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# Vascular Effects of Phytoestrogens and Alternative Menopausal Hormone Therapy in Cardiovascular Disease

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## Abstract

Phytoestrogens are estrogenic compounds of plant origin classified into different groups including isoflavones, lignans, coumestans and stilbenes. Isoflavones such as genistein and daidzein are the most studied and most potent phytoestrogens, and are found mainly in soy based foods. The effects of phytoestrogens are partly mediated via estrogen receptors (ERs): ERα, ERβ and possibly GPER. The interaction of phytoestrogens with ERs is thought to induce both genomic and non-genomic effects in many tissues including the vasculature. Some phytoestrogens such as genistein have additional non-ER-mediated effects involving signaling pathways such as tyrosine kinase. Experimental studies have shown beneficial effects of phytoestrogens on endothelial cells, vascular smooth muscle, and extracellular matrix. Phytoestrogens may also affect other pathophysiologic vascular processes such as lipid profile, angiogenesis, inflammation, tissue damage by reactive oxygen species, and these effects could delay the progression of atherosclerosis. As recent clinical trials showed no vascular benefits or even increased risk of cardiovascular disease (CVD) and CV events with conventional menopausal hormone therapy (MHT), phytoestrogens are being considered as alternatives to pharmacologic MHT. Epidemiological studies in the Far East population suggest that dietary intake of phytoestrogens may contribute to the decreased incidence of postmenopausal CVD and thromboembolic events. Also, the WHO-CARDIAC study supported that consumption of high soybean diet is associated with lower mortalities from coronary artery disease. However, as with estrogen, there has been some discrepancy between the experimental studies demonstrating the vascular benefits of phytoestrogens and the data from clinical trials. This is likely because the phytoestrogens clinical trials have been limited in many aspects including the number of participants enrolled, the clinical end points investigated, and the lack of long-term follow-up. Further investigation of the cellular mechanisms underlying the vascular effects of phytoestrogens and careful evaluation of the epidemiological evidence and clinical trials of their potential vascular benefits would put forward the use of phytoestrogens as an alternative MHT for the relief of menopausal symptoms and amelioration of postmenopausal CVD.

### Keywords

estrogen; endothelium; smooth muscle; calcium; blood pressure

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## INTRODUCTION

Estrogen (E2) deficiency during menopause is associated with perimenopausal symptoms such as hot flushes and night sweats which prompt women to seek menopausal hormone therapy (MHT). In addition to the relief of menopausal hot flushes and vaginal dryness, MHT may improve sleep quality and social well-being, retard bone loss and minimize osteoporotic fractures [1,2].

The risk of cardiovascular disease (CVD) also increases after menopause, suggesting vascular benefits of endogenous E2 [3-5]. Estrogen receptors (ERs) have been identified in the vasculature, and E2 has been shown to promote beneficial effects on the endothelium, vascular smooth muscle (VSM) and extracellular matrix (ECM) [3,4,6-8]. The vascular benefits of E2 observed in experimental studies have suggested potential benefits of MHT in CVD.

Studies of the vascular benefits of female sex hormones have mainly focused on natural and synthetic estrogens. Initial population-based observational studies showed 35% reduction in mortality and a 50% reduction in CV events among women using MHT [9]. Also, a meta-analysis of results from different studies demonstrated overall improvement of atherosclerotic biomarkers and suggested CV benefit of MHT [10]. However, randomized clinical trials (RCTs) did not demonstrate a decrease in CV events and instead showed increased risk of thromboembolic events. As of 2001, only 38% of postmenopausal women (Post-MW) in the United States used MHT [11]. This has prompted investigations of the possible causes of the discrepancies between the experimental vascular benefits of E2 and the results of the clinical trials. Other investigations have focused on alternative MHT.

In the past two decades, there has been an increasing interest in phytoestrogens as natural alternatives to MHT [12]. Phytoestrogens, or “dietary estrogens”, are a heterogeneous group of naturally occurring compounds with structural similarities to E2 that allow them to mimic the effects of E2. Phytoestrogens have several potential applications in different diseases. Phytoestrogens decrease bone resorption and delay the progression of osteoporosis in Post-MW [13], exert anti-androgenic effects which could be useful in benign prostatic hypertrophy [14], and may have protective effects in prostate and breast cancers [15], and neuroprotective effects that could improve cognitive functions of the brain [16]. Phytoestrogens also showed a potential to improve CV function and to decrease the risk of CVD associated with menopause [17].

Epidemiological evidence suggests potential protective effects of phytoestrogens. The incidence of CVD, diabetes, obesity and breast cancer are less in Asian than Western populations. Also, the incidence of hot flushes is 70-80% in menopausal Western women compared to 14-15% in Asian women [18]. Migration studies of the Japanese population moving to the United States showed that they developed an increased incidence in “Western Diseases” – mainly CV- after two generations. These observations suggest that the factors contributing to CVD are not only genetic, but could also involve environmental factors such as the diet. One important difference between Asian and Western diets is the high content of soy-rich in phytoestrogens- in the Asian diet (20-150 mg/d) compared to the Western diet (1-3 mg/d) [12]. In a study examining the relation between coronary artery disease (CAD) and dietary habits of 61 populations in 25 countries, the 24 hour urinary excretion of taurine and isoflavones, which are abundant in fish and soybean diets, was inversely related to mortality rates from CAD [19]. These dietary differences may contribute to the lower incidence of CAD among the Asian populations.

Research on the CV effects of phytoestrogens has progressed steadily, and the beneficial vascular effects demonstrated in some studies have suggested potential applications in CVD.

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Also, being natural, phytoestrogens have less side effects. However, phytoestrogens are a diverse group of compounds with different modes of metabolism, bioavailability and *in vivo* effects. Thus, after decades of research there is no definitive agreement as to the vascular effects of phytoestrogens and their benefit in CVD. In effect, some studies have suggested that phytoestrogens may not have any benefit in CVD, and other studies attributed the benefits of the soy-rich diet to food components other than phytoestrogens [20]. Also, most of the clinical studies of phytoestrogens have been limited in terms of the number of subjects enrolled, the compounds studied, the duration of dietary intake and the long-term follow-up of the participants.

This review discusses reports from the Pubmed database and highlights the sources, classification, and chemical structure of phytoestrogens, and their interaction with ERs, signaling pathways and vascular effects on the endothelium, VSM and ECM. Other vascular effects of phytoestrogens on lipid profile, angiogenesis, and inflammation, and how these effects could retard the progression of atherosclerosis will also be discussed. We will then highlight some of the clinical trials that evaluated the vascular effects of phytoestrogens, and their implications in CV medicine. Throughout the review we will discuss the reported benefits of phytoestrogens and suggest areas that need further investigation. To facilitate comparison, we will briefly describe the effects of E2 followed by the data on phytoestrogens.

## Sources and Classification of Phytoestrogens

Phytoestrogens are polyphenolic non-steroidal compounds of estrogenic activity. While phytoestrogens are generally plant in origin, resorcylic acid lactones, which are produced by molds, exhibit estrogenic activity and hence termed mycoestrogens [21]. Major classes of phytoestrogens include isoflavones, lignans and coumestans. Other phytoestrogens include stilbenes, flavanones, flavonols, and flavones (Fig. 1). The most abundant, most studied and most potent phytoestrogens are isoflavones. There are more than 1000 types of isoflavones including genistein, daidzein, genistin, daidzin, formononetin, biochanin-A and equol. The most commonly studied isoflavones are genistein, daidzein and its metabolite equol.

Isoflavonoids are a subclass of flavonoids, where one phenolic ring has migrated from C-3 to C-2. Isoflavones are found in legumes such as soy, chickpeas, clover, lentils and beans (Table 1) [22]. Unextracted soy protein contains onaverage 1.105 mg genistein and 0.365 mg daidzein/g soy proteins isolate. However, total isoflavone content may vary up to 3-fold with growth of the same soy cultivar in different geographical areas and different years. Biochanin-A and formononetin are precursors of genistein and daidzein, respectively, and also have estrogenic properties. Formononetin is abundant in *Astragalus mongholicus* Bunge and *Curcuma comosa* Roxb. Glycitein and its conjugates are minor isoflavones in soybean cotyledons, but are major components in dietary supplements and foods made from the soybean hypocotyls.

Lignans are common in the plant kingdom and are the building block of lignin found in the plant cell wall. Food containing lignans include flaxseed, lentils, whole grains, beans, fruits, and vegetables (Table 1). Enterolactone and enterodiol are major lignans produced by the action of intestinal bacteria on matairesinol and secoisolariciresinol, respectively [23].

Coumestans such as coumestrol and 4-methoxycoumestrol are found in mung bean sprouts, brussel sprouts and spinach. Coumestrol, the most important coumestan consumed by humans, is found in clover sprouts, alfalfa sprouts, and other legumes.

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The stilbenes family of phytoestrogens includes reseveratrol and pterostilbene which are commonly found in red wine and peanuts. Resveratrol has estrogenic activity only in the Trans form [24].

Flavanones include eriodictyol, naringenin, pinocembrin and are mainly found in citrus fruits. Flavonols include kaempferol, myricetin, quercetin, and quercetagetin, and are found abundantly in green tea and to a less extent in dark tea and chocolate. Flavones include apigenin, baicalain, chyrisin, norwogenin and are found mainly in cereals and herbs.

## Phytoestrogens Metabolism

Phytoestrogens are present in plants as inactive glycosidic conjugates. In the intestine, they are hydrolyzed by the action of UDP-glucuronosyltransferase secreted by intestinal bacteria to the active forms aglycones (Fig. 2). The aglycones are then absorbed by the intestinal tract. On entering the circulation, aglycones may undergo extensive metabolism to other compounds through various reactions including demethylation, methylation, hydroxylation, chlorination, iodination, and nitration [25]. These metabolites are then transported to the liver where they undergo conjugation to form β-glucuronides and to a less extent sulfate esters. In the liver some glucuronides undergo further fermentation into other metabolites that vary depending on the class of phytoestrogen. The glucuronides are excreted in bile and partially reabsorbed via the enterohepatic circulation. Phytoestrogens are excreted in bile and urine as conjugated glucuronides and in feces in the unconjugated form (Fig. 2) [25,26].

As with other phytoestrogens, isoflavones in food are bound to glucose. When ingested, they are enzymatically cleaved in the gut into active aglycones. Genistein and daidzein, the most active forms of isoflavones, are produced both by hydrolysis of their biologically inactive glucoconjugates, as well as from the demethylation of their precursors biochanin A and formononetin, respectively. The aglycone forms of isoflavones are easily transported across the intestinal epithelial cells to the blood or are further metabolized in the intestine [26]. In humans consuming soy-free diets, the plasma concentration of isoflavones is usually in the nanomolar range, ≤40 nM. Acute ingestion of dietary soy leads to a rapid increase in the plasma concentration of isoflavones up to the micromolar range [27]. The isoflavone serum concentration shows variability in different populations. In serum samples of Japanese men, the average concentration of genistein is 276 nmol/L and of daidzein is 107 nmol/L [28].

The majority of the genistein and daidzein consumed is eliminated from the body within 24 hours [26]. Genistein is transformed to dihydrogenistein and is further metabolized in the colon to 4-ethyl phenol. Daidzein is metabolized to dihydrodaidzein, which is further metabolized to both equol and O-desmethylangolensin (O-DMA). Genistein, daidzein, equol and O-DMA are the major isoflavones detected in blood and urine of humans and animals [29]. Interestingly, only 30-40% of humans –mostly Asians and vegetarians - are able to metabolize daidzein into equol, and the ability to produce equol may be associated with an increased benefit of isoflavones on bone mineral density and a lower risk of breast cancer [30,31].

## Factors Affecting the Metabolism of Phytoestrogens

The metabolism and excretion of isoflavones after soy consumption show considerable variation among individuals. The average time taken after ingesting the aglycones to reach peak plasma concentration is 4–7 hr, and is delayed to 8–11 hr for the corresponding glycosidic conjugates. This suggests that the rate-limiting step for absorption is the initial hydrolysis of the glycosidic moiety. The half-lives of genistein and daidzein are 7.1 and 9.3 hr, respectively [32].

