. They will have a baseline and three-year coronary angiogram to assess any changes in lesions in their coronary arteries. Since the results of this clinical trial may have implications for future clinical practice, potential conflicts of interest will be addressed in this document.

The WAVE investigators recognize that bias is a potential concern for any clinical trial, and the study design has incorporated a number of safeguards against the introduction of bias. These include the use of randomization into one of the four treatment strategies, masking of all clinical personnel to study group assignment, the management and analysis of data by a Study Coordinating Center, central and masked interpretation of key data by Core Laboratories, and the use of an independent Data and Safety Monitoring Committee (DSMB).

Despite these safeguards, the WAVE investigators realize that concerns about real or potential conflicts of interest may arise. Even with a well designed multicenter trial comparing treatment groups using common drugs, it may be impossible to entirely eliminate any possible appearance of conflict of interest, as this would essentially require the investigators to give up many routine professional activities. Where potential conflicts exist, the WAVE investigators endorse the task of rational management of conflict according to pre-agreed guidelines and principles. Therefore, the WAVE investigators have agreed to a policy on conflict of interest which has few specific restrictions, but a broad indication for disclosure of potential conflicts of interest. The WAVE investigators wish to endorse the spirit and content of the 21st Bethesda Conference: Ethics in Cardiovascular Medicine (JACC 16:1-36, 1990) dealing with these issues,

and seeks to make this policy consistent with the record of that conference, and the American College of Cardiology guidelines on conflict of interest.

POLICY FOR CONFLICT OF INTEREST FOR THE WOMEN'S ANGIOGRAPHIC VITAMIN AND ESTROGEN (WAVE) TRIAL

To address actual or perceived conflict of interest in the WAVE Trial, the participating investigators voluntarily agree to abide by the guidelines described in this policy statement.

INDIVIDUALS TO BE GOVERNED BY THESE GUIDELINES

Members of the investigative group who will be governed by these guidelines include the Principal Investigator at each Clinical Unit, the Principal and Co-Investigators at the Study Coordinating Center (SCC), and the Principal Investigators of the associated Core Laboratories for the WAVE Trial. Co-Investigators at Clinical Units or affiliated hospitals playing a major role in the conduct of the study will also be governed by these guidelines if deemed appropriate by the Clinical Unit's Principal Investigator. The Principal Investigator for each Clinical Unit, the SCC, CAL and CBL will each submit a list of individuals who will be governed by these guidelines at the beginning of the Trial.

TIME PERIOD OF THE POLICY

The guidelines set forth in this policy will commence at the start of patient recruitment and will terminate at the time of initial public presentation of the principal results. Investigators not privy to endpoint data and discontinuing participation in the trial during recruitment will be subject to these guidelines until their departure from the study.

FINANCIAL GUIDELINES

1. The investigators agree not to own, buy or sell stock or stock options during the aforementioned time period in any of the pharmaceutical companies (Wyeth Ayerst, BASF/Knoll) with products being tested in this trial, or who have provided financial support for the study.

2. The Study Coordinating Center will maintain conflict of interest statements updated annually from each investigator.

Activities not explicitly prohibited, but to be reported annually to the Study Chairman and maintained by the Study Coordinating Center include:

1. Consultant relationships to companies providing drugs or financial support to the trial.

2. Participation of investigators in educational activities that are supported by the companies.

3. Participation of investigators in other research projects supported by the companies

4. Financial interests in these companies, over which the investigator has no control, such as mutual funds or blind trusts.

REPORTING OF FINANCIAL DISCLOSURES AND OTHER ACTIVITIES

The investigators agree to update their financial disclosures and related activities as described above on an annual basis and submit these data to the Study Chairman, who will forward them to the Study Coordinating Center for storage. The SCC will maintain the confidentiality of these records and present them to a review committee, to be constituted by the Study Chairman. In the case of actual or perceived conflict of interest, the Study Chairman will bring it to the attention of the NHLBI.

REVIEW OF POLICY STATEMENT

The investigators agree to review on a regular basis these guidelines and take any additional steps to insure that the scientific integrity of the trial remains intact.

RELATIONSHIP TO INSTITUTIONAL POLICIES ON CONFLICT OF INTEREST

Since existing policies on conflict of interest may vary between participating institutions, in addition to the above policy, it is expected that each investigator will comply with the policies on conflict of interest which exist within their individual participating institutions (medical schools and hospitals). This is the responsibility of each individual investigator.

INITIAL FINANCIAL DISCLOSURE STATEMENT

The undersigned certifies that:

1. Neither I, nor my spouse or dependent children own or will buy or trade stock or stock options in Wyeth Ayerst or BASF/Knoll, who are providing medication and/or financial support to the WAVE trial, as of September 4, 1996.

2. I agree to disclose financial interests as outlined in the WAVE Policy on Conflict of Interest during my participation in the WAVE Trial.

