Current knowledge and future direction of research on soy isoflavones as a therapeutic agents

V. Kalaiselvan, M. Kalaivani[1], A. Vijayakumar[1], K. Sureshkumar[1], K. Venkateskumar[1]

Indian Pharmacopoeia Commission, Sector 23, Raj Nagar, Ghaziabad- 201002, Uttar Pradesh, [1]KMCH College of Pharmacy, Kovai Estate, Kalapatti road, Coimbatore-42, Tamil Nadu, India.

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ABSTRACT

Isoflavones, the most abundant phytoestrogens in Soy beans, are structurally similar to 17beta-estradiol. The antioxidant property of the soy isoflavones, namely, genistein and daidzein is well established in different experimental models and also in clinical studies. The compounds have been found effective in the management of diabetes by acting on peroxisome proliferator-activated receptors. It reduces the risk of coronary heart disease by reducing the level of low-density lipoprotein and triglycerides. Soy isoflavones have the potential in the treatment of osteoporosis to act on osteoclasts further to inhibit tyrosine kinase. Among the soy isoflavones, genistein is the potential compound found effective in the treatment of cancer by acting on androgen receptor further to inhibit tyrosine kinases. In this article, various aspects of the diverse biological activities of soy isoflavones and their potential clinical implications with mechanism of action, especially in the treatment and prevention of diabetes, cardiovascular diseases, cancer, osteoporosis, neuroprotection, and also future area of research on soy isoflavones are reviewed and discussed.

Key words: Antioxidant, daidzein, genistein, isoflavones, neuroprotection, osteoporosis, soy beans

INTRODUCTION

Legumes play an important role in the traditional diets of many regions of the world. *Glycine max* (Leguminosae), commonly known as soy bean or soya bean (SB) is unique among the legumes. It is an annual plant that may vary in growth, habit, and height. It may grow prostrate, not growing to higher than 20 cm (7.8 inches), or even stiffly erect up to 2 m (6.5 feet) in height. The mature seed is rich in proteins and is a concentrated source of isoflavones.[1-3] For centuries, SBs and soy protein have been consumed as a staple food and are an important source of high-quality proteins.[4,5] The rich history of soy began 5000 years ago on the windy plains of Eastern Asia. According to the Chinese tradition, SB was one of the five sacred crops named by the Chinese emperor Sheng-Nung, who reigned 5000 years ago.[6] Historians maintain that Sheng-Nung mentioned SB in his "Ben Tsao Gang Mu," written in the year 2838 B.C. Soy was once cultivated by Chinese farmers, but it spread gradually across China, Korea, Japan, India, and European countries, and finally to America.[7]

Address for correspondence:
Dr. V. Kalaiselvan,
Indian Pharmacopoeia Commission, Sector 23,, Raj Nagar,
Ghaziabad- 201 002, Uttar Pradesh, India.
E-mail: vivekarts@rediffmail.com

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As SBs have many important nutritious components, such as proteins, fats, carbohydrates, and α-tocopherol, long-term continuous intake of this legume might effectively prevent senility.[8] Over the last few years, research has focused on SB constituents, such as isoflavones, phytosterols, saponins, water- and fat-soluble vitamins, and minerals.[3] The isoflavones, namely, genistin and daidzin, are metabolized to genistein and daidzein.[9] They are considered to be essential for the healthy functioning of bowels, heart, kidney, liver, and stomach.[10,11] Soy proteins have many beneficial effects in humans: reduction of serum cholesterol levels and reduction in the risk for coronary heart disease (CHD), reduction in the risk for breast cancer, reduction in the risk for osteoporosis in women, and alleviation of the disturbances caused by menopause.[12] This review article highlights the health benefits and lacunae of soy isoflavones as major nutraceutical compounds.

COMPOSITION

SB contains about 39.6% good-quality proteins, 20% cholesterol-free oil, 33% carbohydrates, and reasonable amounts of minerals, including calcium and iron, as compared with conventional source of proteins. SBs are largely replacing milk and other sources of animal proteins, which are expensive and not readily available, as suitable substitutes for high-quality proteins.[13] Soy does not have lactose. Hence, soy milk is suited for lactose-intolerant people.[14] SBs contain a variety of bioactive components,

including saponins, protease inhibitors, phytic acid, all essential amino acids, and isoflavones [Figure 1]. Isoflavones belong to a class of compounds generally known as phytoestrogens, plant compounds that have estrogen-like structures.[15,16]

