Health Promotion Effects of Soy Isoflavones

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Summary Soybeans contain several physiologically active ingredients, such as soy phytosterol, soyasaponin, soy protein, and lecithin, and are therefore expected to express the functionalities of said ingredients. Among them, soy isoflavones have been studied in recent years for their various functions, including their obesity-preventing effect, blood glucose level reducing effect, osteoporosis and breast cancer risk reduction, and anti-oxidative effect, and several health promoting effects and disease preventing effects are expected. For example, it has been determined that soy isoflavones reduce body and fat weight in experiments in which mice were fed a diet containing soy isoflavones in studies on anti-obesity. Epidemiologic studies with humans have also shown that women who consume more soybeans have lower BMI than those who consume less. We previously found that soy isoflavones may have anti-obesity effects in myoblasts through the activation of transcriptional coactivator PGC-1β, which increases energy expenditure. In recent studies, a decrease in blood glucose level due to soy isoflavone was seen in an experiment in which diabetic model mice were fed a diet containing soy isoflavone. It has also been suggested that soy isoflavone intake may increase bone mineral density in postmenopausal women and reduce the risk of breast cancer. This review focuses on the actions of soy isoflavones known to date, including their anti-obesity and anti-diabetic effects, bone loss preventing effects, and cancer risk reduction effects, and introduces reports on the health promotion and disease prevention effects of soy isoflavones.

Key Words isoflavone, obesity, diabetes, antioxidant, osteoporosis, breast cancer, skeletal muscle

Soy Isoflavones

Isoflavones have structures similar to that of the female hormone estrogen (Fig. 1) and show a weak female hormone-like action by binding to the estrogen receptor (8)—that is why they are called “phytoestrogens.” The isoflavones contained in soybeans are mainly daidzein and genistein, and approximately 95% or more are contained in the form of glycosides (genistin and daidzin) (9). Genistin and daidzin are hydrolyzed into aglycones (genistein and daidzein) by β-glucosidase of intestinal bacteria and then absorbed. Some aglycones are metabolized further by intestinal bacteria and absorbed into the body. It is important for glycoside isoflavones to become aglycones in order to efficiently absorb glycoside isoflavones.

It has also been suggested that the effects of soy isoflavones on humans depend on the individual isoflavone metabolic capacity. Equol, is a metabolite of daidzein which is metabolized by intestinal bacteria and has a high estrogen activity. Bacterial strains capable of producing equol from daidzein have been isolated, and their related enzymes and metabolic pathways have been reported (Fig. 2) (10). Equol producers contain the equol-producing bacteria in the intestines. However, an epidemiological study reported that only about 50% of Japanese women can produce equol (11). Furthermore, other epidemiological studies have suggested that equol-producing ability may be affected by dietary habits. There have been reports that equol production...
increases when energy intake from carbohydrates is high (12) and that dietary fiber intake also increases equol production (13). Fujii et al. determined that the ingestion of cellooligosaccharides, which are indigestible oligosaccharides, increases equol production. Also the combination of isoflavones and cellooligosaccharides ameliorates the reduction of bone mass and strength in ovariectomized (OVX) postmenopausal osteoporosis model mice (14). On the other hand, it has been reported that equol production capacity decreases when energy intake from lipids is high (12). It was also shown that kanamycin administration reduced the ability to produce equol and inhibited the protective effect of daidzein against bone loss due to OVX in a study with OVX mice where the antibiotic kanamycin and daidzein were ingested in combination (15).

The reported amounts of soy isoflavones contained in food are 154.53 mg/100 g for raw soybeans, 41.45 mg/100 g for miso (fermented soybean paste), 82.29 mg/100 g for natto (fermented soybeans), and 13.1 to 34.78 mg/100 g for tofu (soybean curd) (16). Most soy isoflavones exist as glycosides in foods, but soybean fermented foods such as miso contain large amounts of isoflavone aglycones (e.g., approximately 90% in miso). Furthermore, the daily intake of soy isoflavones in each region is 0.73 to 3.3 mg in the United States, 0.37 to 4.5 mg in Europe, 6.2 to 75.7 mg in China, 22.6 to 54.3 mg in Japan, and 14.88 mg in South Korea (16), which is an indication that soy isoflavone consumption is high in Asian countries.

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**Fig. 1.** Chemical structures of genistein, daidzein, and an estrogen (17β-estradiol). Genistein and daidzein are the main soy isoflavones. They are similar in structure to the female hormone 17β-estradiol and weakly bind to estrogen receptor.

