Table 2. Subgroup analyses of the effects of soy isoflavones on spine BMD[†]

Variables	No. of trials	Sample size	*p* for heterogeneity	Fixed effect model			Random effects model	
				WMD (95% CI)	*p*-value	*p*-value (diff)	WMD (95% CI)	*p*-value
Intervention duration								
6 months	6[16-18, 21, 27, 29]	522	< 0.00001	17.72 (14.03, 21.41) mg/cm^2	< 0.00001	= 0.0002	18.74 (1.25, 36.23) mg/cm^2	0.04
			< 0.00001	1.81 (1.40, 2.21) %	< 0.00001	= 0.03	2.31 (0.16, 4.47) %	0.04
1 year	5[8, 22-25]	718	< 0.00001	8.74 (5.90, 11.58) mg/cm^2	< 0.00001		22.64 (1.54, 43.74) mg/cm^2	0.04
			< 0.00001	1.23 (0.88, 1.58) %	< 0.00001		2.52 (0.17, 4.87) %	0.04
Isoflavone dose								
≤ 75 mg/d	6[8, 22, 23, 25, 27, 29]	818	< 0.00001	11.70 (9.10, 14.30) mg/cm^2	< 0.00001	= 0.57	20.79 (1.48, 40.09) mg/cm^2	0.03
			< 0.00001	1.53 (1.20, 1.85) %	< 0.00001	= 0.59	2.59 (0.26, 4.92) %	0.03
> 75 mg/d	5[16-18, 21, 24]	422	< 0.00001	13.21 (8.73, 17.69) mg/cm^2	< 0.00001		19.49 (2.64, 36.34) mg/cm^2	0.02
			< 0.00001	1.37 (0.91, 1.83) %	< 0.00001		2.10 (0.31, 3.90) %	0.02
Region of participants								
Asian	7[17, 18, 22-24, 27, 29]	535	< 0.00001	9.01 (6.44, 11.59) mg/cm^2	< 0.00001	< 0.00001	15.06 (0.89, 29.23) mg/cm^2	0.04
			< 0.00001	1.17 (0.86, 1.49) %	< 0.00001	= 0.0006	1.85 (0.16, 3.54) %	0.03
Western	5[8, 16, 21, 25]	705	< 0.00001	21.97 (17.34, 26.60) mg/cm^2	< 0.00001		31.46 (0.56, 62.37) mg/cm^2	0.05
			< 0.00001	2.20 (1.71, 2.68) %	< 0.00001		3.56 (0.13, 6.99) %	0.04
Basal spine BMD								
Normal bone mass	3[18, 21, 24]	319	< 0.00001	12.31 (7.42, 17.20) mg/cm^2	< 0.00001	= 0.92	17.06 (−7.55, 41.66) mg/cm^2	0.17
			< 0.00001	1.27 (0.78, 1.76) %	< 0.00001	= 0.33	1.78 (−0.74, 4.29) %	0.17
Osteopinia or osteoporosis	8[8, 16, 17, 22, 23, 25, 27, 29]	921	< 0.00001	12.02 (9.48, 14.55) mg/cm^2	< 0.00001		21.70 (5.43, 37.97) mg/cm^2	0.009
			< 0.00001	1.56 (1.24, 1,87) %	< 0.00001		2.64 (0.69, 4.60) %	0.008

[†]BMD, bone mineral density; WMD, weighted mean difference; *p*-value, test for overall effect of each subgroup; *p*-value (diff), test for subgroup differences.

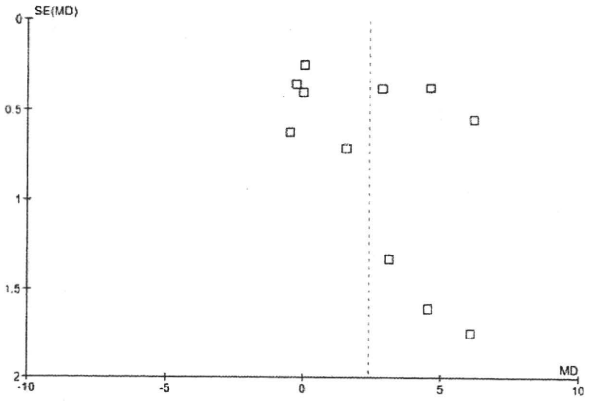

Figure 3. Funnel plots of effects of soy isoflavones on spine BMD (%). MD, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; SE (MD), standard error of MD; fixed, fixed effect model.

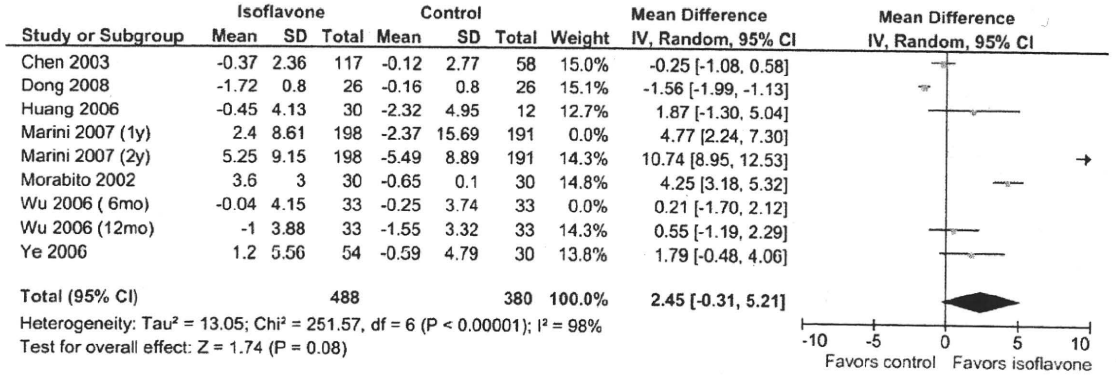

Figure 4. Effects of soy isoflavones on femoral neck BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of femoral neck bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

baseline and follow-up values to be 0.75 and 0.5 and using data set of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on trochanter BMD.

DISCUSSION

The present meta-analysis found that ingestion of about 82 mg of extracted soy isoflavones (in the aglycone form) per day for 6 months to 1 year significantly increased lumbar spine BMD by 2.38% compared with controls without isoflavones, in menopausal women. Results of sensitivity analyses indicated that the effect of soy isoflavone extracts in increasing lumbar spine BMD was robust. This magnitude of beneficial effect of soy isoflavones appears to almost completely offset naturally occurring postmenopausal bone loss. Effect of soy isoflavones in increasing femoral neck BMD seems to take more time than spine BMD. Our meta-analysis did not reveal significant effects on total hip and trochanter BMD, which might be due to the limited number of five trials.

An intake of 82 mg soy isoflavones/day (in the aglycone form) is approximately equivalent to 1.7 times the amount consumed habitually in Japan (mean: 47.2 mg/day).[40] The mechanism mediating the improvement of BMD at these skeletal sites by soy isoflavones is not well understood, but it may be a result of their chemical and biological similarity to mammalian estrogens, which are known to increase BMD in menopausal women.[1,4]

Results of subgroup analyses indicated that the varying effects of soy isoflavone extracts on spine BMD across the 11 trials were associated with study characteristics of intervention duration, region of participants, and basal BMD. The heterogeneity of effects of soy isoflavones on spine BMD across the 11 trials might also be induced by differences in habitual dietary intake of soy isoflavones,[28] time since menopause,[3] intervention duration,[25] isoflavone dosage,[17,41] chemical forms and proportions of individual soy isoflavones,[42-44] and participants' ethnicity. Isoflavone glycosides are not absorbed intact across the enterocytes of healthy adults, and their bioavailability requires initial hydrolysis by intestinal β-glucosidases for uptake into the peripheral circulation.[44] Asian and Western populations are reported to have differences in the capacity of intestinal flora to convert daidzein to its metabolite, equol.[45] Equol is easily absorbed and possesses substantial estrogenic activity because of its affinity for both the estrogen α and β receptors.[43] Equol is suggested to be the single most important factor that influences the clinical efficacy of soy isoflavones in preventing bone

loss.[46] Because of the limited number of trials and insufficient data available, our meta-analysis was also unable to evaluate possible influences on the varying effects of soy isoflavones on spine BMD across trials of dietary intake of soy isoflavones, time since menopause, chemical forms and proportions of individual soy isoflavones, blood isoflavone concentration, urinary isoflavone excretion, and equol producer status.