SOY ISOFLAVONES

Isoflavones are found in varying amounts in legumes, but their significant source is SBs.[17] Isoflavones are phytoestrogens and have a chemical structure similar to that of estrogen [Figure 2]. The two primary isoflavones in SB are genistein and daidzein, along with other glycosides. A method for the quantification of daidzein, genistein, glycitein, and other glucosides and acetylglucosides in soy extracts has been developed and validated.[18] Isoflavones are metabolized by gut microflora; their sugar portion is removed from the glycosides, forming the active compounds genistein and daidzein, which are then absorbed in the intestine. Daidzein is more bioavailable than genistein, because it has a longer half-life in the intestine. The genistein is degraded about twice as fast and is therefore less absorbed. However, the isoflavones are also degraded by microflora to other metabolites, including the isoflavonoid equol.[15] Together with isoflavones, there are two other main classes of phytoestrogens: lignans and coumestans.[19] Isoflavones are associated with the protein fraction; hence, they are present only in the whole SB and other high-protein secondary products.[20,21] Soy isoflavones are being studied intensively to clarify the physiologic effects they exert. In some cases, the isoflavones may be one of the key factors in SB that have a disease-fighting potential.

ANTIOXIDANT ACTIVITY OF SOY ISOFLAVONES

A considerable amount of research is being done to investigate the potential role of antioxidants in reducing cell damage that can contribute to the development of cancer. Genistein has been shown to have antioxidant properties. It was found that genistein has a potential antioxidant effect on hydrogen peroxide production by 12-O-tetrade-canoylphorbol-13-acetate-activated HL-60 cells. Feeding genistein to mice for 30 days also significantly enhanced the activity of antioxidant enzymes in the skin and small intestine.[22,23] *In vivo* soy isoflavones (150 and 250 ppm) showed antioxidant property by elevating the antioxidant enzyme activity, such as catalase and superoxide dismutase, in various organs of rat where as tofu containing approximately 50 ppm isoflavones had better effects than the soy isoflavones. This indicated that molecules other than isoflavones may have a synergistic effect on *in vivo* antioxidant enzyme inductions of tofu.[24] Another study reveals that daidzein enhances catalase promoter activity at 100 µmol/L in a reporter gene assay and at 200 µmol/L in Northern blot experiments but shows only little antioxidant capacity.[25]

In another study, stroke patients were given a soy creme product (10.6% protein) that reduced lipid peroxidation in molecules of low-density lipoprotein (LDL) cholesterol, very-low-density lipoprotein cholesterol, and high-density lipoproteins (HDL).[26] Another study found that genistein inhibited the oxidation of LDL cholesterol in the presence of copper ions or superoxide/nitric acid radicals *in vitro*. In addition, this research suggested that genistein effectively protected human endothelial cells from damage by oxidized lipoproteins.[27] Another study was conducted on six healthy volunteers who consumed three soy bars per day for 2 weeks; each bar contained 12 mg of genistein and 7 mg of daidzein. Serum LDL cholesterol, which was isolated at baseline, after 2 weeks of soy bar consumption, and after soy was discontinued, was subjected to copper-mediated oxidation *in vitro*. Compared with off-soy values, during soy intake, the lag phases of LDL cholesterol oxidation curves were prolonged by a mean of 20 min ($P < 0.02$), which indicated a reduced susceptibility to oxidation.[28] Studies also found that the oxidative metabolites of genistein and daidzein showed higher antioxidant activities than the positive controls of quercetin and ascorbic acid in different *in vitro* assays.[29-32]

