Statistical Analyses

All data were output in Excel format and transferred to SPSS 14.0 for statistical analysis.[17] Two-way ANOVA was performed; in the case of a significant F value, a post hoc test using the Newman-Keuls method was performed to identify any significant differences in mean values. All data are given in the form mean ± SE; the median value is quoted where the doubled standard deviation was greater than mean. Statistical significance was set *a priori* at $p < 0.05$ for all comparisons.

Results

Profiles of the CKD patients are given in Table 1. Three of the patients maintained a very low protein diet (VLPD) for more than 10 years, two for 8 years, three from 4 to 6.5 years, and one each for one and two years, respectively. All of them started VLPD at CKD stage 3 (eGFR < 30 ml/min/1.73m^2). Basic diseases underlying the CKD were varied; most of the patients received anti-hypertensive drugs, anti-uremic acid, and/or anti-hyperlipidemia. Two patients took $CaCO_3$, but none used erythropoietin or other supplements.

One patient and two family members were male, and two of the eight female CKD patients had recently entered hemodialysis (hereafter "the hemodialysis group"). Six other female patients (hereafter "the LPD group"), 6 female family members ("the family group") and 11 female dietitians ("the dietitian group") completed the study and were subjected to detailed analysis. Although the number of hemodialysis patients was only two, they continued lower protein intake (0.8g/kg body weight) compared to the ordinary hemodialysis patients (1.1-1.2 g/kg), so these two were remained for the analysis.

One male patient and two male family members showed similar trends to the female groups, but were not included in the detailed analysis.

Dietary intake

Dietary intakes, calculated from the 3-day dietary records, are summarized in Table 2 into 6 groups including the males. Daily intake was 2046±8 kcal for male and

1782±340 kcal for female patients. On average, female intake was 32.7 kcal/kg body weight. Daily protein intake by the LPD group was 0.39±0.08 g/kg body weight, compared to 0.55±0.1 g in the hemodialysis group. The three control groups (male and female family members, and dieticians) took more than 1g per kg of protein daily. Intakes of lipid soluble retinol, carotene, vitamin A, water soluble vitamin K, folic acid, and vitamin C by the VLPD group were less than half that of family and dietitians, but no patients showed clinical manifestation of associated vitamin deficiencies. Intakes of P, K, Ca, and Mg by VLPD were also less than one third that of family and dietitians. NaCl, K and P intakes were kept in the low range within allowable limits.

Protein intake calculated from 24 hr urinary urea excretion using the equation of Kopple and Mitch[2,18] was found to be significantly correlated with actual intake ($R=0.6$, $p<0.01$), but dietary amino acid showed no correlation with either plasma or urinary amino acids (data not shown).

Laboratory data

Laboratory data of the CKD group showed moderate anemia, low hematocrit, and higher MCV and MCH compared to the family and dietitian groups (Table 3).
The patients' group showed significantly higher values for gamma-GTP, creatinine (Cr), BUN/Cr ratio, uric acid (UA) and triacylglycerol (TG) and lower HDL cholesterol.

ProBNT was remarkably high in one case of IgA glomerulonephritis, and was over 200 mg/dl in 7 out of 9 CKD patients, but less than 100 mg/dl in the control groups (36.9±20.8). K was slightly higher in CKD group, and plasma Ca level did not vary significantly from controls.

Dual-Energy X-ray Absorptiometry (DEXA)

Body composition by DEXA showed no significant differences between the LPD and healthy groups. Lean body mass and bone density values were similar across 4 groups (Table 4).

eGFR and urinary findings

eGFR of the LPD group was 27.6±22.0 ml/min (median=20.0), while those of the family and dietitian groups were 83.3±14.8 and 91.5±12.6 ml/min/1.73m^2, respectively

(Table 5).[16] Median values of the latter two groups were over 90 ml/min/1.73m^2. Daily cumulative urinary albumin and β_2-microglobulin values were significantly high in the CKD group. pH was high in the hemodialysis group, but the median value was similar to that of the dietitian group.

Amino acid profile

Amino acid profiles in both serum and 24-hour urine on the 3rd day are given in Table 6. The high plasma urea in CKD patients resulted in significantly high citrullin in the urea cycle. Arg did not vary among the 4 groups, but ornithin was high in hemodialysis group. Urinary excretion of these amino acids in the urea cycle was less in CKD patients, and very low in the dialysis group. Tyr and Thr showed similar trends in dialysis patients.

In general, hydrophobic amino acids were excreted less in CKD patients. Although the amino acid with the highest excretion was Gly, it was less in urine of CKD patients with accumulation in the blood. Lower urinary amino acid excretion was related to the low plasma levels of Ala, Tyr, Leu, Ile, Glu etc. in the LPD group. Plasma levels of some amino acids such as Thr, Phe, Ser, Asp, Pro, etc., were not excreted into the urine and remained on a par with healthy controls. Urinary Lys was lower in CKD patients, while plasma Lys level remained the same.

In the methionin cycle, only Cys and taurin appeared in urine, with some accumulation in plasma. Homocystine and cystathionin were not present in either plasma or urine in VLPD group.

There was no significant change in Glu or Gln, but there was an absence of alpha-adipinate in the urine of CKD patients. His was only present in the plasma of all groups, but its metabolites 3-methyl histidine and carnosin (beta-alanyl-L-histidine) appeared in urine, being less in CKD patients with higher plasma concentration.

Metabolites such as beta-amino-isobutyrate, beta-alanin and hydroxyprolin were also higher in the plasma of the CKD group. In the hemodialysis group, excretion of branched chain amino acids such as Val, Leu and Ile were markedly higher in urine but lower in plasma.

Discussion

Low protein diet is considered to be most effective to save the function of GFR, which tends to decrease according to aging. However, degree of protein restriction is still in debate.

Menon et al.[19] recently reported an increased risk of death from very low protein diets (VLPD) based on a 7-year follow-up after the MDRD study. Their study B group took 0.28 g/kg body weight protein and amino-keto acid supplements (about 0.28 g/kg body weight). They listed several reasons for the increased risk of death. We evaluated their study and considered that the notably low energy intake (30 kcal/kg body weight was ordered, but 25 kcal/kg at midterm and 22 kcal/kg at the end) may be the cause of malnutrition and resulting death. Actual protein intake of control and VLPD groups during the MDRD trial was 0.73 g/kg and 0.66 g/kg, respectively; hence, protein deficiency should not occur if the energy intake is sufficient.

