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Phytoestrogen-Rich Dietary Supplements in Anti-Atherosclerotic Therapy in Postmenopausal Women

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Abstract: Cardiovascular diseases remain the leading cause of morbidity and mortality among postmenopausal women in western societies. There are still no specific and highly efficient methods of preservation of women's vascular health in modern preventive medicine. For many years physicians have assumed that hormone replacement therapy prevents the development of atherosclerosis in menopausal women. However, the results of the largest international trials involving thousands of women have completely destroyed this hope. The modern perspective for the development of effective and safe drugs to enhance the quality of life and to prevent atherosclerosis progression in postmenopausal women may be the use of phytoestrogens, the substances of plant origin possessing estrogen-like effects, and possibly providing anti-atherosclerotic and anti-climacteric action. Phytoestrogens are often considered as a possible alternative to hormone replacement therapy, since they are believed to alleviate some symptoms of menopause. However, until now there is no exact evidence to consider phytoestrogens as the substances that protect women from atherosclerosis. It should be noted that the data from clinical studies with inconsistent results are mainly inconsistent per se, as most of the studies have serious limitations due to the study design and the participants' compliance. Nevertheless, there is a substantial evidence that phytoestrogens have the potential to address several conditions and diseases associated with the menopausal transition. Phytoestrogens, at least, can potentially reduce atherosclerosis and atherosclerosis-related diseases through multiple mechanisms, by regulating serum lipid metabolism, arterial vessels, cytokine levels, and coagulation/fibrinolysis system. However, a skepticism exists concerning the true potential of phytoestrogens to beneficially modify these processes. An analysis of findings from supplementing the diet with phytoestrogens has failed, in general, to confirm them as the agents responsible for beneficial cardiovascular effects. Fortunalely, now there is a growing interest to the use of phytoestrogens for primary prevention of cardiovascular disease in postmenopausal women. Clinical and epidemiologic data indicate that phytoestrogens possess anti-atherosclerotic effects and may be used to prevent and treat cardiovascular diseases, and that adding phytoestrogens to the diet can contribute to the health of postmenopausal women. This review discusses the effects of phytoestrogens possibly beneficial for cardiovascular health, and how these effects could retard the progression of atherosclerosis, as well as the areas that need further investigation.

Keywords: Atherosclerosis, coronary heart disease, menopause, prevention, treatment, phytoestrogens.

INTRODUCTION

The cardiovascular diseases remain the leading cause of morbidity and mortality among postmenopausal women in western societies. From clinicians' point of view, atherosclerosis is considered as a local narrowing of the arteries, which leads to myocardial infarction, cerebral stroke, gangrene of the lower limbs and other severe pathological conditions. Women of young and mature age with preserved endocrine function are reliably protected against atherosclerosis. In menopausal state, the risk of atherosclerosis and atherosclerotic disease is sharply increased and accompanied with significantly reduced quality of life. In the 60-year-old women, the severity and prevalence of atherosclerosis are comparable with those in men. According to the CIS Interstate Statistical Committee, atherosclerotic disease in women is an absolute leader among the causes of death and accounts for about 73% of cases, whereas in men only for about 51% [1]. Despite the significantly lower rates of overall mortality in women, the absolute number of deaths from cardiovascular disease in them is even higher than in men. For about 55% of women, the reduced life expectancy is determined by the increased mortality from diseases of cardiovascular system, and the situation in urban and rural areas differs insignificantly. Therefore, it is strongly required to develop new approaches that combine effective prevention of atherosclerosis with improving the quality of life in women.

There are still no specific and highly efficient methods of preservation of women's vascular health in modern preventive medicine. For many years physicians have assumed that hormone replacement therapy (HRT) prevents the development of atherosclerosis in menopausal women. However, the results of the largest international trials involving tens of thousands of women have completely destroyed this hope. It has been established that HRT not only reduces the risk of cardiovascular diseases, but also even increases it in some specific groups of women [2-5]. Additionally, the other adverse effects of such therapy, e.g., increased risk of malignant hormone-dependent tumors, have also produced a negative impact on the perspectives of HRT [6-8]. As the beneficial effects of HRT, the prevention of osteoporosis and effective elimination of the symptoms of menopause remained, thus clearly improving the quality of life [9-11].

So, at the beginning of the XXI century the medicine was forced to admit that it does not have a reliable tool to delay the development of atherosclerosis in postmenopausal women. The root of this problem is that the science for decades has paid close attention to the prevention of atherosclerosis in men, while the mechanisms of atherogenesis in women remained out of the spotlight. As a result, with the respect of medical and social aspects, women remained the least protected category of the population.

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The modern perspective for the development of effective and safe drugs to enhance the quality of life and to prevent atherosclerosis progression in postmenopausal women may be the use of phytoestrogens, the substances of plant origin possessing estrogen-like effects, and possibly providing anti-atherosclerotic and anti-climacteric action. Indeed, phytoestrogens are often considered as a possible alternative to hormone replacement therapy, since they are believed to alleviate some symptoms of menopause. However, until now there is no exact evidence to consider phytoestrogens as the substances that protect women from atherosclerosis.

Phytoestrogens have a structural similarity to endogenous estrogens and are close to them in molecular weight, and possess some estrogen-like effects [12]. Most phytoestrogens are the members of the class of flavonoids. Their biological activity is several hundred times lower than the activity of endogenous estrogens; however, their long-term use as a non-pharmacological remedies in a course of prevention therapy may lead to an adequate biological effect. Phytoestrogens may also act as anti-estrogenic agents while blocking estrogen receptors but possessing weaker effect as compared to estrogens. Phytoestrogens are known to decrease estrogenic activity in target organs, for example, in the breast tissue, thus reducing the risk of developing breast cancer [13]. To some extent, the effects of phytoestrogens on cardiovascular risk factors, in particular the level of blood cholesterol, are known [14]. However, the data on antiatherosclerotic action of phytoestrogens are rather scarce, and there is no convincing evidence for their use in prevention of atherosclerosis and cardiovascular disease.

HORMONE REPLACEMENT THERAPY FOR ATHEROSCLEROSIS PREVENTION IN WOMEN: THE LOST HOPES

At the beginning of 1990th, a consensus existed that postmenopausal hormone replacement therapy with estrogens can be able to markedly reduce both the burden and mortality associated with coronary heart disease. However, the decade later that consensus has become very debatable, as several randomized prospective trials failed to find benefits from estrogen replacement therapy.