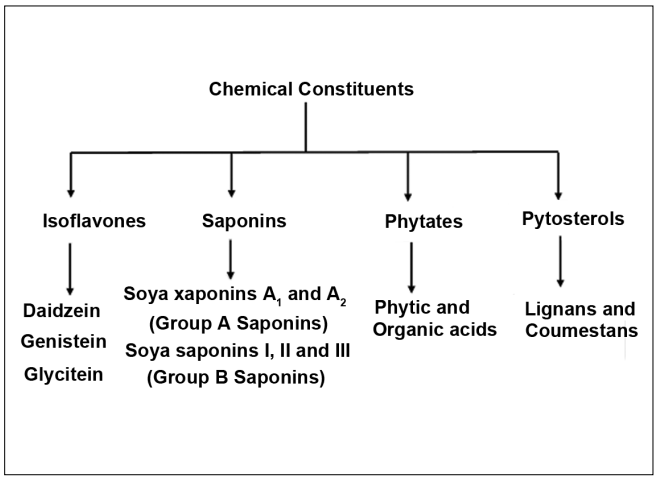

Figure 1: Different chemical constituents in soya bean

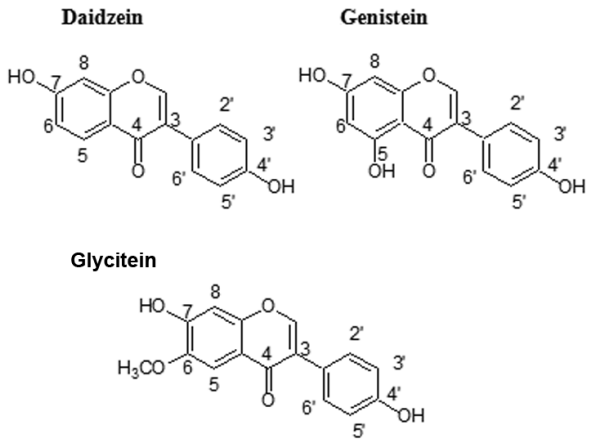

Figure 2: Chemical structure of soy isoflavones, such as daidzein, genistein, and glycitein, Daidzein is 4', 7- dihydroxyisoflayone, genistein is 4'5, 7-trihydroxy isoflavones and glycitein is 7,4' - dihydroxy-6-methoxyisoflayone.

EFFECT IN THE MANAGEMENT OF DIABETES

Consuming foods with a low glycemic index (GI) has been shown to improve blood sugar control and blood lipid values in patients with non-insulin-dependent diabetes. Legumes, especially SBs, have a very low GI and are valuable foods to be included in a diabetic diet.[33-36] Studies also found that isoflavones in SBs significantly reduced the risk of type II diabetes by decreasing GI.[37] This has been attributed to many factors, including their fiber, tannin, and phytic acid contents.[38-40] In support of this statement, there are findings from a prospective study showing that women who consumed diets with a high GI were 40% more likely to develop diabetes than those consuming low-GI diets, even after controlling for several diabetes risk factors.[41] Thus, beans may be a particularly important food for individuals with diabetes and those with an elevated risk of developing diabetes.

Blood sugar control may also be improved by choosing carbohydrates that are high in soluble fiber. Some researchers believe that fiber has no measurable benefit unless it is added to the diet in very large amounts.[42] Soy fiber is extremely fermentable in humans, and therefore, may have more physiologic benefits than some other types.[43] A study using 25 g of supplemental soy fiber per day found that it significantly reduced insulin response to an oral glucose challenge—and reduced total plasma cholesterol in hypercholesterolemic patients.[44] Soy isoflavones improve lipid and glucose metabolism by acting as antidiabetic peroxisome proliferator-activated receptors, which are promiscuous nuclear receptors that regulate the transcription of genes involved in lipid and glucose homeostasis and lipid metabolism within the cell.[45] Soy isoflavones increase insulin secretion without any change in the glucose disposal as well as decrease the plasma adiponectin concentrations.[46] Studies also found that genistein increases glucose-stimulated insulin secretion in cell lines and mouse pancreatic islets at micromolar concentrations via a cAMP-dependent protein kinase mechanism.[47]

EFFECT ON CARDIOVASCULAR DISEASE

Diabetes raises the risk for heart disease and atherosclerosis. Cardiovascular disease is 2–4 times as common in diabetic patients as in the general population. CHD is the major cause of death in most developed countries and is rapidly increasing in prevalence in the developing countries. More than one in four Americans have some form of cardiovascular disease.[48] In the United States, death rates from cardiovascular disease exceed 1 million annually and the total cost is estimated to exceed $120 billion, the largest disease-related cost to health. Although many risk factors, such as cigarette smoking and hypertension, contribute to the risk for CHD, lipid abnormalities are the major factors. LDL plays a central role in the atherosclerotic process. LDL penetrates the walls of blood vessels where it is oxidized by free radicals and accumulates as a gruel-like material that blocks the blood vessel to cause thrombosis. HDL cholesterol has a protective effect and acts to prevent LDL oxidation and remove the cholesterol that accumulates in the blood vessel wall.[49] Soy protein exerts several antiatherogenic effects. First, it decreases LDL cholesterol levels significantly. Second, it tends to increase HDL cholesterol levels. This is rather unique since most dietary interventions, such as oat bran intake or decreased saturated fat intake, significantly decrease HDL cholesterol levels.[50] Third, soy isoflavones, plant chemicals unique to SB, have antioxidant properties that prevent LDL from oxidation.[32]