In the present study, the CKD group maintained a VLPD (less than 0.4 g/kg body weight) for 7 years on average, and eGFR appeared to remain at the low level over many years. Three patients began hemodialysis, but have since maintained a low protein diet, compensating for the protein estimated to be lost in dialysis. They opted for only two weekly sessions of hemodialysis instead of the normal three sessions. One patient with a polycystic kidney started restriction of dietary protein early in their course of PKD, which seemed to slow disease progression.[12]

Good awareness by patients and families about the importance of protein restriction and sufficient calorie intake ensured a high level of compliance to the low protein diet. Energy requirements were calculated in the form [body weight x 0.4] energy units, where one energy unit is defined as the energy needed to melt 1 kg of ice (80 kcal).[20,21] Instruction in cooking methods using low-protein foodstuffs, particularly low-protein rice (containing $1/25^{th}$ of the protein in ordinary rice) and bread and noodles made from the low protein rice powder, was found to be helpful.[22] Japanese people consume on average 60 g of protein per day according to the National Dietary Survey,[23] about 30 g of which is derived from rice, so substitution of low protein rice can easily be used to reduce the protein intake. The amino acid score of rice is 85, higher than bread made from wheat powder which has a score of 45-50. Rice and soy-based foods form the bulk of the Japanese diet, making it simple to achieve a lower protein intake than in Western countries. Suitable amounts of protein-containing foods, such as meat, fish and eggs,

can be used to make the diet more acceptable.

Long term VLPD at 0.4 g/kg body weight was found not to cause sarcopenia or osteoporosis in our CKD patients. They led normal daily lives, and did not have a lean body mass different from control groups. Only slight anemia occurred. CKD patients usually suffer from hyperkalemia, hyperphosphatemia, hyperuremia, high uric acid etc., but values remained within normal levels for the VLPD. Vitamin and mineral intakes were lower than controls, but no recognizable symptoms from nutrient deficiency occurred. The values were far less than the corresponding dietary reference intakes (DRIs), indicating the importance of tailor-made nutrition based on the individual non-deficient level. Common deficiencies in CKD patients are said to include 1,25 dihydroxycholecalciferol, vitamin B_6, folic acid, vitamin C, iron and possibly carnitine and zinc.[18] These values are often compared with the corresponding DRIs, although the adequate or recommended doses are typically set high to avoid deficiencies in these nutrients. Minimum requirements have not been proposed yet. Tailor-made nutrition based upon individual requirements is thought to be necessary for CKD patients, whether or not they require supplements.

Another factor proposed by Menon et al.[19] in their long term follow-up study of MDRD was toxicity of amino acid-ketoacid supplements. Amino acid supplement was often used for VLPD[24-26].

Comparison of free amino acid profiles of plasma and urine showed interesting findings. In general, excretion of most amino acids in urine was lower, with some completely absent. It is not determined whether this was due to VLPD, or to changes in secretion from the glomeruli and/or increased reabsorption from the renal tubules.

High citrulin concentration in both plasma and urine may reflect increased production of ammonia inside the body and lowered ability to excrete urea. Citrulin appears to be a rate-limiting amino acid in the urea cycle; hence, ornithin supplementation as in MDRD study should increase overloading of the cycle. Increased urine excretion of BCAA and a corresponding decreased plasma level is also noteworthy, suggesting increased activity of alpha-keto valerinate dehydrogenase, deficiency of which is known to cause maple syrup urine disease[27].

Decreased histidine in both plasma and urine, increased methyl derivatives and absence of detectable carnosin (β-alanyl-L-histidine) and anserine (β-alanyl N(p)

methyl-L-histidine) demonstrates changes in histidine metabolism by CKD. Carnosine is a dipeptide of the amino acids β-alanine and histidine, with a number of antioxidant properties that may be beneficial[28]. β-Alanine is formed *in vivo* from the degradation of carnosine; its supplementation has been shown to increase carnosine concentration in muscles, decrease fatigue in athletes and increase total muscular work output. It is a component of the naturally occurring peptides carnosine and anserine, and also of pantothenic acid (vitamin B_5) which itself is a component of coenzyme A. It was only present in plasma of hemodialytic patients.

Ethanolamine, low in hemodialytic patients, is the second-most-abundant head group in membrane phospholipids. Monoethanolamine is produced by reacting ethylene oxide with aqueous ammonia. Aminoisobutyric acid is a strong helix inducer in peptides; 2-aminoisobutyric acid may be synthesized from acetone cyanohydrin, by reaction with ammonia followed by hydrolysis.[29] An increase in aminoisobutyric acid was observed in both urine and plasma of CKD patients.

Further study is necessary to clarify the metabolic changes of amino acids in CKD at critical levels of protein restriction. Many changes are thought to occur inside the body, so administration of amino acid supplements should be more carefully considered.

In addition to the increased creatinine, proBNT was very high among the patients. It is believed to be a risk factor for cardiac disease,[30] but it is necessary to clarify whether proBNT is a biomarker for kidney damage, like β_2 microglobulin.

Conflicts of interest

We affirm there to be no conflicts of interest with any company in relation to this study.

Acknowledgement

The authors appreciate members of "Adequate Protein Intake Group", and thank Ms. K. Motegi and Li for revising our manuscript. A part of this work was supported by the Nutritional therapy panel of the Society of Anti Aging Medicine.