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In addition to the inter-individual variations in phytoestrogen metabolism, sex may also play a role, with women metabolizing phytoestrogens more efficiently than men [12]. Other factors that could influence isoflavone bioavailability include the chemical composition, the administered dose, intestinal transit time, intestinal microflora and the individual ability to produce equol [26]. The source of the isoflavones and hence the food matrix in which the compound is delivered plays a minor role in their bioavailability. The effect of age on the bioavailability of isoflavones was also investigated, but no difference was found in the pharmacokinetics of either genistein or daidzein between Pre- and Post-MW [33]. Also, the frequency of ingestion does not appear to cause significant difference in the bioavailability of isoflavones [34].

## Estrogen Receptor

ER has two major subtypes ERα and ERβ that differ in their C-terminal ligand-binding domain and in the N-terminal transactivation domain [3,35]. Several splice variants of ER subtypes have also been identified. The diversity among ER variants could be due to epigenetic changes, methylation of the genes encoding ERs, alternative RNA splicing leading to multiple ER mRNA isoforms, and multiple sites for initiation of translation of ER mRNA [36]. The two nuclear ER genes are located on separate chromosomes. ER1, the gene that encodes ERα, is located on chromosome 6q(25.1) and ER2, the gene that encodes ERβ, is located on chromosome 14q(23-24.1) [37]. Although E2 release patterns and plasma levels change with aging, little is known about the age-associated changes in ER expression and subtypes.

Similar to other members of the nuclear receptor superfamily, ERα and ERβ share a common structure with five functional domains A/B, C, D, E and F [3]. Domain A/B is involved in protein-protein interactions and transcriptional activation of target gene expression. Domain C is involved in DNA binding and ER dimerization. Domain D is the hinge domain linking domain C and E and is responsible for nuclear localization of ER. Domain E is the ligand-binding domain. Domain F contains co-factor recruitment regions [3]. Two acidic activation factors, AF-1 and AF-2, mediate the ligand-dependent transcriptional activity of ER. AF-1 is located within the N terminus. AF-1 in ERα is very active on a variety of E2-sensitive promoters whereas its activity in ERβ is minimal. Hormone-dependent AF-2 is located in the ligand-binding domain [38]. AF-1 and AF-2 may also be required for ligand independent receptor functions, including growth factor activation by AF-1 and cAMP activation by AF-2.

Nuclear ERs are 40 times more abundant than membrane ERs. The same DNA sequence is responsible for coding both nuclear and membrane ERs, but post-translational protein modifications are likely to be responsible for targeting ER to either the nucleus or plasma membrane [39]. ERα and ERβ have overlapping but not identical tissue distribution and expression levels, suggesting distinct biological roles. ERα is expressed abundantly in the uterus, vagina, ovaries, mammary gland, and hypothalamus [3,40]. ERβ is more active in the prostate and ovaries, with smaller number in the lungs, brain, and bones [3]. ERs have also been identified in ECs and VSM [3,6,41,42].

G protein-coupled receptor (GPR30) also termed G protein-coupled ER (GPER) is a novel membrane receptor that binds E2. GPER comprises 375 amino acids and shares little homology with the classical ERs. The gene coding for GPER is located on chromosome 7p22.3 and consists of three exons which code for three domains; an N-terminal domain, a 7-transmembrane domain and a C-terminal domain [43]. GPER is widely distributed in the brain and peripheral tissues and may play a functional role in the vasculature. GPER has been localized in the endoplasmic reticulum [44], and plasma membrane [45]. However, the cellular localization of GPER appears to vary depending on the cell type.

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ERs display marked differences in binding affinity and activation by natural and synthetic ligands [37,46]. Endogenous natural estrogens are C18 steroids and include estrone (E1), estradiol (E2), and estriol (E3). They have 4 rings A, B, C, D, a hydroxyl group at C3, and either a hydroxyl or ketone group at C17. The phenolic A ring is responsible for selective high-affinity binding to ER. Only 5 chemicals without aromatic rings were found to be active. These 5 chemicals possess H-bond capability with a rigid hydrophobic backbone that matches the A, B and C rings of E2 [3,47].

## Phytoestrogens and ERs

Different classes of phytoestrogens have distinct chemical structures that could allow them to bind to ERs (Fig. 1). The key structural elements which are essential for the estrogenic effects are the phenolic rings, low molecular weight, and optimal hydroxylation patterns [3]. Phytoestrogens could modulate ER function in several ways, including having both agonist and antagonist effects. Phytoestrogens bind both ERα and ERβ, and activate ER-dependent gene transcription. The affinity of most phytoestrogens to ERs is 1/100 to 1/10000 that of E2 but they may reach concentrations up to 10000 times that of E2 in the human body. Phytoestrogens also have different binding affinities to ER subtypes with generally higher affinity for ERβ than ERα, which explains why they may act differently from E2 [12,16]. Genistein has high affinity for ERβ, almost identical to that of E2, while its affinity for ERα is only 6% of E2. Daidzein has very weak binding affinity for both ERα and ERβ, but its relative affinity for ERβ is still higher than that for ERα. One study estimated that the maximal activity induced by isoflavone phytoestrogens is about half the activity of E2. Coumestrol has very high binding affinity for human ERα, but still a slightly higher affinity for ERβ. The actions of phytoestrogens at the cellular and molecular level are influenced by many factors including the phytoestrogen concentration, ER status, presence or absence of endogenous estrogens, and the type of target organ or cell [35,48].

## ER-Mediated Genomic and Nongenomic Effects

Although life is possible without either or both ERs, the reproductive functions are severely impaired [49]. ERs also mediate multiple vascular, hematologic and metabolic effects through stimulation or inhibition of gene expression (genomic pathways) and via other pathways which do not involve gene transcription or new protein synthesis (nongenomic pathways).

E2/ER activate genomic pathways that regulate many transcriptional processes and require relatively longer time to show their effects. Upon binding E2, ER undergoes conformational changes resulting in the formation of a homo- or heterodimer with high affinity for E2 and DNA. This is followed by nuclear translocation of ER, binding to specific estrogen response elements (ERE) and regulation of target gene expression. Depending on the cell and promoter context, the DNA-bound ER exerts either positive or negative effects on the expression of downstream target gene(s). Ligand-bound ER may also interact with other transcription factor complexes to influence transcription of genes whose promoters do not harbor ERE [3,5].

The overall effects of E2 depend on the ratio between ERα and ERβ in different tissues. However, ERβ stimulation can produce some ERα effects in some organs [49]. Also, ERβ may interact in a ligand independent manner with EREs of target promoters and attenuate the ligand dependent transcriptional activity of ERα [50].

ERs can also regulate gene transcription without binding directly to DNA and thus regulate the expression of a large number of E2-responsive genes that do not contain ERE. The

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receptors in such cases are tethered through protein-protein interactions to a transcription factor complex that contacts the DNA [51].

ER function can also be modulated by extracellular signals in the absence of E2. Polypeptide growth factors such as epidermal growth factor and insulin-like growth factor-1 can activate ER and increase the expression of ER target genes. The mechanisms by which the E2 and growth factor pathways converge are not clear, but these pathways appear to be dependent on each other for the full manifestation of the ligand-mediated response [52].

Non-genomic effects are rapid responses that occur too quickly to be mediated by gene transcription, are independent of protein synthesis, and typically involve modulation of membrane bound and cytoplasmic regulatory proteins. For example, following E2 binding to GPER, the Gα-GTPase subunit dissociates from the G-protein complex and activates adenylyl cyclase and phospholipase C, which in turn generates second messengers such as cAMP, $IP_3$ and $Ca^{2+}$ [53]. Other E2-activated pathways include mitogen-activated protein kinase (MAPK), phosphatidylinositol trisphosphate kinase $PI_3K$/Akt, and alteration of ion channel fluxes [3,54].

## Phytoestrogens and Endothelium

Like E2, phytoestrogens may have beneficial effects on the CV system partly through effects on the vascular endothelium.

**Effects of Phytoestrogens on Endothelial Cell Growth and Permeability—**E2 stimulates endothelial cell (EC) proliferation via cytosolic and nuclear ERs [55]. E2 is also important for the integrity of the endothelium and consequently the vascular permeability. In cultured human umbilical vein endothelial cells (HUVECs) E2 has a biphasic effect on vascular permeability; at nanomolar concentrations E2 decreases the permeability, but at micromolar concentrations E2 increases it [56]. Animal studies also support a role of E2 on EC integrity and permeability. Studies have shown that the permeability of the blood brain barrier is 500% greater in ovariectomized (OVX) than intact female rats, and E2 replacement restores barrier properties [57]. The E2-induced decrease in EC permeability may be related to regulation of prostaglandin E2 (PGE2) levels [58]. Similar to E2, phytoestrogens regulate EC proliferation, maintain EC integrity and decrease vascular permeability. Several studies have shown that genistein regulates the proliferation of human endometrial ECs [59]. Genistein derivatives protect HUVEC-12 from $H_20_2$ induced apoptosis [60]. Genistein also inhibits TNF-α-induced apoptosis in human aortic ECs [61]. Phytoestrogens also maintain the integrity and decrease the permeability of the endothelium. For example, in HUVECs equol improves EC function by reducing the generation of reactive oxygen species (ROS) [62]. Equol also has a protective effect against EC dysfunction induced by ritonavir, an antiprotease drug used in HIV patients [63]. Low concentrations of biochanin A also inhibit cell proliferation in the human EC line ECV304 [64]. Also, long-term oral administration of genistein inhibits retinal vascular leakage in experimentally-induced diabetes in rats, possibly via tyrosine kinase (TK) inhibition [65]. Genistein, also via TK inhibition, modulates bradykinin- and substance P-induced increase in macromolecular efflux from the hamster cheek pouch microcirculation [66]. In mouse skin, genistein inhibits vascular endothelial growth factor (VEGF)-induced increase in vascular permeability, possibly by inhibiting TK-mediated local production of NO and arachidonic acid metabolites [67]. Also, pretreatment of bovine aortic ECs with genistein inhibits thrombin-induced increase in EC permeability via activation of the PKA/cAMP pathway [68]. Thus, phytoestrogens regulate EC proliferation, maintain vascular integrity and decrease endothelial permeability; and further studies are needed to define the mechanisms and pathways involved.

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**Phytoestrogens and EC Function—**Vascular tone is controlled by the ratio between vasodilators such as NO, $PGI_2$ and EDHF and vasoconstrictors such as angiotensin II (Ang II) and endothelin (ET). Experimental data have shown beneficial vascular effects of E2. ERs mediate endothelium-dependent vascular relaxation, and E2 promotes NO, $PGI_2$ and EDHF production and decreases ET release [5,8,69,70]. Phytoestrogens promote endothelium-mediated vascular relaxation via similar mechanisms.