Since there was significant heterogeneity in effects of soy isoflavones on spine BMD, we preferably presented the results by incorporating heterogeneity into the random effects model in this meta-analysis. A random effects meta-analysis model involves an assumption that the effects being estimated in the different studies are not identical, but follow some distribution. The model represents our lack of knowledge about why real, or apparent, treatment effects differ by considering the differences as if they were random.[19]

The magnitude of effect of soy isoflavone extracts in increasing spine BMD by 20.25 mg/cm^2 revealed in our present meta-analysis, were consistent with the results (by 20.6 mg/cm^2) from the previous meta-analysis that included 10 RCTs testing both extracted soy isoflavones and isolated soy protein containing isoflavones.[15] Thus, soy isoflavones ingested either alone in extracted form or as constituent part of isolated soy protein have been demonstrated to exert a mild but significant effect in increasing lumbar spine BMD in menopausal women. Our meta-analysis also revealed that ingestion of soy isoflavones for 6 months appears to be enough to exert beneficial effect on spine BMD in menopausal women. The present meta-analysis did not reveal influences of isoflavone dosage on the effect on spine BMD, possibly due to the fact that trials tested various forms and compositions of soy isoflavones likely possessing different bioavailability and effects on bone mass; other explanations might be the limited number of trials or of some other factors inducing the heterogeneity.

CONCLUSION

The effect of soy isoflavones in increasing spine BMD in menopausal women are not as strong as those of approved pharmacologic therapies involving estrogen or bisphosphonates.[1,4,47,48] However, the present meta-analysis revealed that soy isoflavone extract supplements did result in a significant improvement of lumbar spine BMD with good tolerance and no induction of notable adverse events. Our meta-analysis suggested that soy isoflavone supplements can be used not only to offset the bone loss that occurs naturally in women after menopause, but are also applicable for complementary or alternative use in patients with postmenopausal osteopenia or osteoporosis who are unable to tolerate the side effects of estrogen or/and bisphosphonate therapies. Furthers studies are needed to address factors affecting the magnitudes of the effect of soy isoflavones on spine BMD and to verify the effect on hip BMD.

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AUTHOR DISCLOSURES

Kyoko Taku, Melissa K. Melby, Jun Takebayashi, Shoichi Mizuno, Yoshiko Ishimi, Toyonori Omori and Shaw Watanabe, disclose no conflicts of interest.

REFERENCES

1. Nielsen TF, Ravn P, Bagger YZ, Warming L, Christiansen C. Pulsed estrogen therapy in prevention of postmenopausal osteoporosis. A 2-year randomized, double blind, placebo-controlled study. Osteoporos Int. 2004;15:168-74.
2. Seeman E. Estrogen, androgen, and the pathogenesis of bone fragility in women and men. Curr Osteoporos Rep. 2004;2:90-6.
3. Pouilles JM, Tremollieres F, Ribot C. Effect of menopause on femoral and vertebral bone loss. J Bone Miner Res. 1995; 10:1531-6.
4. Bone HG, Greenspan SL, McKeever C, Bell N, Davidson M, Downs RW et al. Alendronate and estrogen effects in postmenopausal women with low bone mineral density. Alendronate/Estrogen Study Group. J Clin Endocrinol Metab. 2000;85:720-6.
5. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003;362:419-27.
6. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet. 1997;350:1047-59.
7. Anderson GL, Judd HL, Kaunitz AM, Barad DH, Beresford SA, Pettinger M, Liu J, McNeeley SG, Lopez AM. Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. JAMA. 2003;290:1739-48.
8. Morabito N, Crisafulli A, Vergara C, Gaudio A, Lasco A, Frisina N et al. Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. J Bone Miner Res. 2002;17:1904-12.
9. Salamone LM, Pressman AR, Seeley DG, Cauley JA. Estrogen replacement therapy. A survey of older women's attitudes. Arch Intern Med. 1996;156:1293-7.
10. Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med. 1997;29:95-120.
11. Somekawa Y, Chiguchi M, Ishibashi T, Aso T. Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. Obstet Gynecol. 2001;97:109-15.
12. Zhang X, Shu XO, Li H, Yang G, Li Q, Gao YT, Zheng W. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. Arch Intern Med. 2005;165:1890-5.
13. Messina MJ. Soy foods and soybean isoflavones and menopausal health. Nutr Clin Care. 2002;5:272-82.
14. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998;139:4252-63.
15. Ma DF, Qin LQ, Wang PY, Katoh R. 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.
16. Harkness LS, Fiedler K, Sehgal AR, Oravec D, Lerner E. Decreased bone resorption with soy isoflavone supplementation in postmenopausal women. J Womens Health (Larchmt). 2004;13:1000-7.
17. Ye YB, Tang XY, Verbruggen MA, Su YX. Soy isoflavones attenuate bone loss in early postmenopausal Chinese women:

a single-blind randomized, placebo-controlled trial. Eur J Nutr. 2006;45:327-34.
18. Gao YP, Li RH, Guo SZ. The effect of soy isoflavone on bone metabolism in postmenopausal women. J Shanxi Med Univ. 2006;37:633-5. (in Chinese)
19. The Cochrane Collaboration [Internet]. Cochrane Handbook for Systematic Reviews of Interventions Version 4.2.6. September 2006 [cited 2009/05/29]; Available from: http://www.cochrane.org/resources/handbook/Handbook4.2.6Sep2006.pdf
20. Liu J, Ho SC, Su YX, Chen WQ, Zhang CX, Chen YM. Effect of long-term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized controlled trials. Bone. 2009;44:948-53.
21. Brink E, Coxam V, Robins S, Wahala K, Cassidy A, Branca F. Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. Am J Clin Nutr. 2008;87:761-70.
22. Chen YM, Ho SC, Lam SS, Ho SS, Woo JL. Soy isoflavones have a favorable effect on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized, controlled trial. J Clin Endocrinol Metab. 2003;88:4740-7.
23. Dong J, Zhen WH, Piao JH, Li F, Zeng J, Gong J, Yang XG. Relationship between estrogen receptor gene Px haplotype and the effect of calcium and soy isoflavone supplementation on bone mineral density of Chinese postmenopausal women. Chin J Prev Med. 2008;42:329-34. (in Chinese)
24. Huang HY, Yang HP, Yang HT, Yang TC, Shieh MJ, Huang SY. One-year soy isoflavone supplementation prevents early postmenopausal bone loss but without a dose-dependent effect. J Nutr Biochem. 2006;17:509-17.
25. Marini H, Minutoli L, Polito F, Bitto A, Altavilla D, Atteritano M et al. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. Ann Intern Med. 2007;146:839-47.
26. Uesugi T, Toda T, Okuhira T, Chen JT. Evidence of estrogenic effect by the three-month-intervention of isoflavone on vaginal maturation and bone metabolism in early postmenopausal women. Endocr J. 2003;50:613-9.
27. Wu J, Oka J, Higuchi M, Tabata I, Toda T, Fujioka M et al. Cooperative effects of isoflavones and exercise on bone and lipid metabolism in postmenopausal Japanese women: a randomized placebo-controlled trial. Metabolism. 2006;55:423-33.
28. Wu J, Oka J, Tabata I, Higuchi M, Toda T, Fuku N et al. Effects of isoflavone and exercise on BMD and fat mass in postmenopausal Japanese women: a 1-year randomized placebo-controlled trial. J Bone Miner Res. 2006;21:780-9.
29. Xin Y, Yang HY. Influence of daidzein tablets on climacteri syndrome and bone mineral density of women. Chin J Osteoporos. 2006;12:149-51. (in Chinese)
30. Nelson HD, Vesco KK, Haney E, Fu R, Nedrow A, Miller J, Nicolaidis C, Walker M, Humphrey L. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA. 2006;295:2057-71.
31. Linde K, Jonas WB, Melchart D, Willich S. The methodological quality of randomized controlled trials of homeopathy, herbal medicines and acupuncture. Int J Epidemiol. 2001;30:526-31.
32. Balk E, Chung M, Chew P, Ip S, Raman G, Kupelnick B et al. Effects of Soy on Health Outcomes: Evidence Report/Technology Assessment No. 126. Rockville, MD: Agency for Healthcare Research and Quality; 2005. AHRQ Publication No. 05-E024-2.
33. Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA. 1995;273:408-12.
34. Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.0.1 [updated September 2008]. The Cochrane Collaboration, 2008. Available from www.cochrane-handbook.org.
35. Taku K, Umegaki K, Sato Y, Taki Y, Endoh K, Watanabe S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. Am J Clin Nutr. 2007;85:1148-56.
36. Marini H, Minutoli L, Polito F, Bitto A, Altavilla D, Atteritano M et al. OPG and sRANKL serum concentrations in osteopenic, postmenopausal women after 2-year genistein administration. J Bone Miner Res. 2008;23:715-20.
37. Huang ZW, Dong J, Gong J, Zeng J, Li WD, Yang XG. Changes of bone mineral density after one-year nutrition supplement in postmenopausal Chinese women. Chinese Journal of Health Education. 2007;23:426-7. (in Chinese)
38. Ye YB, Su YX. The attenuating effect of soy isoflavones extract on bone loss in postmenopausal women. Ying Yang Xue Bao. 2004;26:49-53. (in Chinese)
39. Grinspoon S, Thomas E, Pitts S, Gross E, Mickley D, Miller K, Herzog D, Klibanski A. Prevalence and predictive factors for regional osteopenia in women with anorexia nervosa. Ann Intern Med. 2000;133:790-4.
40. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr. 2000;130:2243-50.
41. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW, Jr. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr. 1998;68:1375S-9S.
42. Morito K, Aomori T, Hirose T, Kinjo J, Hasegawa J, Ogawa S, Inoue S, Muramatsu M, Masamune Y. Interaction of phytoestrogens with estrogen receptors alpha and beta (II). Biol Pharm Bull. 2002;25:48-52.
43. Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T, Ogawa S, Inoue S, Muramatsu M, Masamune Y. Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol Pharm Bull. 2001;24:351-6.
44. Setchell KD, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, Kirschner AS, Heubi JE. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr. 2002;76:447-53.
45. Arai Y, Uehara M, Sato Y, Kimira M, Eboshida A, Adlercreutz H, Watanabe S. Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake. J Epidemiol. 2000;10:127-35.
46. Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr. 2002;132:3577-84.
47. Ravn P, Clemmesen B, Riis BJ, Christiansen C. The effect on bone mass and bone markers of different doses of ibandronate: a new bisphosphonate for prevention and treatment of postmenopausal osteoporosis: a 1-year, randomized, double-blind, placebo-controlled dose-finding study. Bone. 1996;19:527-33.
48. Reid IR, Wattie DJ, Evans MC, Gamble GD, Stapleton JP, Cornish J. Continuous therapy with pamidronate, a potent bisphosphonate, in postmenopausal osteoporosis. J Clin Endocrinol Metab. 1994;79:1595-9.