References

1. Mitch WE. Proposed mechanisms for changes in progression by dietary manipulation. In Hatano M, ed. *Nephrology.* Tokyo: Springer-Verlag; 1991:775-782.
2. Kopple JD, Massry SG. *Nutritional Management of Renal Disease.* Baltimore: Williams & Wilkins; 1996.
3. Bernhard J, Beaufrère B, Laville M, et al. Adaptive response to a low-protein diet in predialysis chronic renal failure patients. *J Am Soc Nephrol.* 2001;12(6):1249-1254.
4. Fouque D, Laville M, Boissel JP, Chifflet R, Labeeuw M, Zech PY. Controlled low protein diets in chronic renal insufficiency: meta-analysis. *BMJ.* 1992;304(6821):216-220.
5. Fouque D, Laville M, Boissel JP. Low protein diets for chronic kidney desease in non diabetic adults. *Cochrane Database Syst Rev.* 2006;(2):CD001892.
6. Klahr S, Levey AS, Beck GJ, et al. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. *N Engl J Med.* 1994;330(13):877-884.
7. Kopple JE, Levey AS, Greene T, et al. Effect of dietary protein restriction on nutritional status in the Modification of Diet in Renal Disease Study. *Kidney Int.* 1997;52(3):778-791.
8. Levy AS, Greene T, Beck GJ, et al. Dietary protein restriction and the progression of chronic renal disease: what have all of the results of the MDRD study shown? Modification of Diet in Renal Disease Study Group. *J Am Soc Nephrol.* 1999;10(11):2426-2439.
9. Japan Nephrology Society. Evidence-based Guideline for the Treatment of CKD. Tokyo Igaku-sha, Tokyo, 2009
10. Ideura, T, et al. Protein intake of more than 0.5 g/kg BW/day is not effective in suppressing the progression of chronic renal failure. Contrib Nephrol. Karger, 2007; 155: 40-49.
11. Watanabe S, FFF database. http://www.nihn.go.jp/
12. Haarbo J, Gotfredsen A, Hassager C, and Christiansen C. Validation of body composition by dual energy X-ray absorptiometry (DEXA). *Clin Physiol.* 1991;11(4):331-341.
13. Miyachi M, Tanaka H, Yamamoto K, Yoshioka A, Takahashi K, Onodera S. Effects of one-legged endurance training on femoral arterial and venous size in healthy humans. *J Appl Physiol.* 2001;90(6):2439-2444.
14. Sanada K, Miyachi M, Tabata I, et al. Muscle mass and bone mineral

indices: does the normalized bone mineral content differ with age? *Eur J Clin. Nutr.* 2009;63(4):465-472.

15. Miyatani M, Kawano H, Maswani K, Gando Y, Yamamoto K, Tanimoto M, Oh T-W, Usui C, Sanada K, Higuchi M, Tabata I, Miyachi M. Required muscle mass for preventing lifestyle-related diseases in Japanese women. BMC Public Health 2008; 8:291. http://biomedcentral.com/1471-2458/8/291
16. Matsuo S, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009; 53:982-992.
17. SPSS for Windows Ver 14. Mariya J. Norusis, SPSS Inc. Tokyo, 200
18. Mitch WE, Ikizler TA. *Handbook of Nutrition and the Kidney.* 6th ed. Philadelphia, PA: Lippincott, William & Wilkins, a Wolters Kluwer Business; 2009.
19. Menon V, Kopple JD, Wang X, et al. Effect of a very low-protein diet on outcomes: Long-term follow-up of the Modification of Diet in Renal Disease (MDRD) Study. *Am J Kid Dis.* 2009;53(2):208-217.
20. Watanabe S. Tailor made nutrition (2). A simple food scheme for representation of personal energy intake. Clin Funct Nutr 2009; 1(2):
21. Watanabe S. Tailor-made nutrition (3). Easy access for the individual energy requirement. Clin Funct Nutr 2009; 1(3):
22. Watanabe S. Low Protein Diet for saving Kidney Function. Shufu-no-tomo, Tokyo, 2009,
23. Ministry of Health, Labour and Welfare, National Nutrition Survey 2005, Tokyo, http://www.nih.go.jp/
24. Coresh J, Walser M, Hill S. Survival on dialysis among chronic renal failure patients treated with a supplemented low-protein diet before dialysis. *J Am Soc Nephrol.* 1995;6(5):1379-1385.
25. Vendrely B, Chauveau P, Barthe N. Nutrition in hemodialysis patients previously on a supplemented very low protein diet. *Kidney Int.* 2003;63(4):1491-1498.
26. Walser M, Hill S. Can renal replacement be deferred by a supplemented very low protein diet? *J Am Soc Nephrol.* 1999;10(1):110-116.
27. Wendel U, Ogier de Baulny H. Branched-chain organic acidurias/acidemias. In: Fernandes J, Saudubray J-m, van den Berghe G, Walter JH, eds. *Inborn Metabolic Diseases: Diagnosis and Treatment.* 4th ed. New York, NY: Springer; 2006:chap 19.

28. Babizhayev MA, Seguin MC, Gueyne J, Evstigneeva RP, Ageyeva EA, Zheltukhina GA. "L-carnosine (beta-alanyl-L-histidine) and carcinine (beta-alanylhistamine) act as natural antioxidants with hydroxyl-radical-scavenging and lipid-peroxidase activities". *Biochem. J.* 1994; 304 (Pt 2): 509–16.

29. Rawn JD. Chapter 18. Resolution of Amino Acid and Urea Cycle. In; Biochemistry, Carolina Biological Supply Co. Ltd., 1989

30. Ross G, Bever F, Uddin Z, Devireddy L, Gardin J. "Common scenarios to clarify the interpretation of cardiac markers". *J Am Osteopath Assoc* 2004; 104 (4): 165–76.

Table 1

Table 2

Table 3

Table 4

Original Article

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

Kyoko Taku PhD[1], Melissa K Melby PhD[2], Jun Takebayashi PhD[3], Shoichi Mizuno PhD[4], Yoshiko Ishimi PhD[3], Toyonori Omori PhD[5], Shaw Watanabe PhD[2]

[1]*Information Center, National Institute of Health and Nutrition, Tokyo, Japan*
[2]*Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan*
[3]*Food Function and Labeling Program, National Institute of Health and Nutrition, Tokyo, Japan*
[4]*Center for Collaboration and Partnership, National Institute of Health and Nutrition, Tokyo, Japan*
[5]*Director for Research Coordination and Evaluation, National Institute of Health and Nutrition, Tokyo, Japan*

This study was conducted to clarify the effect of ingesting soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. PubMed, CENTRAL, ICHUSHI, CNKI, Wanfang Data, CQVIP, and NSTL were searched for randomized controlled trials published in English, Japanese, or Chinese reporting the effects of soy isoflavone extracts on lumbar spine or hip BMD in menopausal women. Trials were identified and reviewed for inclusion and exclusion eligibility. Data on study design, participants, interventions, and outcomes were extracted. Eleven, seven, five, and five trials were finally selected for estimation of the effects on spine, femoral neck, hip total, and trochanter BMD, respectively. Meta-analysis including data from1240 menopausal women revealed that daily ingestion of an average of 82 (47–150) mg soy isoflavones (aglycone equivalent) for 6–12 months significantly increased spine BMD by 22.25 mg/cm^2 (95% CI: 7.62, 32.89; p=0.002), or by 2.38% (95% CI: 0.93, 3.83; p=0.001) compared with controls (random-effects model). Subgroup analyses indicated that the varying effects of isoflavones on spine BMD across trials might be associated with study characteristics of intervention duration (6 vs. 12 months), region of participant (Asian vs. Western), and basal BMD (normal bone mass vs. osteopenia or osteoporosis). No significant effects on femoral neck, hip total, and trochanter BMD were found. Soy isoflavone extract supplements increased lumbar spine BMD in menopausal women. Further studies are needed to address factors affecting the magnitudes of effect on spine and to verify the effect on hip.

