Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials

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Objective: To clarify the effects of isoflavone intake on bone resorption and bone formation.

Methods: We identified randomized controlled trials related to urinary deoxypyridinoline (Dpyr, a bone resorption marker) and serum bone-specific alkaline phosphatase (BAP, a bone formation marker) listed on MEDLINE (January 1966–April 2006), the Cochrane Controlled Trials Register, EMBASE (1985–January 2006), Science Citation Index and PUBMED (updated till April 2006).

Results: Nine studies with a total of 432 subjects were selected for meta-analysis. The urinary Dpyr concentration in subjects who consumed isoflavones decreased significantly by −2.08 nmol/mmol (95% confidence interval CI): −3.82 to −0.34 nmol/mmol) in comparison with those in subjects who did not consume isoflavones. Isoflavone intake vs placebo intake significantly increased serum BAP by 1.48 μg/l (95% CI: 0.22–2.75 μg/l). Decreases in the urinary Dpyr concentration with isoflavone intake of < 90 mg/day and with treatment lasting less than 12 weeks were −2.34 nmol/mmol (95% CI: −4.46 to −0.22 nmol/mmol) and −2.03 nmol/mmol (95% CI: −3.20 to −0.85 nmol/mmol), respectively.

Conclusions: Isoflavone intervention significantly inhibits bone resorption and stimulates bone formation. These favorable effects occur even if < 90 mg/day of isoflavones are consumed or the intervention lasts less than 12 weeks.


Keywords: soy; isoflavone; osteoporosis; bone metabolism; deoxypyridinoline; bone-specific alkaline phosphatase

Introduction

With increases in life expectancy, osteoporosis has become a common disease in post-menopausal women. Although hormone replacement therapy (HRT) is the first choice of treatment for hormone-related osteoporosis, some results of randomized controlled trials (RCTs) of this treatment showed no bone protection or a significant reduction in the risk of hip fracture (Cauley et al., 2001; Rossouw et al., 2002). Furthermore, HRT has some negative side effects such as an increased risk of cardiovascular disease and breast cancer (Recker, 1993). Thus, new bone protection options are needed.

In recent years, isoflavones have received much attention in the medical and scientific literature. It is well known that the incidence of osteoporosis-related fracture is significantly lower in Southern and Eastern Asian women than in Western women (Ho et al., 1993; Tham et al., 1998). One possible reason for this difference is high intake of phytoestrogens; Asian people consume soy 10–20 times more than Western people (Kimura et al., 1998; Ho et al., 2003). Soy isoflavones comprise mainly genistein, daidzein and glycitein, which have structures similar to that of 17β-estradiol, a potential alternative to HRT (Knight and Eden, 1996). However, the effects of isoflavones on bone metabolism appear inconsistent in RCTs. Thus, a statistical method of combining...
these diverse data is needed to evaluate the usefulness of isoflavone therapy. Meta-analysis combines or integrates the results of several studies to provide increased statistical power for the quantitative identification of trends (Brockwell and Gordon, 2001). In the RCTs, urinary deoxyypyrindinoline (Dpyr) was generally used as a bone resorption marker, and serum bone-specific alkaline phosphatase (BAP) was the most commonly used index of bone formation. Therefore, we identified all reported RCTs related to the effects of isoflavones on Dpyr or BAP and analyzed the effects of isoflavones on bone metabolism quantitatively.

Materials and methods

MEDLINE (January 1966–April 2006), the Cochrane Controlled Trials Register, EMBASE (1985–January 2006), Science Citation Index and PubMed (updated till April 2006) were used to search articles that described RCTs investigating the effect of isoflavones on bone metabolism. Titles, abstracts and subject headings in the databases were searched with the help of the following keywords: bone, osteoporosis, bone metabolism, phytoestrogens, soy, isoflavones, genistein, daidzein, Dpyr or BAP. We also examined all references of related reviews and papers identified by the search. Additionally, we contacted the experts for the obtaining of unpublished data. Studies were selected for analysis if they met all of the following criteria: (1) subjects were limited to female; (2) subjects ingested soy products or isoflavones for at least 4 weeks; (3) the RCT included a parallel control group; and (4) Dpyr or BAP was used as an index of bone metabolism. If the study sample was found to overlap with that in another article or if two articles described aspects of the same study, only the publication with the largest sample group was used.

