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Effect of Soy Isoflavone Protein and Soy Lecithin on Endothelial Function in Healthy Postmenopausal Women

Mary Pearson

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ABSTRACT

EFFECT OF SOY ISOFLAVONE AND SOY LECITHIN ON ENDOTHELIAL FUNCTION IN HEALTHY POSTMENOPAUSAL WOMEN

Meg Pearson, Valentine Njike, Marian Evans, Martha Huxley, and David L. Katz. Department of Epidemiology and Public Health, Yale University, School of Medicine, New Haven, CT.

The purpose of this study was to assess the effects of soy isoflavone protein concentrate and soy lecithin on endothelial function measured as flow mediated dilation (FMD) of the brachial artery in healthy postmenopausal women. In this randomized, double-blind, placebo controlled crossover trial, twenty-five subjects (mean age 61 years; BMI 25.46 kg/m^2) were recruited from the general population of southwestern Connecticut. Subjects underwent endothelial function testing using high frequency ultrasound at baseline and following 4 weeks of each randomly assigned treatment with intervening 4-week washout periods. Treatment assignment included: soy isoflavone protein (SP, 25gm/day) or soy lecithin (SL, 20gm/day) alone with placebo for the alternative treatment; both active treatments; or double placebo. Main outcome measures were endothelial function, assessed as flow mediated dilation (FMD) of the brachial artery, and serum lipids.

Twenty-two women completed the trial. Baseline FMD (pre-treatment FMD) was 8.60 $\pm$ 7.20. No statistically significant ($P > 0.05$) difference was seen in FMD between treatment assignments. A trend was suggested, however, with FMD highest after treatment with both soy protein and lecithin (7.50 $\pm$ 9.85), followed by soy protein and placebo lecithin (5.51 $\pm$ 10.11), placebo protein and soy lecithin (5.35 $\pm$ 6.13), and lowest after double placebo (4.53 $\pm$ 7.84). Soy isoflavone protein and soy lecithin significantly ($P < 0.05$) increased HDL/LDL relative to baseline value (soy isoflavone protein and soy lecithin, 0.64 ± 0.19; soy isoflavone protein and placebo lecithin, 0.58 ± 0.17; placebo protein and soy lecithin, 0.65 ± 0.18; baseline, 0.49 ± 0.15).

In this sample of healthy postmenopausal women, soy isoflavone protein and soy lecithin significantly improved the lipid profile. A favorable influence on endothelial function by soy isoflavone and soy lecithin was suggested but could not be confirmed statistically, possibly due to small sample size, timing of testing, dose, or delivery vehicle. Although soy protein consumption is generally recommended as part of a heart-healthy diet, its favorable effects on cardiovascular disease risk factors such as endothelial function and lipids have not been consistently demonstrated in clinical trials, and further investigation into its effects on specific cardiovascular outcomes is necessary before a substantial cardioprotective role for soy protein can be asserted.

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TABLE OF CONTENTS

INTRODUCTION

DEFINITIONS

Terminology related to postmenopausal hormone therapy is inconsistent and potentially confusing. The following terminology will be used in this paper, as proposed in the October 2004 position statement by the North American Menopause Society.[1]

ET	estrogen therapy
EPT	combined estrogen-progestogen therapy
HT	hormone therapy (encompassing both ET and EPT)
CC-EPT	continuous-combined estrogen-progestogen therapy (daily administration of both estrogen and progestogen)
CS-EPT	continuous-sequential estrogen-progestogen therapy (estrogen daily, with progestogen added on a set sequence)
progestogen	encompassing both progesterone and progestin

OVERVIEW OF MENOPAUSE

Menopause is the permanent cessation of menstruation and ovarian follicular production of estrogens and progesterone. The mean age at menopause is 51 years, and 95% of women reach menopause between the ages of 45 and 55.[2] The climacteric is the epoch of endocrinal, somatic, and psychological changes accompanying the menopausal transition (MT). The onset of the MT is defined as the first break in menstrual cyclicity, in a woman whose cycles have previously been regular. Early menopausal transition continues up until the third consecutive month of amenorrhea, at which point a woman is said to have entered the late menopausal transition.[3] In most women, the final menstrual period (FMP) occurs within 4 years of the beginning the late menopausal transition.[4] The FMP marks the beginning of postmenopause, although it is not officially recognized until 12 month of amenorrhea have elapsed. The term perimenopausal includes the menopausal transition and the first 12 months of postmenopause.[5]

The volume of the ovary and its follicle pool decline throughout reproductive life, with significant declines beginning after the age of 30 and continuing until the age of 70.[6, 7] When the follicle cohort diminishes to a critical level, cycle irregularities ensue, signaling the onset of the MT. During the early MT, when the ovary still contains sufficient follicles, it is considered to be in a state of compensated failure. The elevated FSH levels can result in a shortened follicular phase and hyperestrogenemia. The late phase of the MT is characterized by increasing levels of FSH and low levels of estrogen, termed hypergonadotrophic hypogonadism.[7]

MENOPAUSAL SYMPTOMS

Symptoms directly related to declining ovarian endocrine function include vaginal dryness and vasomotor symptoms such as hot flashes and night sweats. Symptoms suggestive of normo- or hyperestrogenemia, such as breast tenderness, tend to occur with greatest frequency during the early MT. Urogential atrophy and vasomotor instability are suggestive of low estrogen levels, and are associated with the late MT.[8] The etiology of hot flashes is unknown, but it is thought that estrogen withdrawal leads to a centrally-mediated thermoregulatory dysfunction at the level of the hypothalamus.[9]

Menopausal estrogen loss is associated with at least two pathologies: osteoporosis and cardiovascular disease. A detailed discussion of osteoporosis is beyond the scope of this project, but a brief overview of the etiology and clinical management of menopause-related bone loss is warranted.

OSTEOPOROSIS

Osteoporosis is characterized by global skeletal fragility in conjunction with low bone mineral density, susceptibility to fracture with minimal trauma, and disruption of skeletal micro-architecture. Osteoporosis results from multiple causes, including physical, genetic and nutritional factors, in addition to estrogen loss with menopause. Skeletal status in adulthood is determined by the peak bone mass at skeletal maturity (age 28) minus the bone mass that is lost thereafter.[10]

The daily loss of calcium among premenopausal women is 20 mg, and it increases to approximately 60 mg per day as women enter the menopausal transition.[11] The estrogen loss with menopause results in increased bone resorption and decreased bone formation, as well as increased urinary excretion and decreased intestinal absorption of calcium. Decreased estrogen stimulates osteoclast activity, leading to increased bone resorption and elevated serum calcium. Hypercalcemia inhibits parathyroid hormone release, thereby decreasing synthesis of 1,25-dihydroxyvitamin D by the kidney and absorption of calcium in the intestine.

Treatment for all postmenopausal women should include a daily intake of 1500 mg of elemental calcium and 800 mg vitamin D (most women require supplements of 1000mg calcium and 400 IU vitamin D to reach this), exercise for at least 20 minutes daily, and smoking cessation. For many years, the first-line pharmacologic treatment for menopause-related bone loss was hormone therapy (HT), but concern for possible increased risks of breast cancer and cardiovascular disease with HT (discussed in detail below), has led to a change in clinical practice. First-line treatment of postmenopausal osteoporosis is usually a bisphosphonate, such as alendronate or risedronate, whose efficacy in preventing and reversing bone loss is half that of hormone therapy. The selective estrogen receptor modulator (SERM) raloxifene is also approved for prevention and treatment of postmenopausal osteoporosis, although its efficacy is less than estrogen's. Other options include calcitonin and parathyroid hormone.[12]

CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among postmenopausal women in westernized societies.[13, 14] CVD accounts for nearly half of all deaths in women, and 50% of these deaths are due to coronary heart disease (CHD).[15, 16] Women are largely protected from CHD before menopause, but their risk increases rapidly following menopause.[17] Clinical and experimental studies indicate that the increased risk may be largely attributable to the decline of endogenous estrogens.[18] The Framingham study showed that the incidence of CVD is higher among postmenopausal than in premenopausal women, even among

women of the same age. Women who experience early menopause, both naturally and surgically (bilateral oophorectomy), also have increased risk of coronary events.[19]

Cardiovascular disease comprises three major areas of disease: coronary heart disease (manifested clinically as myocardial infarction, angina pectoris, heart failure, and coronary death), cerebrovascular disease (manifested as stroke and transient cerebral ischemia), and peripheral vascular disease (manifested as intermittent claudication). The primary pathological process behind these disease states is atherosclerosis, a type of arteriosclerosis (hardening of the arteries) characterized by irregular deposition of lipids in the intima of large and medium-sized arteries.

The atherosclerotic process begins in childhood with the development of fatty streaks, which progress to fibrous plaques that may ultimately cause ischemic damage through thrombotic occlusions. Fatty streaks are focal regions of intimal thickening consisting of foam cells (lipid-laden macrophages and monocytes), T lymphocytes, and vascular smooth muscle cells that have proliferated and migrated within the intima. Further accumulation of smooth muscle cells, macrophages, connective tissue, and lipid deposits transforms the fatty streak into a fibrous plaque. Advanced lesions are characterized by reactive fibrous caps, revascularization, and a necrotic core consisting of leukocytes, lipids and debris.

Atherosclerosis is caused by numerous, interrelated factors, including dyslipidemia, endothelial dysfunction, smoking, and inflammatory factors and oxidative damage. Of these, dyslipidemia and endothelial function are of particular relevance to postmenopausal women and are the focus of this study.

Dyslipidemia is known to be associated with atherosclerosis in both men and women, and has also been linked to menopause. High levels of low density lipoprotein (LDL) and low levels of high density lipoprotein (HDL) are established risk factors for atherosclerosis. Circulating LDL is taken up by macrophages and monocytes to form foam cells that contribute to the formation of plaque. When oxidized, LDL particles are more readily phagocytosed by

macrophages.[20] In addition to oxidizing LDL particles, oxygen-derived free radicals also inactivate NO in the vessel wall.[21] CVD is associated with both increased levels of oxidized LDL and increased risk of *in vitro* LDL oxidation.[22] Premenopausal women have low LDL and high HDL cholesterol levels compared to men of the same age. After menopause, women's LDL cholesterol and lipoprotein (a) levels climb, and HDL cholesterol levels fall, giving them a more atherogenic lipid profile.[18, 23, 24] These unfavorable changes in lipid profile at menopause are independent of BMI, age, and other potential confounding variables,[23] and may contribute to the increased incidence of CVD in postmenopausal women.

OVERVIEW OF ENDOTHELIAL FUNCTION

Menopause is also associated with impaired endothelial function (EF).[25] The endothelium, the single layer of cells lining blood vessels, performs a wide array of vital homeostatic functions. A healthy endothelium regulates vascular tone and proliferation, thrombosis and thrombolysis, and platelet and leukocyte interactions with the vessel wall.[26, 27] A dysfunctional endothelium has been shown in experimental and clinical studies to lose these salutary effects,[26] leading to thrombosis, vasospasm and vasoconstriction, and abnormal vascular proliferation. Endothelial dysfunction is associated with traditional cardiac risk factors, including hypertension, hypercholesterolemia, cigarette smoking, and diabetes mellitus.[28] It is widely viewed as an indicator of coronary risk.[29] Risk factor modification, including reduction of LDL levels, smoking cessation and blood pressure reduction has been shown to improve endothelial function.[28]

Endothelial function refers to arterial vasomotor responses mediated by the release of chemicals including nitric oxide (vasodilating) and endothelin (vasoconstricting) from the vascular endothelium.[30] Impaired release of nitric oxide (NO) results in endothelial dysfunction, in which vessels tend to constrict and impede flow in response to stimuli that should lead to dilatation and flow augmentation.[31] NO is an endothelial-derived vasodilator factor (EDVF) that

is synthesized from L-arginine by nitric oxide synthase. Synthesis and release of NO is stimulated by increased blood flow (causing sheer stress on the endothelium) and circulating agents such as bradykinin, thrombin and acetylcholine that activate specific endothelial cell membrane receptors. NO acts by increasing intracellular cGMP release in smooth muscle cells, resulting in decreased intracellular calcium and smooth muscle relaxation. In addition to its role as an EDVF, NO may also have other antiatherogenic properties. NO has been shown to decrease platelet aggregation and adhesion, limit vascular smooth muscle proliferation, inhibit monocyte adhesion and chemotaxis, and inhibit leukocyte adhesion to the endothelium[21, 27]

Clinical assessment of endothelial function has been reported in the literature for about two decades, beginning with Ludmer's study of coronary EF in the mid 1980's.[32] In this study, coronary angiography was used to measure the change in diameter of the coronary arteries in response to intracoronary infusion of the vasoactive drugs acetylcholine and nitroglycerin. In healthy endothelial cells, acetylcholine triggers the release of NO, resulting in vasodilation. In an unhealthy endothelium, acetylcholine produces vasoconstriction via a direct effect on smooth muscle cells. Endothelial-dependent vasodilation can be distinguished from endothelium-independent vasodilation using nitroglycerin, an exogenous source of NO that acts directly on smooth muscle cells to facilitate dilation.

Methods for the clinical assessment of endothelial function have evolved over the past few decades to include a variety of pharmacologic and physiologic stimuli as well as noninvasive measures of response. A noninvasive assessment of EF in the brachial artery known as flow-mediated dilation (FMD) was developed in the 1990's and has become a standard assessment method. Hyperemic flow is induced in the brachial artery, which subjects the endothelium to an increase in sheer stress, causing dilation of the vessel. This dilation is then measured using high-resolution ultrasound. Brachial artery FMD has been shown to correspond strongly with invasive assessment of coronary EF.[29, 33] The major limitations of this method are that it is subject to intra-

and interobserver variability and that the ultrasonography requires substantial technical expertise.[34, 35]

According to a 2005 review of EF assessment methods,[35] the two other methods used most commonly today are Venous Occlusion Plethysmography (VOP) and Laser Doppler Iontophoresis (LDI). The VOP method measures the increase in forearm volume (following venous occlusion of the forearm), which represents vascular resistance, a function of normal endothelial function. Vasoactive drugs can be injected locally into the brachial artery, thereby minimizing systemic effects. VOP is a relatively invasive method of EF measurement but is nonetheless considered by some to be the gold-standard of EF measurement because of its superior accuracy and reproducibility.[35]

In the LDI method, tiny quantities of vasoactive drugs are administered to forearm skin microvessels using small electric currents, and blood flow alterations (which reflect EF at the microvascular level) are assessed using laser Doppler imagers. LDI is a relatively simple, painless, non-invasive technique that is not observer-dependent, but reports of accuracy and reproducibility are conflicting. The main problem with the technique is that the applied current itself can cause vasodilatation independent of drug administration.[35]

There is currently no consensus as to which technique is optimal, and method selection appears to be largely determined by experience and access to equipment. The degree of correlation between measurements of endothelial function in different vascular beds has been the subject of several small studies, but results have thus far been inconclusive.[35] The current study assessed endothelial function using brachial artery FMD.