The Heart and Estrogen / Progestin Replacement Study (HERS), determined whether daily HRT would reduce coronary heart disease events in 2763 post-menopausal women with pre-existing coronary heart disease. No reduction in coronary events by the hormone treatment was shown during an average of 4.1 years of follow-up; moreover, during the first year of the trial there were more coronary events in the hormone-treated group, but in an extended 6.8 years post-trial observational follow-up, there was no evidence of differences in CHD outcomes between the two treatment groups [2, 3]. In addition to the events trial, a carotid ultrasound substudy of 362 participants was conducted concurrently to determine whether HRT affects the progression of the underlying atherosclerotic process; intima-media thickness progressed both in the hormone treatment and placebo groups, without statistical difference between the rates [15].

The Estrogen Replacement Atherosclerosis (ERA) Trial was aimed to examine the effects of postmenopausal hormone therapy on the progression of existing coronary artery atherosclerosis in 309 women, who had experienced a previous myocardial infarction. After 3.5 years of follow-up, the repeated angiography did not detect any differences in disease progression between active treatment groups and placebo recipients [16].

The Women's Health Initiative (WHI) was a large randomized study evaluating the effects of hormone replacement therapy in postmenopausal women [17]. WHI had two arms; one arm studying the impact HRT or placebo in primary prevention in 27,347 women with or without a uterus. This study was initially scheduled for completion in 2005, but was prematurely stopped in July 2002, after a mean of 5.2 years of follow-up, because the Data and Safety Monitoring Board concluded that the evidence of breast cancer harm, along with some increase in CHD, stroke, and pulmonary emboli, outweighed the evidence of benefit for bone fractures and possible benefit for colon cancer. The general outcome of WHI was that neither estrogen nor estrogen/progestin decreased cardiovascular disease [18]. Thus, the data from WHI were similar as seen in HERS and ERA trials.

Finally, Kronos Early Estrogen Prevention Study (KEEPS) was established to assess atherosclerosis progression and cardiovascular risk factors after menopause hormone therapy initiated in early menopause. It was a randomized controlled trial settled in nine U.S. academic centers, and involved 727 randomly assigned healthy menopausal women aged 42-58 years between 6-36 months from last menses without prior cardiovascular events, who had a coronary artery calcium score less than 50 Agatston units and had not received estrogen or lipid-lowering therapy for at least 90 days before inclusion in the study. The primary end point was annual change in carotid artery intima-media thickness, and secondary end points included changes in markers of CVD risk. Mean carotid intima-media thickness increases were similar across groups. Additionally, the percentages of participants in whom CAC score increased did not differ significantly across groups. Thus, four years of early menopause hormone therapy did not affect progression of atherosclerosis despite improving some markers of cardiovascular risk [19].

Taken together, the results of major prospective studies together with the newest findings form KEEPS support the American Heart Association recommendation that women with established coronary disease should not initiate hormone therapy with an expectation of atherosclerotic benefit, and there is neither a compelling reason to initiate hormone therapy in a woman for the sole purpose of primary CHD prevention nor a compelling reason to discontinue it if she is doing well with therapy [20].

SEARCHING FOR AN ALTERNATIVE: PHYTOESTROGENS FOR ANTI-ATHEROSCLEROTIC THERAPY

In the past decades there has been an increasing interest in phytoestrogens as natural alternatives to hormone replacement therapy [21, 22]. It can be stated that there exists a strong theoretical and experimental background for the use of phytoestrogens in anti-atherosclerotic therapy. Phytoestrogens are a heterogeneous group of naturally occurring compounds of plant origin with structural similarities to estrogen (E2), which can mimic estrogenic effects. It is presumed that the effects of phytoestrogens are partially mediated via estrogen receptors (ERα and ERβ), and possibly G protein-coupled estrogen receptor (GPER). Similarly to endogenous estrogens, phytoestrogens were shown to delay the progression of osteoporosis and decrease bone resorption in postmenopausal women [23, 24], provide anti-androgenic effects in prevention of benign prostatic hypertrophy [25], provide protective effects in prostate and breast cancers [26-28], and even to improve cognitive functions of the brain [29, 30]. The recent meta-analysis of randomized controlled trials have shown that flavonoids and flavonoid-rich foods have a potential to decrease cardiovascular risk, including that one associated with menopause [31].

Phytoestrogens are generally classified into different groups including major classes (isoflavones, stilbenes, lignans, and coumestans), and minor classes, like flavanones, flavonols, and flavones. Isoflavones are found mainly in soy based foods, and also in clover, lentils, beans and chickpeas. Such phytoestrogens as genistein, daidzein and its metabolite equol are the most studied and most potent ones; the other isoflavones with known estrogen-like activity are genistin, daidzin, formononetin, and biochanin-A; the last two are the precursors of genistein and daidzein, respectively. The stilbenes with known estrogen-like activity include trans-resveratrol and pterostilbene, commonly found in red wine, grape seeds and peanuts. Lignans are the components of lignin in the plant cell wall and are found mainly in fruits and vegetables, whole grains, flax-

seed, lentils and beans. Coumestans with known estrogen-like activity such as coumestrol and 4-methoxycoumestrol are found mainly in sprouts of clover, mung bean and alfalfa. Since the term «phytoestrogen» is commonly applied to the soy isoflavones genistein, daidzein and glycitein, just isoflavones found in soybean are the most extensively studied phytoestrogens [26, 28]. Therefore, the use of soy isoflavones dominates in experimental and clinical studies of biological effects of phytoestrogens.

Different classes of phytoestrogens have distinct chemical structures that could allow them to bind to estrogen receptors. The key structural elements which are essential for the estrogenic effects are the phenolic rings and certain hydroxylation patterns [32]. Phytoestrogens can bind both to ERα and ERβ, and further activate ER-dependent gene transcription. They can modulate estrogen receptor function in several ways, providing both agonist and antagonist effects. Of course, the affinity of most phytoestrogens to estrogen receptors is 100-10,000-fold lower that of endogenous estrogen; but the effect may be compensated due to high concentrations of phytoestrogens in circulation, which may be 10,000-fold higher than that of endogenous estrogen. Phytoestrogens also known to have higher affinity for ERβ, which explains their action different from endogenous estrogen [21, 30]. Since estrogen receptors participate both in genomic and postgenomic pathways, they can mediate multiple vascular, hematologic and metabolic effects, which are closely related to the processes of atherogenesis, through stimulation or inhibition of gene expression and other pathways which do not involve gene transcription or new protein synthesis. Therefore, the effects of phytoestrogens on the functioning of estrogen receptors may also affect atherogenesis; this hypothesis represents theoretical background for the use of phytoestrogens as anti-atherosclerotic drugs.