**Fig. 2.** Equol biosynthetic pathway from daidzein by intestinal bacteria. Daidzein is metabolized to (R)-dihydrodaidzein by daidzein reductase. (R)-dihydrodaidzein is metabolized to cis-tetrahydrodaidzein by dihydrodaidzein reductase or to (S)-dihydrodaidzein by dihydrodaidzein rasemase. (S)-dihydrodaidzein is metabolized to trans-tetrahydrodaidzein by dihydrodaidzein reductase and further metabolized to (S)-equol by tetrahydrodaidzein reductase. Tetrahydrodaidzein reductase metabolizes trans-tetrahydrodaidzein to (S)-equol and cis-tetrahydrodaidzein to (R)-dihydrodaidzein, but not trans-tetrahydrodaidzein to (S)-dihydrodaidzein.
Anti-Obesity Effect

One of the expected effects of soy isoflavone is its anti-obesity effect. A decrease in body weight and blood cholesterol level was observed when a diet containing daidzein (50 mg/kg, 100 mg/kg) was fed to mice fed a high-fat diet for 6 wk that were showing obesity (17). It has been reported that body weight, visceral fat weight, and visceral adipocyte area decreased, and inhibition of visceral fat synthesis and promotion of lipid hydrolysis were observed when high-fat diets containing soy isoflavone (150 mg/kg and 450 mg/kg) were given to high-fat diet-induced obesity rats (18). An epidemiological study in humans also determined that adult women (>20 y of age) who consumed more (>2 servings/wk) soy had lower BMI compared to women who consumed less (0 or <1 servings/wk) soy (19).

We previously investigated the anti-obesity effect of soy isoflavones using cultured cells. As a result, genistein and daidzein activated transcriptional coactivator PGC-1α, which is highly homologous to transcriptional coactivator PGC-1β and acts in the promotion of mitochondrial biogenesis and fatty acid oxidation. PGC-1α is deacetylated by deacetylase SIRT1, and its transcriptional activity increases (24). It has been reported that genistein and daidzein increase SIRT1 and deacetylase PGC-1α, and promote mitochondrial biosynthesis (increase the protein level of ATP synthase β and ND6 (NADH Dehydrogenase Subunit 6)) (25). Therefore, genistein and daidzein may act as anti-obesity agents through SIRT1/PGC-1α.

These studies suggest that soy isoflavones may be effective in preventing and improving obesity.

Prevention or Improvement Effects on Diabetes

Blood glucose control effect

Control of blood glucose level has also been reported as one of the many functions of soy isoflavones. When a soy isoflavone-containing diet (334 mg/kg) was fed to a type 1 diabetic model (streptozotocin (STZ)-induced and insulin-dependent diabetes mellitus) rat, blood glucose level decreased and plasma insulin level increased (26). In a similar experiment, a soy isoflavone diet (600 mg/kg) increased the level of glucokinase, which is a glycolytic enzyme, and decreased the level of glucose-6-phosphatase, which is an enzyme involved in gluconeogenesis (27). Moreover, in an experiment in which genistein (200 mg/kg) or daidzein (200 mg/kg) was administered to type 1 diabetes model mice, they reduced the activity of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, which are liver enzymes involved in gluconeogenesis, in addition to the decrease in blood glucose level (28). A study using ob/ob mice, which are type 2 diabetes and obesity model mice, reported that weight loss was observed when a genistein-containing diet (600 mg/kg) was administered, and serum glucose levels were reduced by 18% in females and 43% in males (29).

Studies have also been conducted to investigate how soy isoflavones work in lowering blood glucose levels. An experiment using pancreatic β cells (INS-1) showed that adding genistein activated adenylate cyclase increased intracellular cAMP accumulation, activated protein kinase A (PKA), and increased secreting insulin (30). In another study, the addition of genistein also increased cAMP accumulation, activated PKA and ERK1/2, and increased INS-1 cell proliferation (31). Thus, it has been suggested that genistein may activate the cAMP/PKA signaling pathway. Horiuchi et al. suggest that the addition of equol also increases the amount of cAMP in INS-1 cells, activates PKA signaling, and increases INS-1 cell survival (32).

Anti-oxidative effect

Antioxidant effects are thought to be involved in reducing the risk of diabetes. Soy isoflavones have been reported to have antioxidant effects. In intestinal epithelial cells (Caco-2), genistein and daidzein increased gene expression of metallothionein, which acts to eliminate reactive oxygen species (33). Daidzein also induced catalase activity in Caco-2 cells (34).
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Hepatic superoxide dismutase (SOD), catalase, and glutathione peroxidase activities were significantly reduced in STZ-induced diabetic rats compared to control rats. However, feeding genistein to STZ-induced diabetic rats significantly increased these enzymatic activities (27). In an experiment in which exercised rats were fed a diet containing soy isoflavones (a mixture of genistein, daidzein, glycitein, and their glycosides), it was determined that soy isoflavones reduced exercise-induced increases in thiobarbituric acid-reactive substances (i.e., increased oxidative stress) (35). They also increased the activities of the hepatic antioxidant enzymes SOD and catalase (35). To date, studies have been conducted on the mechanism by which soy isoflavones act on antioxidants (36), but the entire mechanism has not yet been clarified.

Such research results support that soy isoflavones have the possibility of reducing blood glucose levels and have preventive or improvement effects for diabetes in diabetic model animals. Further elucidation of the mechanisms through which soy isoflavones show anti-diabetic effects is expected in the future.