REVIEW OF LITERATURE: TREATMENT OF THE POSTMENOPAUSAL WOMAN

MENOPAUSAL SYMPTOMS

Alleviation of menopausal symptoms such as hot flashes and night sweats is the primary reason women seek menopausal treatment. Vasomotor symptoms are estimated to occur in 14-

51% of premenopausal women, 35-50% of perimenopausal women, and 30-80% of postmenopausal women.[5] Hot flashes persist for more than a year in over 80% of cases. Untreated, hot flashes usually stop spontaneously within 6 years, but an estimated 9% of women continue to have hot flashes into their seventies.[36] Treatment options include hormone therapy and alternatives to traditional HT, such as antidepressants, phytoestrogens, meditation, herbal remedies, and nutritional supplements.

Hormone therapy

Hormone therapy seeks to alleviate menopausal symptoms and improve pathological sequelae of estrogen loss by replacing the estrogen, with the ultimate goal of promoting overall health in postmenopausal women. Estrogen therapy (ET) has been used in the US to manage menopausal symptoms since the 1940's; and combined estrogen-progestogen therapy (EPT), since the 1990's. The role of the progestogen is endometrial protection, for unopposed ET carries a known risk of endometrial hyperplasia and adenocarcinoma.[37] Estrogen is by far the most effective therapy available for the treatment of symptoms due to vasomotor instability and urogenital atrophy.[2] A meta-analysis of 21 double-blind, randomized, placebo-controlled trials of oral HT demonstrated a 77% reduction in frequency of hot flashes compared to placebo, as well as significant reduction in symptom severity.[38] Conjugated equine estrogen (CEE) and 17 beta-estradiol (both oral and transdermal) were found to have comparable effects on the treatment of hot flashes.[39]

Other pharmaceutical agents

Antidepressants such as venlafaxine, paroxetine, and fluoxetine have been shown to improve hot flashes and are frequently employed as first-line agents in women who cannot or choose not to use estrogen.[40] Clonidine, an alpha-2 adrenergic agonist used primarily as an anti-hypertensive agent, is also used to treat hot flashes, both in the form of a transdermal patch and an oral medication. Megestrol acetate, a progestin used primarily to treat endometrial and breast

cancer and as an appetite stimulant, is a highly effective treatment for hot flashes but can only be used for several months due to side effects such as weight gain.[41]

Complementary and alternative therapies

Women are increasingly seeking alternatives to traditional hormone therapy, including nutritional supplements, vitamins and minerals, herbal remedies, and meditation. 80% of women aged 45-60 report the use of non-prescription therapies for relief of menopausal symptoms.[42] Increasing use of complementary and alternative medicine (CAM) for menopausal symptoms is reflective of the growing popularity of alternative therapies for a wide variety of medical conditions. Alternative therapies are defined functionally as interventions not typically available in US hospitals or taught in medical schools and include herbal medicine, homeopathy, vitamins, massage, self-help groups, relaxation techniques, and acupuncture. The proportion of Americans using alternative therapies increased from 33.8% in 1990 to 42.1% in 1997, and total annual visits to alternative medicine practitioners were estimated to be 629 million, more than the total visits to all primary care physicians.[43]

In spite of the paucity of data on their efficacy and long-term safety, herbal menopausal treatments are widely held to be safe and effective. A survey of white, middle-aged, college-educated women found that most respondents believe “natural” hormones are safer, cause fewer side effects, and are at least as effective as standard HT for symptom management.[44] In a study of 500 women aged 40-60, 79% reported the use of botanical dietary supplements, yet only 30% had informed their doctor and only 3% had obtained information from healthcare professionals about these supplements.[45]

<u>Herbal remedies</u>

Herbal remedies used by American women for the management of menopausal symptoms include black cohosh, dong quai, chaste tree berry, evening primrose oil, ginseng, motherwort, red clover, and licorice. A 2002 recent review of 10 randomized clinical trials of herbal therapy for primary outcome measures, including hot flashes, joint pain, fatigue, sleep

disturbances, and forgetfulness, reports that only black cohosh has been shown to improve menopausal symptoms.[46]

Three out of four trials of Remifemin, a standardized formulation of black cohosh made by GlaxoSmithKline, show significant improvement in menopausal symptoms, especially hot flashes. The active compounds and mechanism of action of black cohosh remain unknown, and there are no data on the safety of long-term treatment. Of particular concern is the potential for estrogenic stimulation of endometrial and breast tissue. Long-term use of alternative therapies such as black cohosh for relief of menopausal symptoms cannot be recommended in the absence of published data regarding safety, and patients must be counseled that "natural" therapies cannot be presumed to be without adverse effects on health.[46]

Single trials of other herbs have found no significant benefit with dong quai, evening primrose oil, or ginseng on hot flashes.[46] These few studies are of small size and short duration, and further investigation is necessary before any conclusions can be reached.

Dietary phytoestrogens

The most popular alternative to traditional HT is phytoestrogens,[47] non-steroidal, plant-derived compounds with estrogenic activity.[48, 49] Phytoestrogens bind to both alpha and beta estrogen receptors,[50] but are much weaker than endogenous estrogens, with only 1/100 to 1/10,000 the activity.[49] Phytoestrogens' estrogenic activity was first noted in 1926 and gained prominence in the 1940's with the outbreak of reproductive disturbances among sheep grazing on clover-rich pastures in Western Australia, dubbed "Clover disease."[51]

Phytoestrogens are divided into three main classes: isoflavones, coumestans, and lignans. Isoflavones are the most common types of phytoestrogens and are found most abundantly in legumes (soybeans, lentils, chick peas and haricot, broad, kidney, and lima beans) and in products containing most or all of the whole soybean (soy meal, soy grits, soy flour, tofu and soy milk). Soy ingredients are also added to a number of manufactured foods, and these so-called second-generation soy foods (such as tofu yogurt and soy noodles) contain fewer isoflavones. Of note,

soy sauce also has diminished isoflavone content. Lignans are found in high concentration in flax seed and in lesser concentration in whole grain cereals, fruits and vegetables.[51] In humans, the two major isoflavones consumed are genistein and daidzein. After consumption, plant phytoestrogens undergo enzymatic metabolic conversions in the gastrointestinal tract, yielding heterocyclic phenols structurally similar to estrogens.[51]

Equol, a potent metabolite of daidzein, has received a fair amount of attention recently due to the discovery that many people are unable to make it, presumably because of differences in intestinal flora. The possible existence of two subpopulations within soy trials, “equol-producers” and “non-equol producers,” could help explain the inconsistencies seen in the data with respect to isoflavones’ cardiovascular effects (described below). Accordingly, "bacterio-typing" individuals for their ability to make equol may prove useful in upcoming studies of the effects of soy isoflavones.[52]

Epidemiological studies report that only 10-20% of Asian women experience hot flashes, compared with 70-80% of women in western countries.[53-55] It has been proposed that the high dietary intake of isoflavones found in soy products could contribute to the decreased prevalence of menopausal symptoms in Asian populations.[56] Average soy consumption in the US is less than 5 g/day, compared to as much as 55 g/day in Japan.[57] Among many Asian populations, dietary consumption of genistein alone is 20-80 mg/day, compared to 1-3 mg/day in the US.[58] A cohort study of Japanese women found that hot flashes were significantly inversely associated with consumption of both total soy products and isoflavones.[59]

A survey of 886 American women aged 45-65 found that 22.9% use dietary soy and that 7.4% use it specifically for management of menopausal symptoms.[60] Clinical trials have shown only a modest improvement in menopausal symptoms with soy products and soy isoflavone supplementation.[61-71] A review[46] of 8 randomized studies with at least 6 weeks of treatment showed significant relief of hot flashes in 3 of the trials.[61, 67, 68] It is possible that six weeks may be inadequate for assessing the efficacy of vasomotor interventions, although even the longest

study (24 weeks) was among the studies with negative findings.[62] Of note, nearly all of the studies showed symptom improvement in both the treatment and placebo groups, of up to 60%.[46]

A 2004 review of 25 trials lasting a minimum of 4 weeks reported that phytoestrogens in the form of soy foods/beverages/powders, soy extracts, and red clover extracts do not relieve hot flashes or other menopausal symptoms.[72] This review included a large randomized, double-blind, placebo-controlled trial of 252 symptomatic menopausal women, which found that 2 isoflavone supplements (Promensil and Rimostil), both extracts from red clover, did not significantly improve hot flashes or other symptoms of menopause relative to placebo.[73] Similarly, a 2006 American Heart Association (AHA) consensus statement states that soy protein and isoflavones have not been shown to improve menopausal vasomotor instability.[74]

Other treatments

In their systematic review of RCT's of alternative treatments of menopausal symptoms, Kronenberg and Fugh-Berman report that vitamin E, wild yam cream and acupuncture were ineffective for hot flashes. Behavioral therapies such as paced respiration (slow, deep breathing) have been shown to be effective in a few small studies, seem to be safe, and warrant further investigation.[46]

PREVENTION OF CARDIOVASCULAR DISEASE

Hormone therapy

In addition to treatment of menopausal symptoms, hormone therapy has also been routinely prescribed over the past two to three decades for long-term prevention of cardiovascular disease and osteoporosis. This practice was based on vast observational data and basic research findings suggesting estrogen's protective effects on the heart and bone. However, a series of large, randomized, controlled clinical trials have challenged the notion that hormone therapy is cardioprotective.

Basic research findings

Basic research findings and clinical trials using intermediate markers of CHD suggest a cardioprotective role for estrogens.[75, 76] In ovariectomized monkeys, ET significantly reduces the development of dietary atherosclerosis.[77, 78] Adams et al found coronary artery atherosclerosis to be reduced by approximately half in diet-induced atherosclerotic ovariectomized monkeys with 17 beta-estradiol treatment, both with and without progesterone.[79] In a subsequent study, Adams found that conjugated equine estrogen (CEE) treatment of coronary artery atherosclerosis led to a 72% reduction in coronary artery plaque size.[80] Plausible biological mechanisms of estrogen's cardioprotective effect include benefits in lipid profile changes, antioxidant activity, coagulation, fibrinolysis, inflammation, and vascular reactivity.

Lipid Profile

Estrogen therapy has been shown in several studies, including large, randomized, controlled trials[81] to reverse adverse changes in the lipid profile associated with menopause. In these studies, oral estrogens decrease total serum cholesterol, LDL, and lipoprotein (a) concentrations and increase HDL and triglycerides.[82-87] Transdermally delivered estrogen does not have the first-pass hepatic effects of oral estrogen, and therefore causes minimal to no increases in HDL and triglycerides.[82] The addition of progestins can also diminish estrogen's effect on the lipid profile, to varying degrees depending on the progestin type.[81]

Antioxidant activity

Antioxidant properties have also been attributed to estrogens. Both short-term and long-term administration of 17 beta-estradiol decrease the oxidation of LDL cholesterol in postmenopausal women,[88] possibly through regulation of gene encoding enzymes responsible for generation and degradation of superoxide.[89]

Coagulation, fibrinolysis, and inflammation

Estrogen affects the hepatic expression of several genes involved in coagulation and fibrinolysis. Estrogen has been shown to increase fibrinolytic potential in postmenopausal women by decreasing concentrations of plasminogen-activator inhibitor type 1 (PAI-1), an

antifibrinolytic protein.[90, 91] Estrogen demonstrates anticoagulant properties by decreasing fibrinogen and factor VII concentrations, but also promotes coagulation by reducing levels of anticoagulant proteins antithrombin III and protein S. Hormone therapy has divergent effects on inflammatory markers as well: anti-inflammatory effects include reducing fibrinogen, PAI-1 and endothelial cell adhesion molecules concentrations, while pro-inflammatory effects include increasing C-reactive protein,[92] matrix metalloproteinase,[93] albumin, and D-dimer.[94]

Vascular Reactivity: Arterial compliance

An important component of arterial function is arterial compliance, which pertains to the dilation and constriction of arteries with systole and diastole. Arterial compliance is dependent upon arterial wall components such as proteoglycans, elastin, and smooth muscle cells. The Rotterdam Study, a population-based study, found a significant association between arterial stiffness and atherosclerosis.[95]

Aging is associated with decreased arterial compliance. Several studies have shown that estrogen enhances arterial function in post-menopausal women.[96-98] Women receiving HT showed increased systolic arterial compliance (SAC) and decreased pulse wave velocity (PWV), indicating that estrogen may decrease stiffness of the aorta and large arteries in postmenopausal women.[98]

Vascular Reactivity: Endothelium-mediated vasodilation

Estrogen directly affects endothelial cells and has been shown to improve endothelial function in both non-human primates and postmenopausal women. Estrogen receptors are found on endothelial cells, vascular smooth muscle cells, and adrenergic nerve endings and mediate the vasodilatory effect of 17-B estradiol, via synthesis of nitric oxide (NO).[99] Estrogen has rapid, direct effects on endothelium-dependent coronary dilation: intravenous injection of ethinyl estradiol leads to dilation of atherosclerotic coronary arteries within 20 minutes in monkeys.[100] Replacement of estrogen enhances endothelial-dependent vasodilation in normocholesterolemic ovariectomized rabbits[101] and in atherosclerotic ovariectomized monkeys.[77] Addition of cyclic or

continuous medroxyprogesterone acetate (MPA) to the conjugated equine estrogen regimen blunts the endothelium-dependent vasodilation of CEE alone.[102]

Estrogen administration in postmenopausal women induces positive effects on endothelial function and blood flow in normal and atherosclerotic arteries.[103-107] Estrogen infusion acutely potentiates endothelium-dependent vasodilation of coronary arteries[108] and the brachial artery,[105] and it also attenuates abnormal coronary vasomotor responses to acetylcholine.[104, 109] Oral administration of estradiol and CEE has also been shown to improve endothelium-dependent flow-mediated vasodilation.[106, 107]

Observational Studies

Extensive observational data, from more than 40 studies, suggest that women who take hormone therapy have a 35-50% reduction in the risk coronary heart disease compared to non-users.[110] Particularly compelling is the Nurses' Health Study (NHS), with its enrollment of over 70,000 postmenopausal women, sound methodology, and twenty years of follow-up. Results demonstrate a 40% reduction in CHD risk in women taking estrogen or combined estrogen-progestogen compared with women who have never used HT.[111-114] Surprisingly, no dose-response or duration-response effects were demonstrated in the NHS. In fact, coronary benefits diminished with longer duration of HT.[114] Cardiovascular benefit of HT was found to be especially striking for secondary prevention in women with CHD, with 35-80% risk reduction.[115-117]

Randomized Clinical Trials

As mentioned above, results of recent, large, randomized, placebo-controlled trials have challenged the use of HT for the prevention of CVD. Data from the Heart and Estrogen/Progestin Replacement Study (HERS-I and –II), the Women's Heath Initiative (WHI), other smaller controlled trials, and meta-analyses fail to confirm a cardioprotective role for HT. Significant

confusion exists among patients and providers regarding how to apply these findings to clinical practice.

Primary prevention of CVD

The WHI included two parallel trials[118, 119] designed to evaluate HT for the primary prevention of cardiovascular and other diseases in healthy postmenopausal women ages 50-79. Both arms were prospective, 8-year trials scheduled for completion in 2005.