Key Words: meta-analysis, isoflavones, dietary supplements, menopause, bone density

INTRODUCTION

Osteopenia and osteoporosis are major health problems in postmenopausal women, who experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling.[1,2] The yearly decline in bone mineral density (BMD) of the lumbar spine and hip in postmenopausal women is reported to be at least 1% and up to 2.4%.[1,3] Although hormone replacement therapy (HRT) has positive effects in increasing BMD in postmenopausal women with low bone mass,[1,4] it is associated with a higher risk of hormone-related cancer[5-7] and other unfavorable adverse events.[8,9]

Epidemiological studies indicate that women who have high soy intake have a lower risk of osteoporosis than women who consume a typical Western diet.[10-12] Consequently, many menopausal women use phytoestrogens to maintain their BMD because they are unlikely to cause the undesirable effects associated with steroid hormones.[8,13] The primary dietary phytoestrogens ingested are soy isoflavones, which have structures similar to that of estrogen.[14]

A meta-analysis of randomized controlled trials (RCTs) has estimated the effect of ingesting soy isoflavones on lumbar spine BMD.[15] This included 10 RCTs of both soy isoflavone tablets and isolated soy protein containing isoflavones, and revealed a significant increase of BMD by 20.6 mg/cm^2 (magnitude in term of percentage and effect on hip not presented) resulting from soy isoflavones. Given the result in units of mg/cm^2, whether the magnitude of increase can prevent the naturally occurring postmenopausal bone loss remains unclear. Subgroup analysis of three trials testing isoflavone tablet revealed no significant effect, however one trial testing soy isoflavone extract was mistakenly included in the isolated soy

Corresponding Author: Dr Kyoko Taku, Information Center, National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan.
Tel: +81-3-3203-5721; Fax: +81-3-3202-3278
E-mail: takuk@nih.go.jp
Manuscript received 29 May 2009. Initial review completed 8 September 2009. Revision accepted 5 October 2009.

protein subgroup.[16] In addition, two[17] and three[18] comparisons from the same trial respectively with two and three soy isoflavone groups compared to the same control group were included simultaneously as separate studies in the meta-analysis. This is not recommended because it is considered to induce a serious unit-of-analysis problem.[19] Another recently published meta-analysis included 10 RCTs of soy isoflavones supplementation of at least one year duration (four RCTs testing isoflavones extracts), and did not find significantly beneficial effects of soy isoflavones on spine and hip BMD.[20]

Supplements of soy isoflavone extracts were easily ingested by the people who want to benefit from soy isoflavones, but are unable to usually consume and/or do not like to intake products of soy protein or soy foods containing isoflavones. In addition, the beneficial effects of soy protein might require synergistic reactions between isoflavones and other soy components.[15] Thus, clarifying the effects of extracted soy isoflavones (not as a constituent part in soy protein) is of more clinically important. However, both the two meta-analyses failed to reveal significant effects of soy isoflavone extracts in subgroup analysis, which might be due to the fact that only data from four RCTs were included.[15,20] We have identified 12 RCTs of soy isoflavone extracts (not of soy protein or foods containing isoflavones) that reported effects on spine BMD in menopausal women,[8,16-18,21-29] and performed the present meta-analysis to clarify the effects of soy isoflavone extract both in terms of change (mg/cm^2) and percentage change (%) from baseline for lumbar spine and hip BMD, without influence on the same parameters by soy protein per se or other components in soy protein.

MATERIALS AND METHODS

PubMed (1966–2008), CENTRAL (1966–2008), ICHUSHI (1983–2008), and CNKI (1979–2008) were searched for relevant studies that had been published by September 2008. We also searched Wanfang Data, CQVIP and NSTL, which are other major search engines in China. Reference lists of relevant studies were manually searched. Studies were eligible for inclusion if they met all of the following criteria: (1) randomized parallel-group controlled trials published in English, Japanese, or Chinese; (2) trials with a crossover design that contained data for the first period;[19,30] (3) tested the effects of ingesting supplements of soy isoflavone extracts (not of soy protein or foods containing isoflavones) on lumbar spine or hip (femoral neck, total hip, or trochanter) BMD in menopausal women; and (4) BMD data were measured by dual X-ray absorptiometry. When duplicate data were reported for the same study subjects, only the article with the largest sample was included.[19] Two reviewers independently reviewed and evaluated the studies, and consensus was reached by discussion when there were disagreements.

Data on study design, number of participants, interventions, and outcomes for BMD were also independently extracted by two reviewers and confirmed by each other. When necessary, data on outcomes for BMD were obtained from graphs. If possible, we obtained necessary data not reported in the articles by contacting to the authors. We calculated mean change (follow-up − baseline) and percentage change [(follow-up − baseline) ÷ baseline × 100%] from baseline in BMD, when the data were not directly available. We primarily determined missing SD of the changes if statistical analyses comparing the changes themselves were presented (e.g., confidence intervals, standard errors, t values, p values, F values). Alternatively, we imputed them by computing mean correlations between the baseline and final values from included trials in which SD for change, as well as for baseline and final measurements were available.[19] Standard deviation for percentage change was calculated by dividing SD for change with mean baseline value.