Two researchers extracted data independently. A data collection form was designed, and data were entered into the form twice to reduce input errors. The items entered in the form included participant characteristics, treatment duration, interventional dosage, and values of relevant indices before and after isoflavone or placebo treatments. Jadad scores were used to measure the quality of the RCTs (Jadad et al., 1996). Two reviewers rated study quality independently, there was (90%) agreement on Jadad scores. If the reviews disagreed, a final score was reached by discussion.

In this meta-analysis, we obtained the mean differences from the post-randomization baseline and post-treatment values for each trial, and calculated the pooled standard deviation of the mean differences according to the method of Yeung and Yu (2003). Weighted mean difference was calculated by subtracting the mean difference of the control group from that of the treatment group. The inverse variance method was used to pool the weighted mean difference with Review Manager 4.2 software (Nordic Cochrane Center, Oxford, England). Because the estimate from the random-effects model considers both intra- and inter-study variations, it is more conservative and hence more appropriate than an estimate from the fixed-effects model for an analysis such as this (Zhuo et al., 2004). Thus, we report results from the random-effects model. To assess the heterogeneity (apparent diversity in weighted mean differences across studies), we conducted a test based on \( \chi^2 \) distribution. The funnel plot was performed to detect publication bias.

Results and discussion

The trial flow chart is illustrated in Figure 1. Our literature search identified 31 RCTs. Twenty-two studies were excluded because of lack of indices of interest (Agnusdei et al., 1997a, b; Gambacciani et al., 1997a, b; Potter et al., 1998; Aleksi et al., 2000; Wangen et al., 2000; Clifton-Bligh et al., 2001; Katase et al., 2001; Anderson et al., 2002; Chiuchi et al., 2002; Lucas et al., 2002; Chen et al., 2003, 2004; Jones et al., 2003; Schult et al., 2003; Atkinson et al., 2004; Harkness et al., 2004; Olsen et al., 2004; Mori et al., 2004a, b), non-randomization (Dalais et al., 1998), lack of a control group (Agnusdei et al., 1997a, b), insufficient original data or baseline values (Gambacciani et al., 1997a, b; Khalil et al., 2002). Thus, nine studies with a total of 432 subjects were included in this meta-analysis, in which five studies had quality score of five; three studies had quality score of four and one study had quality score of three (Morabito et al., 2002; Uesugi et al., 2002; Yamori et al., 2002; Arjmand et al., 2003, 2005; Dalais et al., 2003; Brooks et al., 2004; Mori et al., 2004a, b; Nikander et al., 2004) (Table 1). In five of these studies, isolated soy protein that contained mainly isoflavones was used, and isoflavone tablets were used in other studies. Isoflavone intake varied from 37.3–118 mg/day in the various treatment groups. The duration of treatment also varied widely, ranging from 4 to 48 weeks including three studies with a duration exceeding 12 weeks. Six studies were performed in Caucasian women and three studies in Asian women. Seven of these nine studies were carried out in

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**Figure 1** Results of search for eligible studies.

- Potentially relevant articles identified and screened for retrieval (n = 873)
- Articles excluded because not RCTs (n = 642)
- RCTs retrieved for more detailed evaluation (n = 31)
- RCTs excluded: did not meet the inclusion criteria (n = 22)
- RCTs included in meta-analysis (n = 9)
- RCTs withdrawn because did not report:
  - Deoxyypyrindinoline (n = 2)
  - Serum bone-specific alkaline phosphatase (n = 4)
- RCTs with usable information on:
  - Deoxyypyrindinoline (n = 9)
  - Serum bone-specific alkaline phosphatase (n = 5)
post-menopausal women. The age of the female being studied ranged from 51 to 62.4 years. In all RCTs, subjects were healthy and were not undergoing any other therapy for osteoporosis; common soy diets such as tofu and natto were restricted for the treatment duration; and the daily nutrient intake assessed by food-frequency questionnaire or 3-day dietary record for subjects in both treatment groups was similar before and after the treatment period. All studies reported no significant differences regarding baseline characteristics such as age, body mass index, urinary isoflavones and Dpyr excretion or serum BAP between groups. There were also no significant weight changes or negative side effects reported.

Bone metabolism is a complex process involving bone remodeling and bone modeling. Dpyr is a small cross-linking peptide of the type I collagen molecule. During bone remodeling, Dpyr is released into the circulatory system and excreted in the urine. It is thought to be more specific than other classical bone resorption markers (Demers, 1992).