Like endogenous estrogen, phytoestrogens may provide beneficial effects on cardiovascular system through the effects on vascular endothelium, vascular smooth muscle cells, intracellular cholesterol metabolism, extracellular matrix synthesis, platelet aggregation, lipid profile, vascular inflammation, etc.

Similar to estrogen, phytoestrogens regulate proliferation of endothelial cells, maintain their integrity and decrease vascular permeability. As an example, in cell culture studies genistein regulates the proliferation of human endometrial endothelial cells [33], protects endothelial cells from H_2O_2- and TNF-α-induced apoptosis [34, 35]. Genistein also inhibits retinal vascular leakage in diabetic rats [36], modulates bradykinin- and substance P-induced increase in macromolecular efflux from the hamster cheek pouch microcirculation, possibly via tyrosine kinase inhibition [37]. Genistein inhibits vascular endothelial growth factor-induced increase in vascular permeability by inhibiting tyrosine kinase-mediated local production of NO and arachidonic acid metabolites [38]. The pretreatment of bovine aortic endothelial cells with genistein inhibits thrombin-induced increase in cellular permeability [39]. Equol improves endothelial function by reducing the generation of reactive oxygen species [40], and provides a protective effect against endothelial dysfunction [41]. Biochanin-A can inhibit cell proliferation [42]. De Andrade *et al*. evaluated an extract of soybeans in which polyphenol glucosides were biotransformed to aglycone forms on production of nitric oxide, prostaglandin E2 and endothelin-1 *in vitro* in human endothelial cells, comparing it with a non-fermented extract. Bioconverted soybean extracts enhanced endothelin-1, nitric oxide and prostaglandin E2 production, while the unfermented extract enhanced endothelin-1 production only. Thus, the aglycone-rich forms of soybean extracts were able to increase nitric oxide and prostaglandin E2 production, demonstrating that, in endothelial cells *in vitro*, they may be usable as therapeutic agents against the development of atherosclerosis [43]. Zhang *et al*. have used a model of oxidized low-density lipoprotein-induced injury in on human umbilical vein endothelial cells to evaluate the protective role of genistein. Pretreatment with genistein markedly reduced ox-LDL-induced MCP-1, VCAM-1 and ICAM-1 secretion and mRNA transcription, which was further decreased by the inducer of HO and reversed by the inhibitor of HO; additionally, the effects were accompanied with upregulating HO-1 mRNA and protein expression and markedly abolished with Nrf2 siRNA. So, they nave demonstrated anti-inflammatory effect of genistein on endothelial cells, which may be associated with the activation of Nrf2/HO-1 pathway [44]. In human umbilical vein endothelial cells stimulated with lipopolysaccharide, phytoestrogen extracts from Glycine max soy bean, genistein, formononetin, biochanin A and daidzein, as well as a mixture of these extracts, reduced the expression of adhesion molecules, VCAM-1, ICAM-1 and E-selectin on cell surface and in culture supernatant, thus acting as preventive agents as well as therapeutic agents [45]. Phytoestrogens can promote endothelium-mediated vascular relaxation via the mechanisms similar to those of estrogens, via NO, PGI_2 and EDHF production and decrease of endothelin release [46-48].

Genistein stimulates NO release in human aortic endothelial cells and human umbilical vein endothelial cells [49], acting through a protein kinase A (PKA)-dependent pathway [50]. In the same cell types, equol stimulates phosphorylation of ERK1/2 and PI3 K/Akt, leading to the activation of NOS and increased NO production [51]. Biochanin A, formononetin and their metabolites genistein and daidzein, respectively, increase eNOS promoter activity and NO release [52]. Animal studies supported the hypothesis that phytoestrogens increase NO production by increasing eNOS expression and activity [53]. In mouse aorta, both red wine polyphenols and ERα agonists stimulate endothelium-dependent NO pathway via activation of ERα [54]. Red clover extracts stimulated NO synthesis in cultured human endothelial cells by recruiting ERβ [55]. It was suggested that daidzein and estrogen may reduce the expression of caveolin-1 and increase the expression of calmodulin, thereby increasing eNOS activity [56]. Phytoestrogens may have cGMP-mediated vascular effects, which are important, since biological signaling by NO is primarily mediated by cGMP. It has been shown that in human coronary smooth muscle cells, resveratrol enhances cGMP formation approximately 2-fold higher than estradiol [57]. Treatment of and human umbilical vein endothelial cells with serum from postmenopausal women taken after diet supplementation with isoflavones from soy and red clover increased the capacity of cultured cells to produce prostacyclin PGI_2, a prostaglandin that promotes endothelium-dependent vascular relaxation [58]. Phytoestrogens may affect prostaglandins other than PGI_2; it was shown that in spontaneously hypertensive rats aorta isoflavones inhibited endothelium-dependent reaction to acetylcholine by reducing the release of PGH_2 and its vasoconstrictor effect [59]. Phytoestrogens may also enhance adenylate cyclase activity and affect cAMP-dependent pathways in endothelial and vascular smooth muscle cells, thus affecting cAMP-dependent mediation of some vascular effects of prostaglandins. In bovine aortic endothelial cells, genistein increased intracellular cAMP by enhancing adenylate cyclase activity [39]. In porcine coronary artery, genistein caused vascular smooth muscle relaxation via a cAMP-dependent mechanism [60]. Finally, phytoestrogens can mimic the effects of estradiol on endothelin: it was shown that genistein decreases endothelin production in rat arteries via estrogen receptors, probably inhibiting the expression of endothelin converting enzyme-1 [61]. However, it is necessary to note that vasoconstriction-related effects of phytoestrogens described in experimental studies do not deal much with the mechanisms of atherogenesis.

Vascular smooth muscle cells (VSMC) proliferation is involved in many pathological processes including atherosclerosis. Phytoestrogens were shown to inhibit VSMC proliferation. In cultured human VSMCs, the red clover-derived metabolites of isoflavones inhibited PDGF-induced extracellular receptor kinase activation and cell proliferation [62]. Kim *et al*. investigated the apoptotic effects of genistein on TNF-alpha-induced proliferation in human

aortic smooth muscle cells. The apoptotic effects of genistein were assessed to determine the mechanism(s) of its antiproliferative activity. The results showed that genistein significantly reduced cell proliferation and intracellular nuclei staining with DAPI in a dose-dependent manner. These findings indicated that genistein regulates the activation of apoptosis-related molecules in TNF-alpha-induced proliferation in human aortic smooth muscle cells, leading to the suppression of proliferation and induction of apoptosis [63]. In endothelium-denuded rabbit aorta both genistein and daidzein inhibited VSMC proliferation via an effect independent from inhibition of TK activity by genistein [64]. In aortic VSMCs of spontaneously hypertensive rats, several isoflavones (genistein, daidzein and glycitein) inhibited both VSMC proliferation and DNA synthesis [65]. In cholesterol-fed mice, neointimal proliferation was selectively attenuated by dihydrodaidzein, possibly by inhibiting VSMC migration and proliferation and/or enhancing endothelial proliferation and function [66]. In rat aortic VSMCs genistein inhibited PDGF-induced proliferation [67].