We used the Jadad scale to assess the quality of included RCTs, a score of < 3 indicating low quality.[31] We also used a 3-category grading system (A, B, C) to denote the methodological quality of each study.[32] Category A studies have the least bias and results are considered valid; B studies are susceptible to some bias, but not sufficient to invalidate the results; and C studies have significant bias that may invalidate the results. We arbitrarily defined category C as of low quality. Concealment of treatment allocation in RCTs was assessed as adequate, inadequate or unclear.[33] Two reviewers independently assessed the studies, and consensus was reached by discussion when there were disagreements.

We performed meta-analysis to determine the overall treatment effect of soy isoflavones on BMD, using the weighted mean difference method in Review Manager (version 5.0.20; Nordic Cochrane Center, Oxford, England). Treatment effect of each trial was estimated as the mean difference between changes (or percentage changes) from baseline in BMD for each comparison group (i.e., the change from the baseline for participants ingesting soy isoflavones minus that for controls). When data of more than one time points for the same trial were reported in one article or reported separately in two articles, we primarily used the data set for the short duration in order not to induce unit-of-analysis error. The data set for other time points were used for sensitivity analysis to prevent reporting bias. For trials had more than one isoflavone group compared with one control group, we combined the multiple isoflavones groups into a single group for each of these trials without inducing unit-of-analysis error.[34]

We used both a fixed effect model or a random effects model to calculate weighted mean differences (WMD), 95% CIs for each comparison, a combined overall effect with p-value, and the p-value for testing heterogeneity ($p < 0.1$ was considered significant); when there was significant heterogeneity across included trials, the results based on the random effects model were shown.[19,30,35]

We conducted sensitivity analyses to evaluate the effects of degree of correlation between baseline and final values, time point of measurement (using data for long duration instead of data for short duration in trials with multiple time points of evaluation), study design (selecting only placebo-controlled trials), and study quality (eliminating low-quality trials). If at least 10 trials were available, subgroup analyses and meta-regressions were performed to investigate possible factors that might related to varying effects of soy isoflavones on BMD across trials, on the basis of pre-specified factors of intervention

duration, isoflavone dosage, region of participants, and basal BMD.[15,20] We used a cut-off point of 75 mg/day in subgroup analysis for isoflavone dosage, because daily isoflavone intake of up to 75 mg (aglycone form) is considered safe by the Japan Food Safety Commission. Significant tests based on test for heterogeneity, chi-squared statistics, were performed to investigate differences between two subgroups.[19,34] We examined potential publication bias by using funnel plots and by performing Egger's test to assess the asymmetry of funnel plots. Meta-regressions and Egger's test were respectively performed with the use of user-written "metareg" and "metabias" commands for Stata 10.1 for Windows (StataCorp LP, College Station, Tex).

RESULTS

The search strategy (Figure 1) yielded 16 potentially appropriate reports of RCTs to be included in the meta-analysis. After excluding one article[36] reporting only duplicate femoral data that had appeared in another article,[25] and two articles[37,38] describing a smaller sample than that analyzed in another article,[23,17] 13 articles on 12 trials were included for meta-analysis.[8, 16-18, 21-29] Two articles reported outcomes for durations of six months[27] and one year[28] for the same trial participants.

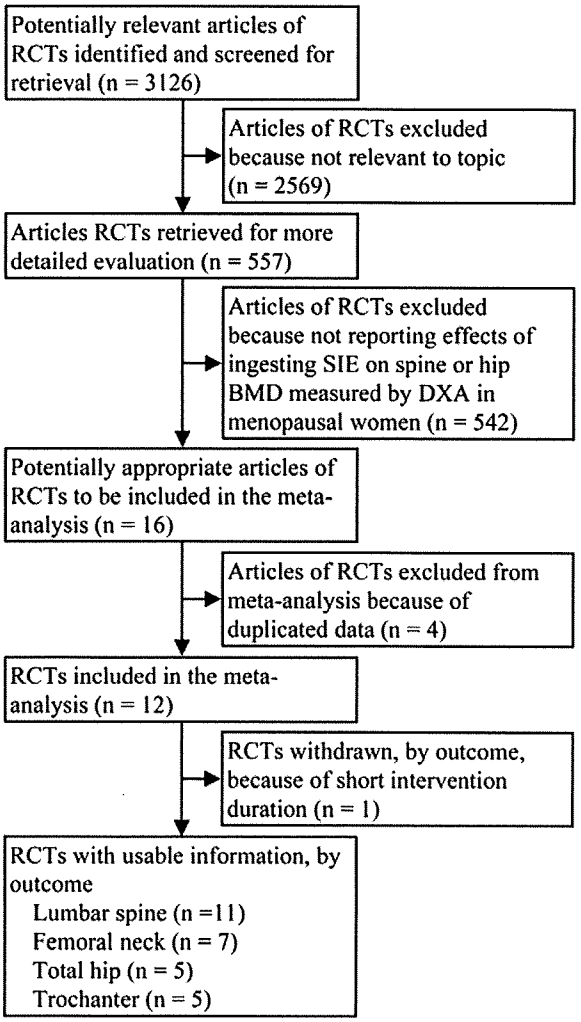


Figure 1. Search and selection of trials. Abbreviations: RCTs, randomized controlled trials; BMD, bone mineral density; SIE, soy isoflavone extracts, DXA, dual X-ray absorptiometry.

The characteristics of 12 trials are summarized in Table 1. Two articles for each trial contained data for two time points.[21,25] Three trials tested two isoflavone groups[17,22,24] and one tested three isoflavone groups[18] compared with one identical control group. One trial did not address the form and composition of soy isoflavones tested,[18] we assumed the dose as aglycone equivalent to calculate the mean dosage. Four, six, and two trials included participants of normal bone mass (T-score > –1 SD, corresponds to BMD > 0.937g/cm^2), low bone mass or osteopenia (–1 SD ≥ –2.5, corresponds to 0.937 g/cm^2 ≥ BMD ≥ 0.772 g/cm^2), and osteoporosis (T-score < –2.5 SD, corresponds to BMD < 0.772 g/cm^2) on the basis of averaged basal spine BMD, respectively.[39] Only one trial was assessed as "adequate" for concealment of treatment allocation,[22] and the remaining trials were assessed as "unclear" due to insufficient information. Participants in the comparison groups had similar dietary intakes of soy isoflavones, calcium, and vitamin D and physical activities. Most of the studies were designed to maintain the participants' usual diets, lifestyle and body weight. Adverse events were generally similar for both the isoflavone and control groups and no serious adverse events were noted in the included trials, although they were not well addressed in several trials.

