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Pawlowski JW, Martin BR, McCabe GP, et al. Impact of equol-producing capacity and soy-isoflavone profiles of supplements on bone calcium retention in postmenopausal women: a randomized crossover trial. *American Journal of Clinical Nutrition*. 2015;102(3):695-703.

What We Know, Think We Know, or Are Starting to Know

Women display a higher risk for osteoporosis compared to men, with up to 2-4-fold higher risk for osteoporosis-related fractures depending on the fracture site [(1)]. Menopause serves as a major catalyst for change in risk profile for osteoporosis due to the decline in oestrogen production [(2)].

Bone cells contain oestrogen-receptors, and the menopausal deficiency in oestrogen results in less oestrogens binding to these receptors to stimulate bone formation activity and suppress bone resorption rates [where bone tissue is broken down and absorbed back into the circulation] [(2,3)].

Historically, women in South-East Asian populations were noted to have significantly lower osteoporosis rates compared to Caucasian populations in Western countries, however, with ageing populations and other factors, osteoporosis rates in Asian countries are trending toward Western incidence rates [(4,5)].

Ok, so far, we know that the reason for greater osteoporosis risk in women, particularly in the post-menopausal period, is due to the important roles that oestrogen plays in bone turnover. And we know that, at least historically, women in Asian countries exhibited lower risk of osteoporosis. The combination of these factors led prior research to look at soy foods, as an important part of the diets of East Asian countries with lower osteoporosis risk [(6)].

Soy foods have several properties that may be beneficial to bone health; the capacity of soy isoflavones to bind the oestrogen-receptor, the protein content of soy foods, the anti-inflammatory effects of isoflavones, and the influence of isoflavones on calcium uptake and retention in bone [(6)]. There is also the potential for the influence of soy foods to be mediated by the capacity of gut bacteria to convert the soy isoflavone, daidzein, into a metabolite known as equol.

The Study

This study was conducted as a randomised, double-blind, crossover trial testing the effects of 5 different soy isoflavone supplements on bone calcium retention in postmenopausal women in the United States, and to compare whether any effect was mediated by equol-producing ability [see ***Geek Box**, below]. The control for the study was a bisphosphonate drug, risedronate, which inhibits bone resorption. Calcium retention was compared to baseline calcium status measured over 100-days.

The different interventions reflected differences in isoflavone content and composition:

- **Low-dose mixed isoflavones**: 105mg total isoflavones [46mg genistein, 44mg daidzein, 14mg glycitein]
- **High-dose mixed isoflavones**: 219mg total isoflavones [95mg genistein, 92mg daidzein, 31mg glycitein]
- **Low-dose genistein**: 53mg total isoflavones [44mg genistein, 8mg daidzein, 0.5mg glycitein]
- **High-dose genistein**: 113mg total isoflavones [93mg genistein, 18mg daidzein, 1.5mg glycitein]
- **High-dose mixed isoflavones + genistein**: 161mg total isoflavones [91mg genistein, 54mg daidzein, 15mg glycitein]

The different compositions, allowed for the effects of either total isoflavones or genistein to be determined. For example, the low-dose isoflavone supplement and the low-dose genistein supplement contained the same absolute level of genistein [~45mg], however, the mixed isoflavones supplement also contained substantially higher daidzein and glycitein.

Prior to the intervention, participants collected a morning urinary sample after consumption of supplement enriched in the soy isoflavone daidzein, 3-days before. Urinary levels of equol were used to distinguish equol-producers vs. non-producers. This resulted in 8 equol-producers and 16 non-producers.

Participants were then randomised based on their equol-producing status. All participants began the intervention with the low-dose mixed isoflavone supplement to compare calcium retention responses based on equol-producing status of participants, which was the primary outcome of the study. The order of the remaining 4 supplements, and the control drug, were randomised. Each treatment period was 50-days, followed by a 50-day washout period between interventions. Participants were also provided with 500mg/d calcium and 600IU/d vitamin D as supplements.