There is a great deal of evidence that soy protein helps lower blood cholesterol levels.[51-54] Replacing animal protein with soy protein in the diet lowers the total and LDL cholesterol levels in people with high cholesterol levels. A meta-analysis of 38 research studies concluded that soy protein lowers total and LDL cholesterol and triglycerides, without lowering HDL cholesterol in hypercholesterolemic humans. In these studies, the average consumption of soy protein was 47 g per day. The greatest decreases in cholesterol were seen in those with the highest starting levels. Even adding soy protein to an omnivorous diet has been shown to produce this effect, which also emphasized that 25 g of soy protein per day may be enough to lower cholesterol levels.[55-57] The research study with rhesus monkeys found that isoflavones associated with soy protein enhance the cholesterol-lowering effects.[58] Another study suggests that soy protein inhibits LDL oxidation.[26]

A meta-analysis of 27 studies concluded that replacing dietary saturated fat with unsaturated fat lowers total cholesterol levels and also improves the ratio of HDL to LDL cholesterol.[59] Various protein fractions and isoflavone components were actively studied for antiatherogenic effects and the demonstration expressed that estrogen receptor-α is required for atheroprotection by soy protein; this provides an important new mechanistic insight.[60] Soy protein isoflavones stimulate the transcription factor sterol regulatory element–binding protein. The reduction of this factor decreases the expression of several lipogenic enzymes, thus decreasing the serum and hepatic triglycerides, as well as LDL cholesterol.[61]

Cardioprotective effects of soy isoflavones may be due in part to a variety of actions, including a favorable effect on the blood lipid profile and inhibition of LDL cholesterol oxidation. The precise mechanism by which soy isoflavones the blood lipid profile is unknown. One possible mechanism is altered hepatic metabolism, with enhanced removal of LDL cholesterol and very-low-density-lipoprotein cholesterol by hepatocytes.

EFFECT ON CANCER

Cancer is the second leading cause of death in the United States. Scientific evidence indicates that one third of cancer deaths in the United States are due to dietary factors.[62] SB compounds, namely, isoflavones, saponins, phytates, protease inhibitors, and phytosterols, are identified as anticancer drugs.[63] Isoflavones are weak estrogens. They can function as antiestrogens by binding

with estrogen receptors in place of the potent physiologic estrogens, thus blocking them from exerting their effects.[64] Theoretically, this would reduce the risk for cancers, which are stimulated by estrogen, such as certain breast cancers. Genistein arrests cell cycle progression at the G2–M transition, includes apoptosis, has antioxidant properties, modifies eicosanoid metabolism, and inhibits angiogenesis.[65]

Pubertal exposure to genistein that is administered via either injections or fed has consistently been found to reduce the incidence and/or multiplicity of subsequent carcinogen-induced, estrogen-dependent mammary tumors.[66] Genistein inhibits the proliferation of MDA-MB-231 human breast cancer cells in cell culture and the probable mechanism is inhibition of the cell cycle at G2–M.[67] *In vitro* studies have demonstrated that genistein and daidzein were shown to inhibit the proliferation of a number of murine and human neuroblastoma cell lines.[68-70] Soy isoflavones has been shown to act on androgen receptor further to inhibit tyrosine kinases, and thereby block the growth and proliferation of cancer cells[71] [Figure 3]. Genistein selectively inhibits cancer cells, and leaves normal cells alone, and also inhibits angiogenesis; it is also a potent antioxidant that blocks the formation of oxygen free radicals, which are involved in cancer promotion.[72,73]

EFFECT ON OSTEOPOROSIS

Osteoporosis is characterized by reduced bone mass and structural deterioration of bone tissue; it occurs in women and is primarily related to aging and hormone deficiency.[74] Drugs that act on resorptive pathway (responsible for bone loss) and to build new and improved skeletons are specifically recommended to treat bone loss.[75] Experimental studies on genistein showed a positive effect on osteoporotic bone by decreasing osteoclastic resorption factor, such as collagen C-telopeptide, and increasing osteoblastic formation markers, such as bone-alkaline phosphatase.[76] Genistein has also shown to selectively antagonize the bone catabolic effects of parathyroid hormone in osteoblasts by reducing parathyroid hormone-induced increases in soluble receptor activator of nuclear factor-κB ligand and reversing decreases in osteoproteger in expression *in vitro*.[77,78]

The mechanism of action of isoflavones seems to be to act independently on osteoclasts via nonestrogenic mechanisms because there are no estrogen receptors in the nuclei of osteoclasts. Isoflavones act on osteoclasts by inhibition of tyrosine kinase.[79] Although the mechanisms of isoflavones on osteoporosis are still not completely known, evidence from *in vitro* studies suggests that they act in multiple ways, via genomic and nongenomic pathways and via both osteoclasts and osteoblasts. Soy isoflavones stimulate the activity and proliferation of bone-building cells, namely, osteoblasts, to maintain bone mass against the action of osteoclast cells, which release acid and enzyme to dissolve bone. The osteoblast cells produce a collagen core and coat it with an adhesive substance, which is present in the bone and finally calcium adheres to the collagen to form a new bone tissue[80] [Figure 4].