The combined estrogen-progestogen arm of the WHI (CC-EPT vs. placebo in over 16,000 women) was prematurely discontinued in 2002 by the data safety monitoring board according to predetermined cut-offs for "hazard ratio" and "global risk" to subjects. A hazard ratio is an annual calculation of risk relative to placebo, while global risk is a subjective risk/benefit evaluation. The study was halted because of the invasive breast cancer hazard ratio in year 5. Hazard ratios greater than 1 were also found for stroke, cardiovascular events, and thromboembolic events, but were below the threshold for study termination.

The effects of the WHI CC-EPT regimen (0.625 mg/day CEE and 2.5 mg/day MPA) on cardiovascular risk factors were as follows: increased rate of CHD events, with greatest increase in year 1; increase in stroke; and increase in venous thromboembolic events. The risk of breast cancer was also increased, while the risk of fracture and colorectal cancer were significantly decreased.[118]

The unopposed estrogen arm of the WHI study (ET vs. placebo in nearly 11,000 women with prior hysterectomy) was terminated in early 2004 by the National Institutes of Health due to increased incidence of stroke and a lack of cardioprotection. The ET regimen (0.625 mg/day CEE) failed to demonstrate a cardioprotective role for ET, but in contrast to the combined trial, did not show an increased risk of CHD. A subgroup analysis of women 50-59 suggested a decreased risk of CHD, but this result was not statistically significant. As with the combined arm, there was an increase in stroke and venous thromboembolism, and decrease in hip fracture.

No significant difference was found in the rate of colorectal cancer. Breast cancer rates actually decreased with CEE use, but this finding fell just short of statistical significance (P=.06).[119]

The WHI also included the Women's Health Initiative Randomized Controlled Dietary Modification Trial,[120] which studied the cardiovascular effects of a diet low in fat and high in fruits, vegetables, and grains intended to reduce cancer. The intervention group received intensive behavior modification in individual and group sessions aiming to reduce total fat to 20% of calories and increase vegetable/fruit consumption to 5 servings/day and grains to 6 servings/day, and the comparison group received dietary education materials. Over a mean of 8.1 years, the low-fat dietary intervention did not have a significant effect on CHD, stroke, or CVD in postmenopausal women aged 50-79 years. The low-fat intervention did not distinguish between different types of fat, such as saturated and polyunsaturated and trans fats, and therefore is not a test of the current dietary guidelines for CVD prevention, which recommend a plant-based, high-fiber diet high in fruits, vegetables, whole grains, nuts, legumes, fish, and low-fat dairy products and substitution of saturated and trans fats with mono- and polyunsaturated fats.[121]

Secondary prevention of CVD

In the HERS-I trial, 2763 postmenopausal women younger than 80 years with known coronary disease were randomized to CC-EPT regimen (0.625 mg/day CEE and 2.5 mg/day MPA or placebo. Data revealed no significant differences between study groups in CHD events or any secondary cardiovascular outcomes, despite an 11% decrease in LDL levels and a 10% increase in HDL levels. Within the overall null effect, a pattern of increased CHD risk in year 1 and a decreased risk in years 3 to 5 relative to placebo was observed. Treatment with CC-EPT also significantly increased the rate of thromboembolic events and gallbladder disease.[122]

HERS-II was an unblinded follow-up for 2.7 years of 93% of surviving HERS-I subjects, designed to investigate whether the CHD risk reduction in years 3-5 of HERS persisted with additional years of follow-up. The reduction in CHD events among women taking hormones did

not persist; thus, after a total of 6.8 years, HT did not decrease CHD risk events among postmenopausal women with coronary disease.[123]

The Estrogen Replacement in Atherosclerosis (ERA) trial found no benefit with either combination therapy or estrogen alone in women with known coronary disease.[124]

A 2002 meta analysis of HT for primary prevention of CVD concludes that benefits of HT include prevention of osteoporotic fractures and colorectal cancer, harms include increased risk of CHD, thromboembolic events, stroke, cholecystitis, and breast cancer with 5 or more years of use, and uncertainties include dementia.[125] Another meta-analysis designed to evaluate potential explanatory variables of the relationship between HT, CVD, and CHD confirms the findings of randomized trials that HT is not effective for secondary or primary prevention of CVD events.[126]

It is important to note that the increases in CVD and breast cancer risks associated with the WHI CEE-MPA regimen were quite small. The absolute excess risk of serious adverse events (defined as CHD, stroke, pulmonary embolism, breast cancer, hip fracture, and colorectal cancer) due to HT was 19 events per 10,000 women per year (or approximately 2 events per 1000 person-years). The absolute excess risk is presumed to be much smaller for younger women: 1 event per 1000 person-years for 50-year-old-women, for whom estimates of disease rates are estimated to be about half of those of 60-year-old-women.[127] In the estrogen-only arm, the absolute excess risk of adverse events was a non-significant 2 events per 10,000 person-years.[119]

Interpreting the Data

While data from clinical trials are consistent with observational data with respect to nearly all outcomes measured (including stroke, thromboembolic events, hip fracture, breast cancer and colorectal cancer), they show striking divergence with respect to CHD.[128] A number of factors have been proposed to explain the inconsistency of CHD results, including

methodologic issues, differences in hormone regimens, and characteristics of the patient populations.

Methodologic factors

Methodologic factors that may have contributed to the CHD risk reduction in the observational studies include the "healthy user" bias (failure to adequately control for the fact that hormone users are generally healthier than nonusers); the compliance bias (women who adhere to HT tend to adhere to other health-promoting behaviors as well); and the incomplete capture of early events (many women enrolled in the observational trials were long-time rather than new hormone users). If the observational studies were subject to confounding variables such as the "healthy user" and compliance biases, however, one would have expected the data for other diseases such as stroke, which shares many lifestyle and behavioral factors with CHD) to be similarly affected.[128]

Hormone Regimens

Another possible explanation for the discordant data relates to variations in hormone regimens from study to study, including the type of estrogen used and the addition of a progestogen.

The endogenous form of estrogen in premenopausal women and the most bioavailable exogenous form is 17 beta-estradiol. The formulations most commonly used by women in the United States, and those used in HERS and WHI trials, are conjugated equine estrogens, which are a mixture of estrogens derived from pregnant mares' urine. Results of clinical trials using 17 beta-estradiol are mixed.

The Estrogen in Prevention of Atherosclerosis Trial (EPAT) randomly assigned 222 healthy postmenopausal women to either 17 beta-estradiol or placebo and found that ET decreased the risk of atherosclerosis, assessed by carotid artery intima-media thickness (IMT).[129] A study of 321 healthy postmenopausal women in which a progestogen (gestodene) was added to 17-beta estradiol found no reduction in the progression of subclinical atherosclerosis by IMT.[130]

Two studies of secondary prevention using 17 beta-estradiol in women with CHD show no benefit relative to placebo. Data from the oEStrogen in the Prevention of ReInfarction Trial (ESPRIT) indicate that unopposed estradiol valerate (17 beta-estradiol) does not reduce cardiac events among postmenopausal women who have survived a first myocardial infarction.[131] In the Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial (WELL-HART) 17 beta-estradiol alone or with sequentially administered MPA failed to effect the progression of atherosclerosis among older postmenopausal women with established coronary artery atherosclerosis. [132]

Another factor that may influence the cardiovascular outcome of HT is the addition of a progestogen to the hormone regimen. Many clinical trials have used CC-EPT regimens, while the majority of women in observational studies were treated with estrogen alone. It is believed that some formulations of progestogens may mitigate the beneficial effects of estrogen on cardiovascular health, but there is currently no consensus regarding the recommended progestogen regimen.[37] The WHI results, in which elevated risk of CHD was seen in the combined trial but not in the unopposed trial, are supportive of a blunting effect for progestogens.[133]

A study in rats used a fluorescent imaging technique to examine acute peripheral and cerebrovascular responses to progesterone, synthetic progestins (MPA and norethindrone), and estrogens (CEE and 17 beta-estradiol), and found that synthetic progestins, but not progesterones or estrogens cause rapid vascular toxicity. The toxic effects included endothelial disruption, margination and adherence of leukocytes and platelets to vessel walls, platelet aggregation, and formation of large and small thrombi, all of which are early events in atherogenesis.[134]

Patient populations

Other possible explanations for the discordance between observational and clinical studies pertain to characteristics of the patient populations in each--namely, body weight, age, and the number of years since menopause. The mean BMI in the Nurses' Health Study was 24.3,[128]

while those in the WHI trials were 28.5 and 30.1.[118, 119] A large cohort study of almost 300,00 women found that the correlation between HT use and decreased CHD risk was most pronounced in women whose BMI was less than 22.[135]

In the observational studies, nearly all women were less than 55 years old and were taking hormones for menopausal symptoms. In the Nurses' Health Study, for example, the age range was 30-55, and 80% were less than 2 years postmenopausal.[128] By contrast, the average ages of the women in the ERA, HERS and WHI trials were 65, 67 and 63, respectively,[118, 119, 122, 124] and only about 10% of the women in the WHI were symptomatic.[136] Women in the CC-EPT arm of the WHI were an average of 12 years post-menopausal; the average number of years post-hysterectomy was not provided in the publication of the unopposed arm. A power analysis of the CC-EPT arm reveals that the study was 10-fold underpowered to detect cardioprotection of HT administered during the menopausal transition of the magnitude demonstrated by the Nurses' Health Study.[137]

Differences in CHD outcomes between early- and late-start HT are likely related to the extent of underlying atherosclerosis. The progression of atherosclerosis in women is such that active formation of coronary artery lesions occurs between the ages of 45 and 55, and clinical complications from these lesions develop on average by the age of 65.[128] Animal studies suggest that estrogen may have favorable effects in the early stages of atherosclerosis but little to no impact on complicated plaques and coronary events.[76] Studies of cynomolgus monkeys show that those with minimal atherosclerosis showed 70% inhibition of coronary artery atherosclerosis with CEE treatment compared to placebo;[80, 138] those with moderate atherosclerosis, only 50%; and those with a substantial delay in initiation of treatment (comparable to 6 human years) and therefore advanced atherosclerosis, showed no effect on the extensiveness of disease.[139]

Because the patient populations in WHI and HERS were significantly older and further from menopause than those in epidemiologic studies, the data cannot be generalized to younger women initiating HT during the menopausal transition for management of menopausal symptoms.

In order to test the hypothesis that *early* initiation of hormone therapy, in newly menopausal women, confers a cardioprotective effect, a new study of HT in younger women closer to menopause is underway.[140] KEEPS is a primary prevention trial that will examine the effect of CEE and transdermal estradiol, both with cyclic oral, micronized progesterone, on two intermediate markers of CHD--carotid intimal medial thickness and coronary calcium--in women who are between the ages of 42-58 and within 36 months of their FMP.

Changes in clinical practice in response to the WHI

In response to the WHI, the USFDA has mandated that all estrogen products carry a warning of the risk of cardiovascular disease and cancer.[141] The US Preventive Services Task Force, American College of Obstetricians and Gynecologists, American Heart Association, North American Menopause Society, and Canadian Task Force on Preventive Health Care have all issued statements recommending against the use of HT for the prevention of chronic diseases in postmenopausal women. The general guiding principle of most professional organizations is that HT be prescribed at the lowest dose and for the shortest duration possible in consideration of the treatment goals of the individual woman.

The International Menopause Society takes a different view of the matter by arguing that the clinical trials to date do not answer the question whether HT initiated during the menopausal transition is effective for primary prevention of CVD. The IMS therefore recommends "continuation of the presently accepted global practice, including the use of estrogen + progestin, or estrogen alone in the case of women who have undergone hysterectomy, for the relief menopausal and urogenital symptoms, avoidance of bone-wasting and fractures, and atrophy of connective tissue and epithelia."[136]

In the year following the WHI publication in July 2002, prescriptions for hormone therapy dropped by 38% overall, and by 74% for Prempro in particular (the CEE-MPA drug used in the combined trial).[142] The percentage of postmenopausal women taking HT had increased from 33% to 42% between 1995 and 2001, but fell to 28% by July 2003.[143]

SERMs

Due in large part to the findings of HERS and WHI, women are increasingly seeking alternatives to traditional HT, including SERMS and phytoestrogens. As the name suggests, selective estrogen receptor modulators bind with great affinity to estrogen receptors and have agonist effects on particular tissues and antagonist effects on others.

Raloxifene is a SERM that has activity in bone and other systems, but not in reproductive tissue. The Multiple Outcomes of Raloxifene Evaluation (MORE) trial examined 7705 osteoporotic women younger than 81 who were randomly treated with either raloxifene or placebo. Treatment with raloxifene resulted in a 90% decrease in the risk of estrogen receptor (ER)-positive breast cancer, as well as a decrease in vertebral fracture and an increase in bone mineral density in the spine and femoral neck. Raloxifene did not increase the risk of endometrial cancer, but it did increase the risk of thromboembolic disease and hot flashes.[144, 145]

Raloxifene's effect on CVD is unclear, and is the subject of the RUTH trial (Raloxifene Use for The Heart), an ongoing study of over 10,000 postmenopausal women at risk of CHD.[146] Secondary analysis of the MORE data showed no effect on CVD risk overall, but decreased risk of CVD events in women with elevated CVD risks.[147]

Studies of intermediary markers of CVD show that raloxifene reduces serum LDL, homocysteine, lipoprotein (a), and plasma fibrinogen but has no effect on HDL, triglycerides, CRP, or plasminogen-activator inhibitor concentrations.[148-151] Raloxifene has been shown to enhance endothelial function in postmenopausal women relative to both placebo[152] and HT.[153]

Tamoxifen is a SERM used primarily for treatment or prevention of ER-positive breast cancer. Like raloxifene, it has been shown to have beneficial effects on osteoporosis and intermediate markers of CVD, and adverse effects on thromboembolic disease and hot flashes. Unlike raloxifene, however, tamoxifen causes endometrial hyperplasia and endometrial cancer, and its clinical use is therefore limited to treatment and prevention of breast cancer.[154]

Soy Isoflavones

Epidemiological studies indicate that increased phytoestrogen consumption in Asian countries and in vegetarians may be associated with a lower incidence of cardiovascular disease and breast and prostate cancer.[51, 57, 155] Experimental data are also supportive of a cardioprotective role for phytoestrogens.

A significant body of evidence suggests that soy protein in place of animal protein (casein) decreases progression of atherosclerosis. Anthony et al randomized monkeys to diets containing casein protein, soy protein with isoflavones extracted (SPI-), or soy protein with isoflavones intact (SPI+). They found the worst coronary artery atherosclerosis in the casein group, intermediate disease in the SPI- group, and least in the SPI+ group, with ~85% and ~50% reduction compared to casein and SPI- groups, respectively.[156] A subsequent trial in monkeys showed significant and comparable reduction in carotid artery stenosis with both soy phytoestrogens and conjugated equine estrogens, but not with phytoestrogen-depleted soy protein.[138] Possible mechanisms of soy isoflavones' antiatherosclerotic effects include alteration of lipid profile, antioxidant properties, antithrombotic and anti-inflammatory effects, antiproliferative and antimigratory effects on smooth muscle cells, and promotion of normal vasoreactivity.[157]

<u>Lipids</u>

A substantial body of evidence in both animals in humans,[158-161] as well as a meta-analysis of 38 trials,[162] initially indicated a lipid-lowering role for soy protein, which led to the 1999 approval by the USFDA of a health claim that "25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."[163] Recent studies of soy isoflavones' impact on lipid levels have been inconsistent, however, and have generated controversy regarding the appropriateness of the health claim.[164] These findings will be discussed in greater detail in the Discussions section.