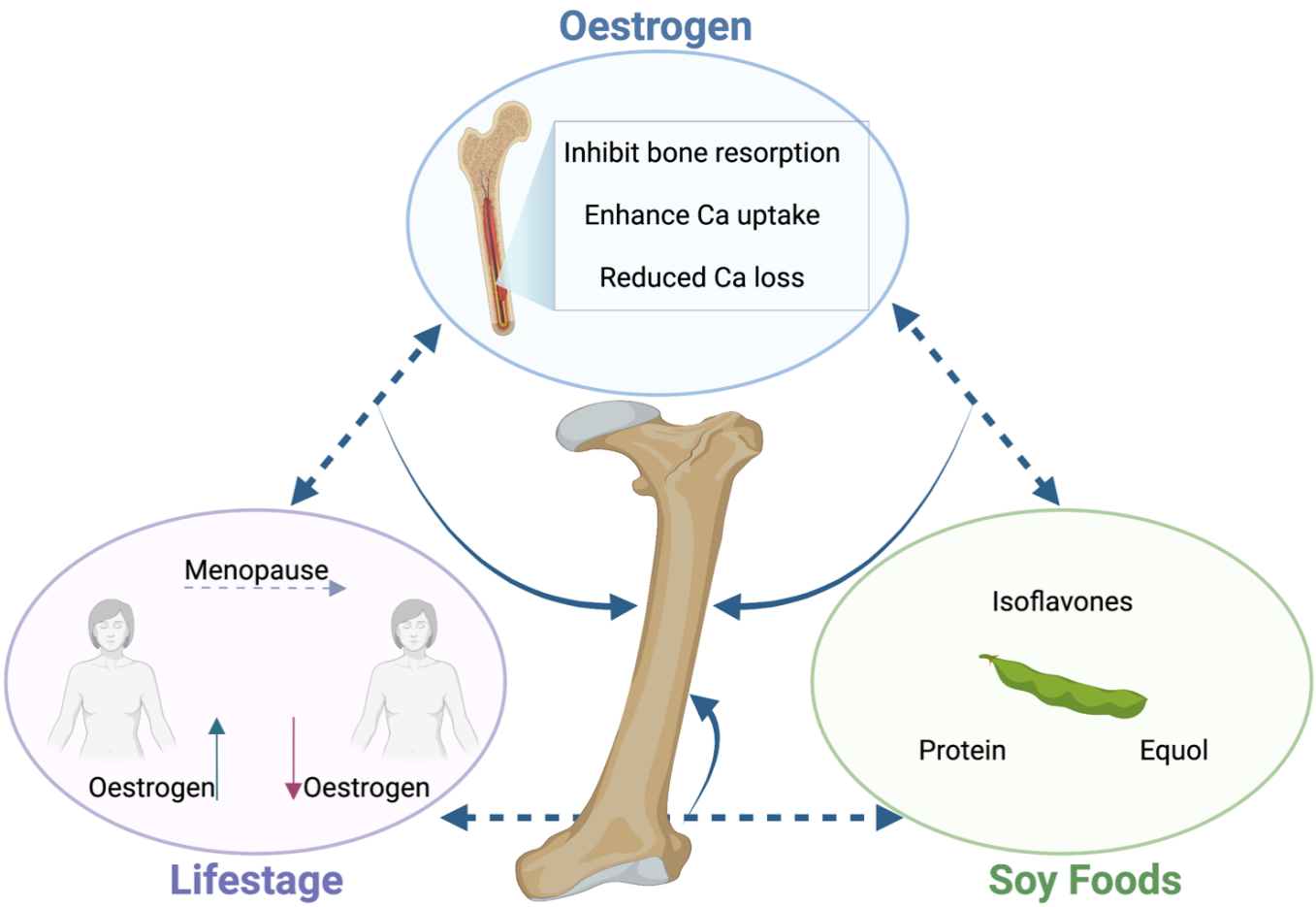

Figure *illustration of the interaction between lifestage, oestrogen production and influence on bone, and soy foods. Oestrogen confers a range of benefits on bone, from inhibiting the breakdown of bone tissue [resorption] to enhancing calcium uptake in bone [among other mechanisms], thus maintaining overall bone turnover. The shift in lifestage in women over menopause leads to loss of ovarian oestrogen production, and the deficiency in oestrogen thus influences bone turnover, tipping the balance toward net bone mineral density loss. Soy foods may exert beneficial effects on bone, particularly in the postmenopausal period, due to the affinity for soy isoflavones and equol to bind to the oestrogen-receptor. Isoflavones may also influence calcium retention, independent of the oestrogen-receptor effects.*

*Geek Box: Equol Producing Capacity

While soy is generally recognised for the phytoestrogen activity of its main isoflavones, it is the metabolism of daidzein to equol that may be of particular interest for the purported health effects of soy foods. Before they can be fully absorbed, soy isoflavones are metabolised by gut bacteria; this is a step that is now recognised as a critical stage in metabolism and ultimate bioactivity of all (poly)phenolic compounds.