Phytoestrogens may inhibit MAPKs, which are serine/threonine-specific protein kinases that regulate various cellular activities such as gene expression, mitosis, differentiation, proliferation, cell survival/apoptosis, and some of the processes contributing to VSMC contraction. Gene expression profiling revealed that MAPK signaling is one of the biological pathways, which may be affected by genistein. In human aortic VSMCs, several phytoestrogens inhibited or downregulated MAPK activity in a concentration-dependent manner and in the certain descending order of potency, namely, biochanin A - genistein - equol - daidzein - formononetin [68].

Extracellular matrix is considered as a major component of arterial architecture, and plays an important role in the control of vascular wall integrity and vascular remodeling. Pathogenic changes in the extracellular matrix in atherogenesis are closely related to elevated TGF-β levels, enhanced oxidative stress, and especially lipid accumulation. Phytoestrogens were described to affect various components of extracellular matrix, such as collagens, elastin, glycoproteins, glycosaminoglycans and proteoglycans. Phytoestrogens can suppress the synthesis of new collagen fibers. Cao *et al*. has shown that genistein inhibits proliferation of hypertrophic scar fibroblasts and collagen synthesis [69].

Current concept of atherogenesis implies the presence of low-grade vascular inflammation, and phytoestrogens may affect the development of atherosclerosis by inhibiting vascular inflammation. The anti-inflammatory effect of several medicinal herbs could be due to their phytoestrogen content [70, 71]. Genistein protects against inflammatory factor-induced endothelial dysfunction and inhibits leukocyte-endothelium interaction [72]. 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 [73]. Genistein also inhibits the activity of the key inflammatory enzyme secretory phospholipase A2 in mice [70]. However, in contrast to isoflavones, resveratrol may have a vascular pro-inflammatory activity as shown in normoglycemic and diabetic rat aortic VSMCs [74].

Intracellular and extracellular lipid deposition in arterial wall, plays a central role in atherogenesis. Phytoestrogens were shown to improve the lipid profile. In mice fed a high fat diet with an addition of daidzein derivative, the daidzein-treated animals showed a reduction in body and fat pad weight, and also an improvement of diet-induced hyperlipidemia, the effect being due to inhibiting the activity of both pancreatic lipase and lipoprotein lipase [75]. Genistein was shown to inhibit the oxidation of LDL in human and bovine aortic endothelial cells [76]. Beneficial effects on lipid profile were observed with dietary fibers and lignans [77]. Asgary *et al*. have investigated the effect of red clover, a phytoestrogen-rich member of the legume family, on the development of atherosclerosis in male hyperlipidemic rabbits. Dietary use of red clover in hyperlipidemic rabbits significantly decreased C-reactive protein, triglyceride, total cholesterol and LDL-cholesterol, whereas HDL-cholesterol was significantly increased in those animals. Fatty streak formation was also significantly lower in aorta and left and right coronary arteries in the same animals due to use of dietary red clover supplementation [78]. To clarify the possible role of flavonoids in the prevention of atherosclerosis, Safari and Sheikh investigated the effects of some of these compounds on the susceptibility of low-density lipoprotein to oxidative modification. Six flavonoids (apigenin, genistein, morin, naringin, pelargonidin and quercetin) were examined. Among flavonoids used, quercetin and morin significantly and dose-dependently prolonged the lag time before initiation of oxidation reaction. These two flavonoids suppressed the formation of lipid peroxides and TBARS more markedly than others. Their ability to prolong lag time and suppression of lipid peroxides and TBARS formation resulted to be in the following order: quercetin > morin > pelargonidin > genistein > naringin >apigenin, abd LDL exposed to flavonoids *in vitro* reduced oxidizability [79]. Similar study has been performed by Naregi *et al*. Morin, genistein, apigenin and biochanin A, naringin and quercetin were used at different concentrations. These flavonoids significantly inhibited *in vitro* LDL oxidation, and genistein, morin and naringin have stronger inhibitory activity against LDL oxidation than biochanin A or apigenin [80]. Thus, most of the experimental evidence suggests beneficial effects of phytoestrogens on lipid metabolism.

Some studies suggest that dietary supplementation of phytoestrogens can prevent the progression of atherosclerosis. It has been shown that grape phytoestrogens prevent cholesterol accumulation in cultured monocytes from postmenopausal women [81]. Animal studies also support the anti-atherogenic properties of phytoestrogens. Genistein inhibited atherogenesis in hypercholesterolemic rabbits mainly by improving endothelial dysfunction [82]. Compared with genistein, its derivative 7-difluoromethyl-5,4′-dimethoxygenistein provided a better protective effect against endothelial damage in rabbits [83]. Resveratrol exhibited multiple anti-atherogenic effects [84] including inhibition of intimal hyperplasia [85], attenuation of platelet aggregation [86] and inhibition of LDL oxidation [87].

The above results of experimental studies demonstrate that phytoestrogens have a potential in anti-atherosclerotic therapy, because they are able to modulate practically all cellular and molecular mechanisms of atherogenesis, namely, cellular proliferation, lipidosis, extracellular matrix synthesis, vascular permeability, LDL modification and vascular inflammation. However, whether phytoestrogens can improve the clinical course of atherosclerosis and whether their effects are consistent throughout the different models and different atherosclerotic stages, needs to be further examined in depth.

ANTI-ATHEROSCLEROTIC THERAPY WITH PHYTOESTROGENS: CLINICAL CONSIDERATIONS

After decades of research there is no definitive agreement as to the vascular effects of phytoestrogens and their benefit in cardiovascular disease. Some studies have suggested that phytoestrogens may not have any benefit in atherosclerotic disease, and other studies attributed the benefits of the soy-rich diet to food components other than phytoestrogens [88]. Importantly, 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 duration of follow-up.

Most of clinical studies were focused on the effects of phytoestrogens on plasma lipid profile, and practically none of them had atherosclerosis progression or clinical outcomes as the endpoint. In general, the results of those clinical trials supported the hypothesis that phytoestrogens decrease LDL cholesterol, total cholesterol and triglycerides, and increase HDL cholesterol. In any way, those beneficial effects were related to major cardiovascular risk factors,

thus allowing to discuss only indirect anti-atherosclerotic action of phytoestrogens.