Antioxidant effects

Multiple clinical studies have demonstrated an association between the consumption of soy protein and reduction of LDL oxidation.[165-169] In three studies of soy isoflavone extracts (without soy protein), no resistance to LDL oxidation was seen.[170-172]

Antiproliferative and antimigratory effects

The proliferation and migration of smooth muscle cells is an integral part of the formation and progression of atherosclerosis. Several studies have demonstrated soy isoflavones' ability to inhibit smooth muscle cell proliferation and migration *in vitro*.[173-175] Genistein in particular has been shown to inhibit DNA synthesis, extracellular matrix synthesis, and migration of smooth muscle cells via its interactions with the beta estrogen receptor.[173]

Coagulation, fibrinolysis and inflammation

A study of the effects of phytoestrogens on the hemostatic system found no evidence of biologically significant estrogenic effects on fibrinolysis or the coagulation cascade. No significant changes in levels of D-dimer, vWF, PAI-1, or soluble fibrin were found.[176] Genistein was found in one study to inhibit thrombin formation and platelet activation *in vitro*.[177]

Vascular Reactivity: Arterial compliance

Soy isoflavones also have favorable effects on another important cardiovascular risk factor, arterial compliance. Nestel reported that isoflavones, from both soy and red clover, significantly improve systemic arterial compliance, but not blood pressure or lipid profile, in menopausal and perimenopausal women.[171, 178]

Vascular Reactivity: Endothelial function

The effect of soy protein isoflavones on endothelial function in postmenopausal women is the subject of the current study. Soy isoflavones have been shown to influence endothelial function positively in primates,[159] but studies in postmenopausal women have produced conflicting results. Four studies reported a significant increase in flow-mediated vasodilation of the brachial artery with isoflavone treatment,[179-182] while six studies reported no effect on

endothelial function. [183-188] Further study is warranted to elucidate the effects of soy isoflavone protein on endothelial function in postmenopausal women.

Lecithin, a phosphatidylcholine-containing compound, has been shown to lower cholesterol in hyperlipidemic animals[189-192] and humans,[193] but not in normolipidemic animals and humans. [193, 194] Choline is a component of lecithin and is a precursor to acetylcholine, which leads to vasodilation in healthy endothelium and vasoconstriction in unhealthy endothelium.[32] To our knowledge, soy lecithin's effect on endothelial function has not been studied.

STATEMENT OF PURPOSE

Fear of adverse effects of hormone therapy and the perception that alternative therapies are safer than HT have influenced many women to pursue alternatives to conventional hormone therapy. More and more women are consuming soy products and supplements, yet the effects of soy isoflavone protein on endothelial function and overall cardiac risk in postmenopausal women are poorly delineated.[44, 46, 60] Reliable information about the benefits and risks of soy isoflavone protein is needed in order for women and their health care providers to make informed decisions about menopausal health. Management of menopausal symptoms and prevention of cardiovascular disease and osteoporosis in perimenopausal women are matters of great public health significance and warrant further clinical investigation. We therefore conducted a randomized, double-blind, placebo controlled crossover trial of soy isoflavone protein and soy lecithin products on endothelial function in healthy postmenopausal women. Endothelial function, measured as flow-mediated dilation of the brachial artery, is the primary outcome measure. Other measures include lipid panel.

We hypothesize that soy isoflavone and soy lecithin will improve endothelial function, as measured by brachial artery flow-mediated dilation. With respect to isoflavones, we base this hypothesis on isoflavones' demonstrated favorable effects on vascular reactivity in non-human primates, as well as on estrogen's improvement of endothelial function in both animal and human

models, coupled with the knowledge that phytoestrogens are structurally similar to estrogens and have activity at alpha and beta estrogen receptors. Soy lecithin's effect on endothelial function has not been studied to date, but as a source of choline and therefore acetylcholine, lecithin could be expected to increase endothelial-dependent vasodilation.

MATERIALS AND METHODS

Subjects

Healthy postmenopausal women were recruited from the general population of Southwestern Connecticut (CT), primarily through mass media (newspaper advertisements, press releases) and posters. A subject flow diagram is provided in Figure 1. Those subjects (*n = 89*) who responded to recruitment efforts were prescreened using a semi-structured telephone interview. Inclusion criteria were healthy, postmenopausal (defined as absence of menses for at least one year, FSH>40 miu/ml, and estradiol<25 pg/ml) women not currently using hormone replacement, who were normotensive (<140/90) and normolipidemic by laboratory assay (total cholesterol < 240). Exclusion criteria were smoking, history of cardiovascular disease, breast or endometrial cancer, vasoactive medication use, daily prescription drug use, regular use of high dose vitamin C>250 mg, vitamin E>400iu or fiber supplements, and failure to meet inclusion criteria or anticipated inability to complete the study protocol.

Subjects who met initial prescreening criteria (*n* = 33) underwent a clinical screening examination (height, weight, body mass index [BMI] and blood pressure measurements) and laboratory testing (fasting cholesterol, HDL, LDL, triglyceride levels, estradiol, FSH, choline/phosphatidylcholine and isoflavone). Subjects provided written informed consent prior to performing screening and study procedures. The study was approved by the Institutional Review Board of Griffin Hospital, Derby, CT.

Study protocol

Subject management

After meeting eligibility criteria, twenty-five subjects underwent a baseline ultrasound scan and were randomly assigned to treatment groups by the data manager using block randomization. Subjects were randomly assigned to a sequence of 4 sustained treatment phases, each 28 days (4 weeks) in duration with a 28 day (4 weeks) wash-out in-between. Treatment products were consumed twice daily (in the form of packets of powder and packets of granules) and included: soy isoflavone protein (SP, 25g/day) and soy lecithin (SL, 20g/day); soy isoflavone protein (25g/day) and placebo lecithin (PL); placebo protein (PP) and soy lecithin (20g/day); and double placebo.

Subjects were instructed throughout the study to consume one package of powder (mixed with water to create a beverage shake, chocolate and vanilla flavored) and one package of granules twice a day. Treatments were taken in the morning and then later in the day. Patients were instructed to consume any missed treatments as soon as they remembered, unless it was time for the next treatment, in which case they were instructed to return the unused doses at the next scan.

On the last day of each treatment period, following an overnight fast (nothing to eat or drink after midnight) and timed to precede the scheduled scan time by 2 hours, subjects ingested (at home) the final morning treatment dose with water only, and then reported to the PRC for compliance adherence review, endothelial function testing, and phlebotomy.

Compliance Adherence Review

All participants were contacted by telephone every two to four weeks and two days prior to the end of each treatment phase to monitor compliance, inquire about any study related issues, significant side effects, reinforce compliance, serve as a reminder for A.M. previsit testing instructions, and answer potential questions. At each visit, adherence to A.M. pretesting instructions were verified with the subjects before each scan. Subjects returned any unused

treatment product, which was used to corroborate self-reported compliance. Compliance was defined as >80% use of treatment.

Brachial Artery Reactivity Studies (BARS) methodology

The brachial artery reactivity studies (BARS) methodology is comparable to those of other leading labs, as described in "Guidelines for ultrasound assessment of endothelial-dependent flow–mediated vasodilation of the brachial artery,"[34] enhanced by the use of software (Brachial Analysis Tools, Medical Imaging Applications) to automate the brachial artery diameter measures. The BARS procedure is designed to measure flow-mediated dilatation in the brachial artery as a percent of resting vessel diameter.[34] Our methods have been published previously.[195] In brief, brachial artery reactivity was evaluated at baseline (week 0) and after each treatment (weeks 4, 12, 20, 28). Endothelial function was measured non-invasively in the right brachial artery by means of a high frequency ultrasound machine (Philips Medical Systems: Sonos 4500, Andover, MA) in accordance with published guidelines.[34] Subjects were required to lie at rest in a quiet, temperature-controlled, softly lit room for at least 15 minutes before scanning was initiated. Subject comfort was enhanced with the addition of an angled knee cushion, which relieved back strain.

To create a flow stimulus in the brachial artery, a sphygmomanometer cuff is placed on the upper arm proximal to the transducer. The reference diameter of the brachial artery was measured from two-dimensional ultrasound images using a high frequency, 10-15 MHz, vascular ultrasound transducer (Philips Medical Systems, 15-6L L7540 linear array transducer, Andover, MA). The brachial artery was imaged at a location 3-7 cm above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous 2D gray scale imaging. The transmit (focus) zone was set to the depth of the near wall because of difficulty in differentiating the near from the far wall "m" line (the interface between media and adventitia). Diameter measurements were taken from the anterior to the posterior "m" line, over a consistent segment of vessel at least 10-

15 mm in length in diastole. Arterial flow-velocity was measured by means of a pulsed Doppler signal at a 70° angle to the vessel, with the range gate in the center of the artery. Flow was determined automatically by multiplying the arterial cross-sectional area (πr^2) by the Doppler flow velocity. A reference blood flow and diameter are recorded. Arterial occlusion is created by cuff inflation to 50 mmHg above the systolic blood pressure. The cuff is inflated for 5 minutes. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms.[34] Cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. A pulsed Doppler signal is obtained within 15 seconds of cuff release to assess hyperemic velocity, and a longitudinal image of the artery is recorded continuously from 20 seconds to 2 minutes after cuff deflation.

The timing of each image frame with respect to the cardiac cycle was determined with simultaneous ECG gating during image acquisition via the high-quality mainframe ultrasound system. Images were acquired on videotape and magnetic optical disk for evaluation and analysis. All BARS were completed prior to 12 noon.

Handling of BARS Images and Data

Measures of vessel diameter and flow velocity were generated after each scanning session. Velocity measures were generated automatically using the pulsed wave Doppler. Diameter measurements were obtained by automatic identification using edge-detection software (Brachial Analysis Tools, Medical Imaging Applications 2001,Iowa City, IA), an automated method for near and far wall detection and vessel diameter measurements in brachial ultrasound image sequences. [196] The method automatically learns properties for the analyzed vessel in one frame of a sequence that is analyzed under training parameters. The vessel properties were reflected in the cost function used in a graph-search-based border detection process. This automated method decreased variability by generating an average diameter measurement derived

from multiple measures obtained along a segment of the vessel. Several automated quality control steps are incorporated to improve accuracy and reproducibility. These computerized readings are charted on a standardized scan form, which and scanned into the database for analysis. Measurement of vessel diameter and flow velocity was conducted by a dedicated vascular research specialist who was blinded to treatment assignment. A random sample of 30 BARS was provided to the clinical research specialist for a blinded second reading. The resultant coefficient of intra-observer reliability was 0.98; $P<.0001$.

Serum assays and analysis

Venous whole blood was drawn from each subject for serum assays for lipids, choline/phosphatidylcholine, and isoflavones (Equol, Daidzein, Dihydrodaidzein, O-desmethylangolensin, genistein), which were assessed at baseline and following each treatment period. Serum for choline and phosphatidylcholine analysis was sent to the University of North Carolina, and serum for isoflavone analysis was sent to the University of Alabama (Dr. S. Barnes).

Adverse effects

An adverse effects questionnaire was administered at the end of each treatment assignment to compare side effects of soy lecithin, soy isoflavone protein, and placebo.

Statistical analysis

Statistical analysis was performed by Dr. Valentine Njike. The sample size was determined to allow for approximately 10% attrition and noncompliance rate and provide at least 80% power to detect a minimal difference of 3.5% in FMD between isoflavone and placebo. Data were analyzed using SAS software (Version 8.2 of the SAS System for Windows; SAS Institute Inc., Cary, NC). Endothelial function was assessed as flow-mediated dilation; this was calculated as the percent change in diameter post-occlusion of brachial artery at 60 seconds relative to the measurement at baseline before cuff inflation. To account for variability in the strength of the

stimulus that triggered endothelial reactivity (i.e., hyperemic flow), FMD was divided by flow at 15 seconds post-cuff deflation to create a stimulus-adjusted response measure (SARM).[195] Other measures include plasma analytes.

Paired t-testing was performed to assess change in FMD, SARM, serum measures, and blood pressure from baseline. The paired t-test was also used to compare baseline measures of FMD, SARM and serum measures to the values obtained at the end of each washout period to demonstrate complete washout. To adjust for any potential carryover effect following washout, treatment sequence (timing of each treatment) was entered as a control variable in multivariable analysis. The change in FMD, SARM and serum measures between the treatment groups was assessed using repeated measures ANOVA. The combined effects of independent variables and treatment assignment on endothelial function were assessed with multivariable models using ANCOVA. A two-tailed alpha of less than 0.05 was considered statistically significant.

Role of student

As a student researcher, I participated in the planning of the study, the recruitment and selection of study subjects, including telephone prescreenings, review of literature relevant to the study, and drafting of the abstract and manuscript for publication. From June through August of 2002, I worked ~40 hours per week at the study site, the Yale Prevention Research Center, during the subject recruitment phase of the study. Although I was not able to be physically on-site during the implementation of the study, I remained in contact with the study team throughout. During the winter of 2003, I took two weeks away from clinical rotations to research and write drafts of this thesis, as well as the study manuscript for publication. I took an additional two-week block of research time in early 2006 to update the literature review and complete this thesis project.

RESULTS

Subjects

Subjects ranged in age from 46 to 76. Mean age was 61.5 years, BMI was 26.34 kg/m^2, and the mean time from menopause was 118.91 months. Demographic data for the study population are provided in Table 1. A total of 22 of 25 eligible, recruited, and randomized participants completed the study. Two women withdrew from the study due to incompatibility with treatment (dietary preferences, and menopausal symptoms); one woman withdrew for reasons unrelated to the study.

Brachial artery flow-mediated vasodilation

Table 2 provides a summary of the results of the vascular reactivity studies. The mean baseline FMD (pre-treatment FMD) was 8.60 $\pm$ 7.20. FMD did not change significantly ($P > 0.05$) from baseline with any of the 4 treatments. No statistically significant ($P > 0.05$) difference was seen in FMD between treatment assignments. A trend was suggested, however, with FMD highest after treatment with both soy protein and lecithin (7.50 $\pm$ 9.85), followed by soy protein and placebo lecithin (5.51 $\pm$ 10.11), placebo protein and soy lecithin (5.35 $\pm$ 6.13), and lowest after double placebo (4.53 $\pm$ 7.84). Our results persist after controlling for respondent characteristics. A similar trend was observed with SARM.