Original Article

Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials

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大豆異黃酮抽取物的補充劑對停經後婦女骨質密度的效果：隨機對照試驗的後設分析

本研究旨在確認攝取大豆異黃酮抽取物(並非大豆蛋白或含有異黃酮的食品)對停經後婦女骨質密度(BMD)的效果。我們從 PubMed，CENTRAL，ICHUSHI，CNKI，Wanfang Data，CQVIP，和 NSTL 檢索，以英語，日語，或中文發表，並報告大豆異黃酮抽取物對停經後婦女腰椎或髖關節 BMD 效果的隨機對照試驗論文。依照納入和排除標準，對試驗論文進行鑑別和評閱來判定是否採用。有關研究設計，對象，介入，和結果的數據被抽取出進行分析。最終分別有 11、7、5、和 5 個試驗被採用來評估對腰椎、大腿骨頸部、髖關節全體、和股骨大轉子 BMD 的效果。包括 1240 名停經後婦女的後設分析(隨機效果模型)顯示，與對照組相比，每日平均攝取 82 (47-150) mg 的大豆異黃酮(苷元當量)持續 6-12 個月，顯著地提高腰椎 BMD 22.25 mg/cm^2 (95%信賴區間: 7.61，32.89；p=0.002)，或提高 2.38% (95%信賴區間: 0.93，3.83；p=0.001)。亞組分析顯示，不同試驗間大豆異黃酮對腰椎 BMD 的效果各異，可能與介入期間(6 或 12 個月)，對象的區域(亞洲或西方)，和基礎 BMD(正常骨質或骨質減少症或骨質疏鬆症)的研究特徵相關。我們的後設分析沒有發現對大腿骨頸部，髖關節全體，和股骨大轉子 BMD 的效果。大豆異黃酮抽取物的補充劑提高了停經後婦女的腰椎 BMD。需要更深入的研究去闡明影響其對腰椎效果程度的因素，以及驗證其對髖關節的效果。

關鍵字：後設分析、異黃酮、膳食補充劑、停經、骨密度

Life-style Related Diseases

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Soybean Isoflavones in Bone Health

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Abstract

Soybean isoflavones are structurally similar to estrogen, bind to estrogen receptors, and exhibit weak estrogenic activity. It has been reported that isoflavones play an important role in the prevention of hormone-dependent diseases, including osteoporosis, cardiovascular diseases, cancer, and postmenopausal syndrome. There are many researches indicating isoflavones prevent bone loss caused by estrogen deficiency in animal models. Furthermore, it has been demonstrated that a combination of isoflavone treatment and exercise cooperatively prevented bone loss in the estrogen-deficient status. Epidemiological studies demonstrated the relationship between the lower incidence of osteoporosis in Asian women and a diet rich in soy foods. Although a number of observational studies confirm the findings from the animal studies, the results from intervention studies are still controversial. One of the potential reasons for these inconsistencies could be individual differences in the isoflavone metabolism. Recently, it has been suggested that the clinical effectiveness of isoflavones might partly depend on the ability to produce equol, a gut bacterial metabolite of daidzein showing stronger estrogenic activity than the predominant isoflavones. Several candidate bacteria responsible for equol production have been suggested, for example *Lactococcus* 20-92 strain. From these findings, food factors enhancing equol production have received great deal of attention recently. On the other hand, safety assessment of isoflavones has been conducted by the Japanese Food Safety Commission. Further studies are required to address the numerous questions on the potential benefits, mechanisms of action, and safety of isoflavones.

Soybeans have been consumed in Asia since ancient times. Compared with Caucasians, the low incidence of heart disease, reproductive cancers, hip fracture, and climacteric symptoms in Asians has been considered to be associated with their high intake of soy foods. Recent growing interest in health and diet has led to an increased focus on soy foods and their functional components, e.g. isoflavones. Soybean isoflavones are structurally similar to estrogen, bind to estrogen receptors, and exhibit weak estrogenic activity. Isoflavones exert beneficial health effects by acting as antioxidants, tyrosine kinase and topoisomerase inhibitors as well as estrogenic activity. It has been reported that they play an important role in the prevention of chronic diseases, including osteoporosis, cardiovascular diseases, hormone-dependent cancer, and postmenopausal syndrome [1–5].

Osteoporosis is a skeletal disorder in which bone strength is compromised by the loss of bone density and bone quality. It is the leading cause of increased morbidity and functional loss in the elderly. Particularly postmenopausal women suffer from osteoporosis, which is part of the postmenopausal syndrome [6]. Although the treatment of postmenopausal osteoporosis is hormone replacement therapy, the reported side effects, such as development of hormone-dependent breast and uterine cancers [7], have prompted the use of alternative therapies. Soybean isoflavones have received considerable attention as alternatives to hormone replacement therapy, since the risk for side effects of isoflavone treatment seem to be low compared with hormone replacement therapy [8].

Epidemiological studies indicate that women who have high soy intake have less risk for osteoporosis than women who consume a typical Western diet [8]. A recent meta-analysis of 10 randomized controlled trials (RCTs) indicated that isoflavone intervention significantly attenuated bone loss in postmenopausal women [9]. However, the results from human studies are still controversial [10]. One of the potential reasons for these inconsistencies could be individual differences in isoflavone metabolism. Recent studies suggest that the clinical effectiveness of isoflavones on bone metabolism might be due to their ability to produce the metabolite equol in the intestine [11].

