

Possible Beneficial Effects of Soy Protein on the Vascular Endothelium in Postmenopausal Women – Future Directions

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Epidemiologic observations, clinical studies, and basic laboratory research suggest that estrogen replacement therapy is associated with beneficial cardiovascular effects in postmenopausal women [1,2]. Estrogen exerts a multitude of biologic effects that may account for this apparent benefit (which remain to be proven in randomized clinical trials), including favourable effects on the lipid profile, a direct effect on the vascular endothelium with increased nitric oxide bioactivity, and improved fibrinolysis [3–5]. However, long-term estrogen therapy increases the risk of breast and endometrial cancers [6]. Thus, intense research has been conducted to find estrogen-like therapies that will have the beneficial effects of estrogen without the unwanted cancer risks and side effects. In this review we focus on the endothelium-dependent effects of soy protein, a prototype of a group of “plant estrogens” (phytoestrogens) that have been claimed to have beneficial cardiovascular effects without risky side effects.

The evidence that natural phytoestrogens protect against several chronic diseases is both observational and experimental. In humans, epidemiologic findings clearly show a higher incidence of some common types of cancer (breast, prostate, and colon) and of coronary heart diseases in western populations exposed to limited amounts of soybean isoflavones (genistein, daidzein) in the diet. Further evidence for cancer and cardiac protection and anti-atherogenic effects resulting from administration of soybean isoflavones has been noted in various experimental animal models. Isoflavones may also prevent postmenopausal bone loss and osteoporosis.

In fact, genistein has been reported to be as active as estrogens in maintaining bone mass in ovariectomized rats. The three major chemical types of phytoestrogens that have been identified are flavones, isoflavones, and coumestans. As for the cardioprotective activity of phytoestrogens, it is well established that a soy diet decreases low density lipoprotein cholesterol and total plasma cholesterol in both animals and humans [7,8]. Moreover, there is a low rate of coronary heart disease in Asian people compared with westerners and among vegetarians compared with omnivores [9].

Mechanism of action

Nitric oxide enhancement

Animal studies have indicated that estrogen may exert vascular effects independently of changes in lipoprotein profile, potentially mediated by estrogen receptors identified in cell cultures of

endothelial and vascular smooth muscle [3,10–12]. Human studies have demonstrated that estradiol infused intra-arterially to achieve physiologic levels in postmenopausal women potentiated endothelium-dependent vasodilator responsiveness to acetylcholine [3,4]. Twenty-four hour transdermal estradiol treatment improved coronary artery diameter and blood flow responses to acetylcholine [13]. Other groups have also shown that acute or chronic administration of estrogen to postmenopausal women improves endothelium-dependent systemic or coronary vasodilator function [5,14–16]. Guetta et al. [17] found that L-NMMA, a specific inhibitor of nitric oxide synthase activity and thus NO synthesis, inhibits the vasodilating effect of estradiol administration on acetylcholine-stimulated epicardial and microvascular coronary vascular responses in postmenopausal women, compatible with augmentation by estradiol of the synthesis and release of NO. Increased NO may reduce levels of the potent constrictor peptide endothelin-1, contributing to vascular relaxation [18,19]. Several groups have reported increases in NO synthase activity in endothelial cell cultures after incubation with physiologic concentrations of estradiol, an effect blocked by estrogen receptor inhibitors [20–22]. With prolonged incubation, increased NO synthase protein was shown by Western blotting [20,21] to be compatible with estrogen responsiveness of the NO synthase gene promoter region at multiple half-palindromic motifs that may function as estrogen response elements [23].

The anti-inflammatory effect

Increased nitric oxide bioactivity as a result of estrogen administration may not only promote smooth muscle relaxation by means of increased cyclic GMP, but also improves other important homeostatic properties of endothelium, such as inhibition of activation of pro-inflammatory genes. In this regard, NO had been found to inhibit the activation of an important pro-inflammatory nuclear transcription factor, NF- κ B [24–26]. In the presence of reduced cytosolic nitric oxide or increased cytosolic oxidant stress, NF- κ B is activated by dissociation from its inhibitor subunit (I κ B) after I κ B phosphorylation in the cytosol. The heterodimer then translocates to the nucleus where it binds to promoter regions of several pro-inflammatory genes, with transcription and synthesis of protein mediators of inflammation, including cytokines, chemokines, and cell adhesion molecules. Inflammatory cells, once

NO = nitric oxide

activated and attracted into the vessel wall by these gene products, exert a variety of pro-atherogenic effects, including the release of reactive oxygen species, growth factors, prothrombotic factors, and in the case of monocytes, transformation into foam cells upon unregulated uptake of oxidized LDL [27]. Cell culture studies may prevent activation of NF- κ B and thus prevent or attenuate transcription of pro-inflammatory proteins [23,26–28]. Because estrogen has both NO stimulatory [20–22] and antioxidant properties [29,30], effects of this hormone on inflammation are of current research interest.