Lipid Panel

Total cholesterol and LDL decreased significantly ($p < .001$) from baseline after all four treatments (see Table 2). The between-treatment cholesterol reductions did not differ significantly ($p>0.05$). Treatment with both soy isoflavone protein plus soy lecithin significantly ($p < 0.05$) increased HDL relative to double placebo. Soy isoflavone protein and soy lecithin significantly ($P < 0.05$) increased HDL/LDL relative to baseline value (soy isoflavone protein and soy lecithin, 0.64 ± 0.19; soy isoflavone protein and placebo lecithin, 0.58 ± 0.17; placebo protein and soy lecithin, 0.65 ± 0.18; double placebo protein, 0.55 ± 0.15; baseline, 0.49 ± 0.15).

Serum Choline

Betaine levels increased significantly after treatment with soy isoflavone protein plus soy lecithin and soy lecithin (98.94 ± 29.63 nmol/ml, $p<.0001$; 92.06 ± 28.00 nmol/ml, $p<.0001$ respectively) relative to baseline (40.57 ± 8.86 nmol/ml). Betaine levels also increased significantly relative to placebo with soy isoflavone protein plus soy lecithin and soy lecithin ($p<0.05$). Betaine levels decreased significantly relative to baseline value in the placebo (33.27 ± 11.19nmol/ml; $p=0.0023$). Choline levels increased significantly with soy isoflavone protein plus soy lecithin (24.31 ± 8.92 nmol/ml; $p<.0001$) and soy lecithin (16.07± 5.27nmol/ml; $p<.0001$) relative to baseline. No treatment assignment provided any meaningful change of phosphatidylcholine levels.

Serum Isoflavone

Baseline total serum isoflavone (pre-treatment total serum isoflavone) was 76.31 ± 108.58 microM. After treatment with soy protein plus soy lecithin and soy protein, total serum isoflavone increased significantly ($p<.0001$) to 1586.83 ± 1045.91 microM and 1604.58 ± 887.34 microM respectively. These increases observed differed significantly ($p<.0001$) compared to soy lecithin (118.88 ± 157.62 microM) and placebo (109.10 ± 244.18 microM). Soy lecithin and placebo treatment increased total serum isoflavone non-significantly ($p>0.05$) from baseline (see Table 2).

Adverse effects questionnaire

A side effects questionnaire administered following each treatment phase is summarized in Table 3. The most common side effects reported were weight gain and headache. Hot flashes were the most commonly reported menopausal symptom pre-treatment.

DISCUSSION

In this study of 22 healthy postmenopausal women, soy isoflavone protein and soy lecithin significantly improved the lipid profile. Soy lecithin, both with and without soy isoflavone protein, also significantly increased serum choline and betaine levels.

Neither soy isoflavone protein nor soy lecithin significantly improved endothelial function. However, this study does suggest a favorable trend for soy isoflavone protein and soy lecithin with respect to endothelial function. While not statistically significant, FMD was highest following treatment with both soy protein and lecithin, lower for each treatment alone, and lowest for double placebo.

In animal models, phytoestrogens have been found to favorably influence vascular reactivity.[159, 197] Honore found that soy isoflavones enhance vascular function in atherosclerotic coronary arteries of premenopausal monkeys. In response to acetylcholine, arteries from females with the low-isoflavone diet (and also from all males, regardless of diet) constricted, whereas arteries from females with the high-isoflavone diet dilated. Furthermore, genistein administered intravenously produced dilation in the formerly constricting females on low-isoflavone diet.[159] Williams et al found a statistically significant interaction between the vasodilatory effects of estradiol and soy consumption. Among surgically postmenopausal monkeys with very low plasma estradiol levels whose coronary arteries constricted in response to acetylcholine, those who received estradiol and soy isoflavones dilated more than those given only the dose of estradiol.[198]

In a study of healthy human subjects, genistein injected into the brachial artery resulted in acute NO-dependent vasodilation of the brachial artery, with potency similar to 17 beta-estradiol. Plasma concentrations of isoflavones were much higher than in other studies with soy supplements.[199]

The literature on soy isoflavone protein's effect on endothelial function in post-menopausal women is mixed. Simons *et al* found no significant effect of 8 weeks of treatment

with 80 mg/day isoflavones in women with evidence of endothelial dysfunction.[185] Three recent studies in postmenopausal women with hypercholesterolemia are discrepant: in one study, treatment with 40 g/day of isolated soy protein containing 80 mg isoflavones for 4 weeks improved flow-mediated vasodilation independent of lipoprotein changes,[181] while two others showed no benefit: treatment with 25 g/day of soy protein containing 85 mg isoflavones for 6 weeks yielded no improvement in endothelial function in one,[200] nor did 6 weeks of treatment with 90mg/day of isoflavones.[187]

Studies in healthy postmenopausal women with neither high cholesterol nor endothelial dysfunction are also contradictory. Teede *et al* found that 3 months of treatment with 40g/day soy protein containing 118 mg isoflavones did not improve vascular function.[186] Hale *et* al reported that 2 weeks of 80 mg/day isoflavone in the form of soy tablets failed to affect vascular reactivity or lipids.[184] A large study of over 200 women used 99 mg/day isoflavones for a relatively long duration of 12 months, and also failed to find a beneficial effect on endothelial function.[188]

On the other hand, three studies in healthy postmenopausal women do show improvement in endothelial function. Steinberg *et al* found that treatment with 25 g/day of soy protein containing 107 mg isoflavones for 6 weeks favorably effects endothelial function, independently of lipid and antioxidant effects.[180] Squadrito *et al* found that one year of genistein therapy (54 mg genistein/day) improves endothelial-dependent flow-mediated vasodilation to the same extent as does estrogen/progestin therapy.[179] Squadrito also reported an increased ratio of NO to endothelin-1 with genistein therapy, suggesting that the endothelial effect is due to NO release.[201] Colacurci *et al* tested the effects of both long duration (6 months) and high dose isoflavone treatment (60 mg/day each of both genistein and daidzein), a dose chosen to be between consumption in the Pacific Rim (25-45 mg/day) and in some regions of Japan (200mg/day). This isoflavone regimen enhanced endothelium-dependent brachial reactivity, and also reduced plasma adhesion molecule levels, but not coagulation parameters.[182] Contributory

factors to the varied outcomes of these studies include the dosage and duration of treatment, the type of product used (soy protein, isoflavone extract, isolated genistein or daidzein) and the presence of underlying endothelial dysfunction or hypercholesterolemia.

As previously mentioned, soy protein's role in lipid-lowering has recently been called into question. The 1995 meta-analysis by Anderson *et al*[162] of 38 controlled clinical trials examining the relation between soy protein consumption (averaging 47 g/day) and serum lipid concentrations demonstrated significant reductions, when compared to control diets, in total cholesterol (9.3%), LDL cholesterol (12.9%), and triglycerides (10.5%). HDL cholesterol levels increased by 2.4%, but this increase was not significant. Despite the wide range in amount of soy protein consumed (18-124 g/day), no dose-response effect was evident. Rather, response to soy protein was found to be directly proportional to the extent of initial hypercholesterolemia, with 20% reductions in blood cholesterol seen in severe hyperlipidemia, down to no significant reductions in subjects with initial cholesterol levels <255 mg/dl. The literature suggesting a role for soy protein in the treatment of hypercholesterolemia contributed to the aforementioned FDA approval for a health claim for food products containing at least 6.25 g of soy protein per serving.[163]

More recent, well-controlled trials have not consistently reported substantial lipid-lowering effects of soy protein and soy-derived isoflavones. The 2006 AHA Science Advisory on soy and CVD[74] reviewed 22 randomized trials of isolated soy proteins with intact isoflavones and found that LDL levels decreased by an average of 3% relative to control (milk or other proteins), with no dose-response effect. This decrease is especially modest considering the high intake of soy protein consumed in the trials, averaging 50 g/day, or about half the typical total daily protein consumption. Although some studies found greater lipid-lowering effects in hyperlipidemic subjects compared to normolipidemic subjects,[202, 203] no overall relation across the 22 trials was seen between initial cholesterol levels and lipid lowering response. No significant effects on HDL, triglycerides, or lipoprotein (a) were found.

Whether soy's proposed hypocholesterolemic effect is attributable to isoflavones has been the subject of much debate. The 2006 AHA Advisory[74] looked at 19 trials that studied the lipid-lowering effects of soy protein with varying quantities of isoflavones and of isoflavone supplements in pill form compared to placebo. The overall effect of soy isoflavones on LDL levels was found to be nil (0%). 3 of the 19 studies found a significant link between isoflavone content and decreased LDL levcls,[204-206]and 3 reported significant decreases in total cholesterol.[202-204] The AHA consensus statement is consistent with a 2003 meta-analysis concluding that changes in isoflavones are unrelated to changes in LDL and HDL levels.[207] In the current study, soy protein with intact isoflavones significantly decreased total and LDL cholesterol relative to baseline, but not relative to placebo protein. This result would have been considered a negative finding by the AHA Science Advisory criteria, but is nonetheless consistent with substantial prior evidence that soy protein with intact isoflavones has a lipid-lowering effect in postmenopausal women.

Favorable effects of soy supplementation on metabolic control in diabetes were reported by Jayagopal et al.[208] In a randomized, double-blind, cross-over study of 32 postmenopausal women with type 2 diabetes, fasting insulin and HbA1c levels improved, along with serum lipids, following 12 weeks of supplementation with soy protein (30 g/day containing 132 mg/d isoflavones). Thus, despite the neutral effects of soy observed in the current study, the possibility of cardioprotective effects, at least in certain population sub-groups, still exists.

Soy lecithin has previously been shown to improve the lipid profile in hyperlipidemic patients, but not in normolipidemic patients.[193] In the current study among normolipidemic women consuming 20g of soy lecithin per day, SL significantly lowered LDL cholesterol relative to baseline and to soy isoflavone protein, but not relative to placebo. The mechanism of lecithin's lipid-lowering effects remains unknown. In this study, soy lecithin also significantly increased levels of choline and betaine. To the best of our knowledge, the effects of lecithin on endothelial function have not previously been studied. Despite the increase in plasma choline levels, which

could be expected to increase endothelial-dependent vasodilation via acetylcholine, the current study failed to demonstrate a significant effect of lecithin on endothelial function.

This study suggests a favorable trend in FMD with soy isoflavone protein and soy lecithin supplementation relative to placebo, but a significant effect on endothelial function could not be confirmed. These findings may be due to study limitations such as small sample size, timing of testing, dose, delivery vehicle, and subject characteristics.

There exists the possibility of a Type II error due to small sample size and limited statistical power. The study was only powered to detect a 3.5% change in FMD, based on previous findings by our group with SERMs.[152] The variability of FMD was unexpectedly high, such that the standard deviation was greater than the mean FMD values. Other investigators have noted increased %FMD variability with upper arm cuff placement relative to lower arm placement.[209]

It is noteworthy that FMD was lower after all treatments, including double placebo, than at untreated baseline. One possible explanation for the decline in FMD from baseline relates to the timing of the testing and the delivery vehicle. The baseline BARS testing was conducted following an overnight fast, but for each of the post-treatment scans, study subjects consumed the AM dose of the treatment two hours prior to the scan (after an overnight fast). Furthermore, the shakes provided to subjects in both treatment and placebo phases had a high glucose content, and hyperglycemia has been shown to inhibit endothelium-dependent coronary vasodilation through oxidative stress.[210-212] It is likely that acute glucose loading prior to the post-treatment scans compromised endothelial function, possibly masking any potential beneficial effects of soy isoflavone protein and soy lecithin.

FMD at baseline was surprisingly robust and could have made it difficult to perceive a favorable effect of soy on EF. The baseline FMD in the current study was 8.6%, whereas most other studies of EF and soy reported FMD's of 5-6%. Given that the study participants were, on average, prehypertensive, overweight, and mildly dyslipidemic, one might have expected to see

mild endothelial dysfunction at baseline rather than a high FMD. It is possible that the baseline FMD measurements in this study were subject to technical error, although there was nothing to suggest such problems.

Another factor that may have contributed to the lack of a statistically significant effect of soy isoflavones on EF was the age and time from menopause of the study subjects. The women in the study had about a 10-year post-menopause mean age (61.5 years), and the time from menopause was very close to 10 years (118 months). As with studies of estrogen and CVD, it is possible that in older women many years past menopause, who likely already have significant atherosclerotic disease, treatments such as soy phytoestrogens may not be able to achieve very much in the way of cardiprotection.

CONCLUSION

In summary, the current study suggests that short-term administration of soy isoflavone proteins or soy lecithin significantly improves the lipid profile but does not improve endothelial function in healthy postmenopausal women. Study limitations include the small sample size, possible hyperglycemic effect, age and time from menopause of study subjects, and possible technique issues with the hyperemic response measurements. Further study is warranted to determine if soy isoflavones and/or soy lecithin confer vascular benefit to particular subgroups of postmenopausal women, notably those with established cardiac or diabetic risk factors. Taking into account limitations intrinsic to the study design, future studies that modify the sugar content of the treatment products, as well as the coordination of the final dose and vascular testing are also warranted.

The clinical use of soy phytoestrogens for the prevention of cardiovascular disease cannot be recommended based on the body of evidence to date. Although soy isoflavones' cardiovascular benefits are biologically plausible and supported by epidemiological evidence, clinical evidence is both limited and conflicting.

The clinical trials of the cardiovascular effects of isoflavones in postmenopausal women to date have examined relatively short-term consumption of isoflavones (usually less than one year) beginning at the time of menopause. It is entirely possible, however, that the epidemiological findings of decreased risks of CVD, menopausal symptoms, and breast cancer associated with soy consumption result from the cumulative effects of lifelong dietary practices rather than perimenopausal consumption alone. Prospective randomized studies of the effect of soy consumption across the lifespan on outcomes such as CVD, breast cancer and menopausal symptoms would be informative, although impractical from a logistical standpoint.

With respect to safety, soy foods have been a staple of Asian diets for centuries, and their consumption can be presumed to be relatively benign, provided that the quantities are reasonable. Dietary intake of isoflavones in Japan, Taiwan, and Korea are estimated to be 20-150mg/day,[69] which is comparable to or even greater than the quantity in most clinical trials.

The safety of isoflavone supplements cannot be presumed, however, and has yet to be thoroughly studied. Investigation into side effects and toxicities of soy isoflavones is currently underway. A study of a single ingestion of high-dose isoflavones in 24 healthy postmenopausal women revealed minimal clinical toxicity.[213] A few isolated episodes of breast tenderness, nausea, and pedal edema were reported and may have been related to the study. Clinical evidence regarding the effect of isoflavones on cancers of the breast, endometrium, and prostate is sparse, but a possible stimulatory effect on these tissues has been noted. Until their safety and efficacy are established, isoflavone supplements should not be recommended for any condition, including menopausal symptoms, CVD, osteoporosis, and breast, endometrial and prostate cancers.