**Phytoestrogens and NO—**Endothelium-derived NO is a key regulator of vascular tone. In ECs, activation of eNOS leads to transformation of L-arginine to L-citrulline and NO production (Fig. 3). E2 upregulates eNOS in human ECs by increasing eNOS promoter activity and enhancing the binding activity of the transcription factor Sp1. E2 also increases EC $Ca^{2+}$, MAPK and $PI_3K$ activity and thereby increases eNOS activity and NO production. E2 also reduces antioxidants which are known to decrease NO bioavailability [71]. Phytoestrogens may have similar effects on NO production and activity. Genistein stimulates NO release in human aortic ECs and HUVECs [72]. In human EA.hy926 EC line, biochanin A and formononetin and their metabolites genistein and daidzein increase eNOS promoter activity and NO release [73]. Studies suggested different mechanisms of phytoestrogen-induced increase in NO production. In bovine aortic ECs and HUVECs, genistein may act through a protein kinase A (PKA)-dependent pathway, as genistein-induced eNOS activation and phosphorylation was abolished by inhibition of PKA by H89 and was not blocked by ER antagonists, MAPK or $PI_3K$/Akt-Kinase inhibitors [74]. In human aortic ECs and HUVECs, equol stimulates phosphorylation of ERK1/2 and $PI_3K$/Akt, leading to the activation of NOS and increased NO production at resting cytosolic $Ca^{2+}$ levels [75]. Animal studies also support that phytoestrogens increase NO production by increasing eNOS expression and activity [76]. In OVX female Sprague-Dawley rats, treatment with E2 reverses EC dysfunction and increases $Ca^{2+}$ dependent NOS activity in lung homogenates, and treatment with genistein increases NOS activity and improves endothelial dysfunction to the same extent [77]. Also, in isolated rat carotid and basilar arteries both equol and daidzein possess vasodilator activity. Interestingly, in hypertensive rats the vasorelaxant response to equol, but not daidzein, is preserved [78]. Also, formononetin relaxes phenylephrine-preconstricted rat aorta via NO-dependent mechanism and other endothelium-independent mechanisms [79]. Several studies support a role of ER in phytoestrogen-induced vasodilatation. In anesthetized pigs, intracoronary infusion of genistein at constant heart rate and BP increases coronary blood flow as assessed by ultrasound flowmeters and induces the phosphorylation of eNOS and NO production through ERK 1/2, Akt and p38 MAPK pathways. The genistein-induced coronary vasodilation appears to involve ERα/ERβ and stimulation of $\beta_2$-adrenoreceptors [80]. In mouse aorta, red wine polyphenols as well as ERα agonists stimulate endothelium-dependent NO pathway via activation of ERα [81]. Also, red clover extracts stimulate NO synthesis in cultured human ECs by recruiting ER-β [82]. In ECs, caveolin-1 is an anchoring protein that binds to eNOS and reduces its activity. The increase in $Ca^{2+}$ together with calmodulin promotes the dissociation of eNOS from caveolin leading to increased eNOS activity. One study suggested that daidzein and E2 may not alter eNOS protein in rat aorta but reduce the expression of caveolin-1 and increase the expression of calmodulin, and thereby increase eNOS activity [83]. Thus phytoestrogens may promote vasodilation by increasing the expression and activity of eNOS and increasing NO production in ECs.

**Phytoestrogens and cGMP—**Biological signaling by NO is primarily mediated by cGMP. cGMP is synthesized by NO-activated guanylyl cyclase and is broken down by phosphodiesterase enzyme. cGMP activates PKG, which phosphorylates many cellular proteins, leading to activation or inactivation of various cellular processes. E2 induces cGMP production especially in ischemic tissues [84], cGMP may also mediate E2-induced

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stimulation of $Ca^{2+}$ activated $K^+$ channels ($BK_{ca}$) in porcine coronary artery [85]. Phytoestrogens may have similar cGMP-mediated vascular effects. In human coronary SMCs, resveratrol enhances cGMP formation and stimulates PKG activity. E2 has 46% lower maximal response than that of resveratrol. The cGMP formation by resveratrol or E2 is attenuated by the ER blocker ICI-182,780, even in endothelium-disrupted coronary arteries. Interestingly, combining E2 with resveratrol shows a competitive rather than an additive response [86].

**Phytoestrogens and Prostacyclin ($PGI_2$)—**$PGI_2$ is a prostaglandin (PG) produced from the metabolism of arachidonic acid by cyclooxygenase enzyme (COX) and in turn promotes endothelium-dependent vascular relaxation. E2-induced vascular relaxation is partly mediated by endothelium-derived $PGI_2$ [87]. E2 induces upregulation of COX-1 expression and $PGI_2$ synthesis in ECs, and increases urinary excretion of the $PGI_2$ stable metabolite 6-keto-$PGF_1\alpha$. Interestingly, deletion of the $PGI_2$ receptor diminishes the vascular protective effect of E2 in OVX female mice [88]. Phytoestrogens may have similar effects on the $PGI_2$ pathway. Treatment of HUVECs with serum from Post-MW whose diet was supplemented with soy isoflavones and red clover increased the capacity of ECs to produce $PGI_2$ [89]. Also, in HUVECs, genistein and daidzein increase $PGI_2$ production through an ER-dependent mechanism involving increased COX-2 protein and activity, but not COX-1 [90]. In mesenteric microvessels isolated from female Wistar rats and preconstricted with norepinephrine, COX inhibitors abolish the vasodilatory effects of genistein, suggesting a role of PGs in genistein-induced vasorelaxation [76]. Phytoestrogens may also affect other PGs. In SHR aorta, isoflavones and E2 inhibit endothelium-dependent contraction to acetylcholine by reducing the release of $PGH_2$ (unstable precursor of PGs and TXs) and its vasoconstrictor response [91]. Thus, phytoestrogens increase $PGI_2$ production and may alter the level of other PGs as well.

**Phytoestrogens and cAMP—**Cyclic adenosine monophosphate (cAMP) is an intracellular second messenger derived from adenosine triphosphate (ATP) by activated adenylate cyclase. cAMP mediates some of the vascular effects of PGs [92,93]. E2 via ER increase cAMP production [94]. cAMP stimulates ER-mediated transcriptional activity in the absence of E2 by direct phosphorylation of the receptor [95]. Phytoestrogens may also enhance adenylate cyclase activity and affect cAMP-dependent pathways in ECs and VSM. In porcine coronary artery, genistein-induced vasodilatation is abolished by the cAMP-dependent protein kinase inhibitor Rp-8-Br-cAMP, suggesting a role of cAMP-dependent signal transduction [96]. Genistein also potentiates β1-adrenoceptor-induced relaxation in rat aortic rings mostly by inhibiting cAMP-phosphodiesterase activity [97]. In bovine aortic ECs, low concentrations of genistein, but not E2, increase intracellular cAMP by enhancing adenylate cyclase activity via a nongenomic mechanism [68]. Also, in porcine coronary artery, genistein causes VSM via a cAMP-dependent mechanism that does not involve Gs proteins or ERs [98]. Collectively, soy isoflavones appear to activate adenylate cyclase, increase cAMP and promote cAMP-dependent pathways. Further studies are needed to examine the effects of other phytoestrogens on cAMP-dependent pathways.

**Phytoestrogens and EDHF—**EDHF plays an important role in acetylcholine-induced endothelium-dependent hyperpolarization and relaxation of VSM [99]. E2 stimulates EDHF release [100]. EDHF may also play a role in phytoestrogen-induced vascular relaxation. In male Sprague-Dawley rats treatment with daidzein or E2 for one week stimulates aortic relaxation via a non-NO, non-PG factor acting through the opening of small conductance $Ca^{2+}$ dependent $K^+$ channels [$SK_{Ca}$] and intermediate $Ca^{2+}$ dependent $K^+$ channels [$IK_{Ca}$], and involving activation of Na/K-ATPase, inward rectifier $K^+$ channel [$K_{IR}$] and CYP450 epoxygenase, suggesting a role of EDHF in daidzein-induced vascular relaxation [83].

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Further studies are needed to investigate the contribution of EDHF to the vasorelaxant effect of phytoestrogens.

**Phytoestrogens and Endothelin (ET)—**ET has three isoforms (ET-1, ET-2, and ET-3) acting on 2 receptors, $ET_A$ receptor which is found in VSMCs and induces vasoconstriction and $ET_B$ receptor which is found mainly in ECs and promotes vascular relaxation [101]. Changes in ET levels and metabolism are thought to contribute to CVD, and ET antagonists are used for treatment of pulmonary hypertension and are being investigated for treatment of other CVD [102]. E2 decreases ET production via an ER-dependent mechanism [70,103]. Phytoestrogens mimic the effects of E2 on ET. For example, genistein via ERs decreases ET production in rat arteries probably by inhibiting the expression of ET converting enzyme-1 [104].

## Phytoestrogens and Vascular Smooth Muscle (VSM)

**Phytoestrogens and Inhibition of VSM Proliferation—**VSMC proliferation is involved in many pathological processes including vascular remodeling, neointimal hyperplasia and atherosclerosis. E2 inhibits VSMC proliferation via cytosolic/nuclear ERs and transcriptional genomic effects [105]. Phytoestrogens also inhibit VSMC proliferation. In human VSMCs, the red clover-derived isoflavone metabolite cis-tetrahydrodaidzein inhibits PDGF-induced extracellular receptor kinase (ERK-1) activation and cell proliferation [106]. Also, genistein regulates the activation of apoptosis-related molecules in TNFα-induced human aortic SMCs, leading to the suppression of proliferation and induction of apoptosis [107]. In endothelium-denuded rabbit aorta both genistein and daidzein inhibit VSMC proliferation via an effect independent from inhibition of TK activity by genistein [108]. Also, in aortic SMCs of stroke-prone SHR, genistein, daidzein and glycitein inhibit naturally and PDGF-induced VSMC proliferation and DNA synthesis [109]. In a study on cholesterol-fed mice, acute neointimal proliferation was induced in the iliac artery by mechanically damaging the endothelium and the damaged arteries were harvested after oral administration of dihydrodaidzein for 4 weeks. The study showed that dihydrodaidzein selectively inhibited neointimal proliferation, possibly by inhibiting VSMC migration and proliferation and/or enhancing endothelial proliferation and function [110]. Also, in rat aortic SMCs genistein inhibits PDGF-induced proliferation by blocking the progression from the G0/G1 to S phase of the cell cycle [111]. It has also been shown that TGF-β-stimulated clone-36, a matricellular protein induced by daidzein, inhibits human umbilical artery SMC proliferation and migration *in vivo* and *in vitro*, and causes accumulation of SMCs in G2 phase of the cell cycle [112]. Collectively, these studies support that phytoestrogens inhibit VSMC proliferation.

**Phytoestrogens and VSM Function—**VSM contraction is triggered by increases in intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) (Fig. 4). Activation of myosin light chain kinase (MLCK), Rho-K, protein kinase C (PKC) and MAPK also contribute to VSM contraction. E2 causes rapid relaxation of endothelium-denuded blood vessels [7,113]. Phytoestrogens may mimic the effects of E2 on VSM. Genistein supplements improve VSM function, vascular motor tone and systemic arterial compliance [61]. Phytoestrogens-induced vasodilation is mediated by different mechanisms. Systemically, phytoestrogens inhibit the renin-angiotensin system and Ang II production. At the cellular level, phytoestrogens regulate $Ca^{2+}$ and $K^+$ ion fluxes and other signaling pathways such as TK, Rho-K, PKC and MAPK.

**Phytoestrogens and *Angiotensin*—**Ang II is a potent vasoconstrictor and an important regulator of electrolyte balance and blood pressure. E2 induces downregulation of vascular Ang II type 1 receptor mRNA and protein [114]. Phytoestrogens also affect the renin-

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angiotensin system. Genistein inhibits the expression of angiotensin converting enzyme (ACE) in rat aortic ECs via ER and ERK1/2 signaling pathway. The downregulation of ACE could, in turn, change the circulating levels of Ang II, the vasorelaxant Ang-(1-7) and bradykinin [115]. Also, in anaesthetized rats, genistein enhances the vasodilator response to bradykinin as a result of ACE inhibition. Genistein decreases ACE activity both *in vivo* and *in vitro* [116]. Further studies are needed to clarify the contribution of ACE inhibition to phytoestrogens-induced vasodilatation.