In observational study by Bairey Merz *et al*., higher blood levels of daidzein were associated with lower TG, higher HDL-C levels, and an improved TC to HDL-C ratio. This beneficial association was evident among the subgroup of women with low blood estradiol levels, regardless of age and lipoprotein levels. The phytoestrogen associations with lipoproteins were incrementally related to the magnitude of daidzein level and independent of other lipoprotein modulators. There were no detectable relationships between the phytoestrogen levels and angiographic coronary artery disease [89]. In randomized controlled trial by Crouse *et al*., isoflavones reduced plasma concentrations of TC and LDL-C, but without statistically significant effect on TG or HDL-C [90]. In a small randomized controlled trial performed by Okamura *et al*. in 19 postmenopausal women, the increase in HDL-C and apolipoprotein A-1, and the decrease in LDL-C, Apo B, and LDL-C/HDL-C ratio was demonstrated [91]. In clinical trial by Blum *et al*., serum TG increased, but TC and LDL levels decreased significantly, while HDL-C showed mild change [92]. In October 1999, the US Food and Drug Administration authorized the use on food labels of health claims associated with soy protein and the reduced risk of coronary heart disease, since several studies have indicated that a total daily intake of 25 g of soy protein with a low-fat diet resulted in clinically important reductions of total cholesterol and low-density lipoprotein cholesterol levels [93].

Some studies did not support beneficial effects of phytoestrogens on lipid profile. In a population-based cross-sectional study in 301 Dutch women aged 60-75 years, dietary isoflavone and lignan intake was assessed with a food frequency questionnaire covering habitual diet during the year preceding enrollment. High intake of isoflavones was associated with lower Lp(a) level, but no relation was found between blood levels and the other plasma lipids, glucose or insulin was found. The results of this study suggested that an effect of dietary phytoestrogen intake at low levels on plasma lipid levels is of limited magnitude [94]. Several clinical trials demonstrated no effect on plasma lipid or lipoprotein levels [95-98].

The major evidence for cardiovascular profit of phytoestrogens still comes from epidemiologic studies. In 34489 healthy postmenopausal women aged 55-69, the analysis of flavonoid food composition data and 16 years follow-up have demonstrated the inverse association between flavanones intake and CHD, and between flavones intake and total mortality. No association between flavonoid intake and stroke mortality was revealed. The list of individual flavonoid-rich foods associated with significant mortality reduction was developed: bran, apples, pears, red wine (CHD and CVD), grapefruit (CHD), strawberries (CVD), and chocolate (CVD) [99]. Recent meta-analysis employing structured search strategy using MEDLINE, EMBASE, and Cochrane databases (133 clinical trial analyzed) provided the data on beneficial effects of chocolate (the increase of FMD after acute and chronic intake, the reduction of systolic and diastolic blood pressure), soy protein isolate (reduction of diastolic blood pressure and LDL cholesterol), and green tea (LDL cholesterol reduction) [100].

Thus, the controversy in the results of clinical and observational studies suggest that it is premature to advise postmenopausal women with low phytoestrogen intake to change their diet towards a phytoestrogen-rich diet with the sole aim to prevent cardiovascular disease. There is still no evidence that phytoestrogens may provide a direct anti-atherosclerotic effects. Current data are insufficient to draw definitive conclusions regarding the use of phytoestrogens for CVD prevention in postmenopausal women. Although epidemiological and basic laboratory studies allude to the possible protective effects of phytoestrogens at specific target tissues, randomized placebo-controlled clinical trials with definite and clinically relevant outcomes are necessary to address these important issues.

THE DEVELOPMENT OF PHYTOESTROGEN-RICH DIETARY SUPPLEMENT FOR DIRECT ANTI-ATHEROSCLEROTIC THERAPY: AN EXAMPLE OF FULL-CIRCLE PROJECT

Atherosclerosis is characterized by a number of morphological, histological and hemorheological changes in large arteries, and is described as an excessive fibro-fatty, proliferative, inflammatory response to damage of the artery wall, involving several cell types, such as smooth muscle cells, monocyte-derived macrophages, lymphocytes and platelets [101]. From clinical point of view, the direct treatment of atherosclerosis must consider at least the prevention of growth of atherosclerotic lesions, diminishing of the lipid core mass and further plaque stabilization. Taken together, these approaches could result in regression of lesions. At the level of arterial wall, the deposition of intracellular lipids, mainly free and esterified cholesterol, and subsequent foam cell formation is a typical feature of early atherosclerotic lesion [102]. Modified low density lipoprotein is generally thought to be the source of accumulating lipids, and intracellular lipid deposition may act as a trigger mechanism for the development of advanced atherosclerotic lesions, implying an excessive production of connective tissue matrix components and, cellular proliferation and, possibly, inflammatory reactions [103, 104]. Subsequent formation of advanced atherosclerotic lesion finally results in widely spread clinical manifestations of atherosclerosis. Several decades ago we have established that low density lipoprotein, as well as the whole serum, taken from atherosclerotic patients, is able to induce lipid accumulation in the primary culture of human intimal aortic cells, thus demonstrating the primary step in atherogenesis at the cellular level [105, 106]. This phenomenon was termed “atherogenicity”. We have proposed that meaningful serum atherogenicity lowering may be the way to prevent lipid deposition in vascular wall, thus inhibiting the initial step of atherosclerosis lesion formation [107-110]. From this point of view, the screening and selection of potentially anti-atherosclerotic compounds of plant origin, including phytoestrogens, could be performed in cell culture studies; the effects of compounds on such parameters as intracellular cholesterol retention, cellular proliferation, and collagen synthesis can be registered in such cell culture-based test system.

To gain the first experimental background for the development of phytoestrogen-rich dietary supplement for anti-atherosclerotic therapy, we have tested several phytoestrogens in cells cultured from atherosclerotic human aortic intima (Table **1**). Several tested phytoestrogens were able to decrease intracellular cholesterol content, as well as to inhibit leucine incorporation, thus could be potential compounds for further development. These experiments supported the idea on the screening and selection of potentially anti-atherosclerotic compound with the use of cell culture.