This chapter focuses on the current topics of research on isoflavones and their relationship to bone health.

Isoflavones and Their Metabolites

The major isoflavones contained in soy-based food products are daidzein, genistein, and glycitein (fig. 1). They exist mainly in the glycoside, acetyl glycoside, or malonyl glycoside forms in soy foods; hence, it becomes necessary to hydrolyze the glycosidic bonds for intestinal absorption to enable physiological activities. These glycosidic bonds are hydrolyzed by glycosidase produced by intestinal microflora such as *Lactobacilli*, *Bacteroides* and *Bifidobacteria* [12]. Furthermore, intestinal microflora affects the metabolism, wherein isoflavones are metabolized to equol, or O-desmethylangolensin (O-DMA) from their precursor daidzein (fig. 1).

Recent studies suggest that the clinical effectiveness of isoflavones might be due to their ability to produce metabolites, such as dihydrodaidzein, tetrahydrodaidzein, equol and O-DMA in the intestine [13]. Particularly, equol, a metabolite of daidzein, has received considerable attention because its biological activities differ from those of its precursor [11]. The metabolites of genistein and glycitein were also primarily found in human urine (genistein: dihydrogenistein, 6′-OH-O-DMA [13], 4-hydroxyphenyl-2-propionic acid and phloroglucinol; glycitein: dihydroglycitein, 5′-methoxy-O-DMA and 6-methoxy-equol). However, the physiological activities of these metabolites are still unclear.

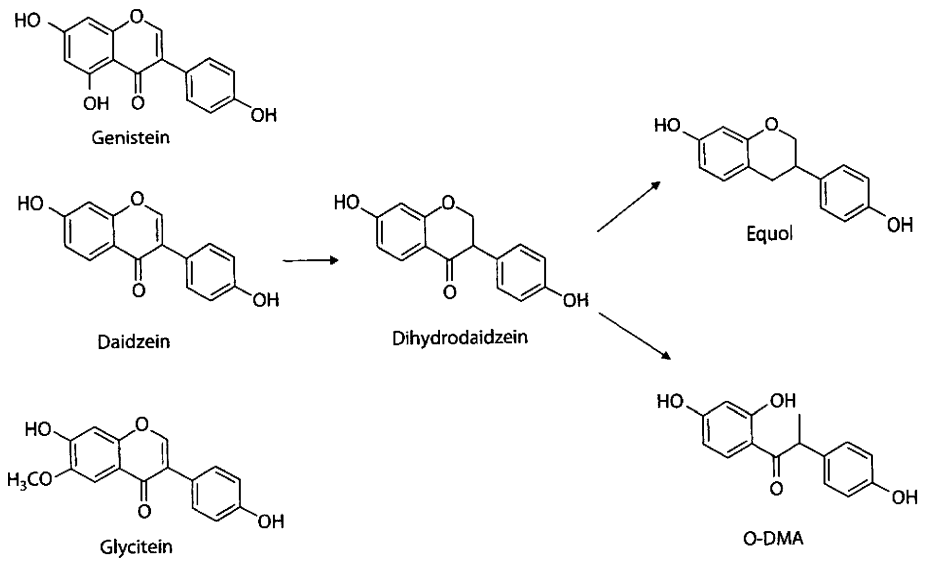

Fig. 1. Molecular structures of isoflavone aglycone and daidzein metabolites.

Equol has higher estrogenicity, stronger antioxidative efficacy, and exhibits anti-androgenic properties [11]. Moreover, equol is a chiral molecule, which exists as enantiomers R (+)-equol and S (−)-equol. In humans, the metabolism of daidzein to equol results in the production of only S-equol. Interindividual variability in equol production may be unique to humans; all the animals including rats, mice, and chimpanzees, tested systematically excrete equol. Although O-DMA was found in 80–90% of a human population, equol was found in only 30–50% of the population [14]. This is because of individual differences in the intestinal microbiota responsible for equol production.

Intestinal bacteria play a key role in isoflavone metabolism; young infants with undeveloped gut microflora do not produce equol, while germ-free animals also do not produce equol or O-DMA. Since some reports suggested a lower disease risk for equol producers than for nonproducers [11], there is a growing interest in certain bacterial strains that can produce equol. A number of strains involved in daidzein metabolism were identified. However, the identification of equol-producing bacteria is complicated. To date, only one lactic acid bacterium (*Lactococcus* 20-92 homologous to *Lactococcus garvieae)* has been identified that produces equol directly from daidzein without producing O-DMA [15]. Interestingly, the strain *Lactococcus* 20-92 can also cleave glycosidic bonds of daidzin; Uchiyama et al. [15] detected *L. garvieae* in the Italian cheese Toma Piemontese.

Estrogen deficiency is associated with increased bone turnover and acceleration of bone loss, which lead to an increased susceptibility to bone fracture. A number of studies have previously reported that soybean isoflavones, genistein and daidzein, dose-dependently inhibited bone loss in both female and male osteoporotic animal models without causing notable effects on the reproductive organs [16]. Ishimi et al. [17] also determined the selective effects of genistein on B-lymphopoiesis and bone loss caused by estrogen deficiency in ovariectomized (OVX) mice. In this study, genistein (0.7 mg/day s.c.) inhibited bone loss due to OVX without any uterine hypertrophy, which has been thought to be a side effect of estrogen administration. The dose which induced uterine hypertrophy was 10 times higher than that which inhibited bone loss. This suggests that genistein may be a natural selective estrogen receptor modulator (SERM). Interestingly, isoflavones inhibited bone loss in androgen-deficient male mice, indicating that soybean isoflavones prevent bone loss due to androgen deficiency in males.

Similarly, equol could also inhibit bone loss due to OVX. Fujioka et al. [18] reported that administration of equol (0.5 mg/day s.c.) inhibited bone loss of the whole body and femur in OVX mice. Although E_2 administration (0.03 μg/day s.c.) prevented OVX-induced bone loss from all regions, uterine hypertrophy occurred in E_2-administered OVX mice [18]. These results suggest that similar to SERMs, isoflavones including equol inhibit bone loss apparently without estrogenic activity in the reproductive organs in estrogen-deficient animals.

It is now recognized that one of the mechanisms by which estrogen deficiency causes bone loss is the stimulation of osteoclast formation, a process enhanced by several inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β. Furthermore, Nakamura et al. [19] recently reported a critical role for the osteoclastic estrogen receptor-α (ERα) in mediating estrogen-dependent bone maintenance in female mice. They selectively ablated ERα in differentiated osteoclasts [ERα(DeltaOc/DeltaOc)] and found that ERα(DeltaOc/DeltaOc) females, but not males, exhibited trabecular bone loss, similar to the osteoporotic bone phenotype in postmenopausal women. Furthermore, estrogen induced apoptosis and upregulation of Fas ligand expression in osteoclasts of the trabecular bones of wild type but not ERα(DeltaOc/DeltaOc) mice. The expression of ERα was also required for the induction of apoptosis by tamoxifen and estrogen in cultured osteoclasts. These results support a model in which estrogen regulates the life span of mature osteoclasts via the induction of the Fas/Fas ligand system, thereby providing an explanation for the osteoprotective function of estrogen as well as SERMs including isoflavones. On the other hand, isoflavones have been reported to inhibit bone resorption via other nonhormonal effects, including antioxidant activity, inhibition of tyrosine kinase and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). In fact, statins, cholesterol-lowering agents that inhibit the activity of HMG-CoA reductase, induce bone formation and inhibit bone resorption both in vitro and in vivo.