Because bone is a slowly responding organ, a complete bone remodeling cycle takes up to 6 months, and therefore a study duration of less than 6 months is not sufficient to evaluate the effect of any intervention on bone BMD.[28] Thus, one 3-month trial of low-quality that reported negative effect of soy isoflavones on spine BMD was then withdrawn.[26] From 3126 relevant articles identified, 11,[8,16-18,21-25,27-29] 7,[8,17,22-25,27,28] 5,[16,17,22,27-29] and 5[17,22-24,27,28] trials were finally selected for estimating the effects on lumbar spine, femoral neck, total hip, and trochanter BMD, respectively (Figure 1, Table 1). Fourteen correlation coefficients between baseline and follow-up values were calculated from 5 reports of 4 trials,[17,23,24,27,28] which were consistent and resulted in a mean value of 0.98 (0.96–1).

Meta-analysis of the 11 trials with 1240 participants using the fixed effect model resulted in significant heterogeneity ($p < 0.001$), and revealed that daily ingestion of an average of 82 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year significantly increased lumbar spine BMD by 12.08 mg/cm^2 (95% CI: 9.83, 14.33 mg/cm^2; $p < 0.001$), or by 1.47% (95% CI: 1.21, 1.74%; $p < 0.001$) compared with controls. Meta-analysis using the random effects model, revealed an significant overall effect of soy isoflavones in increasing spine BMD by 20.25 mg/cm^2 (95% CI: 7.62, 32.89 mg/cm^2, $p = 0.002$), or by 2.38% (95% CI: 0.93, 3.83%, $p = 0.001$; Figure 2). Of the11 selected trials, 7 trials revealed significant positive mean difference between changes or percentage change from baseline in spine BMD for isoflavone and control groups (favors isoflavone). The mean difference was negative at 27-week time point and was positive at 53-week time point in one trial,[21] the mean difference at 2-year duration was about two times of that at 1-year time point;[25] whereas, the

Table 1. Characteristics of included randomized controlled trials

Study	Design[†]	Follow-up	Participants[‡]	Intervention[§]	Baseline mean BMD outcomes (g/cm^2)[¶]	Jadad scale	Quality category
Brink 2008[21]	P; R, DB+, WD	27, 53 wk	N: 300/237 (21%) PoW; mean age: 53 y; TSM = 33 (12–60) mo; non-osteoporotic (spine Z-score ≥ 2); Netherlands, Italy, France	110 mg IAE [25–35% De, 60–75% Ge, 1–5% Gle] vs. placebo	L1–4: 0.990, mean (SD) T-score = −0.0 ± 1.1	4	C (dropout > 20%)
Chen 2003[22]	P; R+, DB+, WD	1 y	N: 203/175 (14%) PoW; mean age: 54.2 y; TSM = 4.1 (1–10) y; Hong Kong	40 and 80 mg IAE [46% De, 15% Ge, 39% Gle] vs. placebo	L1–4: 0.860; FN: 0.682; TH: 0.819; Tr: 0.605	5	A
Dong 2008[23]	P; R, WD	12 mo	N: 60/52 (13%) PoW; mean age: 54.7 y; TSM = 6.2 (≥ 1) y; T-score < −1.5 China	100 mg IC [66 mg IAE: 39% De, 61% Ge, 1% Gle] + calcium vs. calcium only (control)	L2–4: 0.756; FN: 0.719; Tr: 0.552	2	B
Gao 2006[18]	P; R	24 wk	N: 50/50 PoW; age: 48–62 y; TSM ≥ 1y; China	60, 90, and 150 mg IF vs. no-treatment (control)	L1–4: 0.974	1	B
Harkness 2004[16]	CO; R+, DB, WD	6 mo × 2	N: 20/19 (5%) PoW; mean age: 70.6 y; TSM = 19.1 (> 8) y; T-score < 2.5; USA	110 mg IAE [40% De, 52% Ge, 9% Gle] vs. placebo	L1–4: 0.881; TH: 0.800	4	B
Huang 2006[24]	P; R, OL, WD	1 y	N: 43/42 (2%) PoW; mean age: 52.4 y; TSM = 4.4 (1–13) y; Taiwan	100 and 200 mg IAE [29% De, 71% Ge] vs. regular diet only (control)	L1–4: 0.881; FN: 0.812; Tr: 0.715	2	B
Marini 2007[25]	P; R+, DB+, WD	12, 24 mo	N: 389/389 (10, 22%) PoW; mean age: 54.5 y; TSM = 63 mo (≥ 1 y); femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.840; FN: 0.670	5	A, C (dropout > 20%)
Morabito 2002[8]	P; R, DB+	1 y	N: 90/90 PoW; mean age: 51.5 y; TSM = 6.5 (≥ 1) y; femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.925; FN: 0.688	3	A
Uesugi 2003[26]	P; R, WD	3 mo	N: 22/21 (4%) PoW; mean age: 53.7 y; TSM = 6 (5–10) y; non-osteoporosis; Japan	62 mg IC [38 mg IAE: 52% De, 11% Ge, 37% Gle] vs. placebo	L2–4: 1.040	2	C (unclear analyzed N)
Wu 2006a[27], b[28]	P; R, DB+, WD	6, 12 mo	N: 136/128, 108 (6, 21%); mean age: 54.4 y; TSM = 3.2 (1–5) y; Japan	75 mg IC [47 mg IAE: 54% De, 13% Ge, 34% Gle] vs. placebo	L2–4: 0.899; FN: 0.672; TH: 0.782; Tr: 0.595	4	A, C (dropout > 20%)
Xin 2006[29]	P; R, DB	6 mo	N: 76 MW; age: 45–55 y; TSM ≤ 5 y; China	50 mg pure De + calcium vs. calcium only (control)	L2–4: 0.715; TH: 0.643	2	C (unclear analyzed N)
Ye 2006[17]	P; R+, SB, WD	6 mo	N: 90/84 (7%) PoW; mean age: 52.3 (1–5) y; TSM = 2.6 (1–5) y; China	84 and 126 mg IAE [52% D(e), 15% G(e), 33% Gl(e)] vs. placebo	L1–4: 0.864; FN: 0.702; TH: 0.800; Tr: 0.588	3	B