Due to the modifying effect of the gut microbiota, the concept of an "equol producer" has been identified, although the definition appears to be relatively arbitrary [based on absolute thresholds of serum or urinary equol]. Equol-producing capacity may be a combination of genetics and the background diet; however, East Asian populations exhibit greater equol-production capacity compared to Western populations.

It has been suggested that equol provides more of a plausible mechanistic explanation for the association between soy foods consumption and lower risk of chronic diseases, compared to the precursor soy isoflavones. If this is the case, the distinction between "equol producers" and "non-producers" may provide an explanation for certain of the inconsistencies in the literature.

It is important to note that equol is not a phytoestrogen, yet is often misclassified as such in the literature. Equal is exclusively a product of bacterial metabolism of daidzein, and does not appear in urinary excretion [its elimination pathway] unless soy foods are consumed in the diet. Equol is a non-steroidal oestrogen, which can be drugs [e.g., tamoxifen] or naturally occurring compounds that may exert oestrogenic activity.

In this regard, while genistein and daidzein have affinity for binding to the ERβ receptor, the affinity for equol is much greater, and equol exerts greater antioxidant activity compared to the precursor isoflavones. It appears that between genistein and daidzein, genistein has significantly greater affinity for the ER-beta receptor [greater than tamoxifen]. Thus, the purported activity of daidzein may be predicated upon conversion to equol.

Results: 24 participants completed the baseline measurements and the first intervention [low-dose mixed isoflavones]. 14 completed all 5 soy interventions. The women had an average age of 59yrs at baseline, and on average were 14yrs postmenopausal. Baseline dietary calcium intake was 961mg/d [~1,500mg/d after the addition of the supplement].

- ***Bone Calcium Retention:***
  - **Low-dose mixed isoflavones**: 7.6% increase compared to baseline
  - **High-dose mixed isoflavones**: 5.8% increase compared to baseline
  - **Low-dose genistein**: 3.4% increase compared to baseline
  - **High-dose genistein**: No significant increase compared to baseline
  - **High-dose mixed isoflavones + genistein**: 5.5% increase compared to baseline

Except for high-dose genistein, each of the other supplements led to significant increases in bone calcium retention.

The low-dose mixed isoflavone supplement resulted in the greatest magnitude of effect, an increase of 7.6%.

When comparing the composition differences, there was no significant effect of higher doses of genistein when consumed with mixed isoflavones. However, there was no effect of low-dose isolated genistein, indicating that the mixed isoflavones at lower doses of genistein yielded the greatest benefit [more under ***Interesting Finding***, below].