Although the preponderance of evidence to date does not show that soy protein significantly decreases CVD risk as compared to other proteins, the AHA advisory[74] still concludes that soy products such as tofu, soybeans, and soy butter are likely to promote cardiovascular and overall health because they contain polyunsaturated fats, fiber, vitamins and minerals and few saturated fats. Inclusion of soy protein in the diet is likely to displace other

sources of protein that contain saturated fat and cholesterol and thereby promote cardiovascular health. Clinicians should encourage their patients, including perimenpausal women, to include soy products as part of a heart-healthy diet emphasizing plant-based nutrition low in saturated fat and cholesterol. With respect to the FDA-approved health claim regarding the cardioprotective effects of soy, the FDA should reconsider the appropriateness of this claim in light of recent studies that do not substantiate the major cardiovascular benefits of soy protein reported in earlier trials.

Unlike soy protein, the consumption of isoflavone supplements should be discouraged for treatment of menopausal symptoms or any other purpose until their safety and efficacy are established. The safety of isoflavones with respect to breast and endometrial cancers is of particular importance to treatment decisions involving perimenopausal women and is a crucial area for further research.

With respect to cardiovascular health, further investigation into the precise component of the soybean responsible for lipid-lowering is warranted. Available evidence leans toward soy protein and away from isoflavones as the responsible factor, but it could be that another component altogether is the active element. Another area where future research is needed is the effect of high protein diets on cardiovascular health. Studies in which soy protein is consumed in addition to rather than instead of animal protein—thereby reducing the consumption of fats and carbohydrates—are called for. In addition, it would be useful to examine how soy isoflavone consumption during adolescence and puberty affects cardiovascular disease in perimenopausal women, as well as breast and endometrial cancer, and vasomotor instability. Finally, the soy studies that are most needed are those that examine the effects of sustained soy protein and isoflavone consumption on specific cardiovascular outcomes, such as coronary heart disease, stroke, and thromboembolic events. Only then can the cardioprotective role for soy protein suggested by epidemiological and basic research findings be confirmed or denied.

Figure 1: Patient Flow Through Study

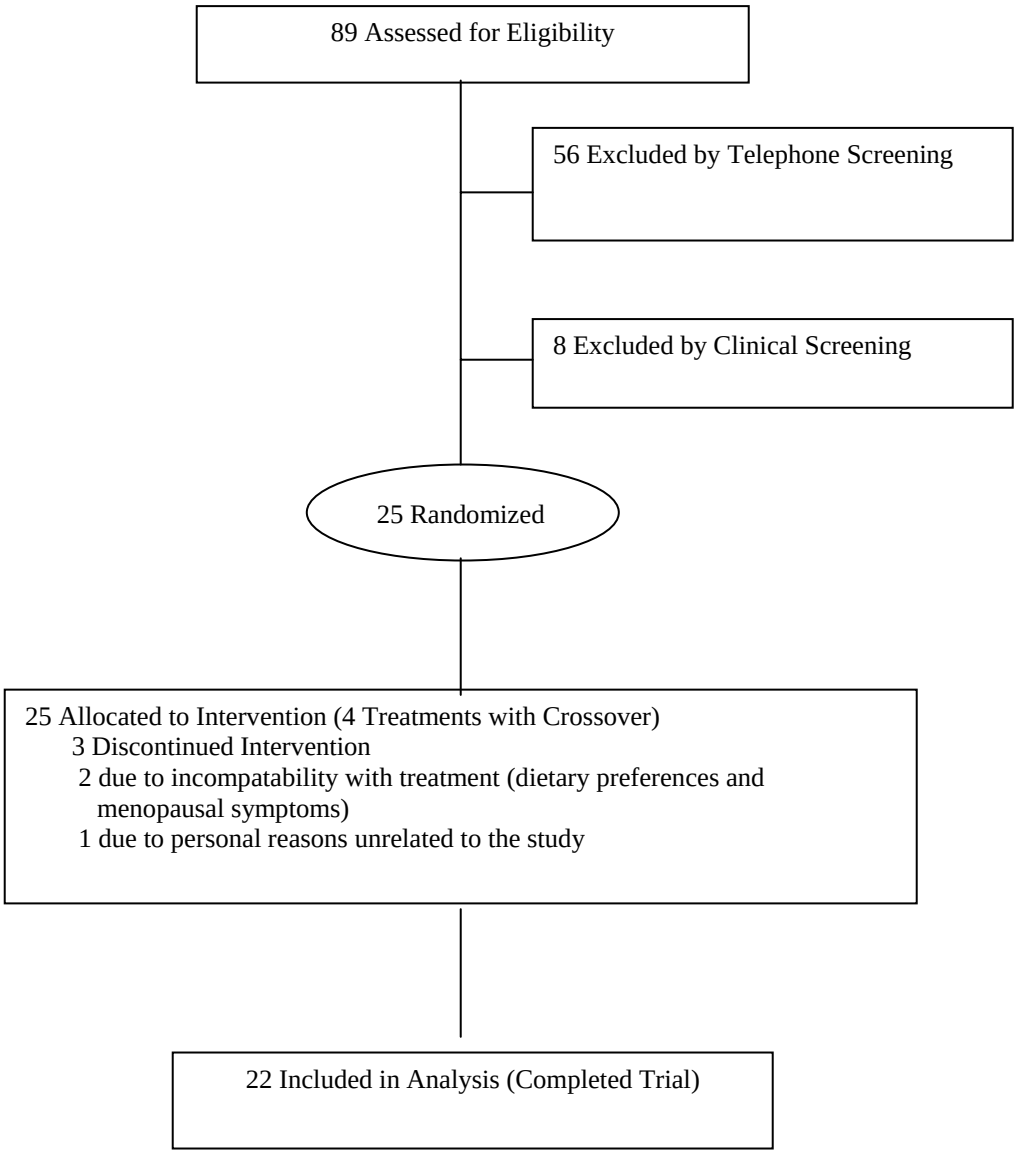

Table 1. Baseline Demographic Characteristics (N=25)

Variable	Mean ± SD
Age (years)	61.50 ± 8.20
Time from menopause (months)	118 ± 107.97
Weight (kg)	70.96 ± 10.10
Height (m)	164.07 ± 4.75
BMI (kg/m^2)	26.34 ± 3.86
Systolic Blood Pressure (mmHg)	135.27 ± 17.00
Diastolic Blood Pressure (mmHg)	77.27 ± 9.43
Pulse Rate (Per Minute)	76.89 ± 9.72
Room Temperature (^oF)	72.71 ± 1.49

Table 2. Flow Mediated Dilation & Plasma Analyte values at Baseline and After Treatment Assignment

Variable	Baseline	Soy Protein plus Soy Lecithin	Soy Protein plus Placebo Lecithin	Placebo protein plus soy lecithin	Double Placebo
Brachial artery Response					
FMD (%)	8.60 ± 7.20	7.50 ± 9.85	5.51 ± 10.11	5.35 ± 6.13	4.53 ± 7.84
SARM (%)	0.056 ± 0.046	0.039 ± 0.050	0.028 ± 0.046*	0.028 ± 0.032*	0.017 ± 0.056*
Lipid panel					
Total Cholesterol (mg/dl)	212.64 ± 29.06	188.55 ± 21.83*	184.32 ± 26.5*	179.73 ± 19.16*	181.83 ± 31.37*
Triglyceride (mg/dl)	99.93 ± 38.96	98.41 ± 44.82	86.91 ± 35.88*	94.45 ± 42.65	89.96 ± 39.52
LDL (mg/dl)	131.21 ± 27.86	104.55 ± 20.50*‡†	107.77 ± 23.78*†	99.36 ± 18.26‡*	107.43 ± 25.4*
HDL (mg/dl)	61.86 ± 12.07	64.73 ± 14.45‡	59.59 ± 14.13	62.00 ± 11.13	56.70 ± 13.25
HDL/LDL	0.49 ± 0.15	0.64 ± 0.19*	0.58 ± 0.17*	0.65 ± 0.18*	0.55 ± 0.15
Choline					
Betaine(nmol/ml)	40.57 ± 8.86	98.94 ± 29.63*	36.50 ± 8.90	92.06 ± 28.00*	33.27 ± 11.19*
Choline(nmol/ml)	7.66 ± 1.73	24.31 ± 8.92*	8.49 ± 2.59	23.84 ± 6.32*	6.89 ± 1.92
Phosphatidylcholine (nmol/ml)	2073.78 ± 195.49	2095.51 ± 223.22	2029.35 ± 263.76	2080.56 ± 220.38	2058.33 ± 250.68
Blood Pressure					
SBP (mmHg)	135.27 ± 17.00	124.95 ± 15.97*	125.33 ± 12.45*	128.35 ± 17.02	121.61 ± 13.69*
DBP (mmHg)	77.27 ± 9.43	70.50 ± 9.26*	71.24 ± 9.67*	71.96 ± 9.18	66.87 ± 7.97*
Isoflavone					
Equol (microM)	1.06 ± 4.03	59.74 ± 101.89*‡†	58.96 ± 103.66*‡†	0.31 ± 1.46	00.00 ± 00.00
Daidzein (microM)	13.60 ± 22.09	409.91 ± 256.96*‡†	413.77 ± 214.20*‡†	28.32 ± 27.07	26.24 ± 59.81
Dihydrodaidzein (microM)	11.66 ± 25.50	119.10 ± 111.90*‡†	86.35 ± 92.64*‡†	15.77 ± 38.37	11.41 ± 38.63
O-DMA (microM)	12.25 ± 25.50	166.77 ± 181.85*‡†	119.31 ± 118.88*‡†	26.70 ± 72.19	10.91 ± 28.23
Genistein (microM)	37.71 ± 56.22	831.31 ± 747.64*‡†	926.18 ± 694.50*‡†	47.78 ± 39.54	60.54 ± 139.98
Total Isoflavones (microM)	76.31 ± 108.58	1586.83± 1045.91*‡†	1604.58 ± 887.34*‡†	118.88 ± 157.62	109.10 ± 244.18

Values are Mean ± SD

*P value < 0.05 in paired ttest; ‡P < value 0.05 versus double placebo; †P value < 0.05 versus soy lecithin plus placebo

FMD= Flow-mediated dilation = % diameter change from reference diameter to response diameter

SARM = Stimulus-adjusted response measure = FMD/flow at 15 s

SBP = Systolic blood pressure

DBP = Diastolic blood pressure

O-DMA = desmethylangolensin

Table 3. Comparison of the Frequency of Occurrence of Side Effects

Side Effect	Soy protein & soy lecithin N (%)	Soy protein & placebo lecithin N (%)	Placebo protein & soy lecithin N (%)	Double Placebo N (%)
Leg cramps	2 (9.1%)	1 (4.5%)	2 (8.7%)	3 (13.0%)
Fever	0 (0%)	0 (0%)	1 (4.3%)	0 (0%)
Hot flashes	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)
Headache	3 (13.6%)	2 (9.1%)	3 (13.0%)	3 (13.0%)
Nausea/vomiting	1 (4.5%)	3 (13.6%)	1 (4.3%)	0 (0%)
Weight gain	5 (22.7%)	4 (18.2%)	5 (21.8%)	6 (26.1%)
Hand or feet swelling	0 (0%)	1 (4.5%)	1 (4.3%)	1 (4.3%)
Muscle aches and pains	0 (0%)	1 (4.5%)	3 (13.0%)	0 (0%)
Joint pain	0 (0%)	0 (0%)	2 (8.7%)	0 (0%)
Lack of sleep	0 (0%)	1 (4.5%)	0 (0%)	2 (8.7%)
Feeling depressed	0 (0%)	0 (0%)	1 (4.3%)	1 (4.3%)
Hoarseness	1 (4.5%)	1 (4.5%)	4 (17.4%)	0 (0%)
Excessive sweating	1 (4.5%)	1 (4.5%)	0 (0%)	1 (4.3%)
Skin rash	1 (4.5%)	1 (4.5%)	0 (0%)	1 (4.3%)
Urinary tract infection	1 (4.5%)	1 (4.5%)	0 (0%)	0 (0%)

REFERENCES

1. Recommendations for estrogen and progestogen use in peri-and postmenopausal women: October 2004 position statement of The North American Menopause Society. Menopause 2004; 11:589-600.
2. Belchetz PE. Hormonal treatment of postmenopausal women. N Engl J Med 1994; 330:1062-71.
3. Research on the menopause in the 1990s. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser 1996; 866:1-107.
4. Taffe J, Garamszegi C, Dudley E, Dennerstein L. Determinants of self rated menopause status. Maturitas 1997; 27:223-9.
5. National Institutes of Health State-of-the-Science Conference statement: management of menopause-related symptoms. Ann Intern Med 2005; 142:1003-13.
6. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992; 7:1342-6.
7. Zapantis G, Santoro N. Ovarian ageing and the menopausal transition. Best Pract Res Clin Obstet Gynaecol 2002; 16:263-76.
8. Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, Burger HG. A prospective population-based study of menopausal symptoms. Obstet Gynecol 2000; 96:351-8.
9. Casper RF, Yen SS. Neuroendocrinology of menopausal flushes: an hypothesis of flush mechanism. Clin Endocrinol (Oxf) 1985; 22:293-312.
10. Marcus R. Post-menopausal osteoporosis. Best Pract Res Clin Obstet Gynaecol 2002; 16:309-27.
11. Heaney RP, Recker RR, Saville PD. Calcium balance and calcium requirements in middle-aged women. Am J Clin Nutr 1977; 30:1603-11.
12. Rosen CJ. Clinical practice. Postmenopausal osteoporosis. N Engl J Med 2005; 353:595-603.
13. Bush TL. The epidemiology of cardiovascular disease in postmenopausal women. Annals of the New York Academy of Sciences. 1990; 592:263-71; discussion 334-45.
14. Gorodeski GI. Update on cardiovascular disease in post-menopausal women. Best Pract Res Clin Obstet Gynaecol 2002; 16:329-55.
15. Mosca L, Manson JE, Sutherland SE, Langer RD, Manolio T, Barrett-Connor E. Cardiovascular disease in women: a statement for healthcare professionals from the American Heart Association. Writing Group. Circulation. 1997; 96:2468-82.
16. American Heart A. Heart and Stroke Facts: Statistical Update. Dallas, Tex: American Heart Association, 2001.
17. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. Jama 1991; 265:1861-7.
18. Kannel WB, Wilson PW. Risk factors that attenuate the female coronary disease advantage. Arch Intern Med 1995; 155:57-61.
19. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med 1976; 85:447-52.
20. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet. 1994; 344:793-5.
21. Gerhard M, Ganz P. How do we explain the clinical benefits of estrogen? From bedside to bench. Circulation. 1995; 92:5-8.
22. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320:915-24.
23. Stevenson JC, Crook D, Godsland IF. Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis 1993; 98:83-90.
24. Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR. Menopause and risk factors for coronary heart disease. N Engl J Med 1989; 321:641-6.
25. Taddei S, Virdis A, Ghiadoni L, et al. Menopause is associated with endothelial dysfunction in women. Hypertension 1996; 28:576-82.
26. Meredith IT, Anderson TJ, Uehata A, Yeung AC, Selwyn AP, Ganz P. Role of endothelium in ischemic coronary syndromes. American Journal of Cardiology. 1993; 72:27C-31C; discussion 31C-32C.