If response is no to question 1 or 2, an explanatory letter is required.

_____   _____

Investigator (type name)   Signature

                                                         _____

                                                         Date

17. APPENDIX D - Brachial Reactivity Protocol

(TO BE INCLUDED)

18. APPENDIX E - dipyridamole SPECT and treadmill study

Substudy Proposal:

Effects of Hormone Replacement Therapy

on Electrocardiographic and Scintigraphic Measures of

Myocardial Ischemia

Substudy Investigators

Eric Peterson MD, MPH, Division of Cardiology/ DUMC

Gary Heller MD, PhD, Division of Cardiology/ Hartford Hospital

Leslee Shaw PhD, Director of Noninvasive Research/ DUMC

Karen Alexander MD, Division of Cardiology / DUMC

ANGIOGRAPHIC TRIAL PARTICIPATING SITES:

George Washington University (Coordinating Center)

Duke University Medical Center

Hartford Hospital

Johns Hopkins University

Medlantic Heart Institute

University of Alabama at Birmingham

18.1 SUBSTUDY PURPOSE

We propose the addition of baseline, three month, and three year gated SPECT exercise
myocardial perfusion imaging as additional study endpoints. The design of the trial is amenable

to the performance of substudies on this population, however this substudy will likely require external sources of funding due to current NIH funding constraints.

18.2 PRIMARY ENDPOINT

- To examine the chronic effect of study drug on changes in perfusion defect size (ischemic burden) from baseline to three year perfusion stress test.

18.3 SECONDARY ENDPOINTS

- To examine the acute effect of study drug on coronary vasomotor tone. This will be done by comparing the degree of ischemic burden between the baseline study and three month nuclear stress test.
- To correlate baseline exercise perfusion study with initial patient symptoms and angiographic findings.
- To determine if initially positive perfusion scans predict subsequent patient symptoms, and clinically important outcomes. To determine the ability of the initial exercise perfusion study to select a subset of patients who receive greater benefits from study drugs in terms of angiographic progression, ischemic burden, symptoms, and cardiac event rates.
- To study the effects of hormone replacement therapy on the ECG changes during standard treadmill exercise testing.
- To examine the acute and chronic effects of study drug on exercise capacity, and to correlate this with patient reported functional status as measured by standardized questionnaires.

18.4 SUBSTUDY PROTOCOL

- All women randomized to participation in the overall trial will be asked to participate in this substudy.
- Women will undergo exercise gated SPECT imaging within 7 days of randomization and prior to initiation of study drug. The exercise protocol will include use of a standard Bruce

protocol with gated SPECT myocardial perfusion imaging. Technetium 99 tomography will be performed at rest and following exercise with reinjection performed in patients with fixed defects.

- At three months, during one of their follow up visits, women will undergo repeat exercise treadmill test. For those women who had perfusion defects initially, a follow up gated SPECT perfusion image will also be obtained at this time point. Those women with negative perfusion scans at enrollment will undergo only exercise ECG testing at the three month time point.

- At completion of the protocol and prior to 3 year recatheterization, all women will undergo repeat Bruce treadmill testing and gated SPECT perfusion imaging. (see Figure 1)

18.5 METHODS

- Treadmill ST segment changes during and immediately following exercise will be analyzed by readers without prior knowledge of patient treatment or clinical history in a treadmill core lab.

- All perfusion imaging will be read and analyzed in a blinded fashion by a nuclear core lab. Gated SPECT was selected for its improved ability to differentiate soft tissue attenuation and to integrate data on resting ventricular function. Data image analysis will include attenuation correction software. Images will be scored both qualitatively and semi-quantitatively. The use of Technetium 99, with a single stress injection, permits simultaneous accurate measurement of LVEF and wall motion through the acquisition of first pass data, and of perfusion from subsequent SPECT imaging. These LVEF and wall motion data have been shown to add incremental information for the diagnosis of significant coronary disease as well as incremental prognostic information over SPECT imaging alone.

18.6 DATA ANALYSIS

The goal of this analysis is to examine the acute and chronic effects of hormone replacement therapy and antioxidants on direct vascular tone, ischemia, and treadmill variables using stress myocardial perfusion and function imaging. Each woman's perfusion defect size will be quantified as a percentage of the total area on a bulls eye plot on enrollment, three month, and three year gated SPECT perfusion studies. We can quantify the changes in magnitude of ischemic burden by treatment group using a simple linear regression model. We hypothesize that some will improve, some will worsen and some will show no change. If our evaluation of the data reveals a large group without change, we will consider a categorical analysis of the data. Additionally, we will control for interim non-protocol drug therapy. Comparing the enrollment study to the three year study will look at the ability of study drug to change ischemic burden by affecting atherosclerotic progression which is the primary endpoint of the substudy. Comparing the enrollment study to the three month study will look at direct effects of study drug on vascular tone and responsiveness.