In the present analysis, nine studies reported values of urinary Dpyr before and after isoflavone or placebo treatments. Three of nine studies did not find a significant effect of isoflavones on bone metabolism and the remainder found that isoflavone intake significantly decreased urinary Dpyr. This discrepancy may be explained by the different chemical forms of isoflavone, various 'equol producer' states of the subjects and the influence of other foods eaten during the study (Cassidy et al., 2006). Moreover, limited sample sizes often prevent the detection of significant effects in individual studies. When we combined the nine studies, isoflavones significantly decreased urinary Dpyr by 2.08 nmol/mmol (95% confidence interval CI = -3.82 to -0.34 nmol/mmol) (Figure 2). Even when we excluded the trial by Morabito et al. (2002), in which the change was much larger than that in other trials, isoflavones were still associated with a significant decrease of -1.22 nmol/mmol (95% CI = -2.41 to -0.02 nmol/mmol).

Further analysis of the effects of isoflavones on urinary Dpyr is shown in Table 2. It is nowadays a general viewpoint that soy protein and isoflavones are needed together for the beneficial effects. Unexpectedly, when the five studies in which isolated soy protein was used as an intervention were...
Table 2  Subgroup analysis of the effects of isoflavones on Dpyr

<table>
<thead>
<tr>
<th>Subgroup outcome</th>
<th>No. of references</th>
<th>No. of subjects</th>
<th>Treatment effect on Dpyr (nmol/mmol)*</th>
<th>Heterogeneity P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Form of intervention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflavone tablet</td>
<td>19, 20, 21, 23</td>
<td>199</td>
<td>-4.59 [-8.35, -0.83]b</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Isolated soy protein</td>
<td>15, 16, 17, 18, 22</td>
<td>233</td>
<td>-0.23 [-1.02, 0.57]</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Isoflavone intake (mg/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤90</td>
<td>15, 16, 17, 19, 20, 22, 23</td>
<td>298</td>
<td>-3.34 [-4.46, -2.22]b</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>&gt;90</td>
<td>18, 21</td>
<td>134</td>
<td>-1.20 [-3.76, 1.36]</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Treatment length (weeks)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>15, 18, 20, 21, 22, 23</td>
<td>282</td>
<td>-2.03 [-3.20, -0.85]b</td>
<td>0.71</td>
</tr>
<tr>
<td>&gt;12</td>
<td>16, 17, 19</td>
<td>150</td>
<td>-3.36 [-8.72, 1.99]</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asian</td>
<td>20, 22, 23</td>
<td>106</td>
<td>-2.79 [-4.55, -1.02]b</td>
<td>0.56</td>
</tr>
<tr>
<td>Western</td>
<td>15, 16, 17, 18, 19, 21</td>
<td>326</td>
<td>-2.03 [-4.27, 0.22]</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Menopausal status</strong></td>
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<td></td>
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</tr>
<tr>
<td>Peri-menopause</td>
<td>20, 22</td>
<td>66</td>
<td>-1.50 [-4.45, 1.45]</td>
<td>0.88</td>
</tr>
<tr>
<td>Post-menopause</td>
<td>15, 16, 17, 18, 19, 21, 23</td>
<td>366</td>
<td>-2.47 [-4.31, -0.52]b</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; Dpyr, deoxypyridinoline.

*95% CI in square bracket.

bStatistically significant.

analyzed collectively, there was no significant effect on urinary Dpyr. This suggests that the components of soy protein are complex and include both favorable and adverse factors (Setchell et al., 1994). Further researches are required to determine the effective components and their mutual reaction with isoflavones. Branca (2003) ever reviewed the available literature and recommended that 90 mg isoflavones per day is required to achieve bone health benefits. In the present analysis, significant reduction in urinary Dpyr did not change when studies with isoflavone intake of more than 90 mg/day were not included. Potter et al. (1998) showed that 90 mg isoflavones was able to attenuate bone loss; however, Dalais et al. (2003) reported that soy protein supplementation with the highest dosage of 118 mg/day isoflavones did not appear to have estrogenic effects on markers of bone resorption. This suggests that isoflavones exert biphasic dose-dependent effects on bone metabolism, stimulating osteogenesis at low concentrations and inhibiting osteogenesis at high concentrations (Dang and Lowik, 2005). Animal studies further indicated that low-dose genistein, a common kind of isoflavone, increased rat femur bone retention, whereas high doses were less effective (Anderson et al., 1998). An intake of 90 mg/day soy isoflavones can be attained by daily consumption of two packs (92 g) of natto, or 249 g miso, amounts that are consumed habitually by the population of countries such as China and Japan (Arai et al., 2000). When the intervention duration was shortened to less than 12 weeks, the effect of isoflavones was still significant. Some studies also indicated that treatment lasting less than 12 weeks may be adequate to produce the effect of isoflavones on indices of bone resorption (Uesugi et al., 2002; Yamori et al., 2002). In the present analysis, we could not judge whether the effects become more obvious when isoflavones are consumed for more than 12 weeks because of the large range of CIs.