27. Celermajer DS. Endothelial dysfunction: does it matter? Is it reversible? Journal of the American College of Cardiology. 1997; 30:325-33.
28. Vogel RA. Coronary risk factors, endothelial function, and atherosclerosis: a review. Clin Cardiol 1997; 20:426-32.
29. Neunteufl T, Katzenschlager R, Hassan A, et al. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. Atherosclerosis 1997; 129:111-8.
30. Luscher TF, Barton M. Biology of the endothelium. Clin Cardiol 1997; 20:II-3-10.
31. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986; 250:H1145-9.
32. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986; 315:1046-51.
33. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995; 26:1235-41.
34. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology. 2002; 39:257-65.
35. Alam TA, Seifalian AM, Baker D. A review of methods currently used for assessment of in vivo endothelial function. [Review] [52 refs]. 2005.
36. Rodstrom K, Bengtsson C, Lissner L, Milsom I, Sundh V, Bjorkelund C. A longitudinal study of the treatment of hot flushes: the population study of women in Gothenburg during a quarter of a century. Menopause 2002; 9:156-61.
37. North American Menopause S. Role of progestogen in hormone therapy for postmenopausal women: position statement of The North American Menopause Society. Menopause. 2003; 10:113-32.
38. MacLennan A, Lester S, Moore V. Oral estrogen replacement therapy versus placebo for hot flushes: a systematic review. Climacteric 2001; 4:58-74.
39. Nelson HD. Commonly used types of postmenopausal estrogen for treatment of hot flashes: scientific review. Jama 2004; 291:1610-20.
40. Kockler DR, McCarthy MW. Antidepressants as a treatment for hot flashes in women. [Review] [35 refs]. 2004.
41. Anonymous. Treatment of menopausal vasomotor symptoms. [Review] [21 refs]. 2005.
42. Kaufert P, Boggs P, Ettinger B, Woods N, Utian W. Women and menopause: beliefs, attitudes and behaviors. The North American Menopause Society 1997 Survery. Menopause 1997; 5:197-202.
43. Eisenberg DM, Davis RB, Ettner SL, et al. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey.[comment]. Jama. 1998; 280:1569-75.
44. Adams C, Cannell S. Women's beliefs about "natural" hormones and natural hormone replacement therapy. Menopause 2001; 8:433-40.
45. Mahady GB, Parrot J, Lee C, Yun GS, Dan A. Botanical dietary supplement use in peri- and postmenopausal women. Menopause 2003; 10:65-72.
46. Kronenberg F, Fugh-Berman A. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. Annals of Internal Medicine. 2002; 137:805-13.
47. Elkind-Hirsch K. Effect of dietary phytoestrogens on hot flushes: can soy based proteins substitute for traditional estrogen replacement therapy? Menopause 2001; 8:154-156.
48. Davis SR, Dalais FS, Simpson ER, Murkies AL. Phytoestrogens in health and disease. Recent Prog Horm Res 1999; 54:185-210.
49. Price KR, Fenwick GR. Naturally occurring oestrogens in foods--a review. Food Addit Contam 1985; 2:73-106.
50. Kuiper G, Lemmen J, Carlsson B, Corton J, Safe S, van der Saag R. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998; 139:4252-4263.
51. Murkies AL, Wilcox G, Davis SR. Clinical review 92: Phytoestrogens. J Clin Endocrinol Metab 1998; 83:297-303.
52. Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr 2002; 132:3577-84.

53. Boulet MJ, Oddens BJ, Lehert P, Vemer HM, Visser A. Climacteric and menopause in seven South-east Asian countries.[comment]. Maturitas. 1994; 19:157-76.
54. Lock M. Menopause in cultural context. Experimental Gerontology. 1994; 29:307-17.
55. Lock M. Contested meanings of the menopause.[comment]. Lancet. 1991; 337:1270-2.
56. Adlercreutz H, Hamalainen E, Gorbach S, Goldin B. Dietary phyto-oestrogens and the menopause in Japan.[comment]. Lancet. 1992; 339:1233.
57. Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 1998; 128:209-13.
58. Barnes S, Peterson TG, Coward L. Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. J Cell Biochem Suppl 1995; 22:181-7.
59. Nagata C, Takatsuka N, Kawakami N, Shimizu H. Soy product intake and hot flashes in Japanese women: results from a community-based prospective study. Am J Epidemiol 2001; 153:790-3.
60. Newton KM, Buist DS, Keenan NL, Anderson LA, LaCroix AZ. Use of alternative therapies for menopause symptoms: results of a population-based survey.[erratum appears in Obstet Gynecol 2003 Jan;101(1):205]. Obstetrics & Gynecology. 2002; 100:18-25.
61. Han KK, Soares JM, Jr., Haidar MA, de Lima GR, Baracat EC. Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. Obstetrics & Gynecology. 2002; 99:389-94.
62. St Germain A, Peterson CT, Robinson JG, Alekel DL. Isoflavone-rich or isoflavone-poor soy protein does not reduce menopausal symptoms during 24 weeks of treatment. Menopause. 2001; 8:17-26.
63. Washburn S, Burke GL, Morgan T, Anthony M. Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. Menopause. 1999; 6:7-13.
64. Van Patten CL, Olivotto IA, Chambers GK, et al. Effect of soy phytoestrogens on hot flashes in postmenopausal women with breast cancer: a randomized, controlled clinical trial.[comment]. Journal of Clinical Oncology. 2002; 20:1449-55.
65. Scambia G, Mango D, Signorile PG, et al. Clinical effects of a standardized soy extract in postmenopausal women: a pilot study.[comment]. Menopause. 2000; 7:105-11.
66. Quella SK, Loprinzi CL, Barton DL, et al. Evaluation of soy phytoestrogens for the treatment of hot flashes in breast cancer survivors: A North Central Cancer Treatment Group Trial.[comment]. Journal of Clinical Oncology. 2000; 18:1068-74.
67. Albertazzi P, Pansini F, Bonaccorsi G, Zanotti L, Forini E, De Aloysio D. The effect of dietary soy supplementation on hot flushes.[erratum appears in Obstet Gynecol 2001 Oct;98(4):702]. Obstetrics & Gynecology. 1998; 91:6-11.
68. Brzezinski A, Adlercreutz H, Shaoul R, Rosler A, Shmueli A, V. T. Short-term effects of phytoestrogen-rich diet on postmenopausal women. Menopause 1997; 4:89-94.
69. Murkies AL, Lombard C, Strauss BJ, Wilcox G, Burger HG, Morton MS. Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. Maturitas. 1995; 21:189-95.
70. Upmalis DH, Lobo R, Bradley L, Warren M, Cone FL, Lamia CA. Vasomotor symptom relief by soy isoflavone extract tablets in postmenopausal women: a multicenter, double-blind, randomized, placebo- controlled study. Menopause 2000; 7:236-42.
71. Dalais FS, Rice GE, Wahlqvist ML, et al. Effects of dietary phytoestrogens in postmenopausal women. Climacteric 1998; 1:124-9.
72. Krebs EE, Ensrud KE, MacDonald R, Wilt TJ. Phytoestrogens for treatment of menopausal symptoms: a systematic review.[see comment]. [Review] [47 refs]. 2004.
73. Tice JA, Ettinger B, Ensrud K, Wallace R, Blackwell T, Cummings SR. Phytoestrogen supplements for the treatment of hot flashes: the Isoflavone Clover Extract (ICE) Study: a randomized controlled trial. Jama 2003; 290:207-14.
74. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy Protein, Isoflavones, and Cardiovascular Health. An American Heart Association Science Advisory for Professionals From the Nutrition Committee. Circulation 2006.
75. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999; 340:1801-11.
76. Mikkola TS, Clarkson TB. Estrogen replacement therapy, atherosclerosis, and vascular function. Cardiovasc Res 2002; 53:605-19.

77. Williams JK, Adams MR, Klopfenstein HS. Estrogen modulates responses of atherosclerotic coronary arteries. Circulation 1990; 81:1680-7.
78. Geary RL, Adams MR, Benjamin ME, Williams JK. Conjugated equine estrogens inhibit progression of atherosclerosis but have no effect on intimal hyperplasia or arterial remodeling induced by balloon catheter injury in monkeys. J Am Coll Cardiol 1998; 31:1158-64.
79. Adams MR, Kaplan JR, Manuck SB, et al. Inhibition of coronary artery atherosclerosis by 17-beta estradiol in ovariectomized monkeys. Lack of an effect of added progesterone. Arteriosclerosis 1990; 10:1051-7.
80. Adams MR, Register TC, Golden DL, Wagner JD, Williams JK. Medroxyprogesterone acetate antagonizes inhibitory effects of conjugated equine estrogens on coronary artery atherosclerosis. Arterioscler Thromb Vasc Biol 1997; 17:217-21.
81. Anonymous. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial.[comment][erratum appears in JAMA 1995 Dec 6;274(21):1676]. Jama. 1995; 273:199-208.
82. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 1991; 325:1196-204.
83. Lafferty FW, Fiske ME. Postmenopausal estrogen replacement: a long-term cohort study. Am J Med 1994; 97:66-77.
84. Hong MK, Romm PA, Reagan K, Green CE, Rackley CE. Effects of estrogen replacement therapy on serum lipid values and angiographically defined coronary artery disease in postmenopausal women. Am J Cardiol 1992; 69:176-8.
85. Sacks FM, McPherson R, Walsh BW. Effect of postmenopausal estrogen replacement on plasma Lp(a) lipoprotein concentrations. Arch Intern Med 1994; 154:1106-10.
86. Nabulsi AA, Folsom AR, White A, et al. Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. The Atherosclerosis Risk in Communities Study Investigators. N Engl J Med 1993; 328:1069-75.
87. Brinton EA. Oral estrogen replacement therapy in postmenopausal women selectively raises levels and production rates of lipoprotein A-I and lowers hepatic lipase activity without lowering the fractional catabolic rate. Arterioscler Thromb Vasc Biol 1996; 16:431-40.
88. Sack MN, Rader DJ, Cannon RO, 3rd. Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women.[comment]. Lancet. 1994; 343:269-70.
89. Arnal JF, Clamens S, Pechet C, et al. Ethinylestradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:4108-13.
90. Koh KK, Mincemoyer R, Bui MN, et al. Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. N Engl J Med 1997; 336:683-90.
91. Gebara OC, Mittleman MA, Sutherland P, et al. Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. Circulation 1995; 91:1952-8.
92. Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE. Hormone replacement therapy and increased plasma concentration of C- reactive protein. Circulation 1999; 100:713-6.
93. Wingrove CS, Garr E, Godsland IF, Stevenson JC. 17beta-oestradiol enhances release of matrix metalloproteinase-2 from human vascular smooth muscle cells. Biochim Biophys Acta 1998; 1406:169-74.
94. Cushman M. Effects of hormone replacement therapy and estrogen receptor modulators on markers of inflammation and coagulation. Am J Cardiol 2002; 90:7F-10F.
95. van Popele NM, Grobbee DE, Bots ML, et al. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke 2001; 32:454-60.
96. Teede HJ, Liang YL, Shiel LM, McNeil JJ, McGrath BP. Hormone replacement therapy in postmenopausal women protects against smoking-induced changes in vascular structure and function. J Am Coll Cardiol 1999; 34:131-7.
97. McGrath BP, Liang YL, Teede H, Shiel LM, Cameron JD, Dart A. Age-related deterioration in arterial structure and function in postmenopausal women: impact of hormone replacement therapy. Arterioscler Thromb Vasc Biol 1998; 18:1149-56.

98. Rajkumar C, Kingwell BA, Cameron JD, et al. Hormonal therapy increases arterial compliance in postmenopausal women. J Am Coll Cardiol 1997; 30:350-6.
99. Caulin-Glaser T, Garcia-Cardena G, Sarrel P, Sessa WC, Bender JR. 17 beta-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca2+ mobilization. Circ Res 1997; 81:885-92.
100. Williams JK, Adams MR, Herrington DM, Clarkson TB. Short-term administration of estrogen and vascular responses of atherosclerotic coronary arteries. J Am Coll Cardiol 1992; 20:452-7.
101. Gisclard V, Miller VM, Vanhoutte PM. Effect of 17 beta-estradiol on endothelium-dependent responses in the rabbit. J Pharmacol Exp Ther 1988; 244:19-22.
102. Williams JK, Honore EK, Washburn SA, Clarkson TB. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. J Am Coll Cardiol 1994; 24:1757-61.
103. Herrington DM, Braden GA, Williams JK, Morgan TM. Endothelial-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. Am J Cardiol 1994; 73:951-2.
104. Collins P, Rosano GM, Sarrel PM, et al. 17 beta-Estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. Circulation 1995; 92:24-30.
105. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO, 3rd. Acute vascular effects of estrogen in postmenopausal women. Circulation 1994; 90:786-91.
106. Lieberman EH, Gerhard MD, Uehata A, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med 1994; 121:936-41.
107. Bush DE, Jones CE, Bass KM, Walters GK, Bruza JM, Ouyang P. Estrogen replacement reverses endothelial dysfunction in postmenopausal women. Am J Med 1998; 104:552-8.
108. Gilligan DM, Quyyumi AA, Cannon RO, 3rd. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. Circulation 1994; 89:2545-51.
109. Reis SE, Gloth ST, Blumenthal RS, et al. Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. Circulation 1994; 89:52-60.
110. Manson JE, Martin KA. Clinical practice. Postmenopausal hormone-replacement therapy. N Engl J Med 2001; 345:34-40.
111. Stampfer MJ, Willett WC, Colditz GA, Rosner B, Speizer FE, Hennekens CH. A prospective study of postmenopausal estrogen therapy and coronary heart disease. New England Journal of Medicine. 1985; 313:1044-9.
112. Stampfer MJ, Colditz GA, Willett WC, et al. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med 1991; 325:756-62.
113. Grodstein F, Stampfer MJ, Manson JE, et al. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease.[comment][erratum appears in N Engl J Med 1996 Oct 31;335(18):1406]. New England Journal of Medicine. 1996; 335:453-61.
114. Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease.[comment]. Annals of Internal Medicine. 2000; 133:933-41.
115. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. Circulation 1987; 75:1102-9.
116. Sullivan JM, El-Zeky F, Vander Zwaag R, Ramanathan KB. Effect on survival of estrogen replacement therapy after coronary artery bypass grafting. American Journal of Cardiology. 1997; 79:847-50.
117. Sullivan JM, Vander Zwaag R, Hughes JP, et al. Estrogen replacement and coronary artery disease. Effect on survival in postmenopausal women. Archives of Internal Medicine. 1990; 150:2557-62.
118. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. Jama 2002; 288:321-33.
119. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. Jama 2004; 291:1701-12.