ADVERSE EFFECT OF SOY ISOFLAVONES

Soy isoflavones in moderation are probably not dangerous, as few studies have indicated adverse effects. However, large doses have been shown to increase apoptosis and cell degeneration, and in some cancer regimes, once the cancer has progressed beyond the hormone-dependent stage, high doses of isoflavones may be contraindicated.[81]

SUMMARY AND CONCLUSION

Most of the studies evaluated the potential of soy isoflavones on various biomarkers or diseases, although, several of the endpoints, such as LDL and bone mineral density, do have known meaningful correlations with clinical outcomes. Cardiovascular surrogate endpoints were assessed by the largest number of

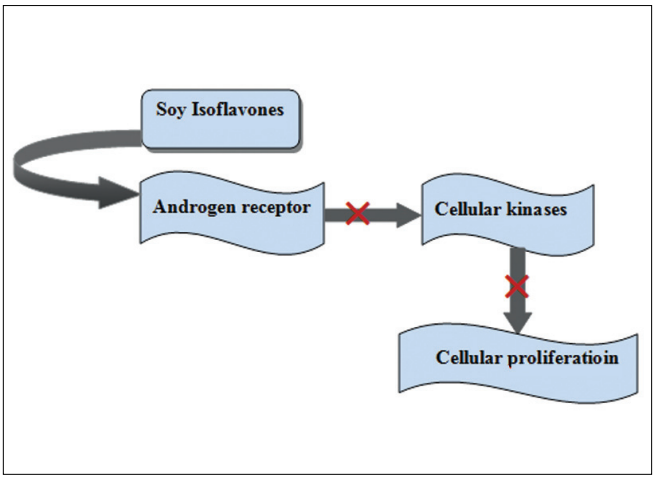

Figure 3: Inhibition of growth and proliferation of cancer cells by soy isoflavones

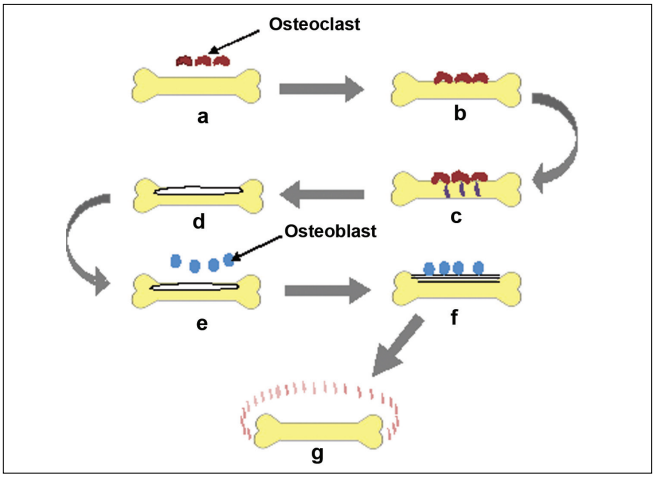

Figure 4: Mechanism of action of soy isoflavones in the treatment of osteoporosis, (a) Osteoclast cells carried via bloodstream to bone, (b) Cells fimly attach themselves to the bone, (c) Cells releases acid and enzymes that dissolve the bone, (d) Osteoclast cells disappears once their function is completed, (e) Soy isoflavones stimulates the activity and proliferation of osteoblasts (bond-building), (f) The cells produce a collagen core and coat it with an adhesive substance, (g) Calcium adheres to the collagen, forming new bone tissue

studies. Overall, soy isoflavone was found to have some effect on lipids. However, the duration of these studies were generally short, and it is uncertain whether the results would be sustained. Furthermore, it is not clear whether the renal protective effects of soy protein are caused by the isoflavones (daidzein and genistein) and lignans or some other component that need to be discussed in future. Till date, even though some epidemiologic data and clinical studies suggest potential protective effects on specific target organs, further controlled, randomized studies are necessary to determine the real benefits of soy isoflavone for the treatment and management of chronic diseases at different dose levels.

Nevertheless, more studies are needed to better understand the exact mechanisms, *in vivo* target sites, stability, and safety, prior to using as a potential therapeutic agent. This review article suggests to healthy individuals as well as patients to include caution in using soy isoflavone supplements until clinical trials are completed. Clinical study with soy isoflavones in patients with chronic diseases should be a high priority.