**Phytoestrogens and *VSM* $Ca^{2+}$—**Activation of VSM by various agonists is associated with increases in $[Ca^{2+}]_i$ due to $Ca^{2+}$ release from the sarcoplasmic reticulum (SR) and $Ca^{2+}$ entry from the extracellular space. E2 mainly inhibits $Ca^{2+}$ influx rather than $Ca^{2+}$ release from the intracellular stores, and a direct effect of E2 on $Ca^{2+}$ channels has been suggested [117,118]. E2 may also decrease $[Ca^{2+}]_i$ by stimulating $Ca^{2+}$ extrusion via the plasmalemmal $Ca^{2+}$ pump [119]. Phytoestrogens may inhibit VSM contraction by inhibiting $Ca^{2+}$ influx or $Ca^{2+}$ release from the SR, or by decreasing the $Ca^{2+}$ sensitivity of the contractile apparatus. In pregnant women who consume soy-derived products in their meals, circulating isoflavones may play a role in the regulation of feto-maternal blood flow, possibly by inhibiting both $Ca^{2+}$ influx and $Ca^{2+}$ release from the SR and decreasing $[Ca^{2+}]_i$ in umbilical SMCs [120]. Also, in swine carotid artery, genistein attenuates histamine-induced $[Ca^{2+}]_I$, myosin light chain (MLC) phosphorylation and isometric stress via TK inhibition [121]. However, the mechanisms by which phytoestrogens decrease $[Ca^{2+}]_i$ may vary in different blood vessels. In rabbit basilar artery, genistein, daidzein, zearalanone and biochanin A cause vascular relaxation by blocking $Ca^{2+}$ entry [122]. Also, in porcine coronary artery, genistein and E2 cause relaxation of KCl-, 5HT- and $CaCl_2$-induced contractions mainly by inhibiting $Ca^{2+}$ influx, and these effects may not be related to ER or classical genomic activities [123]. Other studies suggest that the vasorelaxant effects of phytoestrogens involve inhibition of intracellular $Ca^{2+}$ release. In rat aortic SMCs, genistein mainly suppresses the transient phase of VSM contraction and slightly inhibits the sustained phase, suggesting that genistein decreases $[Ca^{2+}]_i$ by inhibiting TK-linked $Ca^{2+}$ release [124]. Also, in endothelium-denuded rat aorta, the effects of genistein are more pronounced on the norepinephrine-induced phasic contraction in the absence of extracellular $Ca^{2+}$ than on the tonic contraction in the presence of extracellular $Ca^{2+}$, suggesting that genistein inhibits contraction mainly by inhibiting intracellular $Ca^{2+}$ release [125]. Other studies have shown enhanced vascular reactivity in cardiomyopathic hamster aorta possibly due to increased $Ca^{2+}$ sensitivity of the contractile apparatus. The enhanced myofilament $Ca^{2+}$ sensitivity by phenylephrine was markedly inhibited by genistein and to a less extent by daidzein [126]. Thus phytoestrogens appear to cause vasorelaxation mainly by decreasing $Ca^{2+}$ influx and intracellular $Ca^{2+}$ release, and may also decrease the $Ca^{2+}$ sensitivity of the contractile apparatus.

**Phytoestrogens and $K^+$ Channels—**$K^+$ channels play a role in the regulation of VSM membrane potential and consequently the sensitivity to membrane depolarization and contraction. $K^+$ efflux through the opening of $K^+$ channels causes membrane hyperpolarization that closes voltage-gated $Ca^{2+}$ channels, decreases $Ca^{2+}$ entry, and lead to VSM relaxation. $K^+$ channels include large conductance $Ca^{2+}$-activated $K^+$ channels [$BK_{Ca}$], intermediate-conductance [$IK_{Ca}$], small conductance [$SK_{Ca}$], inward rectifier [$K_{IR}$], voltage-dependent [$K_V$] and ATP-sensitive $K^+$-channels [$K_{ATP}$] [127]. E2 and phytoestrogens cause VSM relaxation by activating $K^+$ channels. In human coronary artery SMCs, E2 via ERα induces stimulation of $BK_{Ca}$ causing membrane hyperpolarization and decreased $Ca^{2+}$ influx [128]. Also, in rat aorta, treatment with daidzein or E2 stimulates the opening of $SK_{Ca}$ and $IK_{Ca}$ channels, and activation of Na/K-ATPase and $K_{IR}$ [83]. Also, in rat basilar artery SMCs, daidzein inhibits $BK_{Ca}$ [129]. In rat mesenteric artery precontracted

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with norepinephrine, E2, genistein and daidzein cause relaxation that is antagonized by blockers of $BK_{Ca}$ and $SK_{Ca}$ [130]. Genistein also activates $K_{Ca}$ in rat aortic SMCs [131]. Formononetin and biochanin A activate $BK_{Ca}$ and $K_{ATP}$ in rat aortic SMCs whereas daidzein is less potent [79]. Phytoestrogens may affect $K^+$ channels via different signaling pathways. In rabbit portal vein SMC, TK may play a role in the regulation of $K_{ATP}$ activity [132]. In contrast, in rabbit pulmonary artery genistein inhibits $K_v$ current through a mechanism not involving TK inhibition or PKC activity [133]. Thus, the effects of phytoestrogens on different $K^+$ channels may contribute to endothelium-independent vasodilation. Further studies are needed to define the intracellular signaling pathways mediating the effects of phytoestrogens on $K^+$ channels.

**Phytoestrogens and Tyrosine Kinase (TK)—**Phosphorylation of tyrosine residues in certain proteins affects a wide range of their properties such as enzyme activity, subcellular localization, and interaction between molecules. TK activity in the nucleus is involved in the control of the cell cycle and various transcription factors. E2 and phytoestrogens inhibit TK activity. In OVX female SHR, treatment with E2 and low-dose genistein for 2 weeks is associated with decreased renal artery contraction to Ang II, but not to norepinephrine, KCl or ET-1, and these effects are likely due to TK inhibition [134]. Tyrosine phosphorylation maintains $Ca^{2+}$ channels in a susceptible state for depolarization. In isolated rat portal vein SMC, genistein, which inhibits TK, decreases slow $Ca^{2+}$ current ($ICa_L$) in a concentration-dependent manner while superfusion with daidzein, which does not inhibit TK, had no inhibitory effect even at high concentrations [135]. Also, in rat aorta, genistein inhibits intracellular $Ca^{2+}$ release via TK inhibition [125]. Thus, TK inhibition appears to play a role in the vascular relaxation induced by some phytoestrogens.

**Phytoestrogens and Rho-Kinase—**Rho-K is a downstream effector of the small GTP-binding protein Rho. Rho/Rho-K pathway plays an important role in the regulation of VSM contraction, and may be involved in the pathogenesis of vasospasm, arteriosclerosis, systemic and pulmonary hypertension, and stroke [136]. Studies have shown that long-term inhibition of Rho-K causes regression of coronary arteriosclerosis [137]. Also, in human coronary VSM, inflammatory stimuli such as Ang II and IL-1β, increase the expression and activity of Rho-K possibly via PKC and NF-κB [138]. In rat basilar artery, the Rho/Rho-K inhibitor Y-27632 is 3-fold more potent as vasodilator in males than females. Also, in OVX female rats, the vasodilator response to Y-27632 resembles the response in males, and treatment of OVX rats with E2 normalizes the vasodilator effects of Y-27632 to those observed in intact females. These observations suggest that endogenous E2 inhibits Rho/Rho-K [139]. Phytoestrogens may also inhibit Rho/Rho-K. In male rat aorta, genistein and daidzein cause relaxation of contraction induced by fluoride, a RhoA/Rho-K activator. The phytotestrogen-induced relaxation occurs in the absence of TK inhibition or functional endothelium, and is not antagonized by $BK_{Ca}$ inhibitors, supporting that RhoA/Rho-K inhibition is involved in genistein-induced vasodilation [140].

**Phytoestrogens and PKC—**Protein kinase C (PKC) is a ubiquitous enzyme that comprises a family of $Ca^{2+}$-dependent and $Ca^{2+}$-independent isoforms, expressed in different proportions in VSM of various vascular beds. During cell activation, PKC translocation to the cell surface may trigger a cascade of protein kinases that ultimately interact with the contractile myofilaments and cause VSM contraction [141]. E2 inhibits PKC [142] and decreases the myofilament sensitivity to $[Ca^{2+}]_i$ [143]. PKC activity may also be modulated by phytoestrogens [144], and the effects of phytoestrogens on PKC-dependent pathways need to be further investigated.

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**Phytoestrogens and MAPK—**MAPKs are serine/threonine-specific protein kinases that respond to extracellular stimuli and regulate various cellular activities such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis [145]. MAPK also mediates some of the processes contributing to VSM contraction. Some of the effects of E2 are mediated by inhibiting MAPK [146], and phytoestrogens may also inhibit MAPK. Gene expression profiling revealed that MAPK signaling is one of the biological pathways affected by genistein. In human aortic SMCs, several phytoestrogens inhibit/downregulate MAPK activity in a concentration-dependent manner and in the following order of potency: biochanin A > genistein > equol > daidzein > formononetin [147]. Also, resveratrol inhibits ERK and p38 MAPK phosphorylation causing inhibition of IL-8 secretion in human monocytic cell line [148]. Further studies are needed to define the role of MAPK in phytoestrogens-induced vascular effects.

## Phytoestrogens and Extracellular Matrix (ECM)

ECM is a major component of the blood vessel architecture, and plays an important role in the control of vascular wall integrity and vascular remodeling. Pathogenic changes in the ECM have been linked to elevated TGF-β levels, oxidative stress, and lipid accumulation. ECM proteins also play a role in the formation of the atherosclerotic plaque [149]. E2 plays a role in the regulation of the cellular cytoskeleton, ECM and vascular remodeling. For instance, ERα interacts with the G protein Gα 13 to induce activation of the RhoA/Rho-K pathway and phosphorylation of the actin-regulatory protein moesin, leading to remodeling of the actin cytoskeleton and EC migration [150]. Phytoestrogens also affect various components of ECM including collagens, elastin, glycoproteins, glycosaminoglycans and proteoglycans.

Collagen is secreted by fibroblasts and is present in the form of elongated fibrils mostly in fibrous tissues and also in blood vessels. Collagen is an essential component in the process of fibrosis and in the pathophysiology of atherosclerosis and atherosclerotic events [151]. Phytoestrogens suppress the synthesis of new collagen fibers. Genistein inhibits the proliferation of hypertrophic scar fibroblasts and ECM collagen synthesis via inhibition of TK [152]. In rodent renal mesangial cells cultured in a high-glucose environment, which stimulates collagen deposition, genistein attenuates the synthesis of type IV collagen and fibronectin [153]. However, in SMCs of mature pigs coronary arteries, genistein upregulates matrix protein expression [149].

While collagen fibers in blood vessels bear most of the strength at higher pressures, elastin fibers are essential in determining the mechanical strength of the vessels at lower pressures [3]. Heat-induced ROS may play a role in heat-induced expression of tropoelastin, a precursor of elastin. Pretreatment of human skin with genistein for 24 h inhibits heat-induced expression of tropoelastin in the epidermis [154]. More studies are needed to define the effects of phytoestrogens on the different components of the vascular ECM.

**Phytoestrogens and MMPs—**Matrix metalloproteases (MMPs) are zinc-dependent endopeptidases that play a role in vascular remodeling [155]. MMPs also degrade ECM within the atherosclerotic plaque, and may be involved in plaque instability and CV events. In Sprague-Dawley rats, E2 induces rapid ECM remodeling by upregulating different MMPs [156]. Although MHT E2 downregulates MMPs, it induces acute MMP modulation of vascular function [157]. Phytoestrogens may also regulate vascular remodeling via MMPs. Studies have examined the effects of 4.5 months of genistein treatment on atherosclerosis pattern and MMP expression in hypercholesterolemic rabbits. The average cross sectional area of atherosclerotic lesions in rabbit aortas progressed in rabbits on continuous hyperlipidemic diet (HD), increased mildly in genistein-treated rabbit on HD and decreased

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in rabbits on normal diet. Western blot analysis showed reduction of MMP-3 expression in HD+genistein and normal diet groups than HD group, and suggested that genistein stabilized the atherosclerotic lesions by inhibiting MMP-3 expression [158]. MMP-2 and MMP-9 play a role in the pathogenesis of atherosclerosis. In human aortic SMCs, the naturally occurring flavolignan deoxypodophyllotoxin (DPT) inhibits cell migration and MMP-2/9 activities, and MMP-9 transcription [159].

MMPs also play a role in enhancing cancer metastasis. Studies on cancer cell lines have supported an inhibitory effect of phytoestrogens on MMPs. Treatment of U87MG cancer cells with genistein and biochanin A induced decreases in the enzymatic activity of MMP-9 and the protein levels of MT1-MMP and urokinase plasminogen activation which are involved in the degradation of ECM proteins and subsequent tumor invasion [160]. Phytoestrogens also prevent the degradation of ECM and subsequent tumor invasion in breast cancer cells [161], prostate cancer [162] and melanomas [163]. Thus phytoestrogens regulate various ECM proteins, and further studies are needed to investigate the effects of phytoestrogens on different components of ECM, and the implications of these effects in postmenopausal CVD.