120. Howard BV, Van Horn L, Hsia J, et al. Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. Jama 2006; 295:655-66.
121. Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000; 102:2284-99.
122. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. Jama 1998; 280:605-13.
123. Grady D, Herrington D, Bittner V, et al. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). Jama. 2002; 288:49-57.
124. Herrington DM, Reboussin DM, Brosnihan KB, et al. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. N Engl J Med 2000; 343:522-9.
125. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. Jama 2002; 288:872-81.
126. Humphrey LL, Chan BK, Sox HC. Postmenopausal hormone replacement therapy and the primary prevention of cardiovascular disease.[comment]. Annals of Internal Medicine. 2002; 137:273-84.
127. Grady D. Postmenopausal hormones--therapy for symptoms only. N Engl J Med 2003; 348:1835-7.
128. Grodstein F, Clarkson TB, Manson JE. Understanding the divergent data on postmenopausal hormone therapy. N Engl J Med 2003; 348:645-50.
129. Hodis HN, Mack WJ, Lobo RA, et al. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. Annals of Internal Medicine. 2001; 135:939-53.
130. Angerer P, Stork S, Kothny W, Schmitt P, von Schacky C. Effect of oral postmenopausal hormone replacement on progression of atherosclerosis : a randomized, controlled trial. Arteriosclerosis, Thrombosis & Vascular Biology. 2001; 21:262-8.
131. Cherry N, Gilmour K, Hannaford P, et al. Oestrogen therapy for prevention of reinfarction in postmenopausal women: a randomised placebo controlled trial.[comment]. Lancet. 2002; 360:2001-8.
132. Hodis HN, Mack WJ, Azen SP, et al. Hormone therapy and the progression of coronary-artery atherosclerosis in postmenopausal women.[comment]. New England Journal of Medicine. 2003; 349:535-45.
133. Hulley SB, Grady D. The WHI estrogen-alone trial--do things look any better? Jama 2004; 291:1769-71.
134. Thomas T, Rhodin J, Clark L, Garces A. Progestins initiate adverse events of menopausal estrogen therapy. Climacteric 2003; 6:293-301.
135. Rodriguez C, Calle EE, Patel AV, Tatham LM, Jacobs EJ, Thun MJ. Effect of body mass on the association between estrogen replacement therapy and mortality among elderly US women. Am J Epidemiol 2001; 153:145-52.
136. Naftolin F, Schneider HP, Sturdee DW. Guidelines for the hormone treatment of women in the menopausal transition and beyond. Climacteric 2004; 7:8-11.
137. Naftolin F, Taylor HS, Karas R, et al. The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. Fertil Steril 2004; 81:1498-501.
138. Clarkson TB, Anthony MS, Morgan TM. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. J Clin Endocrinol Metab 2001; 86:41-7.
139. Williams JK, Anthony MS, Honore EK, et al. Regression of atherosclerosis in female monkeys. Arterioscler Thromb Vasc Biol 1995; 15:827-36.
140. Harman SM, Brinton EA, Cedars M, et al. KEEPS: The Kronos Early Estrogen Prevention Study. Climacteric 2005; 8:3-12.
141. www.fda.gov/cder/drug/infopage/estrogens_progestins.
142. Hersh AL, Stefanick ML, Stafford RS. National use of postmenopausal hormone therapy: annual trends and response to recent evidence. Jama 2004; 291:47-53.

143. Haas JS, Kaplan CP, Gerstenberger EP, Kerlikowske K. Changes in the use of postmenopausal hormone therapy after the publication of clinical trial results. Ann Intern Med 2004; 140:184-8.
144. Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. Jama 1999; 281:2189-97.
145. Ettinger B, Black DM, Mitlak BH, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. Jama 1999; 282:637-45.
146. Wenger NK, Barrett-Connor E, Collins P, et al. Baseline characteristics of participants in the Raloxifene Use for The Heart (RUTH) trial. Am J Cardiol 2002; 90:1204-10.
147. Barrett-Connor E, Grady D, Sashegyi A, et al. Raloxifene and cardiovascular events in osteoporotic postmenopausal women: four-year results from the MORE (Multiple Outcomes of Raloxifene Evaluation) randomized trial. Jama 2002; 287:847-57.
148. Delmas PD, Bjarnason NH, Mitlak BH, et al. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. N Engl J Med 1997; 337:1641-7.
149. Walsh BW, Kuller LH, Wild RA, et al. Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. Jama 1998; 279:1445-51.
150. Mijatovic V, van der Mooren MJ, Kenemans P, de Valk-de Roo GW, Netelenbos C. Raloxifene lowers serum lipoprotein(A) in healthy postmenopausal women: a randomized, double-blind, placebo-controlled comparison with conjugated equine estrogens. Menopause 1999; 6:134-7.
151. Walsh BW, Paul S, Wild RA, et al. The effects of hormone replacement therapy and raloxifene on C-reactive protein and homocysteine in healthy postmenopausal women: a randomized, controlled trial. J Clin Endocrinol Metab 2000; 85:214-8.
152. Sarrel PM, Nawaz H, Chan W, Fuchs M, Katz DL. Raloxifene and endothelial function in healthy postmenopausal women. American Journal of Obstetrics & Gynecology. 2003; 188:304-9.
153. Saitta A, Altavilla D, Cucinotta D, et al. Randomized, double-blind, placebo-controlled study on effects of raloxifene and hormone replacement therapy on plasma no concentrations, endothelin-1 levels, and endothelium-dependent vasodilation in postmenopausal women. Arterioscler Thromb Vasc Biol 2001; 21:1512-9.
154. Cosman F, Lindsay R. Selective estrogen receptor modulators: clinical spectrum. Endocr Rev 1999; 20:418-34.
155. Adlercreutz H. Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand J Clin Lab Invest Suppl 1990; 201:3-23.
156. Anthony MS, Clarkson TB, Bullock BC, Wagner JD. Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. Arterioscler Thromb Vasc Biol 1997; 17:2524-31.
157. Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: potential mechanisms. Am J Clin Nutr 1998; 68:1390S-1393S.
158. Anthony MS, Clarkson TB, Hughes CL, Jr., Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996; 126:43-50.
159. Honore EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril 1997; 67:148-54.
160. Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman JW, Jr., Potter SM. Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. J Nutr 1994; 124:213-22.
161. Cassidy A, Bingham S, Setchell KD. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 1994; 60:333-40.
162. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995; 333:276-82.
163. Food labeling: health claims; soy protein and coronary heart disease. Food and Drug Administration, HHS. Final rule. Fed Regist 1999; 64:57700-33.
164. Nestel P. Isoflavones: their effects on cardiovascular risk and functions. Curr Opin Lipidol 2003; 14:3-8.

165. Ashton EL, Dalais FS, Ball MJ. Effect of meat replacement by tofu on CHD risk factors including copper induced LDL oxidation. J Am Coll Nutr 2000; 19:761-7.
166. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci U S A 1998; 95:3106-10.
167. Wiseman H, O'Reilly JD, Adlercreutz H, et al. Isoflavone phytoestrogens consumed in soy decrease F(2)-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 2000; 72:395-400.
168. Jenkins DJ, Kendall CW, Vidgen E, et al. The effect on serum lipids and oxidized low-density lipoprotein of supplementing self-selected low-fat diets with soluble-fiber, soy, and vegetable protein foods. Metabolism 2000; 49:67-72.
169. Jenkins DJ, Kendall CW, Vidgen E, et al. Effect of soy-based breakfast cereal on blood lipids and oxidized low- density lipoprotein. Metabolism 2000; 49:1496-500.
170. Hodgson JM, Puddey IB, Croft KD, Mori TA, Rivera J, Beilin LJ. Isoflavonoids do not inhibit in vivo lipid peroxidation in subjects with high-normal blood pressure. Atherosclerosis 1999; 145:167-72.
171. Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol 1997; 17:3392-8.
172. Samman S, Lyons Wall PM, Chan GS, Smith SJ, Petocz P. The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. Atherosclerosis 1999; 147:277-83.
173. Dubey RK, Gillespie DG, Imthurn B, Rosselli M, Jackson EK, Keller PJ. Phytoestrogens inhibit growth and MAP kinase activity in human aortic smooth muscle cells. Hypertension 1999; 33:177-82.
174. Fujio Y, Yamada F, Takahashi K, Shibata N. Responses of smooth muscle cells to platelet-derived growth factor are inhibited by herbimycin-A tyrosine kinase inhibitor. Biochem Biophys Res Commun 1993; 195:79-83.
175. Shimokado K, Yokota T, Umezawa K, Sasaguri T, Ogata J. Protein tyrosine kinase inhibitors inhibit chemotaxis of vascular smooth muscle cells. Arterioscler Thromb 1994; 14:973-81.
176. Teede HJ, Dalais FS, Kotsopoulos D, et al. Dietary soy containing phytoestrogens does not activate the hemostatic system in postmenopausal women. J Clin Endocrinol Metab 2005; 90:1936-41.
177. Wilcox JN, Blumenthal BF. Thrombotic mechanisms in atherosclerosis: potential impact of soy proteins. J Nutr 1995; 125:631S-638S.
178. Nestel PJ, Pomeroy S, Kay S, et al. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 1999; 84:895-8.
179. Squadrito F, Altavilla D, Crisafulli A, et al. Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. American Journal of Medicine. 2003; 114:470-6.
180. Steinberg FM, Guthrie NL, Villablanca AC, Kumar K, Murray MJ. Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. American Journal of Clinical Nutrition. 2003; 78:123-30.
181. Cuevas AM, Irribarra VL, Castillo OA, Yanez MD, Germain AM. Isolated soy protein improves endothelial function in postmenopausal hypercholesterolemic women. Eur J Clin Nutr 2003; 57:889-894.
182. Colacurci N, Chiantera A, Fornaro F, et al. Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause 2005; 12:299-307.
183. Blum A, Lang N, Peleg A, et al. Effects of oral soy protein on markers of inflammation in postmenopausal women with mild hypercholesterolemia. American Heart Journal. 2003; 145:e7.
184. Hale G, Paul-Labrador M, Dwyer JH, Merz CN. Isoflavone supplementation and endothelial function in menopausal women. Clinical Endocrinology. 2002; 56:693-701.
185. Simons LA, von Konigsmark M, Simons J, Celermajer DS. Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. American Journal of Cardiology. 2000; 85:1297-301.

186. Teede HJ, Dalais FS, Kotsopoulos D, Liang YL, Davis S, McGrath BP. Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. Journal of Clinical Endocrinology & Metabolism. 2001; 86:3053-60.
187. Lissin LW, Oka R, Lakshmi S, Cooke JP. Isoflavones improve vascular reactivity in post-menopausal women with hypercholesterolemia. Vasc Med 2004; 9:26-30.
188. Kreijkamp-Kaspers S, Kok L, Bots ML, Grobbee DE, Lampe JW, van der Schouw YT. Randomized controlled trial of the effects of soy protein containing isoflavones on vascular function in postmenopausal women. 2005.
189. Wilson TA, Meservey CM, Nicolosi RJ. Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate. Atherosclerosis. 1998; 140:147-53.
190. Clark SB, Clark VE, Small DM. Effects of lecithin ingestion on plasma and lymph lipoproteins of normo- and hyperlipemic rats. Am J Physiol 1981; 241:G422-30.
191. Hunt CE, Duncan LA. Hyperlipoproteinaemia and atherosclerosis in rabbits fed low-level cholesterol and lecithin. Br J Exp Pathol 1985; 66:35-46.
192. Wong EK, Nicolosi RJ, Low PA, Herd JA, Hayes KC. Lecithin influence on hyperlipemia in rhesus monkeys. Lipids 1980; 15:428-33.
193. Childs MT, Bowlin JA, Ogilvie JT, Hazzard WR, Albers JJ. The contrasting effects of a dietary soya lecithin product and corn oil on lipoprotein lipids in normolipidemic and familial hypercholesterolemic subjects. Atherosclerosis 1981; 38:217-28.
194. Cobb M, Turkki P, Linscheer W, Raheja K. Lecithin supplementation in healthy volunteers: effect on cholesterol esterification and plasma, and bile lipids. Nutr Metab 1980; 24:228-37.
195. Katz DL, Evans MA, Nawaz H, et al. Egg consumption and endothelial function: a randomized controlled crossover trial. Int J Cardiol 2005; 99:65-70.
196. Frangi AF, Laclaustra M, Lamata P. A registration-based approach to quantify flow-mediated dilation (FMD) of the brachial artery in ultrasound image sequences. IEEE Trans Med Imaging 2003; 22:1458-69.
197. Karamsetty MR, Klinger JR, Hill NS. Phytoestrogens restore nitric oxide-mediated relaxation in isolated pulmonary arteries from chronically hypoxic rats. J Pharmacol Exp Ther 2001; 297:968-74.
198. Williams JK, Anthony MS, Herrington DM. Interactive effects of soy protein and estradiol on coronary artery reactivity in atherosclerotic, ovariectomized monkeys. Menopause 2001; 8:307-13.
199. Walker HA, Dean TS, Sanders TA, Jackson G, Ritter JM, Chowienczyk PJ. The Phytoestrogen Genistein Produces Acute Nitric Oxide-Dependent Dilation of Human Forearm Vasculature With Similar Potency to 17ss- Estradiol. Circulation 2001; 103:258-262.
200. Blum A, Lang N, Vigder F, et al. Effects of soy protein on endothelium-dependent vasodilatation and lipid profile in postmenopausal women with mild hypercholesterolemia. 2003.
201. Squadrito F, Altavilla D, Morabito N, et al. The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. Atherosclerosis 2002; 163:339-47.
202. Lichtenstein AH, Jalbert SM, Adlercreutz H, et al. Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. Arterioscler Thromb Vasc Biol 2002; 22:1852-8.
203. Crouse JR, 3rd, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999; 159:2070-6.
204. Gardner CD, Newell KA, Cherin R, Haskell WL. The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001; 73:728-35.
205. Merz-Demlow BE, Duncan AM, Wangen KE, et al. Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. Am J Clin Nutr 2000; 71:1462-9.
206. Wangen KE, Duncan AM, Xu X, Kurzer MS. Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001; 73:225-31.
207. Weggemans RM, Trautwein EA. Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta-analysis. Eur J Clin Nutr 2003; 57:940-6.

208. Jayagopal V, Albertazzi P, Kilpatrick ES, et al. Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. Diabetes Care 2002; 25:1709-14.
209. Vogel RA, Corretti MC, Plotnick GD. A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. Clinical Cardiology. 2000; 23:571-5.
210. Gross ER, LaDisa JF, Jr., Weihrauch D, et al. Reactive oxygen species modulate coronary wall shear stress and endothelial function during hyperglycemia. Am J Physiol Heart Circ Physiol 2003; 284:H1552-9.
211. Kocsis E, Pacher P, Posa I, Nieszner E, Pogatsa G, Koltai MZ. Hyperglycaemia alters the endothelium-dependent relaxation of canine coronary arteries. Acta Physiol Scand 2000; 169:183-7.
212. Tesfamariam B, Cohen RA. Free radicals mediate endothelial cell dysfunction caused by elevated glucose. Am J Physiol 1992; 263:H321-6.
213. Bloedon LT, Jeffcoat AR, Lopaczynski W, et al. Safety and pharmacokinetics of purified soy isoflavones: single-dose administration to postmenopausal women. Am J Clin Nutr 2002; 76:1126-37.