Bone Loss Inhibitory Effect

Osteoporosis is a disease that reduces bone mineral density and weakens the bones, which leads them to fracture more easily. This is caused by a decrease in estrogen secretion due to aging and a decrease in the ability to absorb minerals in the intestinal tract. Previous studies have suggested that soy isoflavones may be effective in improving osteoporosis. A meta-analysis including a total of 6 studies in postmenopausal women with low bone mass reported that lumbar spine bone mineral density was significantly higher in the group that took an average of 65 mg per day of soy isoflavones for 6 mo to 1 y, compared to the placebo group (37). In another meta-analysis including a total of 19 studies, daily intake of soy isoflavones for 1 mo to 2 y significantly increased bone mineral density and decreased urinary deoxypyridinoline, which is a bone resorption marker (38). Serum osteocalcin, a biomarker for bone formation, increased and mean urinary N-telopeptide (a degradation product of bone and the bone resorption index) decreased in a study including 42 postmenopausal women, with intake of food containing approximately 60 mg of isoflavones daily for 12 wk (39).

In an experiment using OVX mice, OVX-induced reduction of femoral bone mass and density was prevented in mice fed 0.7 mg/d of genistein for 4 wk, which is an effect similar to that in mice fed 17β-estradiol at 0.01 μg/d (40). In other words, it has been suggested that genistein has an estrogen-like effect on bones and may prevent bone loss caused due to estrogen deficiency in women.

Based on the results of these studies, soy isoflavones are believed to be effective for inhibiting bone loss and preventing and improving osteoporosis.

Cancer Risk Reduction Effect

Breast cancer risk reduction effect

Soy isoflavones are expected to have an effect on preventing breast cancer through their anti-estrogen action, because their structures resemble a female hormone. In an epidemiological study which targeted Japanese women, Yamamoto et al. investigated whether there is a relationship between the intake of miso soup, soybean foods, and estimated isoflavones and the risk of breast cancer, and found a significant inverse correlation between breast cancer risk and miso soup consumption or estimated isoflavone consumption (41).

On the other hand, soy isoflavone intake was inversely associated with risk of breast cancer incidence only in the targeted Asian population with high soybean consumption, but not in the Western population with low consumption in a study that included a meta-analysis to examine the relationship between soy isoflavone consumption and the risk of developing breast cancer (42). The above suggests that soy isoflavone intake is associated with a significantly reduced risk of developing breast cancer among Asian populations, but not in Western populations. Further research is needed to clarify whether soy consumption reduces the risk of developing breast cancer.

Potential anti-cancer effect

The inhibitory action of tyrosine kinase. Tyrosine kinase is an enzyme that specifically phosphorylates tyrosine residues of proteins and is involved in signal transduction associated with cell differentiation and proliferation. It is classified into a receptor type on the cell membrane surface and a non-receptor type within the cell. When tyrosine kinase is activated, it specifically phosphorylates itself or the target protein. Several signaling factors that recognize this phosphorylation site bind to the receptor and signal transduction is initiated through autophosphorylation. Phosphorylation of target proteins also activates various intracellular proteins consecutively, resulting in signal transduction.

Genistein has been reported to inhibit tyrosine kinase activity. The tyrosine kinase activity of the receptor tyrosine kinase epidermal growth factor receptor and the non-receptor tyrosine kinases pp60v-src and pp110gag-fes were inhibited by genistein in vitro (43). Soy isoflavones are expected to have anti-cancer effects due to their tyrosine kinase inhibitory action since tyrosine kinase is also involved in the promotion of cancer growth.

Topoisomerase II inhibitory action. It has also been reported that soy isoflavones inhibit topoisomerase II activity. DNA topoisomerase II is an enzyme that alters the helical shape and entanglement of DNA by cleaving and recombining double-stranded DNA. It has been reported that genistein inhibits the action of this DNA topoisomerase II in experiments using cultured cells (44). It is considered that this action is related to anti-cancer activity since inhibition of topoisomerase II in cancer cells prevents them from dividing normally and induces apoptosis.

On the other hand, soy isoflavones were reported to
induce cleavage of the MLL (myeloid-lymphoid leukemia) gene by inhibiting topoisomerase II activity in an experiment with cultured cells. This suggests that soy isoflavones may cause abnormalities (e.g., translocation or rearrangement) in the MLL gene. MLL gene rearrangement is also induced by the anti-cancer drugs, VP16 and doxorubicin, which have an inhibitory effect on topoisomerase II. It is said that MLL gene abnormality is involved in many cases of acute myelogenous leukemia and acute lymphocytic leukemia, and there is concern about the effects on the fetus due to exposure to substances with topoisomerase II inhibitory action during pregnancy.

**Conclusion**

As mentioned here, it has been suggested that soy isoflavones are effective in the prevention and treatment of several diseases, including the prevention and improvement of obesity and diabetes, and osteoporosis and breast cancer in menopausal women. It is also believed that the effects of these soy isoflavones are mainly attributable to their estrogen-like structure, but their estrogenic effects raise concerns about the side effects of overdose. Further basic research and clinical trials are expected to evaluate whether ingestion of soy isoflavones can truly provide the beneficial effects described here, and to eventually clarify how much to ingest and how the mechanisms works.

**Authorship**

SN, MF, and YK performed collection of references, interpretation of the data, and writing of the manuscript.

**Disclosure of state of COI**

No conflicts of interest to be declared.

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