[†]CO, crossover; DB, double-blinded (gives 1 point to Jadad scale); DB+, double-blinded by appropriate method (gives 2 point); OL, open-labeled; P, Parallel; R, randomized (give 1 point); R+, randomized by appropriate method (gives 2 point); SB, single-blinded; WD, withdrawals and dropouts described (gives 1 point).
[‡]BMD, bone mineral density; N, randomize/analyzed number (dropout rate) of participants; MW, menopausal women; PoW, postmenopausal women; TSM, averaged time since menopause.
[§]IAE, isoflavone aglycone equivalents; IC, isoflavone conjugate containing glycoside and aglycone forms; IF, isoflavones (form and composition unknown); D(e), daidz(e)in; De, daidzein; Ge, genistein; G(e), genist(e)in; Gl(e), glycit(e)in; Gle, glycitein.
[¶]FN, femoral neck; L, lumbar spine; TH, total hip; Tr, trochanter.

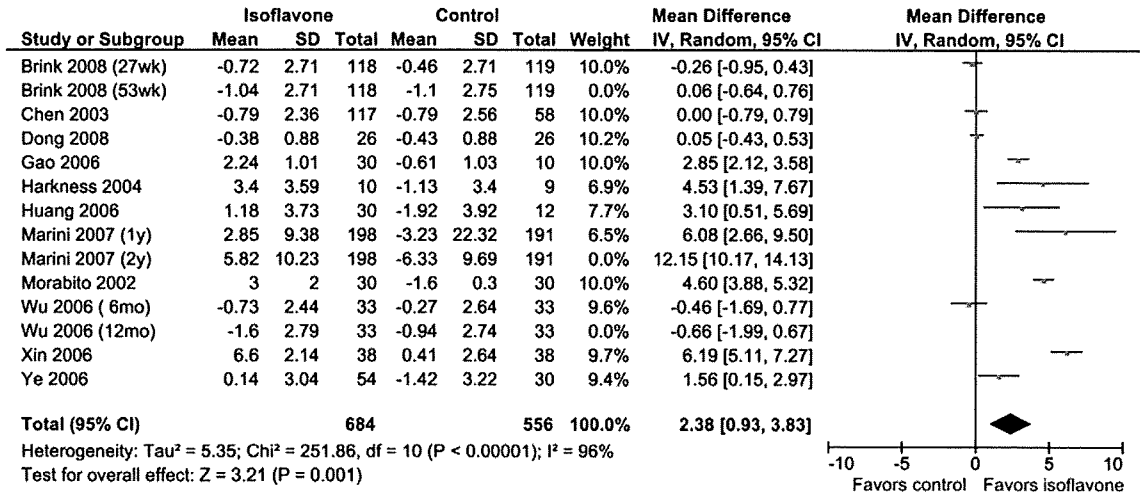


Figure 2. Effects of soy isoflavones on spine BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% Weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

mean difference for 6 months duration[27] was similarly negative to that for 1 year duration.[28]

Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, using data sets of longer duration instead of short duration for trials with two time points of measurements, selecting only placebo-controlled trials, and eliminating low-quality trials (Jadad scale < 3 or Category C) did not result in significantly different overall effects of soy isoflavones on spine BMD.

Results of subgroup analyses of the effects of soy isoflavones on spine BMD were shown in Table 2. Each subgroup analysis resulted in significant heterogeneity and revealed significant effect of soy isoflavones in increasing spine BMD compared with controls using the fixed effect model. Results based on fixed effect model revealed that effects of soy isoflavones on spine BMD in subgroups of 6 months duration and of Asian region were significantly different with the effects in subgroup of 1 year duration and of Western region, respectively. Two subgroups of each subgroup analysis using the random effects model, show similarly significant effects of soy isoflavones in increasing spine BMD, except for a subgroup of participants with normal bone mass at baseline. Meta-regressions analyzing each of or all of the four pre-specified categorical study characteristics (intervention duration, isoflavone dosage, region of participants, and basal spine BMD), did not reveal that these pre-specified factors were significantly associated with the varying effects of soy isoflavones on spine BMD across trials. The funnel plots (Figure 3) and Egger's test of effects of soy isoflavones on spine BMD among the 11 trials (p = 0.251 and p = 0.267 for effects in terms of change and percentage change, respectively) did not indicate any obvious publication bias.

Meta-analysis of the 7 trials with 868 participants using the fixed effect model resulted in significant heterogeneity ($p < 0.001$). Meta-analysis using the random effects model, revealed that daily ingestion of an average of 76 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly increased femoral neck BMD by 10.24 mg/cm^2 (95% CI: −3.73, 24.20 mg/cm^2, p = 0.15), or by 1.48% (95% CI: −0.54, 3.50%, p = 0.15) compared with controls. Sensitivity analysis assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, did not result in significantly different overall effects of soy isoflavones on femoral neck BMD. Whereas, sensitivity analysis using data sets of longer duration for trials with two time points of measurements, found that ingestion of soy isoflavones for 6 months to 2 years tended to increase femoral neck BMD by 16.89 mg/cm^2 (95% CI: −2.34, 36.11 mg/cm^2, p = 0.09), or by 2.45% (95% CI: −0.31, 5.21, p = 0.08; Figure 4) compared with controls (random effects model). Sensitivity analyses selecting only placebo-controlled trials and eliminating low-quality trials were not performed because of the small number of available trials.

Meta-analysis of the 5 trials with 420 participants using the fixed effect model resulted in non-significant heterogeneity ($p \geq 0.1$), revealed that daily ingestion of an average of 74 (47–110) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change total hip BMD by 2.45 mg/cm^2 (95% CI: −1.41, 6.30 mg/cm^2, p = 0.21), or by 0.05% (95% CI: −0.53, 0.63%, p = 0.86) compared with controls. Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5 and using data sets of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on total hip BMD.

Meta-analysis of the 5 trials with 419 participants revealed that daily ingestion of an average of 85 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change trochanter BMD by −0.40 mg/cm^2 (95% CI: −6.58, 5.78 mg/cm^2, p = 0.90), or by −0.07% (95% CI: −1.15, 1.02%, p = 0.91) compared with controls (random effects model). Sensitivity analyses assuming the level of correlation coefficient between

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.