Postmenopausal HRT and a soy protein diet

The vascular effects of hormone therapy were studied in healthy postmenopausal women and compared in premenopausal women. Brachial artery diameter was measured by ultrasonography at rest, during reactive hyperemia following forearm ischemia (with increased flow causing endothelium-dependent dilation because of increased shear stress-release of NO), and after sublingual nitroglycerin (causing endothelium-independent dilation). As compared to premenopausal women, flow-mediated dilation was significantly reduced in both hormone-treated and control postmenopausal groups, and to an equivalent degree, suggesting that hormone therapy had no effect on vasodilator responsiveness [31]. However, several other studies reported beneficial effects of estrogen on vasomotion. Lieberman et al. [5] showed that short-term estrogen replacement therapy improves flow-mediated endothelium-dependent vasodilation in postmenopausal women. McCrohon and colleagues [32] demonstrated that long-term HRT is also associated with improved arterial endothelial function in healthy postmenopausal women. This benefit was observed in both the combined HRT and unopposed estrogen therapy groups. These studies may explain some of the apparent cardioprotective effects of hormone replacement therapy after the menopause.

Future directions

Based on these results and the many other observational studies that have demonstrated significant cardioprotective beneficial effects of estrogen in postmenopausal women (the Nurses' Health Study [1], the PEPI trial [33], etc.), and because long-term estrogen therapy increases the risk of breast and endometrial cancer [6], many research groups looked for a proper substitution, like the selective estrogen receptor modulator (or natural plant estrogens (phytoestrogens). Observational, epidemiologic, and population-based studies have demonstrated cardiac protection and anti-atherogenic effects from soybean isoflavones [34,35]. "Dietary estrogens" are adsorbed from the intestinal tract, transported to the liver and, like endogenous estrogenic steroids, undergo an enterohepatic circulation [36]. The mechanisms through which isoflavones may exert the above-mentioned effects seem to depend on their estrogen agonist/antagonist properties [37]. However, the vascular endothelium-dependent effects of soy protein have not yet

been studied, but based on the estrogen studies that were done it seems to be a promising substitution for estrogen, and could protect the endothelial function and prevent the accelerated progression of atherosclerosis in postmenopausal women without dangerous side effects.

References

- Grodstein F, Stampfer M, Colditz GA, et al. Postmenopausal hormone therapy and mortality. *N Engl J Med* 1997;336(25):1769–76.
- Grodstein F, Stampfer M, Manson JE, et al. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N Engl J Med* 1996;335(7):453–61.
- Gilligan DM, Badar DM, Panza JA, et al. Acute vascular effects of estrogen in postmenopausal women. *Circulation* 1994;90:786–91.
- Gilligan DM, Quyyumi AA, Cannon RO III. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. *Circulation* 1994;89:2545–551.
- Lieberman EH, Gerhard MD, Uehata A, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med* 1994;121:936–41.
- Colditz GA, Stampfer MJ, Willett WC, et al. Prospective study of estrogen replacement therapy and risk of breast cancer in women. *JAMA* 1990;264:2648–53.
- Terpstra AH, Wers CE, Ferris JT, Schouten JA, van der Veen EA. Hypocholesterolemic effect of dietary soy protein versus casein in rhesus monkeys (*Macaca mulatta*). *Am J Clin Med* 1984;39:1–7.
- Lovati MR, Gaddi G, Sirtori CR. Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. *J Clin Invest* 1987;80:1498–502.
- Clarkson TB, Anthony MS, Hughes CL. Estrogenic soybean isoflavones and chronic disease. Risks and benefits. *Trend Endocrinol Metab* 1995;6:11–16.
- Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation* 1994;89:1943–50.
- Karas RH, Baur WE, van Eickles M, et al. Human vascular smooth muscle cells express an estrogen receptor isoform. *FEBS Lett* 1995;377:103–8.
- Kim-Schulze S, McGowan KA, Hubchak SC, et al. Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. *Circulation* 1996;94:1402–7.
- Roque M, Heras M, Roig E, et al. Short-term effects of transdermal estrogen replacement therapy on coronary vascular reactivity in postmenopausal women with angina pectoris and normal results on coronary angiograms. *J Am Coll Cardiol* 1998;31:139–43.
- Reis SE, Gloth ST, Blumenthal RS, et al. Estradiol acutely attenuates coronary vasomotor responses to acetylcholine in postmenopausal women. *Circulation* 1994;89:52–60.
- Herrington DM, Braden GA, Williams JK, et al. Endothelium-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. *Am J Cardiol* 1994;73:951–2.
- Collins P, Rosano GM, Sarrel PM, et al. 17 β -estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation* 1995;92:24–30.
- Guetta V, Quyyumi AA, Prasad A, et al. The role of nitric oxide in coronary vascular effects of estrogen in postmenopausal women. *Circulation* 1997;96:2795–801.
- Best PJM, Berger PB, Miller VM, et al. The effects of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. *Ann Intern Med* 1998;128:285–8.
- Ylikorkala O, Orpana A, Puolukka J, et al. Postmenopausal hormonal replacement decreases plasma levels of endothelin-1. *J Clin Endocrinol Metab* 1995;80:3384–7.
- Hishikawa K, Nakaki T, Marumo T, et al. Up-regulation of nitric oxide synthase by estradiol in human aortic endothelial cells. *FEBS Lett* 1995;360:291–3.