During the next stage of R&D, the search was carried out using Phytochemical and Ethnobotanical Database of Agricultural Research Service of US Department of Agriculture [111], as well as the database of the US National Center for Biotechnology Information [112]. Bioactive components that have estrogen-like activity were identified, as well as plants being the natural source of these components; qualitative and quantitative characteristics of these plants were defined to assess the possibility of their use to replenish the daily needs in phytoestrogens. In brief, pharmacodynamic effects of 27 estrogen-like substances of plant origin were analyzed. The following limitations were taken into account: (1) the subdivision of expected effects into potentially beneficial and potentially adverse effects was rather conditional, since the direction of the effect depended largely on of the compound; (2) most of the data was obtained in *in vitro* studies using cell and animal models; (3) in the studies of various authors, some substances have provided different effects; (4) many substances are still poorly investigated, therefore, the list of known effects was limited.

Table 1. Anti-atherosclerotic effects of phytoestrogens in cells cultured from atherosclerotic human aortic intima.

Compound	Intracellular cholesterol content, % of control			[^{14}C]-leucine incorporation, % of control		
	Concentration, M					
	10^{-6}	10^{-5}	10^{-4}	10^{-6}	10^{-5}	10^{-4}
Biohanin A	128±14	131±10	51±9 *	n.a.	n.a.	n.a.
Cinnaroside	87±5	48±6 *	35±6 *	n.a.	n.a.	n.a.
Formononetin	97±8	101±11	104±9	n.a.	n.a.	n.a.
Orobol	123±15	105±12	96±8	n.a.	n.a.	n.a.
Ononin	94±3	104±4	105±5	96±2	101±4	114±3
Hispidulin	90±6	98±3	toxic	94±7	70±2 *	toxic
Chrysoeriol	98±2	90±5	70±3 *	101±4	102±5	97±4
Haploside C	101±4	86±8	88±8	102±5	103±6	96±4
Tricytene	94±4	86±4 *	85±3 *	98±8	94±8	85±3 *
Genicin	102±3	95±5	84±3 *	94±8	93±10	97±4
Luteolin	97±7	98±8	toxic	100±7	94±7	toxic
Thermopsoside	102±6	99±7	toxic	95±4	87±10	toxic
Kaempherol	143±4 #	153±5 #	toxic	156±8 #	98±5	toxic
Vexibidine	140±5 #	160±10 #	toxic	171±9 #	82±7 *	toxic
Vexibinol	107±	150±8 #	toxic	117±14	120±11	toxic
Pinocembrin	137±10 #	197±12 #	228±16 #	143±6 #	117±7 #	67±6 *
Quercetin	92±7	76±2 *	56±4 *	80±5 *	42±2 *	19±4 *
Myricetin	102±5	100±7	86±2 *	105±6	92±8	90±2
Galangin	96±7	98±5	toxic	94±8	88±6 *	toxic
Morin	126±8 #	134±4 #	124±5 #	131±5 #	127±7 #	50±6 *
Glabranin	107±9	104±8	toxic	95±8	82±3 *	toxic
Isoornithine	96±8	92±5	82±3 *	91±2 *	86±3 *	66±6 *
Limonene	89±9	71±7 *	72±5 *	72±5 *	57±5 *	49±2 *
Allicine	78±5 *	76±6 *	79±6 *	88±3 *	74±3 *	68±5 *
Betaine	93±2	90±3	56±5 *	94±7	82±3 *	34±4 *
Glycyrrhizin	95±3	97±6	65±5 *	92±7	81±2 *	55±5 *
Carvacrol	91±4	90±8	57±5 *	88±5	68±4 *	24±2 *
Lutein	90±6	87±5	68±2 *	78±3 *	76±5 *	47±5 *
Genistein	92±5	83±2 *	60±4 *	78±8 *	73±5 *	55±2 *
Paeonol	103±4	101±8	99±3	90±4	99±3	88±4 *

The means of three independent experiments are shown. Intracellular cholesterol level in control cells accounted for 85±5 to 153±10 μg/mg cell protein, [^{14}C]-leucine incorporation – from 602±38 to 1028±69 dpm/mg cell protein.
n.a. – not assayed
* - significant decrease as compared to control cells, $p<0.05$;
# - significant increase as compared to control cells, $p<0.05$.

The known pharmacodynamic effects could be broadly grouped into the following activities characteristic to estrogen-like substances of plant origin: estrogen-like activity (identification key), antiviral effect, antioxidant effect, antibacterial action, antifungal activity, anti-inflammatory effect, anticancer action, and anti-atherosclerotic effect (only in terms of indirect action through the risk factors). It should also be noted that for any of the identified substances nothing was known about the direct anti-atherosclerotic effects in any of the possible aspects (like the effects on cellular mechanisms of atherogenesis, on experimental atherosclerosis in animal models, on the natural course of atherosclerosis in humans).

Further, the plants containing varying amounts of biologically active estrogen-like compounds were identified. It should be noted that a number of compounds (like anethole, apigenin, beta-sitosterol, diosgenin, genistein, quercetin, kampferol, coumarin, luteolin, naringenin, stigmasterol) are widespread in nature, and many plant sources may be available as raw material base. On the contrary, certain substances (like wogonin, dianetol, irizolon, rapontitsin, embelin) are unique, because are present in a very limited number of plant species that tend to make them unavailable as a source of raw materials. The third group includes compounds that occur in a limited number of plant species (like biochanin A, genistin, glycyrrhizin, glycitein, daidzein, daidzin, kumestrol, resveratrol, tricine, phloretin, formononetin), but these plamts are easily available as a source of raw materials (e.g., soybean, sophora, grapes, clover, licorice, wheat, apple). This subdivision of estrogen-like compounds based on the prevalence in nature and the availability of raw materials was suitable for further development of the phytoestrogen-rich dietary supplement.

At the next step of analysis, the plant geni possessing the greatest estrogen-like potential have been identified. This identification was based on the fact that many plants contain a lot of biologically active substances, thus providing a combination of several components. Therefore, the rating was calculated as the sum of all components having estrogenic or estrogen-like activity. For further development, the key parameter was just rating of genus as the source of estrogen-like biologically active substances.

The important point was the quantitative content of estrogen-like substances in plant sources. Therefore, the next step of screening was performed to characterize the presence of estrogen-like substances in various plant species in the quantitative manner. It was found that for a number of estrogen-like active substances their quantitative content is not established. Therefore, several potentially effective substances, such as anethole, apigenin, dianetol, embelin, irizolon, luteolin, phloretin and tricine, were excluded from further consideration. For further development, only available plants with known quantitative characteristics of estrogen-like substances were selected; additionally, the plants characterized by negligibly low (less than 200 ppm) content of estrogen-like substances were excluded.