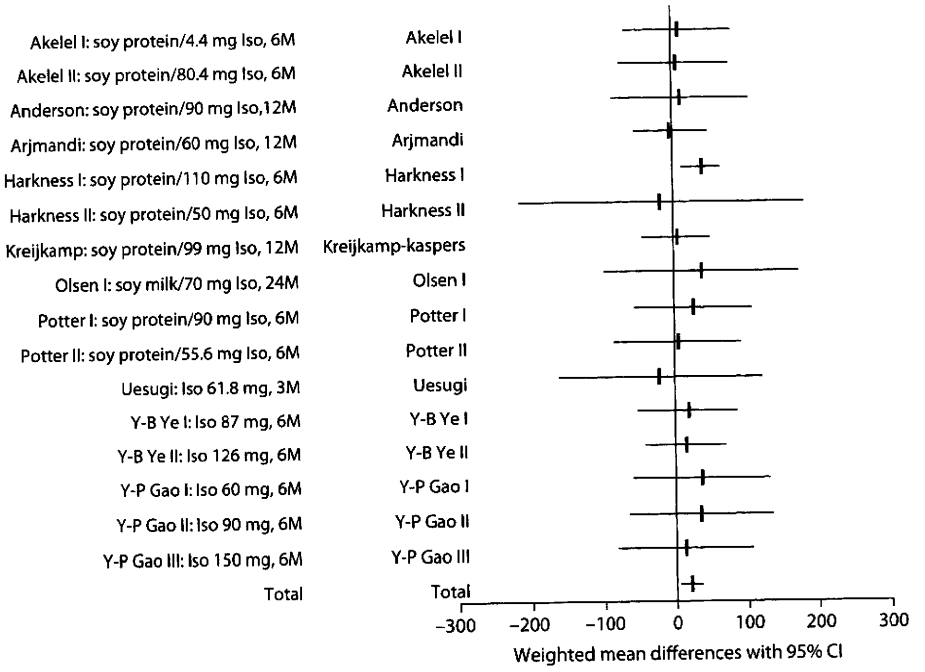

Fig. 2. Meta-analysis of 10 RCTs of isolated isoflavone intervention for BMD of lumbar spine in postmenopausal women [9].

Effects of Isoflavone on Bone Metabolism: Observational and Intervention Studies

Observational studies indicate that women who have high soy intake have less risk for osteoporosis than women who consume a typical Western diet [8, 16]. Recently, increasing numbers of RCTs have tested the effects of soy isoflavones on bone mineral density (BMD) in postmenopausal women. So far, one meta-analysis of RCTs has evaluated the effect on spine BMD of soy isoflavones (fig. 2). This included 10 trials testing both extracted soy isoflavones and isolated soy protein containing isoflavones, and revealed a significantly beneficial effect of soy isoflavone intake on BMD in lumbar spine at more than 90 mg/day [9]. However, the results from human studies are still controversial. Recently, Brink et al. [10] reported that consumption of isoflavone-enriched products (110 mg/day of isoflavone aglycone equivalents) for 1 year did not affect BMD and bone turnover in apparently healthy early postmenopausal white women. Potential reasons for these inconsistencies could include different races, the timing of exposure to isoflavone, duration of intervention, and difference in diet and isoflavone intake from foods between the isoflavone and placebo groups.

Alternatively, interindividual differences in isoflavone metabolism could be a contributing factor. Recent studies suggest that the clinical effectiveness of isoflavones on bone metabolism might be due to their ability to produce the metabolite equol in the intestine [11].

Combined Effects of Isoflavones and Exercise on Bone Metabolism in Estrogen-Deficient Status

It has been shown that running exercise partially prevented bone loss induced by estrogen deficiency. Frost [20] showed that estrogen deficiency increased the 'set point' for the skeleton to respond to loading, causing the skeleton to be less sensitive to mechanical force and decreasing its bone mass. In this regard, it is likely that phytoestrogens can influence the set point of the mechanical loading that affects bone mass. Wu et al. [21] assessed the combined effects of isoflavones and exercise on BMD in postmenopausal Japanese women as well as in OVX mice. The combined intervention of moderate exercise and the submaximal dose of genistein administration showed a cooperative effect in preventing bone loss in OVX mice. Furthermore, they recruited 136 subjects (average age was 55 years), who were postmenopausal within 5 years of natural menopause, and randomly assigned them to four groups: placebo; walking combined with placebo (3 times/week, 6 km/h); isoflavone intake (75 mg conjugates/day; equivalent to 47 mg of aglycone; Fujicco Co. Ltd., Kobe, Japan) in addition to the normal diet, and isoflavone combined with walking exercise [21]. After 1-year intervention, 108 subjects completed the study. BMD of the lumber spine, left hip and subwhole body was assessed by DXA using Hologic QDR-4500 (Hologic Inc., Waltham, Mass., USA) at baseline and after 1 year.

Average daily intake of isoflavone aglycone from soy foods was around 28 mg per day in the subjects in 4 groups at baseline and after 1 year. There were no significant differences in daily intake of isoflavones and other nutrients among the groups at baseline, and between baseline and after 1 year in each group. Of the percent change in BMD, walking showed significant main effects on the preservation of BMD in the total hip region after 1 year. Interventions with isoflavones or walking showed significant main effects on the preservation of BMD at Ward's triangle in the hip after 1 year. Combined intervention of isoflavone intake and walking exercise for 1 year showed a trend for a greater effect on BMD at total hip and Ward's triangle regions than either intervention alone [21].

Since the effect of isoflavone alone was modest compared with those in the Westerners [21], the subjects in the placebo and isoflavone intervention groups were stratified based on their fecal equol production, and serum equol concentrations were measured in order to investigate whether any difference exists in the effects of isoflavone on BMD between equol producers and nonproducers in postmenopausal Japanese women [22].

Possible Role of Equol Status in the Effects of Isoflavones on Bone Health

Equol is one of the main metabolites of the isoflavone daidzein. Equol production depends on the individual's intestinal flora, and research has shown that approximately 30–50 % of individuals in the population studied are capable of producing equol from daidzein [11]. Setchell et al. [11] reported that compared with the control group, equol producers showed a 2.4% increase in their lumbar spine BMD, whereas no significant change in this BMD was observed in the nonproducers after a 2-year isoflavone intervention.

Wu et al. [22] assessed the effects of equol-producing activity on BMD in postmenopausal Japanese women. Fifty-four women (29 in the placebo, 25 in the isoflavone group) completed the 1-year intervention, and their data were used for equol analysis.

The percentage of equol producers in our subjects was 55% by assessment of equol production in fecal suspension incubated with daidzein under anaerobic condition. The number of equol producers and nonproducers was 15 and 14 in the placebo group, and 15 and 10 in the isoflavone group, respectively. Serum daidzein, which was determined by reverse-phase high-performance liquid chromatography, dramatically increased in the isoflavone group after 1 year. However, there was no difference in serum daidzein between equol producers and nonproducers. Conversely, serum equol was increased only in equol producers in the isoflavone group after 1 year. Serum equol did not change in equol producers in the placebo group. The percent change in bone loss at total hip and intertrochanter of the hip of equol producers was significantly lower than that of equol nonproducers in the isoflavone group, as assessed by Student's t test (fig. 3). However, none of the differences between producers and nonproducers was observed in the placebo group. From these results, the effects of isoflavone on bone mass might depend on the equol-producing activity in postmenopausal Japanese women [22].

Isolation of Equol-Producing Bacteria from Human Feces

Intestinal bacteria play a key role in isoflavone metabolism; young infants with undeveloped gut microflora do not produce equol, while germ-free animals also do not produce equol or O-DMA. A number of strains involved in daidzein metabolism were identified. However, the identification of equol-producing bacteria is complicated. Several candidate bacteria responsible for daidzein metabolism have been suggested; for example, a *Clostridium* sp. and *Eubacterium ramulus* metabolized daidzein to O-DMA in vitro, and equol was found in soymilk fermented with some strains of *Bifidobacterium*. Uchiyama et al. [15] and Ishimi et al. [23] were the first to find, mainly four, equol-producing bacteria in human feces; they concluded that the *Lactococcus* 20-92 strain (*Lc.* 20-92) as homologous to *L. garvieae* is the most appropriate bacteria for food usage because we have dietary habit of *L. garvieae*.