REFERENCES

1. Tuladhar B. Comparative study of fish yields with plant protein sources and fish meal. Our Nat 2003;1:26-9.
2. Stürtz M, Lander V, Schmid W, Winterhalter P. Quantitative Determination of Isoflavones in Soy Based Nutritional Supplements by High-Performance Liquid Chromatography. Journal for Consumer Protection and Food Safety 2008;3: 127-36.
3. Polkowski K, Mazurek AP. Biological properties of genistein. A review of *in vitro* and in vivo data. Acta Pol Pharm 2000;57: 135-55.
4. Donovan UM, Gibson RS. Dietary intakes of adolescent females consuming vegetarian, semi-vegetarian and omnivorous diets. J Adolesc Health 1996;18:292-300.
5. Ridout CL, Wharf G, Price KR, Johnson LT, Fenwick GR. UK means daily intakes of saponins-intestine permeabilizing factors in legumes. Food Sci Nutr 1988;42F:111-6.
6. Torres N, Torre-Villalvazo I, Tovar AR. Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. J Nutr Biochem 2006;17:365-73.
7. Messina M, Bennink M. Soy foods, isoflavones and risk of colonic cancer: a review of the *in vitro* and in vivo data. Baillieres Clin Endocrinol Metab 1998;12:707-28.
8. Ishii Y, Tanizawa H. Effects of Soya saponins on lipid peroxidation through the secretion of Thyroid hormones. Biol Pharm Bull 2006;29:1759-63.
9. Greaves KA, Wilson MD, Rudel LL, Williams JK, Wagner JD. Consumption of soy protein reduces cholesterol absorption compared to casein protein alone or supplemented with an isoflavone extract or conjugated equine estrogen in ovariectomized cynomolgus monkeys. J Nutr 2000;130:820-6.
10. Duke JA, Ayensu ES. Medicinal Plants of China. ISBN 0-917256-20-4. Algonac, MI (USA) Reference Publications, Inc ; 1985.
11. Setchell KD, Brown NM, Desai PB, Zimmer-Nechimias L, Wolfe B, Jakate AS, *et al*. Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. J Nutr 2003;133:1027-35.
12. Gutierrez MM, Riquelme Raya R, Campos Martinez AM, Lorite Garzon C, Strivens Vilchez H, Ruiz Rodriguez. Effect of soy beans and soy sauce on vasomotor symptoms during menopause. Rev Enferm 2006;29:16-22.
13. Crouse JR, Morgan T, Terry JG. A randomized trial comparing the effect of casein with that of soya protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999;159:2070-6.
14. Whitten C, Haddad E, Sabate J. Developing a vegetarian food guide pyramid: a conceptual framework. Vegetarian Nutr 1997;1:25-9.
15. Messina MJ, Wood CE. Soy isoflavones, estrogen therapy, and breast cancer risk: analysis and commentary. Nutr J 2008;7:17.
16. Julia RB. The science of soy: What do we really know? Environ Health Perspect 2006;114:1-15.
17. Ravindranath MH, Muthugounder S, Presser N. Anticancer therapeutic potential of soy isoflavone, genistein. Adv Exp Med Biol 2004;546:121-65.
18. Krenn L, Potsch V. An efficient HPLC method for the quantification of isoflavones in soy extracts and soy dietary supplements in routine quality control. Pharmazie 2006;61:582-5.
19. Davis SR, Murkies AL, Wilcox G. Phytoestrogens in clinical practice. Integr Med 1998;1:27-34.
20. Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. J Nutr 1999;129:758S-67S.
21. Amigo-Benavent M, Silván JM, Moreno FJ, Villamiel M, Del Castillo MD. Protein quality, antigenicity, and antioxidant activity of soy-based foodstuffs. J Agric Food Chem 2008;56:6498-505.
22. Wei H, Bowen R, Cal Q. Antioxidant and antipromotional effect of the soy bean isoflavone genistein. Proc Soc Exp Biol Med 1995;208:124-30.
23. Tikkanen MJ, Waahla K, Ojala S. Effect of soyabean phytoestrogen intake on low-density lipoprotein oxidation resistance. Proc Natl Acad Sci USA 1998;95:3106-10.
24. Liu J, Chang SK, Wiesenborn D. Antioxidant properties of soy bean isoflavone extract and tofu *in vitro* and in vivo. J Agric Food Chem 2005;53:2333-40.
25. Kampkötter A, Chovolou Y, Kulawik A, Röhrdanz E, Weber N, Proksch P, *et al*. Isoflavone daidzein possesses no antioxidant activities in cell-free assays but induces the antioxidant enzyme catalase. Nut Res 2008;28:620-8.
26. Kanazawa T, Osanai T, Zhang XS, Uemura T, Yin XZ, Onodera K, *et al*. Protective effects of soy protein on the peroxidizability of lipoproteins in cerebrovascular diseases. J Nutr 1995;125:639S-46S.
27. Kapiotis S, Hermann M, Held I. Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells damage by atherogenic LDL. Arterioscler Thromb Vasc Biol 1997;17:2868-74.
28. Tikkanen MJ, Wahala K, Ojala S. Effect of soy bean phytoestrogen intake on low-density lipoprotein oxidation resistance. Proc Natl Acad Sci USA 1998;95:3106-10.
29. Rüfer CE, Kulling SE. Antioxidant activity of isoflavones and their major metabolites using different *in vitro* assays. J Agric Food Chem 2006;54:2926-31.
30. Kulling SE, Honig DM, Metzler M. Oxidative metabolism of the soy isoflavones daidzein and genistein *in vitro* and in vivo. J Agric Food Chem 2001;49:3024-33.
31. Kulling KE, Honig DM, Simat, Metzler M. Oxidative *in vitro* metabolism of the soy phytoestrogens daidzein and genistein. J Agric Food Chem 2000;48:4963-72.
32. Wei YH, Kao SH, Lee HC. Simultaneous increase of mitochondrial DNA deletion and lipid peroxidation in human aging. Ann. N Y Acad Sci 1996;786:24-43.