## Phytoestrogens and Atherosclerosis

Atherosclerosis is a multifactorial vascular disease. Dysfunctional endothelium recruits different inflammatory pathways leading to intimal hyperplasia, VSMC proliferation, ox-LDL deposition, platelet activation and aggregation resulting in the formation of an atheroma of fat, collagen and elastin with a thin fibrous cap (Fig. 5). Hypertension is a major risk factor of endothelial dysfunction and atherosclerosis. E2 reduces atherogenesis by inhibiting SMC proliferation, LDL oxidation and deposition, and attenuating vascular inflammation by decreasing cell adhesion molecules (CAM), macrophage accumulation and monocyte adhesion [164]. E2-induced vasodilatation may also contribute to its anti-atherogenic properties. However, anti-atherogenic effects of E2 depend on the patient's age and the stage of atherosclerosis. MHT containing E2 given early during perimenopause may decrease the development/progression of the atherosclerotic lesion. In contrast, in already established atheromatous plaques E2 may increase inflammation, MMP expression and neovascularization leading to lesion progression, plaque instability and rupture/hemorrhage. This may explain the reduced vascular benefits of MHT in Post-MW with preexisting CVD. Some studies suggest that dietary supplementation of phytoestrogens prevent the progression of atherosclerosis (Fig. 5). Grape phytoestrogens prevent cholesterol accumulation in cultured monocytes from Post-MW [165]. In HUVECs, genistein reverses homocysteine- and ox-LDL induced decrease in the anti-atherogenic proteins annexin V and lamin A [166]. Animal studies also support the anti-atherogenic properties of phytoestrogens. In the proximal left circumflex coronary artery of atherosclerotic rhesus monkeys, E2 and dietary soy isoflavones enhance the dilator response to acetylcholine [167]. Also, genistein inhibits atherogenesis in hypercholesterolemic rabbits mainly by improving EC dysfunction [158]. Compared with genistein, its derivative 7-difluoromethyl-5,4′-dimethoxygenistein has a better protective effect against EC damage in rabbits [168]. Also, resveratrol exhibits multiple anti-atherogenic effects [169] including inhibition of intimal hyperplasia [170], attenuation of $TXA_2$-induced platelet aggregation [171] and inhibition of LDL oxidation [172]. However, other studies showed that isoflavone treatment of cholesterol fed rabbits failed to exert the same anti-atherogenic effects of E2 [164]. Whether phytoestrogens improve the clinical course of atherosclerosis and whether their effects are consistent throughout the different atherosclerotic stages needs to be further examined. Also, the different factors underlying the potential anti-atherogenic effect of phytoestrogens including inhibition of platelet aggregation, improvement of lipid

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metabolism, attenuation of vascular inflammation, anti-angiogenic and antioxidant effects need to be further examined.

## Phytoestrogens and Platelet Aggregation

Platelet aggregation plays a role in atherogenesis and thromboembolic events. An *ex vivo* study on platelets from 18 Post-MW showed that E2 inhibits the activity of thrombin-activated platelets by inhibiting $Ca^{2+}$ influx and raising cAMP [173]. However, other studies have shown that E2 potentiates thrombin-induced platelet aggregation in platelets from healthy men through ERβ and Src kinase [174]. Phytoestrogens may inhibit platelet aggregation. Genistein suppresses platelet aggregation induced by collagen when administered intravenously in the mouse femoral artery [175]. Genistein also inhibits TXA2- and collagen analogs-induced platelet aggregation [176]. The mechanism of genistein-induced inhibition of platelet aggregation likely involves alteration of the early event signaling pathways involved in platelet activation [177]. NO production could play a role in genistein-induced inhibition of platelet aggregation, since the NOS inhibitor L-NAME suppresses the platelet anti-aggregation effect of genistein in rat aortic strips [178]. The inhibitory effects of phytoestrogens on platelet aggregation could also be due to their ability to compete for binding to the TXA2 receptor [179]. Resveratrol also inhibits collagen- and epinephrine-induced platelet aggregation in acetyl salicylic acid resistant platelets [180]. Thus studies showed inhibitory effects of phytoestrogens on platelet aggregation, but the pathways involved need to be further defined.

## Phytoestrogens and Lipid Profile

Lipid deposition, especially the oxidized form, plays an integral part in atherogenesis. E2 has a favorable effect on the lipid profile [181]. Phytoestrogens may also improve the lipid profile. In mice fed a high fat diet (HFD), and then 6 weeks later either treated or not treated with a daidzein derivative, the daidzein-treated HFD group showed a reduction in body and fat pad weight and an improvement of HFD-induced hyperlipidemia. Daidzein ameliorates HFD-induced hyperlipidemia and reduces body fat by inhibiting the activity of both pancreatic lipase (which promotes lipid absorption) and lipoprotein lipase (which promotes fat tissue deposition). Daidzein also inhibits the differentiation of rat pre-adipocytes and stimulates lipolysis by activating hormone-sensitive lipase [182]. Genistein also inhibits the oxidation of LDL in human ECs and bovine aortic ECs in the presence of copper ions or superoxide/NO radicals [183]. Similar beneficial effects on lipid profile are observed with dietary fiber and lignans [184] possibly due to induction of adiponectin gene expression through an increase in PPAR-γ DNA binding activity [185]. However, a study in cholesterol fed OVX female rabbits found no significant effect of E2 or isoflavones on serum total cholesterol levels [164]. Also, genistein could transform synovial fibroblasts into adipocytes and enhance glucocorticoid-mediated synovial fibroblast adipogenesis [186]. Thus, while most of the experimental evidence suggests beneficial effects of phytoestrogens on lipid metabolism, few studies have not supported these findings.

## Phytoestrogens and Angiogenesis

Angiogenesis involves EC proliferation and differentiation into new vascular capillaries. Angiogenesis is also involved in several disease conditions such as diabetic retinopathy, tumor growth and atherosclerosis [187]. E2 promotes angiogenesis through enhancing the release of VEGF and promoting its angiogenic effects [188]. E2 also disrupts adherens junctions which are important regulators of EC migration and proliferation and thereby enhances the angiogenic effect of VEGF [189]. Some phytoestrogens have similar angiogenic effects. Formononetin promotes early fracture healing and increases the number of vessels and expression of VEGF and VEGF receptor 2 in the early stage of chondrogenesis in rats [190]. Other studies suggest that phytoestrogens may suppress

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angiogenesis. Genistein inhibits cell proliferation, induces apoptosis, and suppresses *in vivo* angiogenesis in human renal carcinoma cells injected into rats [191]. Genistein also inhibits oxLDL-induced angiogenesis in HUVECs [192]. The anti-angiogenic effect of genistein may be due to downregulation of cell adhesion related genes and impairment of cell adhesion [193]. Genistein may also activate anti-angiogenic molecules such as tissue factor, endostatin and angiostatin [194]. Isoflavones may promote anti-angiogenic effects and cell growth arrest by inhibiting TK and targeting growth factors such as FGF, PDGF, EGF and VEGF. Flaxseed lignans may also inhibit E2-induced VEGF secretion in MCF-7 cancer cells [195]. Thus most of the experimental evidence supports anti-angiogenic effects of phytoestrogens which could be of benefit in CVD and neoplastic disease.

## Phytoestrogens and Vascular Inflammation

Low-grade inflammation is implicated in atherogenesis, and E2 may affect the course of atherosclerosis by inhibiting vascular inflammation. E2 attenuates vascular expression of inflammation-associated genes and inhibits adhesion of monocytes to ECs [196]. CD40L activates antigen-presenting cells and regulates B cell function by engaging CD40 on the B cell surface. E2 via an ERα-mediated pathway blocks Interferon-γ induced CD40 and CD40L protein expression and prevents neutrophil adhesion [197]. E2 also attenuates TNF-α-induced mRNA expression of inflammatory mediators [198]. Similar to E2, phytoestrogens may exert vascular anti-inflammatory effects, and the anti-inflammatory effect of several medicinal herbs could be due to their phytoestrogen content [199]. Genistein protects against inflammatory factor-induced EC dysfunction and inhibits leukocyte-endothelium interaction [200]. Genistein, and to a lesser extent daidzein, decrease TNFα-induced secretion of monocyte chemotactic protein-1, a cytokine recruiting white blood cells to sites of inflammation [201]. In human brain microvascular ECs, genistein pretreatment reduces cytokine-mediated upregulation of blood leukocytes transmigration [202]. Also, in HUVECs, genistein inhibits TNFα-induced signaling and plasminogen activator inhibitor (PAI-1) transcription likely due to inhibition of TK because daidzein does not exert the same effect [203]. Genistein also reduces mRNA expression levels of E-selectin, CAM-1 and P-selectin which are elicited by the proinflammatory bacterial LPS [59]. Genistein also inhibits the activity of the key inflammatory enzyme secretory phospholipase A2 in mice [199]. Other studies support the anti-inflammatory properties of phytoestrogens and suggest the utilization of these properties in preventing graft rejection [204], treating arthritis [205], protection against UV rays-induced skin inflammation [206] and treating bronchial asthma [207]. In contrast to isoflavones, resveratrol may have a vascular pro-inflammatory activity as shown in normoglycemic and diabetic rat aortic SMCs [208]. Thus studies support that most phytoestrogens have anti-inflammatory effect in vascular ECs and several other tissues.

## Phytoestrogens as Antioxidants

CVD is partly caused by decreased bioavailability of NO due to increased oxidative stress, ROS production and lipid peroxidation. E2 alters the expression of ROS-generating and -scavenging enzymes and decreases oxidative stress in different cells [209]. Phytoestrogens especially soy isoflavones have multiple protective effects against oxidative stress in vascular ECs [210]. In HUVECs, genistein inhibits the potential of glucose-oxidized LDL to increase tissue factor synthesis [211]. Bcl-2 protein is critical for regulation of cell proliferation and apoptosis under both normal and oxidative conditions. Soy isoflavones prevent oxidative stress-induced apoptosis via ERβ and Bcl-2/Bax expression and modulation of cell survival signaling [212]. Also, treatment of HUVECs with equol reduces $O_2^{\bullet -}$ production by NAD(P)H oxidase [62]. Animal studies have also supported antioxidant effects of isoflavones. Genistein and daidzein through ER-independent mechanisms restore EC function in male SHR by increasing NO production and protection of NO from $O_2^{\bullet -}$-

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driven inactivation [91]. Also, in rat aortic ECs, soy and alfalfa extract potently inhibit the formation of ox-LDL [213]. In porcine coronary artery, the inhibitory effect of genistein and resveratrol on ROS-induced vasoconstriction is greater than that of E2 [214]. 8-Oxo-2′-deoxyguanosine (8-OHdG) is one of the major products of DNA oxidation and its concentration within a cell is used as a measure of oxidative stress. In SHR-Stroke Prone and Wistar-Kyoto rats, genistein, daidzein, and resveratrol decrease the levels of 8-OHdG and prevent oxidative DNA damage induced by advanced glycation end products. Phytoestrogens also increase the levels of the antioxidant glutathione in VSMCs [215]. The antioxidant effects of various phytoestrogens may vary in different tissues and cell types. In rat basilar artery where there is a high $O_2^{\bullet -}$ level, equol exerts weak antioxidant effects, and the effects of daidzein are insignificant [78]. However, in bovine aortic ECs, equol potently inhibits $H_2O_2$-induced cell death by reducing ROS production [216]. Collectively, studies support the antioxidant activity of phytoestrogens which adds to their potential benefits in CVD.

## Epidemiological Evidence and Clinical Trials of Vascular Benefits of Phytoestrogens

The potential vascular benefits of phytoestrogens demonstrated in epidemiological and experimental studies have prompted more observational and interventional studies to further investigate the clinical effects of phytoestrogens on vascular function and CVD. However, the clinical trials have been limited in many aspects and showed inconsistent results.