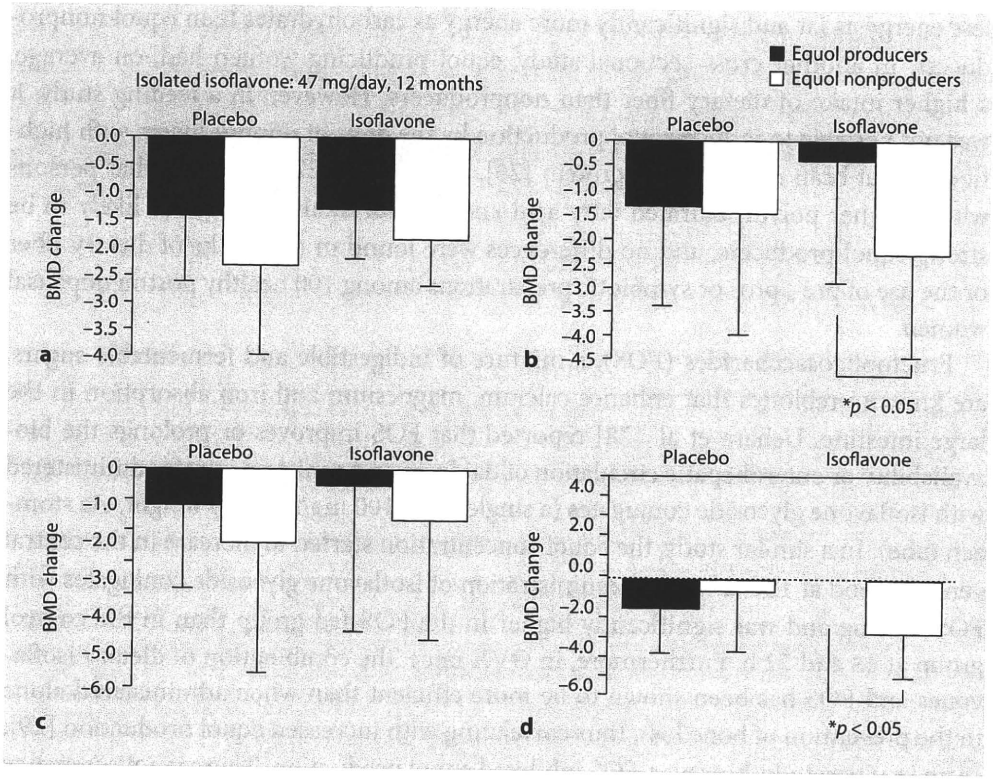

Fig. 3. Percent change in BMD of the whole body and hip analyzed by equol status in postmenopausal Japanese women [22]. **a** Whole body. **b** Total hip. **c** Femoral neck. **d** Intertrochanter. Statistical differences as assessed by Student's t test.

Ishimi et al. [23] detected *Lc.* 20-92 in the feces of 133 of postmenopausal Japanese women using real-time PCR using the particular primer for *L. garvieae.*

The bacteria were detected in 47 of 133 samples (35.3%) [23]. Interestingly, the people with *L. garvieae* were not always equol producers, suggesting that some other bacteria and several factors such as hydrogen gas and short-chain fatty acids, which can affect the environmental conditions in the colon, might be also important for equol production in humans.

Food Factors Affecting Equol Production

It has been suggested that food factors contribute to the ability to produce equol; however, contradictory results were obtained from association studies. For example, Adlercreutz et al. [24] reported a positive association between urinary equol concentration and intake of fat and meat in a Japanese population, whereas in a Western population, Rowland et al. [25] reported that equol producers consumed significantly

less energy as fat and significantly more energy as carbohydrates than equol nonproducers. In another cross-sectional study, equol-producing women had, on average, a higher intake of dietary fiber than nonproducers. However, in a feeding study, it was not possible to induce equol production by the diets of nonproducers with high-fiber wheat bran cereal or soy protein [26]. Bolca et al. [27] suggested that persons with a higher polyunsaturated fatty acid and alcohol intake were more likely to be strong equol producers, and no differences were found in the intake of dietary fiber or the use of pre-, pro- or symbiotic preparations among 100 healthy postmenopausal women.

Fructooligosaccharides (FOS), a mixture of indigestible and fermentable sugars, are known prebiotics that enhance calcium, magnesium and iron absorption in the large intestine. Uehara et al. [28] reported that FOS improves or prolongs the bioavailability or enterohepatic circulation of daidzein and genistein in rats administered with isoflavone glycoside conjugates (a single dose, 100 mg/kg body weight, via stomach tube). In a similar study, the equol concentration started to increase in the central venous blood at 12 h after the administration of isoflavone glycoside conjugates with FOS feeding and was significantly higher in the FOS-fed group than in the control group at 48 and 72 h. Furthermore, in OVX mice, the combination of dietary isoflavones and FOS has been shown to be more efficient than when administered alone in the prevention of bone loss, thus correlating with increased equol production [29]. In an in vitro study, however, FOS inhibited equol production. There is a discrepancy between the results of the in vivo and in vitro studies. In French postmenopausal women, FOS did not increase urinary equol production. However, racial differences might exist with regard to isoflavone metabolism. Therefore, further human studies involving Asian subjects are required. Several factors such as animal species, race, sex, age, and genetic background, including individual variation in intestinal microflora and diet, should be considered with regard to isoflavone metabolism and metabolite production.

Safety Evaluation of Isoflavones in Japan

Soybean isoflavone was approved in 2001 as the principle ingredient in Food for Specified Health Uses (FOSHU) by the Japanese Ministry of Health, Labour and Welfare and is aimed at individuals concerned about bone health. There are tea, soymilk and soft drinks containing 40 mg of isoflavone conjugates, which is equivalent to 25 mg of aglycone form. In 2004, applications of a tablet containing soy isoflavone aglycones as its principal ingredient, and a fermented food containing isoflavone aglycone in amounts exceeding the usual content in FOSHU were filed for approval. Foods with fortified or condensed isoflavones had not been consumed before. And there is a possibility that tablets and capsules would be excessively consumed. Accordingly, the Japanese Food Safety Commission of the Cabinet conducted

Table 1. Effects of 1 year isoflavone intake on serum sex and thyroid hormone concentrations in postmenopausal Japanese Women

		Placebo	Isoflavone	Placebo vs Iso
		(n = 29)	(n = 25)	
Estradiol (pg/mL)	Baseline	12.66 (4.03)	12.32 (3.34)	NS
	After 1 year	12.98 (7.31)	11.98 (2.94)	NS
	% change	6.09 (57.71)	1.20 (23.80)	NS
FSH (U/L)	Baseline	70.36 (26.02)	68.19 (18.66)	NS
	After 1 year	60.00 (19.83)*	58.12 (17.86)*	NS
	% change	−12.36 (8.40)	−14.05 (9.01)	NS
LH (U/L)	Baseline	26.68 (13.87)	27.70 (9.32)	NS
	After 1 year	22.43 (11.12)*	22.33 (7.90)*	NS
	% change	−12.16 (15.98)	−19.10 (14.44)	NS
Progesterone (ng/mL)	Baseline	0.27 (0.11)	0.29 (0.16)	NS
	After 1 year	0.21 (0.10)*	0.24 (0.12)*	NS
	% change	−24.54 (29.47)	−14.07 (18.08)	NS
T3 (ng/ml)	Baseline	1.13 (0.16)	1.08 (0.13)	NS
	After 1 year	1.10 (0.16)	1.03 (0.16)	NS
	% change	−1.60 (60.11)	−3.92 (7.96)	NS
T4 (μg/dL)	Baseline	8.57 (1.12)	8.19 (1.28)	NS
	After 1 year	8.46 (1.13)	7.54 (1.42)*	NS
	% change	−1.85 (7.46)	−4.48 (6.23)	NS
TSH (mU/L)	Baseline	2.34 (1.10)	2.30 (0.74)	NS
	After 1 year	2.75 (2.81)	2.25 (1.10)	NS
	% change	12.71 (69.59)	−9.22 (32.74)	NS

* Significantly different from baseline by paired *t*-test, $p < 0.05$

a safety evaluation on soy isoflavones, and issued a Notice: *'Basic approaches to evaluating the safety of FOSHU containing soy isoflavones'* in 2006. The main contents of the report are summarized in 3 points. Firstly, the upper limit of isoflavone aglycone intake from FOSHU was set at 30 mg/day for additional consumption with a normal diet. This limit represented half of the 57.3 mg soy isoflavones (aglycone equivalent) in the soymilk that was given daily to the premenopausal Japanese women in whom estrogen level tended to decrease and menstrual cycles tended to be longer over 2–3 cycles. Secondly, the maximum recommended level for safe isoflavone aglycone intake in a daily diet is 70–75 mg/day at the present time. This limit was selected on the basis of two findings. One was the National Nutrition Survey in 2002 in Japan that showed

the 95th percentile intake of soy isoflavones at 64–76 mg/day. The other was the result of an experiment in which postmenopausal Italian women taking a 150-mg soy isoflavone aglycone tablet daily showed no effects after 3 years, but showed a significant increase in the occurrence of endometrial hyperplasia after 5 years as compared with a control group. One half of that amount was thus selected as the maximum recommended level, allowing for individual and experimental variations. Thirdly, it was not recommended that pregnant women, infants and children take soy isoflavone from FOSHU [30]. These criteria were also adapted to the so-called Health Foods fortified with isoflavones.