33. Wolever TM, Jenkins DJ, Vuksan V, Jenkins AL, Buckley GC, Wong GS, *et al*. Beneficial effect of low-glycemic index diet in overweight NIDDM subjects. Diabetes Care 1992;15:562-4.
34. Foster-Powell K, Miller JB. International tables of glycemic index. Am J Clin Nutr 1995;62:871S-90S.
35. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 2002;76:274S-80S.
36. Kang MJ, Kim JI, Yoon SY, Kim JC, Cha IJ. Pinitol from Soy beans Reduces Postprandial Blood Glucose in Patients with Type 2 Diabetes Mellitus. J Med Food 2006;9:182-6.
37. Villegas R, Gao YT, Yang G, Li HL, Elasy TA, Zheng W, *et al*. Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health study. Am J Clin Nutr 2008;87:162-7.
38. Lee SH, Park HJ, Chun HK, Cho SY, Cho SM, Hyun SL. Dietary phytic acid lowers the blood glucose level in diabetic KK mice. Nut Res 2006;26:474-9.
39. Venn BJ, Mann JI. Cereal grains, legumes and diabetes. Eur J Clin Nutr 2004;58:1443-61.
40. Zhou W, Fan ZH, Cao Z, Liu F. Health care function of anti nutritional functions in legumes. J Agri Sci Tech 2007;9:61-5.
41. Salmerón J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willet WC. Dietary fiber glycemic load, and risk of non-insulin dependent diabetes mellitus in women. JAMA 1997;277:472-7.
42. Nuttall FQ. Dietary fiber in the management of diabetes. Diabetes 1993;42:503-8.
43. Slavin J. Nutritional benefits of soy protein and soy fiber. J Am Diet Assoc 1991;91:816-9.
44. Hoie LH, Guldstrand M, Sjoholm A, Graubaum HJ, Gruenwald J, Zunft HJ, *et al*. Cholesterol-lowering effects of a new isolated soy protein with high levels of non denaturized protein in hypercholesterolemic patients. Adv Ther 2007;24:439-47.
45. Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. Soy Isoflavones Exert Antidiabetic and Hypolipidemic Effects through the PPAR Pathways in Obese Zucker Rats and Murine RAW 264.7 Cells. J Nutr 2003;133:1238-43.
46. Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life Sci 2006;79:1578-84.
47. Wagner JD, Zhang L, Shadoan MK, Kavanagh K, Chen H, Tresnasari K, *et al*. Effects of soy protein and isoflavones on insulin resistance and adiponectin in male monkeys. Metabolism 2008;57:S24-31.
48. Rosamond W, Flegal K, Friday G. Heart disease and stroke statistics 2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2006;113:e69-171
49. Manson JE, Rimm EB, Colditz GA. The primary prevention of myocardial infarction. N Eng J Med 1992;326:1406-16.
50. Reynolds K, Chin A, Lees KA. A meta-analysis of the effect of soy protein supplementation on serum lipids. Am J Cardiol 2006;98:633-40.
51. Carroll KK. Review of clinical studies on cholesterol-lowering response to soy protein. J Am Diet Assoc 1991;91:820-7.
52. Bakhit RM, Klein BP, Essex-Sorlie D. Intake of 25g of soyabean protein with or without soy bean fiber alters plasma lipids in men with elevated cholesterol concentrations. J Nutr 1994;124: 213-22.
53. Potter SM, Bakhit RM, Klein BP. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. Am J Clin Nutr 1993;58:501-6.
54. Anthony MS, Clarkson TB, Hughes CL. Soy bean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996;126:43-50.
55. Mensink RP, Zock PL, Kester AD. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77:1146-55.
56. Clair RS, Anthony M. Soy, isoflavones and atherosclerosis. Handb Exp Pharmacol 2005;170:301-23.
57. Torres N, Torre-Villalvazo I, Tovar AR. Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. J Nutr Biochem 2006;17:365-73.