LDL = low density lipoprotein

HRT = hormone replacement therapy

21. Hayashi T, Yamada K, Esaki T, et al. Estrogen increases endothelial nitric oxide by a receptor-mediated system. *Biochem Biophys Res Commun* 1995;214:847–55.
22. Caulin-Glaser TL, Garcia-Cardena G, Sarrel P, et al. The effect of 17-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca mobilization [Abstract]. *Circulation* 1994;90(Suppl 1):1–30.
23. Venema RC, Nishida K, Alexander RW, et al. Organization of the bovine gene encoding the endothelial nitric oxide synthase. *Biochim Biophys Acta* 1993;1218:413–20.
24. De Caterina R, Libby P, Peng HB, et al. Nitric oxide decreases cytokine-induced activation: nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995;96:60–8.
25. Peng HB, Rajarashisth TB, Libby P, et al. Nitric oxide inhibits macrophage-colony stimulating factor gene expression in vascular endothelial cells. *J Cell Chem* 1995;270:17050–5.
26. Zeiher AM, Fisslthaler B, Schray-Ulz B, et al. Nitric oxide modulates the expression of monocyte chemoattractant protein-1 in cultured human endothelial cells. *Circ Res* 1995;76:980–6.
27. Berliner PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics. *Circulation* 1995;91:2488–96.
28. Marui N, Offermann MK, Swerlick R, et al. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1993;92:1866–74.
29. Keaney JF, Shwaery GT, Xu A, et al. 17 β -estradiol preserves endothelial vasodilator function and limits low-density lipoprotein oxidation in hypercholesterolemic swine. *Circulation* 1994;89:2251–9.
30. Sack MN, Rader DJ, Cannon RO III. Estrogen and inhibition of oxidation of low density lipoproteins in postmenopausal women. *Lancet* 1994;343:269–70.
31. Sorensen KE, Dorup I, Hermann AP, et al. Combined hormone replacement therapy does not protect women against the age-related decline in endothelium-dependent vasomotor function. *Circulation* 1998;97:1234–8.
32. McCrohon JA, Adams MR, Robyn J, et al. Hormone replacement therapy is associated with improved arterial physiology in healthy postmenopausal women. *Clin Endocrinol* 1996;45:435–41.
33. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors on postmenopausal women. The postmenopausal estrogen/progestin interventions (PEPI) trial. The writing group for the PEPI trial. *JAMA* 1995;273(3):199–208.
34. Anderson JW, Johnstone BM. Meta analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 1995;333:276–82.
35. Anderson JW, Smith BM, Washnock CS. Cardiovascular and renal benefits of dry bean and soybean intake. *Am J Clin Nutr* 1999;70:464–74S.
36. Brandi ML. Natural and synthetic isoflavones in the prevention and treatment of chronic diseases. *Calcif Tissue Int* 1997;61:S5–8.
37. Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* 1987;103:1860–7.

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