In order to examine the effects of isoflavone intake on hormone levels in postmenopausal women, Ishimi et al. [23] evaluated serum concentrations of estrogen, FSH, LH, progesterone and thyroid hormones in their participants. There were no significant differences in estrogenic hormone levels between the placebo and isoflavone treatment groups (table 1). The same was observed with thyroid hormone levels. These results suggest that additional isoflavone intake with a normal diet (total intake was about 75 mg of aglycone equivalent a day) for a year did not affect serum hormone levels in postmenopausal Japanese women [23]. Marini et al. [31] recently reported that 54 mg/day of genistein supplementation for 3 years exhibited a promising safety profile in breast and endometrium with positive effects on bone formation in a cohort of osteopenic postmenopausal women.

It is well known that consuming soy foods has many benefits. For example, they are a good source of protein, calcium; soy protein decreases serum cholesterol, and isoflavones maintain bone health in postmenopausal women. Therefore, the Japanese Ministry of Health, Labour and Welfare set the goal at 100 g/day of beans intake in the Health Japan 21 Program in 2000. Soybeans have been consumed since ancient times in Asia, and there has been no report of any problems even in cases of excessive consumption. Soy isoflavone intake from soy foods in a normal daily diet is therefore considered safe.

Conclusions

Firstly, several factors such as race, age, diet, time of exposure, and individual variations in genetics and intestinal microflora affect the effects of isoflavones on bone health. Secondly, preventive effects of daidzein on bone loss in postmenopausal women might depend on the capacity of an individual to produce equol. Thirdly, 47–54 mg/day of isoflavone supplementation with a normal diet for 1–3 years has not shown any adverse effects at least in postmenopausal women. Further studies are required to address the numerous questions regarding the potential benefits, mechanisms of action and safety of isoflavones.

References

1 Adlercreutz H, Mazur W: Phyto-oestrogens and Western diseases. Ann Med 1997;29:95–120.

2 Adlercreutz H: Phyto-oestrogens and cancer. Lancet Oncol 2002;3:364–373.

3 Lampe JW: Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J Nutr 2003;133:956S–964S.

4 Magee RJ, Rowland IR: Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Br J Nutr 2004;91:513–531.

5 Messina M, Kucuk O, Lampe JW: An overview of the health effects of isoflavones with an emphasis on prostate cancer risk and prostate-specific antigen levels. J AOAC Int 2006;89:1121–1134.

6 Inzerillo AM, Zaidi M: Osteoporosis: trends and intervention. Mt Sinai J Med 2002;69:220–231.

7 Beral V, Bull D, Reeves G: Endometrial cancer and hormone-replacement therapy in the Million Women Study. Lancet 2005;365:1543–1551.

8 Messina MJ: Soy foods and soy isoflavones and menopausal health. Nutr Clin Care 2002;5:272–282.

9 Ma DF, Qin LQ, Wang PY, Katoh R: 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.

10 Brink E, Coxam V, Robins S, Wahala K, Cassidy A, Branca F: Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. Am J Clin Nutr 2008;87: 761–770.

11 Setchell KDR, Brown NM, Lydeking-Olsen E: The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr 2002;132:3577–3584.

12 Xu X, Harris KS, Wang HJ, Murphy PA, Hendrich S: Bioavailability of soybean isoflavones depends upon gut microflora in women. J Nutr 1995;125:2307–2315.

13 Heinone S, Wähälä K, Adlercreutz H: Identification of isoflavone metabolites dihydrodaidzein, dihydrogenistein, 6′-OH-O-dma, and cis-4-OH-equol in human urine by gas chromatography-mass spectroscopy using authentic reference compounds. Anal Biochem 1999;274:211–219.

14 Frankenfeld CL, McTiernan A, Tworoger SS, Atkinson C, Thomas WK, Stanczyk FZ, Marcovina SM, Weigle, DS, Weiss NS, Holt VL, Schwartz SM, Lampe JW: Serum steroid hormones, sex hormone-binding globulin concentrations, and urinary hydroxylated estrogen metabolites in post-menopausal women in relation to daidzein-metabolizing phenotypes. J Steroid Biochem Mol Biol 2004;88: 399–408.

15 Uchiyama S, Ueno T, Suzuki T: Identification of a newly isolated equol-producing lactic acid bacterium from the human feces. J Intestinal Microbiol (Tokyo) 2007;21:217–220.

16 Setchell KD, Lydeking-Olsen E: Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. Am J Clin Nutr 2003;78(suppl):593S–609S.

17 Ishimi Y, Miyaura C, Ohmura M, Onoe Y, Sato T, Uchiyama Y, Ito M, Wang X, Suda T, Ikegami S: Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. Endocrinology 1999;140:1893–1900.

18 Fujioka M, Uehara M, Wu J, Adlercreutz H, Suzuki K, Kanazawa K, Takeda K, Yamada K, Ishimi Y: Equol, a metabolite of daidzein, inhibits bone loss in ovariectomized mice. J Nutr 2004;134:2623–2627.

19 Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, Harada Y, Azuma Y, Krust A, Yamamoto Y, Nishina H, Takeda S, Takayanagi H, Metzger D, Kanno J, Takaoka K, Martin TJ, Chambon P, Kato S: Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. Cell 2007;130:811–823.

20 Frost HM: On our age-related bone loss: insights from a new paradigm. J Bone Miner Res 1997;12: 1539–1546.

21 Wu J, Oka J, Tabata I, Higuchi M, Toda T, Fuku N, Ezaki J, Sugiyama F, Uchiyama S, Yamada K, Ishimi Y: Effects of isoflavone and exercise on BMD and fat mass in postmenopausal Japanese women: a 1-year randomized placebo-controlled trial. J Bone Miner Res 2006;21:780–789.

22 Wu J, Oka J, Ezaki J, Ohtomo T, Ueno T, Uchiyama S, Toda T, Uehara M, Ishimi Y: Possible role of equol status in the effects of isoflavone on bone and fat mass in postmenopausal Japanese women: a double-blind, randomized, controlled trial. Menopause 2007;14:866–874.

23 Ishimi Y, Oka J, Tabata I, Ohtomo T, Ezaki J, Ueno T, Uchiyama S, Toda T, Uehara M, Higuchi M, Yamada K, Wu J: Effects of soybean isoflavones on bone health and its safety in postmenopausal Japanese women J Clin Biochem Nutr 2008;43(suppl 1):48–52.

24 Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T, Okada H: Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am J Clin Nutr 1991;54: 1093–1100.

25 Rowland IR, Wisemen H, Sanders TAB, Adlercreutz H, Bowey EA: Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. Nutr Cancer 2000;36:27–32.
26 Lampe JW, Skor HE, Li S, Wähälä K, Howald WN, Chen C: Wheat bran and soy protein feeding do not alter urinary excretion of the isoflavan equol in premenopausal women. J Nutr 2001;131:740–744.
27 Bolca S, Possemiers S, Herregat A, Huybrechts I, Heyerick A, De Vriese S, Verbruggen M, Depypere H, De Keukeleire D, Bracke M, De Henauw S, Verstraete W, Van de Wiele T: Microbial and dietary factors are associated with the equol producer phenotype in healthy postmenopausal women. J Nutr 2007;137:2242–2246.
28 Uehara M, Ohta A, Sakai K, Suzuki K, Watanabe S, Adlercreutz H: Dietary fructooligosaccharides modify intestinal bioavailability of a single dose of genistein and daidzein and affect their urinary excretion and kinetics in blood of rats. J Nutr 2001; 131:787–795.
29 Ohta A, Uehara M, Sakai K, Takasaki M, Adlercreutz H, Morohashi T, Ishimi Y: A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. J Nutr 2002;132: 2048–2054.
30 The Japanese Food Safety Commission of the Cabinet: Basic approaches to evaluating the safety of Food for Specified Health Uses containing soy isoflavones (in Japanese). 2006; http://www.fsc.go.jp/hyouka/hy/hy-singi-isoflavone_kihon.pdf.
31 Marini H, Bitto A, Altavilla D, Burnett BP, Polito F, Di Stefano V, Minutoli L, Atteritano M, Levy RM, D'Anna R, Frisina N, Mazzaferro S, Cancellieri F, Cannata ML, Corrado F, Frisina A, Adamo V, Lubrano C, Sansotta C, Marini R, Adamo EB, Squadrito F: Breast safety and efficacy of genistein aglycone for postmenopausal bone loss: a follow-up study. J Clin Endocrinol Metab 2008;93:4787–4796.