To identify the plants for the further development of phytoestrogen-rich complex, regression analysis was used, with the following explanatory variables:

- quantitative content of estrogen-like active components (scalar);
- estrogen-like activity of biologically active components normalized by genistein (scalar);
- generic rating of estrogenic potential (ordinal);
- qualitative content of estrogen-like biologically active components (ordinal);
- the likelihood of anti-atherosclerotic and / or anti-atherogenic action (ordinal);
- commercial availability of raw materials (nominal);
- the reliability of suppliers of raw materials (nominal);
- the absence of formal restrictions for the use of given estrogen-like compound (nominal);
- belonging to the natural herbal components of nutrition and / or the use of herbal ingredients in traditional medicine (nominal);
- lack of potential side effects (nominal).

Based on the highest regression beta coefficient, the list of plants intended for further development of the phytoestrogen-rich complex was developed. This list included grapes, soy, sage, carrots, orange, licorice, garlic, onion, hops, tea, fucus, kelp, clover, violet, hawthorn, elder, and calendula.

To evaluate the anti-atherogenic effect of the selected plants, the model "ex vivo" based on primary cultures of monocytes isolated from the blood of healthy donors was used. In this model, the ability of human serum to induce accumulation of cholesterol in cultured cells (serum atherogenicity) was measured, as well as the effect of single dose oral administration of plant extract on serum atherogenicity, as described elsewhere [81, 107-110]. In brief, blood serum was obtained from the volunteers (healthy postmenopausal women, in whom high baseline serum atherogenicity was revealed) immediately before oral administration of plant extract, and at appropriate time intervals (at 2h, 4h, and 6h) after oral administration. The direct anti-atherogenic effects (the ability to decrease serum atherogenicity) of soybeans, grapes, sage, carrots, orange, licorice, garlic, onions, hops, green tea, fucus, kelp, clover, violets, hawthorn, elder, and calendula were studied. Integral measure of anti-atherogenic effects of studied plants is shown in Table **2**.

For further development of anti-atherosclerotic phytoestrogen-rich complex, the plants studied in the "ex vivo" test system were evaluated according to the following criteria:

- the size of anti-atherogenic effect after a single dose oral administration;
- the duration and dynamics of anti-atherogenic action after a single dose administration;
- qualitative and quantitative composition by phytoestrogens content;
- the possibility of use of raw materials in technological process of manufacturing pills;
- the ability to use smaller amounts of raw materials to achieve a sufficient anti-atherogenic effect;
- the commercial availability of raw materials.

Based on these criteria, the following plants (components) were excluded from further development: soybeans, dried grape pomace, crushed fermented grape crests, crushed grape seeds, carrot, orange, licorice, onion, fucus, kelp, clover, violet, hawthorn, elder, and calendula. The remaining plants and compounds (tannins from grape seed, sage, hops, garlic, green tea) formed the list of candidates for the development of natural phytoestrogen-rich anti-atherosclerotic complex.

At the next step of development, dose titration studies were performed using the same "ex vivo" test system [81]. It was found that a lowest single dose of tannin from grape seeds to effectively reduce serum atherogenic potential was 50 mg (integral decrease of serum atherogenicity at 2-6 h after single dose oral administration, 57% of the baseline atherogenicity, S.D.=11); for sage leafs - 250 mg (integral decrease of serum atherogenicity at 2-6 h after single dose oral administration, 35% of the baseline atherogenicity, S.D.=17); for hope cones - 250 mg (integral decrease of serum atherogenicity at 2-6 h after single dose oral administration, 43% of the baseline atherogenicity, S.D.=17); for garlic powder - 100 mg (integral decrease of serum atherogenicity at 2-6 h after single dose oral administration, 73% of the baseline atherogenicity, S.D.=21); for green tea leafs - 100 mg (integral decrease of serum athero-

Table 2. Integral anti-atherogenic effects of phytoestrogen-rich botanicals.

Plant	Integral anti-atherogenic effect (the decrease of serum atherogenicity at 2-6 h after single dose oral administration), % of the baseline serum atherogenicity (S.D.)
Soybeans (35 mg isoflavonoids)	32 (5) *
Grapes (250 mg tannins)	68 (3) *
Dried grape pomace (500 mg)	48 (9) *
Crushed fermented grape crests (500 mg)	36 (8) *
Crushed grape seeds (1000 mg)	94 (12)
Sage leafs (500 mg)	49 (14) *
Dried carrot (1000 mg)	58 (11) *
Orange juice (200 ml)	18 (8)
Licorice root (200 mg)	77 (16) *
Garlic powder (200 mg)	63 (15) *
Onion powder (200 mg)	12 (11)
Hop cones (250 mg)	43 (17) *
Green tea (500 mg)	75 (11) *
Fucus (500 mg)	41 (12) *
Kelp (500 mg)	59 (19) *
Red clover (250 mg)	17 (14)
Viola tricolor (500 mg)	45 (15) *
Hawthorn fruits (500 mg)	67 (20) *
Black elderberry flowers (500 mg)	58 (18) *
Calendula flowers (500 mg)	49 (24) *

* - statistically significant integral anti-atherogenic effect, $p<0.05$.

genicity at 2-6 h after single dose oral administration, 56% of the baseline atherogenicity, S.D.=19).

Based on the results of dose titration studies, qualitative composition of phytoestrogen-rich anti-atherosclerotic complex was defined. Insufficient anti-atherogenic effect of sage leaves (minimum effective single dose required for the development of anti-atherogenic effect was 250 mg), this component has been excluded from the list of candidate compounds. Despite the fact that anti-atherogenic effects of hop cones could also be considered as insufficient (minimum effective single dose required for the development of anti-atherogenic effect was 250 mg), this component had a favorable phytoestrogenic profile, which should help to replenish the daily requirement in phytoestrogens.

Thus, the final list of candidate compounds included tannins from grape seeds, hop cones, green tea leaves, and garlic powder. Mathematical simulation based on exponential regression of dose-response for each of them was used. The resulted assessment of the validity of the developed model is presented in Table **3**.

Given mathematical model allowed the calculation of the optimal quantitative content of these components in combination, designed for single dose administration in order to reach the most pronounced anti-atherogenic effect at minimal doses of the individual components. The estimated composition of the formulation was defined as follows: tannins from grape seeds - 40 mg; green tea leaves - 115 mg; hop cones powder - 160 mg; garlic powder - 100 mg. A single dose of this combination should provide a steady decrease in serum atherogenicity for 6-8 hours after administration. To achieve a steady decline in serum atherogenicity and adequate replenishment of the need in phytoestrogens the optimum mode of administration was defined as 1 pill (tablet) 3 times daily. The estimated daily intake of polyphenolic compounds was 44.6 mg, procyanidin - 27.3 mg, genistein - 2.5 mg, daidzein - 11.8 mg, flavones - 4.6 mg, resveratrol - 3.5 mg.