Human studies examining the effects of phytoestrogens on the endothelium have suggested beneficial effects on endothelial functions and vasorelaxant effects of phytoestrogens (Table 2). However, these results have not been consistent in all studies. Of note, in the studies that showed no beneficial effects of phytoestrogens on endothelial functions, the cohort group had normal endothelial function at baseline.

Studies have also examined the effects of phytoestrogens on plasma lipid profile (Table 3). Observational population-based studies have shown better lipid profile in individuals with high dietary soy intake. However, these beneficial effects should be viewed with caution because individuals who consume soy as a source of protein may have a lower intake of animal proteins, causing further reduction in cholesterol and saturated fat intake [12]. Most clinical studies supported that phytoestrogens decrease LDL-C, total cholesterol and triglycerides, and increase HDL-C. However, some studies did not support beneficial effects of phytoestrogens on lipid profile (Table 3).

Epidemiological studies demonstrated that even in the absence of other risk factors (e.g. diabetes, hypertension, hypercholesterolemia), advanced age increases CV morbidity by enhancing vascular oxidative stress and inflammation [217]. Studies suggest that dietary intake of phytoestrogens reduces vascular inflammation especially in Post-MW (Table 4).

Phytoestrogens may delay the onset of atherosclerotic CVD by reducing vascular inflammation, oxidative stress, platelet aggregation and plasma lipid levels. Also, by improving vascular compliance, phytoestrogens may improve hypertension, a major atherosclerosis risk factor. Epidemiologic studies, Crossover RCT including the WHO-CARDIAC study have shown that phytoestrogens may have beneficial effect on CVD [19,218]. Clinical trials have examined the effect of phytoestrogens on different CVD risk factors and end points, but the results have not been consistent (Table 5).

Thus while several clinical studies have suggested a variety of beneficial vascular effects of phytoestrogens, there have been discrepancies in the results. The causes of these discrepancies may be related to the number of subjects, type of phytoestrogen, study duration, clinical end points, and subjects compliance. The number of subjects involved in

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the interventional trials was too low to extrapolate the results to the general population. The phytoestrogens examined in clinical trials have been mainly isoflavones, and other classes of phytoestrogens could have different or perhaps better outcomes. The duration of the clinical studies has been limited to few weeks or months, and more chronic studies are needed to investigate the long-term effects of phytoestrogens. The clinical studies used different parameters with no clear clinical end points, and used different and incomplete sets of diagnostic tools. Finally, as with other clinical trials involving a change in diet, the compliance of the enrolled subjects is an important issue due to the difficulty of changing dietary habits of a large population consistently over a long period of time.

## Conclusions and Perspectives

Phytoestrogens are currently being evaluated for their potential vascular benefits and as alternatives for MHT. Like E2, phytoestrogens bind to ERs and induce both genomic and non-genomic vascular effects. Phytoestrogens maintain endothelial integrity and decrease vascular permeability, increase NO, $PGI_2$ and/or EDHF release leading to endothelium-dependent vasodilation. Phytoestrogens also inhibit VSM proliferation, and inhibit VSM contraction by activating cAMP- and cGMP-dependent pathways, decreasing $Ca^{2+}$ influx and release, activation of different $K^+$ channels, regulating the RhoA/Rho-K dependent pathway, and inhibition of TK. Phytoestrogens improve lipid metabolism, reduce oxidative stress, inhibit angiogenesis and attenuate vascular inflammation. Several clinical studies support beneficial vascular effects of soy isoflavone extracts, and phytoestrogens may improve endothelial function, the lipid profile and vascular inflammation biomarkers especially in Post-MW. However, the data from clinical studies showed inconsistent results and have been limited due to factors related to the study design and the subjects' compliance.

Thus phytoestrogens may improve the course of several diseases. The vasorelaxant effects of phytoestrogens could be of value in delaying the progression of hypertension in certain populations. Phytoestrogens could also alter the course of diseases characterized by severe vasoconstriction such as pulmonary hypertension and thromboangiitis obliterans. The vasorelaxant and anti-proliferative effects of phytoestrogens could be of benefit in preventing/delaying vascular stent restenosis. The vascular anti-inflammatory effects of phytoestrogens could be of value as a co-therapy in inflammatory vascular diseases and vasculitis. Phytoestrogens could also prevent or delay the progression of atherosclerosis and decrease the incidence of CV events.

Future studies are needed to investigate the effects of phytoestrogens other than isoflavones. Also, some of the signaling pathways of phytoestrogens are not clearly defined. The recently-discovered nongenomic effects of phytoestrogens are one of the areas that need further research. Also, the interactions between different phytoestrogens, E2, SERMs are yet to be investigated.

Future clinical trials need to enroll larger number of subjects, compare different phytoestrogens, include the other dietary components of the population enrolled, study the long-term effects of dietary modifications, and use clear clinical end points (e.g. myocardial infarctions, stroke). The results from these clinical trials could help in answering important questions regarding the relative potencies of different phytoestrogens, the proper quantity of dietary supplement needed to produce measurable effects and the differences in the effects depending on the subjects' ethnicity, age, gender and preexisting CVD. As for now, while studies suggest several beneficial vascular and metabolic effects of phytoestrogens and a tendency to reduce the risk of CVD, there is insufficient evidence to recommend specific quantities or types of phytoestrogens for prevention or treatment of CVD.

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## List of abbreviations

| | |
|---|---|
| **AF** | activation factor |
| **AngII** | angiotensin II |
| **$[Ca^{2+}]_i$** | intracellular free $Ca^{2+}$ concentration |
| **CAD** | coronary artery disease |
| **CVD** | cardiovascular diseases |
| **ACE** | angiotensin converting enzyme |
| **EC** | endothelial cell |
| **ECM** | extracellular matrix |
| **EDHF** | endothelium-derived hyperpolarizing factor |
| **ER** | estrogen receptor |
| **E2** | estradiol |
| **HUVECs** | human umbilical vein endothelial cells |
| **MAPK** | mitogen-activated protein kinase |
| **NO** | nitric oxide |
| **eNOS** | endothelial NO synthase |
| **$PGI_2$** | prostacyclin |
| **PG** | prostaglandin |
| **PKC** | protein kinase C |
| **Post-MW** | postmenopausal women |
| **Pre-MW** | premenopausal women |
| **RCT** | randomized clinical trials |
| **ROS** | reactive oxygen species |
| **SR** | sarcoplasmic reticulum |
| **SHR** | spontaneously hypertensive rat |
| **TK** | tyrosine kinase |
| **VSM** | vascular smooth muscle |

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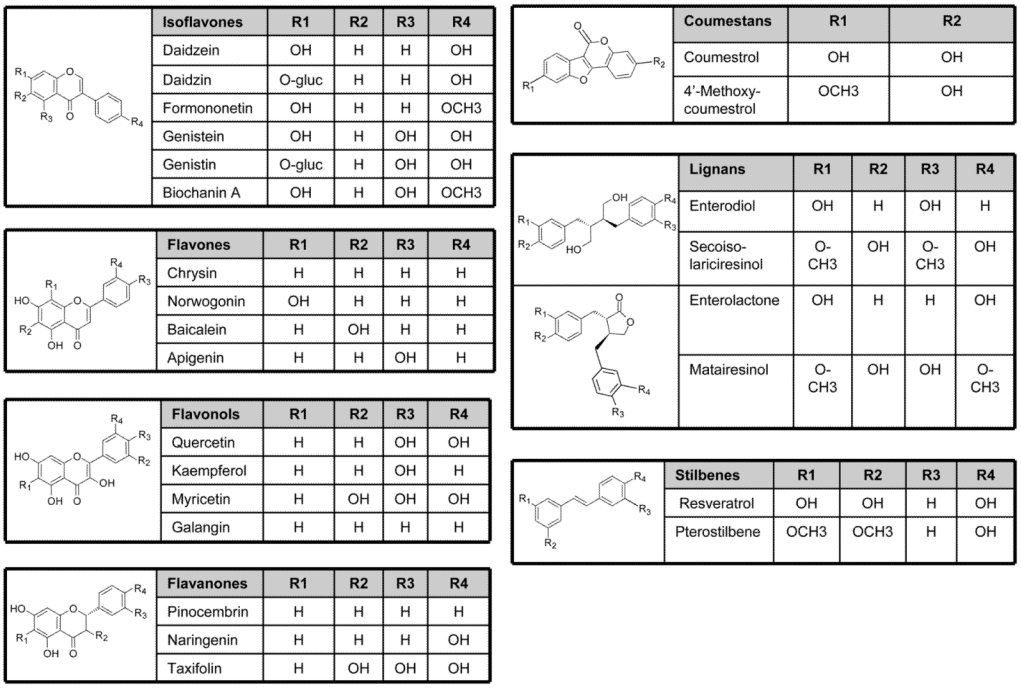

| Isoflavones | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Daidzein | OH | H | H | OH |
| Daidzin | O-gluc | H | H | OH |
| Formononetin | OH | H | H | OCH3 |
| Genistein | OH | H | OH | OH |
| Genistin | O-gluc | H | OH | OH |
| Biochanin A | OH | H | OH | OCH3 |

| Flavones | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Chrysin | H | H | H | H |
| Norwogonin | OH | H | H | H |
| Baicalein | H | OH | H | H |
| Apigenin | H | H | OH | H |

| Flavonols | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Quercetin | H | H | OH | OH |
| Kaempferol | H | H | OH | H |
| Myricetin | H | OH | OH | OH |
| Galangin | H | H | H | H |

| Flavanones | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Pinocembrin | H | H | H | H |
| Naringenin | H | H | H | OH |
| Taxifolin | H | OH | OH | OH |

| Coumestans | R1 | R2 |
|---|---|---|
| Coumestrol | OH | OH |
| 4'-Methoxy-coumestrol | OCH3 | OH |

| Lignans | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Enterodiol | OH | H | OH | H |
| Secoiso-lariciresinol | O-CH3 | OH | O-CH3 | OH |
| Enterolactone | OH | H | H | OH |
| Matairesinol | O-CH3 | OH | OH | O-CH3 |

| Stilbenes | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Resveratrol | OH | OH | H | OH |
| Pterostilbene | OCH3 | OCH3 | H | OH |

**Figure 1.**
Classification of phytoestrogens. Phytoestrogens include isoflavones, flavanones, flavonols, flavones, lignans, coumestans, and stilbenes with different core structure and side chains.