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資　料

「健康食品」中の大豆たんぱく質および大豆イソフラボンの定量と表示に関する調査研究

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Food Labeling and Actual Contents of Soy Protein and Soybean Isoflavones in Health Foods

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Soy bean isoflavones are structurally similar to estrogen, and exhibit weak estrogenic activity. In 2006, Food Safety Commission of the cabinet office set the upper limit of isoflavone (aglycone equivalent) intake from Foods for Specified Health Uses (FOSHU) at 30 mg/day for additional consumption with a normal diet. However, the upper limit for isoflavones was not established in the so-called health foods containing soy protein and isoflavones. We evaluated the respective soy protein and isoflavone contents in 10 health foods including 5 FOSHU by the ELISA and HPLC methods. The soy protein content of eight of these foods was determined to be within 90–118% of that reported on the label, and two protein powders for junior use contained about a half of the soy protein content reported on the label. The isoflavone aglycone content in FOSHU containing soy protein was detected to be 90–122% of the labeled content, while the isoflavone content of two soy protein-fortified foods with no label exceeded 30mg per one serving. It might be necessary to pay attention to junior athletes not excessively consuming these soy protein powder foods.

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Key words: foods for specified health uses (FOSHU), health food, isoflavone, soy protein, upper limit

緒　　言

注）本文でいう「健康食品」は，「保健機能食品」（特定保健用食品および栄養機能食品）および「いわゆる健康食品」をさす。

大豆は古来よりたんぱく質源として利用されてきたが，近年では含有されている大豆たんぱく質および大豆イソフラボン等の機能性成分が注目されている。大豆たんぱく質は血清脂質やコレステロールの濃度を低下させることが多くの研究報告から示されており，米国食品医薬局（FAD）は1999年に大豆たんぱく質を含む食品に対して「25 g／日の大豆たんぱく質の摂取は虚血性心疾患を予防する」旨のリスク低減表示を許可した。大豆イソフラボンは女性ホルモンと類似した構造を有しており，エストロゲンレセプター（ER）と結合するため，生体内で弱いエストロゲン作用を発揮する[1)]。そのため，閉経期女性におけるホルモン代替療法（HRT, Hormone replacement therapy）にかわる食品成分としての利用が期待されている。

現在我が国では，大豆たんぱく質および大豆イソフラボンはともに特定保健用食品の関与成分であり，その他にも多様な「いわゆる健康食品」に利用されている。大

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表1　大豆イソフラボン標準品

分　類	ダイゼイン型 イソフラボン	グリシテイン型 イソフラボン	ゲニステイン型 イソフラボン
配糖体	ダイジン (D; Daidzin)	グリシチン (Gl; Glycitin)	ゲニスチン (G; Genistin)
マロニル配糖体	マロニルダイジン (MD; 6"-O-Malonyldaidzin)	マロニルグリシチン (MGl; 6"-O-Malonylglycitin)	マロニルゲニスチン (MG; 6"-O-Malonylgenistin)
アセチル配糖体	アセチルダイジン (AD; 6"-O-Acetylglycitin)	アセチルグリシチン (AGl; 6"-O-Acetylglycitin)	アセチルゲニスチン (AG; 6"-O-Acetylgenistin)
サクシニル配糖体	サクシニルダイジン (SD; 6"-O-succinyldaidzin)	サクシニルグリシチン (SGl; 6"-O-succinylglycitin)	サクシニルゲニスチン (SG; 6"-O-succinylgenistin)
アグリコン	ダイゼイン (De; Daidzein)	グリシテイン (Gle; Glycitein)	ゲニステイン (Ge; Genistein)

豆イソフラボンについては，内閣府食品安全委員会によりその安全性が検討され，「大豆イソフラボンを含む特定保健用食品の安全性評価の基本的な考え方」（2006年5月）において[2]，特定保健用食品における大豆イソフラボンの上乗せ上限摂取量が30 mg／日と定められた。また，これを受け厚生労働省は，大豆イソフラボンの過剰摂取を防止するために，「大豆イソフラボンを含む特定保健用食品等の取り扱いに関する指針」を通知した（2006年8月）[3]。この指針では，特定保健用食品および大豆イソフラボンを強化した食品について一日当たりの摂取量が30 mgを超えないように一日摂取目安量を設定するとともに，摂取する上での注意事項を表示することが定められている。しかし，大豆たんぱく質を含む「いわゆる健康食品」に対する記述はない。そのため，大豆たんぱく質を強化した「いわゆる健康食品」には相当量の大豆イソフラボンが含まれていると予想されるものの，現在大豆イソフラボン量の表示がなされていない食品が多数流通している。

そこで本研究では，大豆たんぱく質を強化した「健康食品」について大豆たんぱく質および大豆イソフラボンの定量を行った。また，大豆たんぱく質および大豆イソフラボンの表示がなされている健康食品については，実測値と表示値の比較を行った。これらの結果から，大豆たんぱく質を強化した「健康食品」を適切に摂取するための表示の在り方について考察を加えた。

方　　法

「健康食品」中の大豆たんぱく質および大豆イソフラボンの定量

食品は大豆たんぱく質を関与成分とする特定保健用食品および大豆あるいは大豆たんぱく質を原材料としたスポーツ選手対象の健康食品を対象とした。液状食品4品目，粉末状食品6品目の計10品目について，大豆たんぱく質および大豆イソフラボンの定量分析を行った。食品は同ロットのものを3品ずつ分析した。

1. 大豆たんぱく質

大豆たんぱく質の分析は，市販のSoya protein Assay Kit（CAT, No.902001T, Tepnel BioSystems）を用い，キットの説明書に記載されている方法に基づいて行った。

1-1　抽　　出

試料（$n=3$）を精秤後，試料12 g（W1）に対して48.0 g（W2）の割合で0.05 Mトリス-塩酸緩衝液（pH 8.6）を添加し，均一な混合液とした。精密に測った試料混合液2.5 g（W3）に尿素-DTT緩衝液7.5 mlを加え，100℃で1時間抽出を行った。抽出フラスコを50℃に移し，同じ温度に保温しておいたrenaturation溶液20 mlをゆっくりと加えて攪拌後，100 mlのフラスコに移し入れた。室温に戻した後100 mlに定溶して，よく混合した後Whatman No1濾紙で濾過した溶液の，最初の10 mlを回収して抽出溶液とした。

1-2　定量分析

大豆たんぱく質の定量は，Soya protein Assay Kit (Tepnel BioSystems) を用い，酵素免疫測定法（ELISA）で行った。表示に記載されている含有量を参考に，濃いサンプルについては適宜希釈して用い実験に供した。吸光度はマイクロプレートリーダー（コロナ電気製，MTP-650FA）を用い，測定波長450 nmで測定した。

1-3　解　　析

上記の方法に基づき得られたSOYA PROTEIN STANDARDSの吸光度より検量線を作成し，以下の計算式に従って試料中の大豆たんぱく質含量を算出した。

式）

試料中の大豆たんぱく質量（%）

$= (SP \times (W1 + W2) \times W4 \times PV \times 5.71 / SPc \times W3 \times 6.25) \times$ 希釈倍率

SP：検量線から読み取った希釈された試料溶液中のたんぱく量（μg/ml）

SPc：検量線から読み取ったSOYA PROTEIN CON-