Anti-atherogenic effects of the developed combination of phytoestrogen-rich compounds were tested under the same conditions in the "ex vivo" test system [113-115]. Different daily regimens were studied.

The first regimen was 2 pills in the morning, and one pill at the evening. Integral anti-atherogenic effect observed for 24 hours was 67%. The expected 8-h duration of anti-atherogenic effect after single dose administration was reached and sustained by repeated administration every 12 hours. However, a doubling of the dosage in the morning did not result neither in an increase of anti-atherogenic effect, nor in an increase in its duration. Therefore, reduction of serum atherogenicity with this reception mode was uneven, and a significant reductions in atherogenicity alternated with the periods of return of atherogenicity to the baseline level.

Table 3. Mathematical modeling of dose-response assessment for anti-atherogenic activity of candidate compounds.

Compound	R^2	ANOVA	Power
Tannins from grape seeds	0.941	p=0.007	98%
Hop cones	0.886	p=0.018	94%
Green tea leafs	0.778	p=0.029	82%
Garlic powder	0.853	p=0.040	80%

The second regimen tested corresponded to mathematically developed profile (3 pills daily, with 8-h intervals). Using this regimen, integral anti-atherogenic effect observed for 24 hours accounted for 70%. The required duration of anti-atherogenic effect was achieved and sustained by repeated doses taken every 8 hours. The profile of atherogenicity reduction was uniform, and serum atherogenicity did not return to the baseline level throughout the day. Thus, the latter regimen was defined as more favorable, as corresponded well to the desired pharmacodynamics.

After the development of phytoestrogen-rich anti-atherosclerotic complex, it has been officially registered as a dietary supplement ("Karinat", INAT-Pharma, Russia) and was approved for manufacturing and legal use.

A double-blind placebo-controlled 6-months prospective study has been performed in 159 perimenopausal women. This study was aimed to asses anti-climacteric effects of the developed phytoestrogen-rich dietary supplement. It was found that the use of Karinat has provided a statistically significant positive effect on climacteric symptoms, but did not affect the quality of life. There were no adverse effects on the status of internal genitals and mammary glands, body weight, blood pressure, and serum prothrombin level. Karinat was most effective against the symptoms of menopausal syndrome of neurogenic and psycho-emotional nature (paresthesia, myalgia, melancholy, weakness, nervousness, insomnia). Thus, the identified effects reflected the beneficial impact of phytoestrogens on neuropsychological component of somatic menopausal symptoms and general adaptation [116].

Finally, a randomized double-blinded placebo-controlled pilot clinical study on direct anti-atherosclerotic effect of Karinat was performed in 157 asymptomatic postmenopausal women to assess the risks and benefits of phytoestrogen therapy in relation to atherosclerosis progression (ClinicalTrials.gov Identifier, NCT01742000) [117, 118]. The primary endpoint was the annual rate of changes in carotid intima-media thickness (cIMT). The duration of follow-up accounted for 12 months. The was no significant changes during follow-up in body mass index, systolic and diastolic blood pressure, triglycerides and HDL cholesterol in both groups. However, in Karinat recipients there was a statistically significant decrease in total cholesterol by 17 mg/dl, and LDL cholesterol by 13 mg/dl.

In the placebo group, an increase in the mean cIMT of more than 100 μm per year was observed. Thus, the rate of cIMT progression in postmenopausal women was rather high, as it accounted for 13% per year, and the growth of atherosclerotic plaques accounted for 40% per year. On the opposite, in Karinat recipients the mean cIMT did not change; there was statistically insignificant increase of 6 μm per year, i.e. less than 1%. Thus, the results of quantitative measurements of the degree of subclinical atherosclerosis in dynamics showed that the use of Karinat in postmenopausal women almost completely suppresses the formation of new atherosclerotic lesions and slows the progression of existing lesions approximately by 1.5-fold [119].

CONCLUSION

Over the past decades, phytoestrogens have been the subject of a vast amount of research, primarily because they are considered to be an alternative to hormone replacement therapy in postmenopausal women. The basic studies of estrogen-like effects of many bioactive substances derived from natural plants have provided a sufficient bulk of data to support this hypothesis, based on the fact that phytoestrogens have similar molecular structure to that of estrogens. Phytoestrogens have been shown to bind estrogen receptors and to behave as weak agonists or antagonists in both animals and humans. However, clinical studies have demonstrated that phytoestrogens and endogenous estrogen exert differing effects on a variety of health outcomes. It should be noted that the data from clinical studies with inconsistent results are mainly inconsistent per se, as most of the studies have serious limitations due to the study design and the participants' compliance. Nevertheless, there is a substantial evidence that phytoestrogens have the potential to address several conditions and diseases associated with the menopausal transition. Phytoestrogens, at least, can potentially reduce atherosclerosis and atherosclerosis-related diseases through multiple mechanisms, by regulating serum lipid metabolism, arterial vessels, cytokine levels, and coagulation/fibrinolysis system. However, a skepticism has developed concerning the true potential of phytoestrogens to beneficially modify these processes. An analysis of findings from supplementing the diet with phytoestrogens has failed, in general, to confirm them as the agents responsible for beneficial cardiovascular effects. There is now a growing interest in the use of phytoestrogen for primary prevention of cardiovascular disease in postmenopausal women. Again, experimental and clinical background for the wide use of phytoestrogens for primary and secondary prevention of atherosclerosis is still insufficient. But in any way, the clinical and epidemiologic data indicate that phytoestrogens possessing proved anti-atherosclerotic effects may be used to prevent and treat cardiovascular diseases, and that adding phytoestrogens to the diet can contribute to the health of postmenopausal women.

Future clinical trials need to enroll much larger number of study participants, to compare different phytoestrogens, to study the long-term effects of dietary modifications, and to use clear and clinically relevant end points (e.g. myocardial infarctions, stroke, carotid intima-media progression, etc.). The results from such clinical trials would help answering to important questions on the real anti-atherosclerotic potencies of different phytoestrogens and the proper daily quantity of phytoestrogens to produce measurable effects. As for now, there is insufficient evidence to recommend specific quantities or types of phytoestrogens for prevention or treatment of atherosclerosis and cardiovascular disease.

Clinically effective phytoestrogens may be used for further development of phytoestrogen-based pharmaceuticals. The in-depth description of the full cycle of development of phytoestrogen-rich dietary supplement Karinat, which involved theoretical, laboratory, experimental and clinical studies may serve as a successful example.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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