Interestingly, we found that isoflavone intake had significant effects on urinary Dpyr in Asian women but not Caucasian women after sub-group analysis. Isoflavone is converted into its active metabolite equol and inactive metabolite p-ethylphenol in intestinal flora, respectively. Equol is associated with an increased benefit of isoflavones on bone metabolism (Setchell et al., 2002). Approximately one-third of Caucasian women can metabolize isoflavones into equol, whereas more than half of Asian women possess this capacity (Setchell et al., 1984; Watanabe et al., 1998). Because the hormone levels of peri-menopausal women are quite diverse, we pooled results of all trials involving post-menopausal women who have stable lower endogenous estrogen levels. The pooled result from seven trials showed that isoflavones have a slightly stronger favorable effect in post-menopausal women than in peri-menopausal women. In a crossover study, Mei et al. (2001) found that isoflavone intake of 53.3 mg/day was associated with increased bone mineral density in post-menopausal but not in pre-menopausal women. Together with the findings of other studies, this suggests that isoflavones may act as an estrogen agonist under estrogen-depletion circumstances to provide beneficial effects on bone (Xu et al., 1998).

During bone modeling, osteoblasts produce BAP, a non-collagenic protein that is the biochemical marker of bone formation, which is frequently used (Gomez et al., 1995). In five studies that examined serum BAP values, we found a
significant increase of 1.48 µg/l (95% CI = 0.22–2.75 µg/l) in serum BAP, suggesting that isoflavones have a favorable effect on bone formation (Figure 3). Because of the low number of studies, subgroup analysis was not appropriate.

After isoflavone administration, Dpyr and BAP parameters can change quickly; however, bone mineral density and content change slowly. Ravn et al. (1996) observed 979 women and found that high bone turnover is associated with a significantly lower bone mass in menopausal women. Then they further indicated that high bone turnover is a risk factor for osteoporosis (Ravn et al., 1997). Harkness et al. (2004) found that soy isoflavones significantly decreased bone resorption and significantly increased bone mineral density within 24 weeks. Recently, some RCTs reported that isoflavone intake less than 6 months significantly increased bone mineral density or bone mineral content (Alekel et al., 2000; Mori et al., 2004a,b). Thus, the change of Dpyr and BAP is relevant to bone mineral density and content. In our meta-analysis, we found that isoflavone supplementation significantly decreased urinary Dpyr and increased serum BAP. Cassidy et al. (2006) concluded that isoflavone intake may be beneficial for bone in post-menopausal women in a review. The mechanism of the Dpyr-lowering and BAP-increasing effects of isoflavones is not well understood. Likely mechanisms include prevention of urinary calcium loss, beneficial effects on osteoblasts and influences on the secretion of calcitonin, which suppresses bone resorption (Kurzer and Xu, 1997). Isoflavones, as phytoestrogens, may stimulate estrogen receptors. Because estrogen receptors have been found in osteoblasts, isoflavones may cause an alteration in the production of some proteins in bone via the estrogen-receptor pathway (Dang and Lowik, 2005).

The potential for publication bias was examined by construction of a 'funnel plot' of the relation between the reciprocal of standard error and the weighted mean difference. It did not provide strong evidence of publication bias for either index (Figure 4). Generally, studies with significant results were easier to publish than those with nonsignificant results (Qin et al., 2004). Thus, we randomly added three null results appearing in the analysis and calculated a new weighted mean difference. Even if 30 of these null results were added, the pooled results changed little. This suggested that unpublished studies like published studies with nonsignificant results do not seem to influence this combined-effect estimate over a large range.

Although the relatively low number of studies limited the power of our meta-analysis, the results clearly suggested that isoflavones contribute significantly to inhibiting bone resorption and stimulating bone formation, especially in post-menopausal women. These favorable effects are observed even if isoflavones are consumed <90 mg/day or for less than 12 weeks. Further RCTs are required to provide more comprehensive information about the effective amount and duration of isoflavone intake.

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