58. Merrill RM, Capocaccia R, Feuer EJ, Mariotto A. Cancer prevalence estimates based on tumour registry data in the surveillance, epidemiology, and end results (seer) program. Int J Epidemiol 2000;29:197-207.
59. Messina M, Barnes S. The role of soy products in reducing risk of cancer. J Natl Cancer Inst 1991;83:541-6.
60. Messina M, Messina V, Setchell K. The simple soya beans your health. New York: Avery Publishing Group; 1994.
61. Bouker KB, Hilakivi-Clarke L. Genistein: does it prevent or promote breast cancer? Environ Health Perspect 2000;108: 701-8.
62. Merrill RM, Capocaccia R, Feuer EJ, Mariotto A. Prepubertal exposure to zearalenone or genistein reduces mammary tumorigenesis. Br J Cancer 1999;80:1682-988.
63. Santell RC, Kieu N, Helferich WG. Genistein Inhibits Growth of Estrogen-Independent Human Breast Cancer Cells in Culture but Not in Athymic Mice. J Nutr 2000;130:1665-9.
64. Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, Elgavish A. Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate. J Nutr 2002;132:552S-8.
65. Pei RJ, Sato M, Yuri T. Effect of prenatal and prepubertal genistein exposure on N-methyl-N-nitrosourea-induced mammary tumorigenesis in female Sprague-Dawley rats. In Vivo 2003;17:349-537.
66. Pereira MA, Barnes, LH, Rassman VL. Use of azoxymethane-induced foci of abstract crypts in rat colon to identify potential cancer chemopreventive agents. Carcinogenesis 1994;15: 1049-54.
67. Lo FH, Mak NK, Leung KN. Studies on the anti-tumor activities of the soy isoflavone daidzein on murine neuroblastoma cells. Biomed Pharmacother 2007;61:591-5.
68. Zava DT, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells *in vitro*. Nutr Cancer 1997;27:31-40.
69. Akiyama T, Ishida J, Nakagawa S. Genistein, a specific inhibitor of tyrosine-specific protein kinase. J Biol Chem 1987;262: 5592-5.
70. Peterson G. Evaluation of the biochemical targets of genistein in tumor cells. J Nutr 1995;125:3:784S-9.
71. Fotsis T, Pepper MS, Aktas E, Breit S, Rasku S, Adlercreutz H, *et al*. Flavonoids, dietary-derived inhibitors of cell proliferation and *in vitro* angiogenesis. Cancer Res 1997;57:2916-21.
72. Wel H. Inhibition of tumor promoter-induced hydrogen peroxide formation *in vitro* and in vivo by genistein. Nutr Cancer 1993;20:1-12.
73. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. J Natl Cancer Inst 2006;98:459-71.
74. Levine JP. Effective strategies to identify postmenopausal women at risk for osteoporosis. Geriatrics 2007;62:22-30.
75. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. N Engl J Med 2007;357:905-16.

76. Bitto A, Burnett BP, Polito F. Effects of genistein aglycone in osteoporotic, ovariectomized rats: a comparison with alendronate, raloxifene and oestradiol. Br J Pharmacol 2008;155:896-905.
77. Ma DF, Qin LQ, Wang PY. Soy isoflavone intake increases bone mineral density in the spine of menopausal women: meta-analysis of randomized controlled trials. Clin Nutr 2008;27: 57-64.
78. Chen WF, Wong MS. Genistein modulates the effects of parathyroid hormone in human osteoblastic SaOS-2 cells. Br J Nutr 2006;95:1039-47.
79. Williams JP, Jordan SE, Barnes S, Blair HC. Tyrosine kinase inhibitor effects on avian osteoclastic acid transport. Am J Clin Nutr 1998;68:1369S-74S.
80. Atmaca A, Kleerekoper M, Bayraktar M, Kucuk O. Soy isoflavones in the management of postmenopausal osteoporosis: mechanism of action of soy isoflavones Menopause 2008;15:748-57.
81. Cooke GM. A review of the animal models used to investigate the health benefits of soy isoflavones. J AOAC Int 2006;89:1215-27.

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