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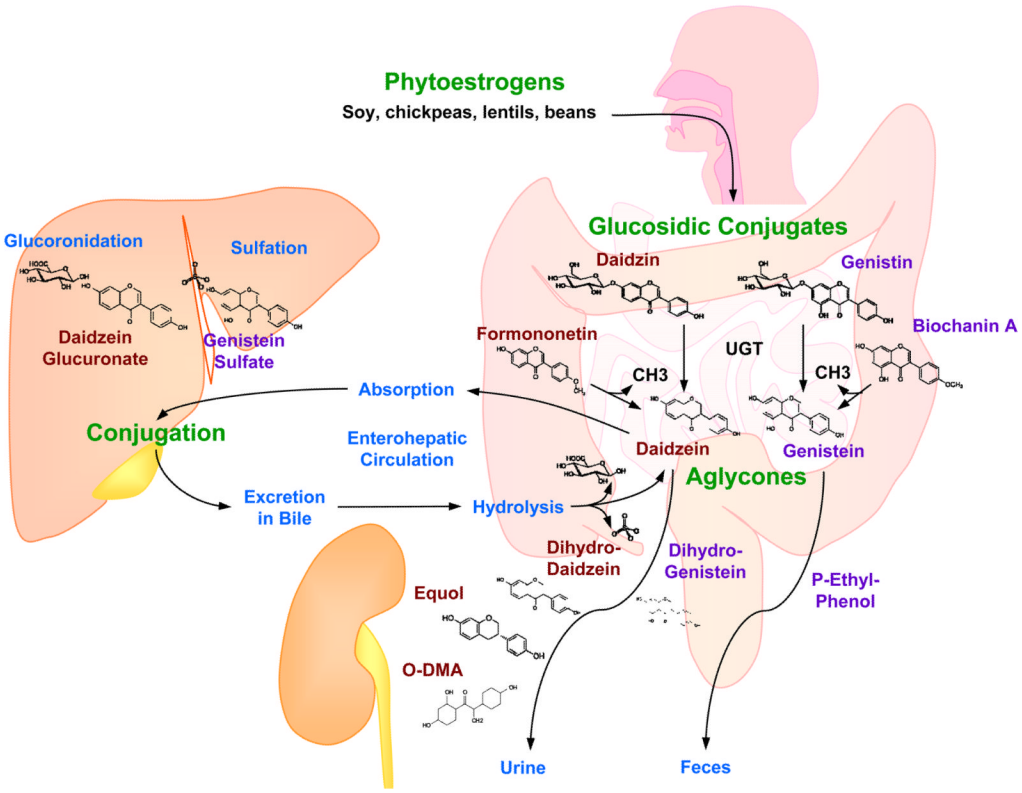


**Figure 2.**

Absorption, metabolism and excretion of isoflavones. Phytoestrogens, found in diet as glucoconjugates (daidzin, genistin), are hydrolyzed in the intestine, by the action of UDP-glucuronosyltransferase (UGT) secreted by intestinal bacteria, into the active forms aglycones (daidzein and genistein). Genistein and daidzein are also produced from the demethylation of their precursors biochanin A and formononetin, respectively. The aglycones are absorbed from the intestinal tract to the liver where they are mainly conjugated with glucuronic acid and sufates. Some of the conjugated aglycones are excreted in the bile where they are hydrolyzed, and some of the unconjugated aglycones are excreted in the feces, while some are reabsorbed to the liver via enterohepatic circulation. In blood, Isoflavones are metabolized mainly into equol and O-desmethylangolensin (O-DMA) which are excreted in urine.

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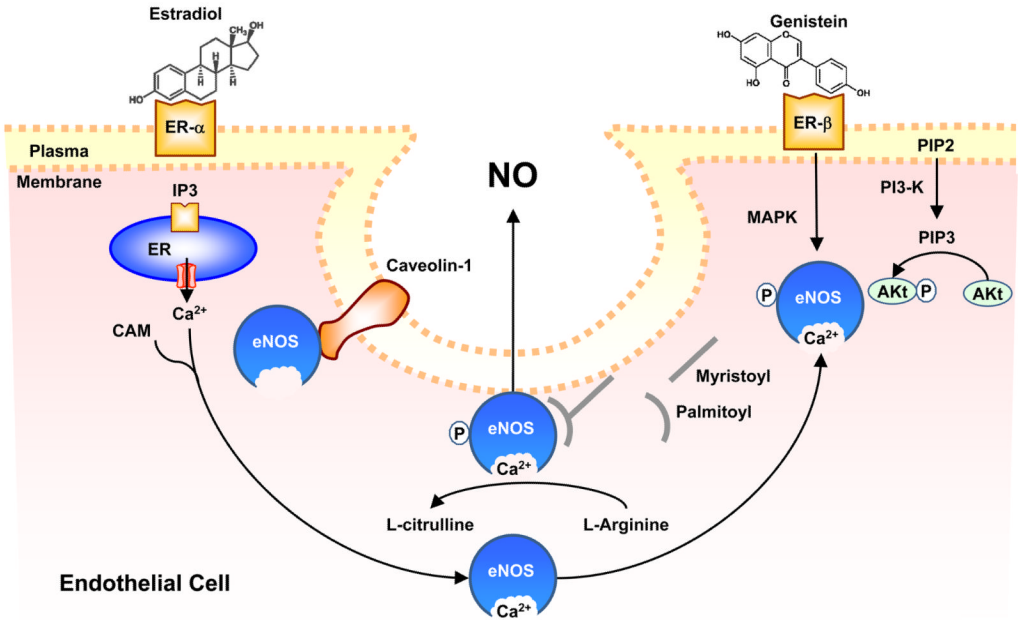


**Figure 3.**

Phytoestrogens-induced NO release from endothelial cells. Like estradiol, phytoestrogens bind to ER on EC and increase the formation of inositol 1,4,5-trisphosphate ($IP_3$), which stimulates $Ca^{2+}$ release from the endoplasmic reticulum. $Ca^{2+}$ forms a complex with calmodulin (CAM), which in turn binds to and causes initial activation of eNOS, its dissociation from caveolin-1, and translocation to intracellular sites. Phytoestrogens may also activate phosphatidylinositol 3-kinase ($PI_3$-K), leading to transformation of phosphatidylinositol-4,5-bisphosphate ($PIP_2$) into phosphatidylinositol 3,4,5-trisphosphate ($PIP_3$), which activates Akt. ER-mediated activation of Akt or MAPK pathway causes phosphorylation of cytosolic eNOS and its second translocation back to the cell membrane where it undergoes myristoylation and palmitoylation, a process required for its full activation. Activated eNOS promotes the transformation of L-arginine to L-citrulline and the production of NO, which is released by EC and causes VSM relaxation.

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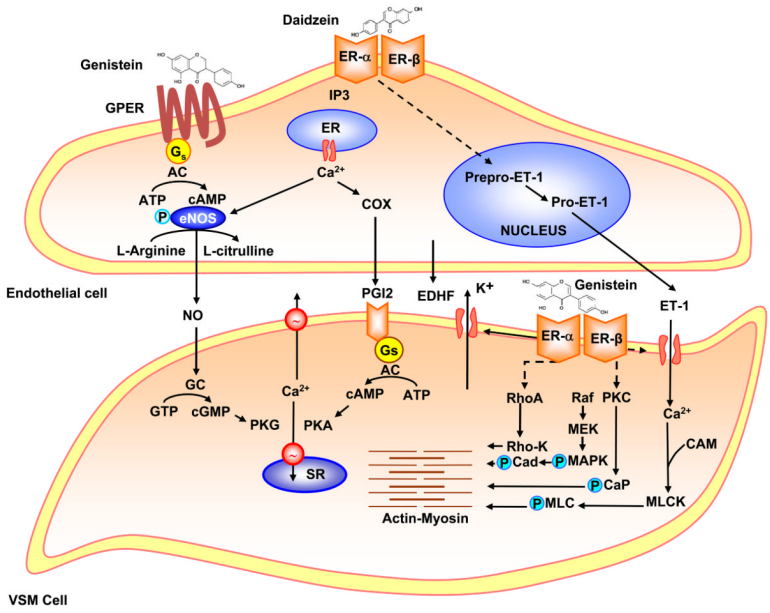


**Figure 4.**

Effects of phytoestrogens on ECs and VSMCs. In ECs, phytoestrogens such as genistein or daidzein bind ERs and GPER and increase $[Ca^{2+}]_i$, $PI_3K$/Akt, MAPK and cAMP which cause phosphorylation and activation of eNOS and promote the production of NO. NO release activates guanylate cyclase (GC) in VSM leading to increased cGMP and stimulation of cGMP-dependent protein kinase (PKG). PKG decreases $[Ca^{2+}]_i$ by stimulating $Ca^{2+}$ extrusion pump in the plasma membrane and $Ca^{2+}$ uptake pump in the sarcoplasmic reticulum (SR) and/or decrease the sensitivity of the contractile myofilaments to $[Ca^{2+}]_i$. Phytoestrogens also activate cyclooxygenase (COX) to produce prostacyclin ($PGI_2$) and in turn activate adenylate cyclase (AC) and the $PGI_2$-cAMP-PKA pathway, leading to VSM relaxation. Phytoestrogens may also induce EDHF release and activate $Ca^{2+}$-activated $K^+$ channels causing hyperpolarization and relaxation of VSM. Phytoestrogens also inhibit ET-1 release and thereby decrease VSM contraction. In VSM, phytoestrogens may activate $K^+$ channels, leading to membrane hyperpolarization, inhibition of $Ca^{2+}$ entry through $Ca^{2+}$ channels, and inhibition of $Ca^{2+}$-dependent MLC phosphorylation and VSM contraction. Phytoestrogens through activation of plasma membrane ERs may also inhibit protein kinase C (PKC), Rho-K and/or the MAPK pathway and thereby further inhibit VSM contraction.

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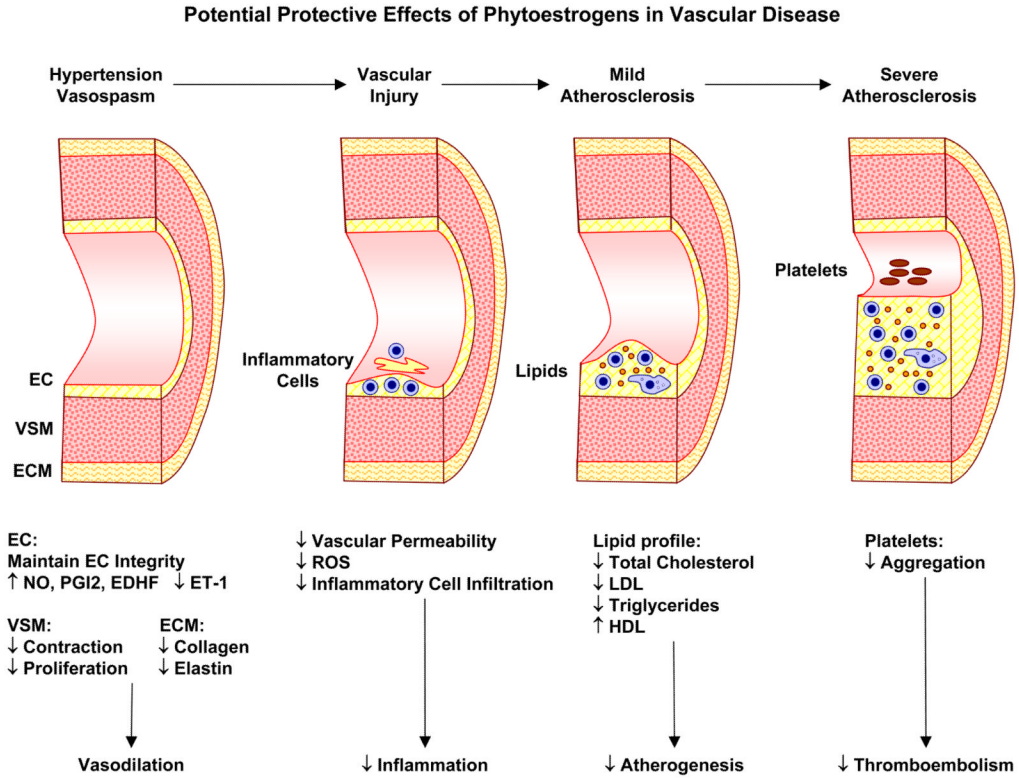


**Figure 5.**

Potential protective effects of phytoestrogens in vascular disease. Increased vascular tone and blood pressure cause hypertension, leading to vascular injury, inflammatory cell infiltration, lipid deposition, atherosclerosis, narrowing of vessel lumen and platelets aggregation. Phytoestrogens promote vasodilation and may ameliorate hypertension. Phytoestrogens could also reduce vascular permeability, reactive oxygen species (ROS), and inflammatory cell infiltration. Phytoestrogens may also retard the progress of atherosclerosis by improving lipid profile, and reduce thromboembolism by decreasing platelets aggregation.