New ECG changes in the absence of perfusion changes at the three month test will be analyzed by treatment group to examine the potential effects of drug therapy alone on ECG parameters. The initial stress perfusion image will also be compared with patient symptoms, and with angiographic findings at enrollment. The degree to which minor luminal narrowings can cause ischemic burden through functional or dynamic stenoses will be examined. Additionally, we will determine if abnormal perfusion scans at enrollment are predictive of future symptoms, angiographic findings, and outcomes. We will look at the ability of perfusion imaging to identify those women who benefit most from treatment in terms of ischemic burden, symptoms, and outcome.

Functional status and exercise duration may be affected by hormone replacement therapy. Enrollment treadmill tests will be compared to the those obtained at the three month and three year endpoint to look at the acute and chronic effects of drug therapy on exercise capacity. We will compare Bruce treadmill variables and estimated metabolic equivalents to responses on functional status questionnaires regarding activity levels in everyday life. Changes in functional status will be compared across treatment groups.

18.7 STATISTICAL POWER

The primary endpoint (change in ischemic burden) can be as a continuous or a dichotomous endpoint. The advantage of the continuous endpoint is that it requires a smaller sample size to reach statistical significance. The disadvantage with this approach, however, is the inability to easily account for those patients who require revascularization or die prior to repeat perfusion imaging. For example, a patient who develops ischemia at two years and receives bypass surgery may have a normal three year perfusion image. One method for handling patients undergoing interval revascularization would be to attempt a repeat perfusion image prior to revascularization. If this is not clinically feasible, we could assign a standardized perfusion deduction based upon the number of diseased vessels revascularized.

Alternatively, one could use a dichotomous approach, defining a successful therapy as one which prevented both the progression of ischemic territory or the occurrence of clinical endpoints (e.g., MI, CV death, revascularization) by three years. For initial sample size calculations, we have estimated 15-35% of our population would reach one of these endpoints. Sample size calculations with this dichotomous endpoint are displayed below.

Table 1. Treatment Effect Needed To Reach Significance Based on Event Rate

Event Rate Placebo Arm	Event Rate Treatment Arm	RR	Power*
15%	6.5%	2.3	80%
20%	10%	2.0	80%
25%	14%	1.8	80%
30%	18%	1.7	80%
35%	22.5%	1.6	80%

*Assumes a sample size of 225 patients per arm.

Table 2. Patients Needed or Study Power Assuming a 1.30 RR Treatment Effect

Event Rate Placebo Arm	RR	Study Power*	Fixing Power	Sample Size Required**
15%	1.3	20%	80%	1160
20%	1.3	25%	80%	955
25%	1.3	30%	80%	781
30%	1.3	35%	80%	651
35%	1.3	41%	80%	548

* Assuming a sample size of 225.

** Assuming a power of 80%.

18.8 DISCUSSION

Coronary artery disease is the number one cause of morbidity and mortality in women, however, the diagnosis of coronary disease in women is complicated. As many as 25-75% of women referred for evaluation of chest pain with insignificant disease will have either abnormal treadmill ECGs or abnormal perfusion imaging. This constellation of positive noninvasive testing with the presence of insignificant coronary disease may indicate dynamic functional stenoses (e.g., microvascular angina, or vasospastic angina), or may simply be false positive studies.

The overall outcome of this trial will be to characterize the ability of hormone replacement therapy and antioxidants to slow the progression of coronary artery disease as assessed by luminal diameter at catheterization. However, angiographic changes are often minimal in regression trials despite marked improvement in patient outcomes. This is due to difficulties in quantification of angiographic stenoses, to the development of more stable plaques, and potentially to the differences in vasomotor tone between normal and diseased vessels. A recent study looked at changes in nuclear perfusion imaging with short term fluvastatin therapy (cholesterol lowering agent). They found a 30% decrease in ischemic territory by perfusion imaging with a drug thought to improve vascular function and known to improve clinical outcome. The current substudy will characterize the effects of treatment on the patient's

ischemic burden with exercise nuclear perfusion imaging. Nuclear perfusion imaging will provide an additional method by which to quantify changes in coronary atherosclerosis and function, and to correlate changes in ischemia with improvements in luminal diameter and patient outcome.

Therefore, in this substudy, by obtaining stress myocardial perfusion images at enrollment, three month return visit, and trial conclusion, we propose to study the effects of study drugs on symptoms, ischemia, functional status, and outcomes. In comparison to the enrollment test, the three month exercise perfusion test will give data on the acute effects of drug on vasomotor tone, exercise duration, and positive ECG responses to exercise. The performance on the initial perfusion scan will be correlated with subsequent cardiac event rates to determine its predictive ability. Finally, the three year perfusion test will yield information on the progression of coronary artery disease by evaluating improvement in degree of ischemia.

18.9 REFERENCES

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