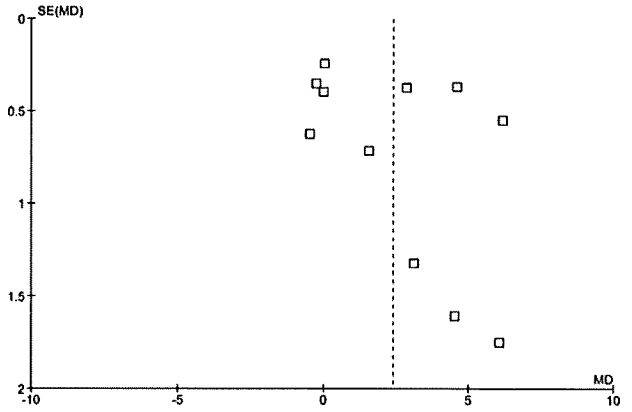


Figure 3. Funnel plots of effects of soy isoflavones on spine BMD (%). MD, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; SE (MD), standard error of MD; fixed, fixed effect model.

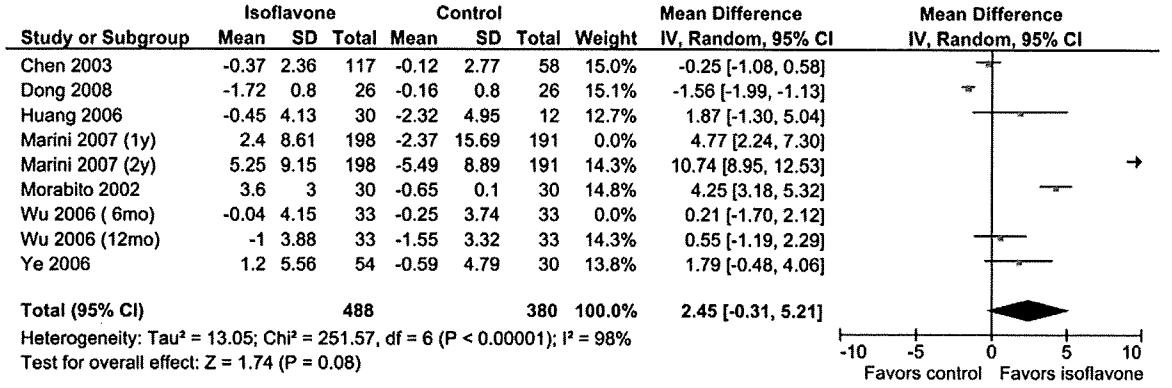


Figure 4. Effects of soy isoflavones on femoral neck BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of femoral neck bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets 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.

ACKNOWLEDGEMENTS

This study was supported in part by a grant from the Fuji Foundation for Protein Research, Osaka, Japan.

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

Kyoko Taku PhD[1], Melissa K Melby PhD[2], Jun Takebayashi PhD[3], Shoichi Mizuno PhD[4], Yoshiko Ishimi PhD[3], Toyonori Omori PhD[5], Shaw Watanabe PhD[2]

[1]*Information Center, National Institute of Health and Nutrition, Tokyo, Japan*
[2]*Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan*
[3]*Food Function and Labeling Program, National Institute of Health and Nutrition, Tokyo, Japan*
[4]*Center for Collaboration and Partnership, National Institute of Health and Nutrition, Tokyo, Japan*
[5]*Director for Research Coordination and Evaluation, National Institute of Health and Nutrition, Tokyo, Japan*

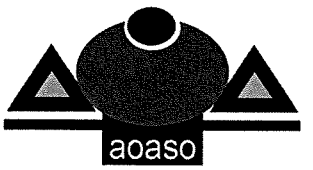
大豆異黃酮抽取物的補充劑對停經後婦女骨質密度的效果：隨機對照試驗的後設分析

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

Obesity Research & Clinical Practice (2009) 3, 179–191

ELSEVIER



http://www.iotf.org/oonet/aoaso.htm

ORIGINAL ARTICLE

Preproghrelin gene polymorphisms in obese Japanese: Association with diabetes mellitus in men and with metabolic syndrome parameters in women

Jun Takezawa, Kouichi Yamada*, Akemi Morita, Naomi Aiba, Shaw Watanabe

Program of Educational Nutrition, The National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan

Received 25 February 2009; received in revised form 3 April 2009; accepted 23 April 2009

KEYWORDS
Diabetes mellitus;
Ghrelin;
LDL cholesterol;
Polymorphism (SNP);
Visceral fat

Summary Preproghrelin gene polymorphisms (SNPs) are possible predisposing factors to obesity and metabolic syndrome. We analysed SNPs in obese Japanese individuals and studied the correlation with diabetes and metabolic syndrome. We recruited 235 subjects (BMI > 28.3) from individuals undergoing periodic medical check-up at Saku Central Hospital. Their SNPs were genotyped using PCR-RFLP method. Frequencies of 5 SNPs in the preproghrelin gene −1500C>G (rs3755777), −1062G>C (rs26311), −994C>T (rs26312), Leu72Met (+408C>A) (rs696217), and +3056T>C (rs2075356) were compared with healthy individuals (data from HapMap Project or Asian population studies). Associations between these SNPs and clinical parameters were investigated. The phenotypes evidently differed between men and women. In men, higher fasting glucose and HbA1c values were observed in the +3056C/C minor homozygotes without leptin or insulin accumulation. The +408C-+3056C haplotype was more frequent in the diabetic subgroup, in which diagnosis was based on fasting glucose, 75gOGTT, and HbA1c values, than normal subgroup. In contrast, in women, a significant correlation was observed between fat metabolism and obesity. The −1062C/C minor homozygotes had higher values of C-peptide, insulin, total and visceral fat area, waist circumference and BMI. The 72Met/Met minor homozygotes showed reduced leptin, total, HDL and LDL cholesterol concentrations and increased value of visceral fat area. Further, in the other SNPs, the minor homozygotes showed a similar trend, and the heterozygotes had intermediate values. Preproghrelin gene polymorphisms in obese Japanese may be predisposing

* Corresponding author. Tel.: +81 3 3203 5723; fax: +81 3 3203 0335.
E-mail address: kouichi@nih.go.jp (K. Yamada).

1871-403X/$ – see front matter
doi:10.1016/j.orcp.2009.04.003