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## Table 1

Sources of Phytoestrogens

| | Isoflavones | | Coumestans | Lignans |
|---|---|---|---|---|
| **Food** | **Daidzein** | **Genistein** | **Coumestrol** | **Secoisolariciresinol** |
| **Soy based foods** | | | | |
| Black bean sauce | 2304.0 | 2486.6 | tr | tr |
| Miso soup | 430.2 | 1009.8 | nd | tr |
| Soy beans | 56621.4 | 44213.4 | tr | 79.1 |
| Soy bean sprouts | 268.3 | 514.6 | nd | tr |
| Soy milk | 921.3 | 1852.2 | tr | tr |
| Soy nuts | 28351.2 | 36264.0 | tr | tr |
| Soy sauce | tr | 100.6 | tr | tr |
| Soy yogurt | 3364.4 | 6565.1 | tr | tr |
| Tempeh | 6974.8 | 10729.6 | tr | tr |
| Tofu | 9337.5 | 17050.2 | tr | tr |
| Veggie burger | 461.5 | 1111.5 | tr | tr |
| **Vegetables and legumes** | | | | |
| Alfalfa sprouts | 151.7 | 117.6 | 105.3 | tr |
| Broccoli | tr | tr | tr | 414.0 |
| Clover sprouts | 71.3 | 70.9 | 97.7 | nd |
| Mung bean sprouts | 91.4 | 135.2 | 136.6 | 97.0 |
| Beans, green | tr | 32.9 | nd | 30.9 |
| Beans, white | tr | 25.3 | tr | 29.9 |
| **Nuts and oil seeds** | | | | |
| Almonds | tr | tr | tr | 70.3 |
| Chestnuts | tr | tr | tr | 172.7 |
| Flaxseed | 58.2 | 173.2 | 46.8 | 375321.9 |
| Hazelnuts | tr | tr | tr | 60.5 |
| Pistachios | 73.1 | 103.3 | tr | tr |
| Sunflower seeds | nd | nd | nd | 127.8 |
| Walnuts | 35.2 | tr | tr | 78.0 |
| Peanut butter | tr | 38.2 | tr | 28.6 |
| **Fruits** | | | | |
| Dried apricots | tr | tr | tr | 147.6 |
| Dried dates | tr | tr | tr | 106.2 |
| Dried prunes | tr | tr | tr | 103.8 |
| Strawberries | tr | tr | tr | 1210.0 |
| Cranberries | tr | tr | nd | 1500.0 |
| Blackberries | tr | tr | nd | 3710.0 |
| **Breads** | | | | |

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| | Isoflavones | | Coumestans | Lignans |
|---|---|---|---|---|
| **Food** | **Daidzein** | **Genistein** | **Coumestrol** | **Secoisolariciresinol** |
| Bread, flax | 85.0 | 212.3 | tr | 7208.3 |
| Bread, rye | tr | tr | nd | 122.0 |
| Bread, multigrain | tr | tr | tr | 4770.4 |
| Bread, whole wheat | 155.8 | 141.8 | tr | tr |
| **Beverages** | | | | |
| Tea, black | na | na | na | 159.0 |
| Tea, green | na | na | na | 246.0 |
| Wine, red | tr | tr | nd | 29.4 |

Phytoestrogen levels are indicated in μg/100 g; tr, trace defined as ≤ 25 μg/100 g; nd, none detected; na, information not available. Also, see references [22,219,220]

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## Table 2

Representative Human Studies Examining the Effects of Phytoestrogens on EC Function

| Phytoestrogen - Clinical Trial | Subjects | Study Design | Outcome | Ref |
|---|---|---|---|---|
| Isoflavones - Observational | 126 High risk CV patients | Dietary questionnaire | Isoflavone intake is associated with enhanced brachial artery FMD and reduced mean carotid intima-media thickness | [221] |
| Genistein - RCT | 79 Healthy Post-MW age 56±4 yr | 1 year of either E2/progesterone or genistein therapy | Genistein increased nitrites/nitrates levels, decreased plasma ET-1 levels in brachial artery and improved EC function to the same extent as E2/ progesterone regimen | [222] |
| Genistein Resveratrol - RCT | 12 Post-MW with CHD & 14 age- matched controls | One hour incubation of resistance subcutaneous arteries with genistein, resveratrol, ERα agonist and E2. | Arterial dilatation to phytoestrogens was enhanced in CHD group as compared to controls. Inhibition of NO synthase had no effect on dilatation induced by the investigated compounds. ERβ expression was enhanced in the vascular wall from CHD women, while ERα predominated in controls. | [223] |
| Dehydroequol - Clinical trial | 6 Healthy males | Brachial artery infusion of dehydroequol in forearm resistance arteries in the absence and presence of eNOS inhibition | Dehydroequol demonstrated potent vasodilator properties in human forearm resistance arteries via a NO-dependent mechanism | [224] |
| Soy - RCT | 22 Healthy Post-MW | 6 wk of either daily raloxifene, soy phytoestrogens 55 mg or placebo in random sequence with intervening 6 wk wash-out periods | No change from baseline endothelial function | [225] |
| Lignan - RCT | 22 Healthy Post-MW | Daily consumption of a low- fat muffin enriched with a lignan complex, providing 500 mg/d of secoisolariciresinol diglucoside for 6 wk periods separated by a 6-wk wash-out intervals. | No difference in FMD and nitroglycerine-mediated endothelium-independent vasodilation, plasma nitrites/nitrates, ET-1, or asymmetric dimethylarginine between the lignan complex intervention period and the placebo period | [226] |
| Isoflavones - RCT | 62 Post-MW age 45-60 yr | 72 mg of soy-derived isoflavones or placebo | No effect on endometrial thickness or the pulsatile index of the uterine and cerebral arteries | [227] |
| Isoflavones - Meta-analysis | | 17 RCTs | Isoflavones can modestly, but significantly improve endothelial function | [228] |
| Isoflavones - Meta-analysis | | 9 RCTs | Isoflavone supplementation improves endothelial function in Post-MW with low baseline FMD levels, but not in women with high baseline FMD levels | [229] |

FMD, flow-mediated dilation

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## Table 3

Representative Human Studies Examining the Effects of Phytoestrogens on Lipid Profile

| Phytoestrogen - Clinical Trial | Subjects | Study Design | Outcome | Ref |
|---|---|---|---|---|
| Soy Phytoestrogens - Epidemiological | 1242 Japanese M, 3592 F | Semiquantitative Food Frequency Questionnaire | Intake of soy products is associated with lower TC levels | [230] |
| Daidzein - Observational | 483 F with CHD risk factors | Blood genistein and daidzein levels, lipoprotein levels, E2 levels, and angiographic CAD | Higher blood levels of daidzein associated with lower TG, higher HDL-C levels, and an improved TC to HDL-C ratio | [231] |
| Isoflavones - RCT | 20 Healthy Post-MW Age 50-70 yr | After 3 wk stabilization on low fat diet, phytoestrogens or placebo intake for 8 wk in random order, separated by 8 wk washout | No effect on BP and plasma lipid or lipoprotein levels | [232] |
| Isoflavones - RCT | 156 Volunteers, mildly high TC | NCEP Step I diet, One of 5 daily diets: 25 g casein or 25 g isolated soy protein containing 3, 27, 37, or 62 mg of isoflavones | Isoflavones reduced plasma concentrations of TC and LDL-C without affecting concentrations of TG or HDL-C | [233] |
| Phytoestrogens - RCT | 19 Post-MW | 2 Month supplementation with *Pueraria mirifica* plant (rich in phytoestrogens) | Increased HDL-C & apolipoprotein A-1, decreased LDL-C and Apo B. and decreased LDL-C/HDL-C. Miroestrol and coumestrol enhanced ERα-& ERβ-mediated transactivation. Daidzein & genistein, preferentially enhanced ERβ-mediated transactivation | [234] |
| Soy Phytoestrogens - Clinical trial | 24 Post-MW with high TC | 25 g soy protein supplement or a mild protein placebo for 6 wk, separated by 4 wk washout. | Serum TG increased, TC and LDL levels decreased significantly, HDL showed mild change | [235] |
| Isoflavones - RCT | 30 Post-MW | Isoflavones or placebo for 3 month interrupted by a 2 month washout period. Cholesterol efflux from cells used as a marker of improved lipid metabolism. | No differences between the isoflavone and the placebo group | [236] |
| Lignans - RCT | 22 Healthy Post-MW | Daily low-fat muffin enriched with a lignan complex for 6 wk separated by a 6-wk washout. Different markers measured at the beginning and end of each intervention. | No effect on plasma lipid concentrations, serum lipoprotein oxidation resistance, or plasma antioxidant capacity | [237] |
| Isoflavones - RCT | 49 Post-MW 47-66 yrs in Brazil | 40 mg of isoflavone (n = 25) or 40 mg of casein placebo (n = 22). Lipid profile monitoring at baseline and after 6 month of treatment | No significant effects of isoflavone on LDL or TC | [238] |

TC, total cholesterol, NCEP, National Cholesterol Education program

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## Table 4

Representative Human Studies Examining the Effects of Phytoestrogens on Vascular Inflammation

| Phytoestrogen - Clinical Trial | Subjects | Study Design | Outcome | Ref |
|---|---|---|---|---|
| Phytoestrogens- - Cross-sectional study | 151 Middle age elderly males and 91 Post-MW | Secoisolariciresinol, matairesinol, pinoresinol, lariciresinol intake was evaluated. Soluble ICAM-1 (biomarker of inflammation involving damage to the endothelium and platelets), insulin, CRP, glucose, TC, HDL-C and triglycerides measured in fasting blood samples. FMD (only in 56 M and 55 F) | Plasma concentrations of soluble ICAM-1 significantly decreased in the whole group. Marked decrease in soluble ICAM-1 accompanied with relevant improvement of FMD only in the matairesinol group. | [239] |
| Isoflavones - RCT | 60 Post-MW | Isoflavone or placebo tablets for 6 month | Significant improvement of endothelium dependent vasodilation in the isoflavone treatment group. Plasma ICAM-1, VCAM-1, and E-selectin decreased in the isoflavone group compared to placebo. | [240] |
| Genistein - Cross-sectional | 30 Males | Either a placebo or high-genistein treatment. Blood samples were collected before and within 5 min, and after 30 min of 80% peak O2 consumption exercise. Measurements were taken before and after 4-week supplementation. | Isoflavones decreased homocysteine levels. Phytoestrogens were shown to have antioxidant effects, but a limited ability to diminish an abrupt surge of oxidative stress due to acute exercise | [241] |
| Isoflavones - Crossover RCT | 117 Healthy Post-MW | Isoflavone-enriched or placebo cereals consumed for 8 wk, with washout of 8 wk | Lower CRP levels but no effect on other inflammatory markers | [242] |
| Lignan - RCT | 22 Healthy Post-MW | Low-fat muffin, with or without a lignan complex, for 6 weeks, separated by a 6-week washout period. | No differences between the lignans and placebo periods in IL-6, TNFα, ICAM-1, VCAM-1, and MCP-1 levels. Lower CRP in the lignans-added period. | [243] |

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## Table 5

Representative Human Studies Examining the Effects of Phytoestrogens on CVD

| Phytoestrogen - Clinical Trial | Subjects | Study Design | Outcome | Ref |
|---|---|---|---|---|
| Flavonoid - Observational | 34489 Healthy Post-MW age 55-69 yr | Flavonoid food composition data, 16 years follow-up | Inverse association between flavanones intake and CHD, and between flavones intake and total mortality. No association between flavonoid intake and stroke mortality. Individual flavonoid-rich foods associated with significant mortality reduction: bran, apples, pears, red wine (CHD and CVD), grapefruit (CHD), strawberries (CVD), and chocolate (CVD) | [244] |
| Lignan - Cross- sectional study | 301 Post-MW, 60-75 yrs in the Netherlands | Food frequency questionnaire covering the year prior to enrollment | Lower systolic & diastolic BP & lower prevalence of hypertension observed with lignan intake, but no association with ankle-arm BP index or EC function. | [245] |
| Biochanin A & formononetin - Clinical trial | 80 Healthy subjects, age 45-75 yr | Biochanin A& formononetin in two 6-week periods. Large artery stiffness, endothelial function, 24-hour ambulatory BP, and total peripheral resistance measured at baseline and after each intervention. | In normotensive men and Post-MW, formononetin reduced arterial stiffness and total vascular resistance, but had no effect on BP | [246] |
| Phytoestrogens - Meta-analysis | 133 Clinical trials | Structured search strategy using MEDLINE, EMBASE, and Cochrane databases | Chocolate increased FMD after acute & chronic intake and reduced systolic & diastolic BP. Soy protein isolate reduced diastolic BP and LDL-C. Acute black tea consumption increased systolic & diastolic BP. Green tea reduced LDL. | [17] |