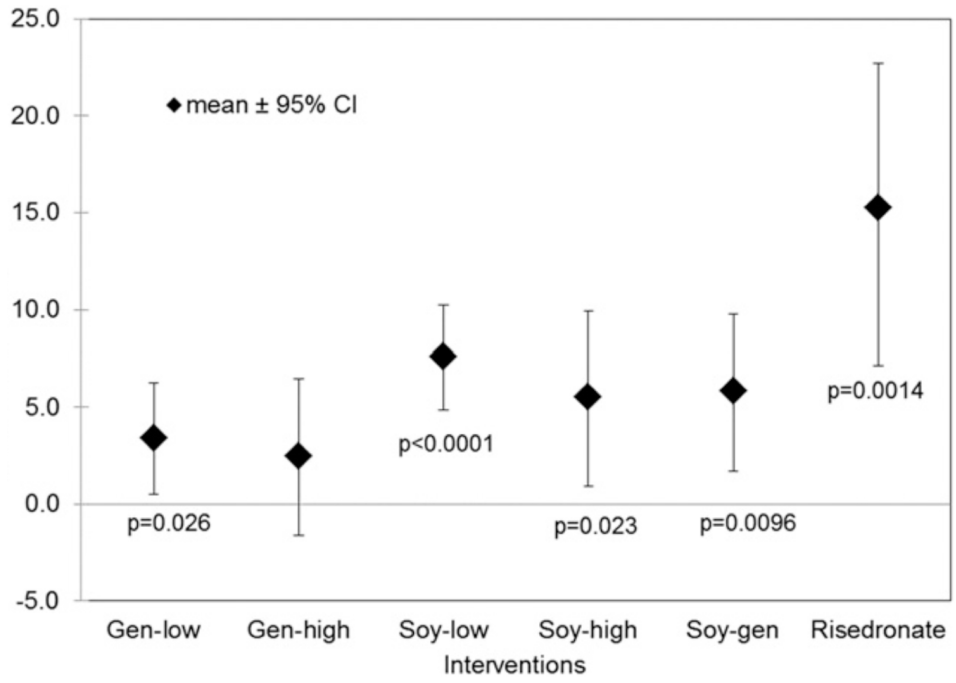

Figure *from the paper illustrating the effect of each of the soy isoflavone interventions, and the risedronate drug control, on calcium retention, over 50-days. "Gen-low" and "Gen-high" are the low-dose and high-dose genistein, respectively. "Soy-low" and "Soy-high" are the low-dose and high-dose mixed isoflavones, respectively, while "Soy-gen" is the mixed isoflavones with high genistein. Obviously, the drug had the greatest magnitude of effect; a 15.3% increase in calcium retention, which is purpose of the drug. Thus, the low-dose mixed isoflavone supplement had half the effect of the drug. Which is not terrible; foods are not drugs.*

- ***Effect of Equol-Producing Status*:** There was no significant effect of equol-producing status on the influence of the interventions on bone calcium retention.

Circulating equol levels were higher in equol-producers during the intervention, however, there was no effect of any soy supplement on serum equol levels in non-producers.

Since equol is metabolised from daidzein specifically, the effect of the interventions on serum equol was analysed relative to level of daidzein in the supplements.

There was a significant increase in serum equol level for each 1mg increase in daidzein in equol-producers, but no effect in non-producers [more under ***Key Characteristic***, below].

The Critical Breakdown

Pros: Participants were stratified by equol-producing status, which was determined prior to the intervention, and randomised to the order of treatment. The crossover design meant each participant served as their own comparison between each intervention. Both participants and study personnel were blinded to the allocation [although there is little detail provided on how blinding was achieved/maintained]. The study tested a range of both total isoflavone content and compositional differences, allowing for detailed comparisons as to independent and related effects of soy isoflavones. The supplement capsules were similar in physical appearance. The study was quite comprehensive in its measurement methods and duration: stable isotopes were used to establish bone calcium turnover, and to assess intestinal calcium absorption, and DEXA scans to determine bone mineral density. Participants were supplemented with 500mg/d calcium and 600IU/d vitamin D, and there were no differences reported in serum vitamin D levels or dietary calcium, thus these two potential effect modifiers were held constant through the study. There was an overall high rate of compliance in a study protocol that required substantial participant adherence to urine sample collections and supplement intake [5 capsules per day].

Cons: Only 8 participants were defined as equol producers, and this low number may have meant a lack of statistical power to detect differences between equol producers vs. non-producers. The final total sample size itself was small for those who completed all soy supplement interventions, $n = 14$, of which 6 were equol producers. Thus, while their power calculations indicate this number was sufficient to detect up to an 8% difference in bone calcium retention, it remains a very small sample size for the comparison of equol-producers vs. non-producers. Equol status was also assessed based on a single morning urinary collection and using an absolute threshold cut-off for urinary equol, rather than urinary daidzein:equol ratio, which would be preferable because it provides a more reliable marker of the conversion of daidzein to equol [7]. Further, a single morning urinary sample rather than a full 24 h urinary collection may have introduced variability between participants [8].

Key Characteristic

Although we note the limited sample size from the **'Cons'** above, having both equol producer phenotypes was an important design characteristic of the present study, given the potential for these distinct phenotypes to explain discrepancies in the research on soy intake and bone health [9]. So, what did we learn from the present study?

The first difference of note was the effect of daidzein on serum equol levels, which as discussed under ***Key Characteristic***, above, only increased in equol-producers. This is, however, consistent with our understanding of this phenotype, and the capacity to produce equol or not appears to relate to differences in composition of gut bacteria [10].

However, the second finding – and perhaps more important relative to the first – is that there was no effect of equol-producing status on bone calcium retention. This highlights an important general principle for thinking about nutrition research, which is that just because we see a change in a circulating biomarker does not by default mean that any effect associated with that biomarker is enhanced [or inhibited].

Thus, in the present study, the conclusion we may arrive at is that the beneficial effect of the soy isoflavone supplements on bone calcium retention were *independent* of equol-producing status and related changes in serum equol levels.

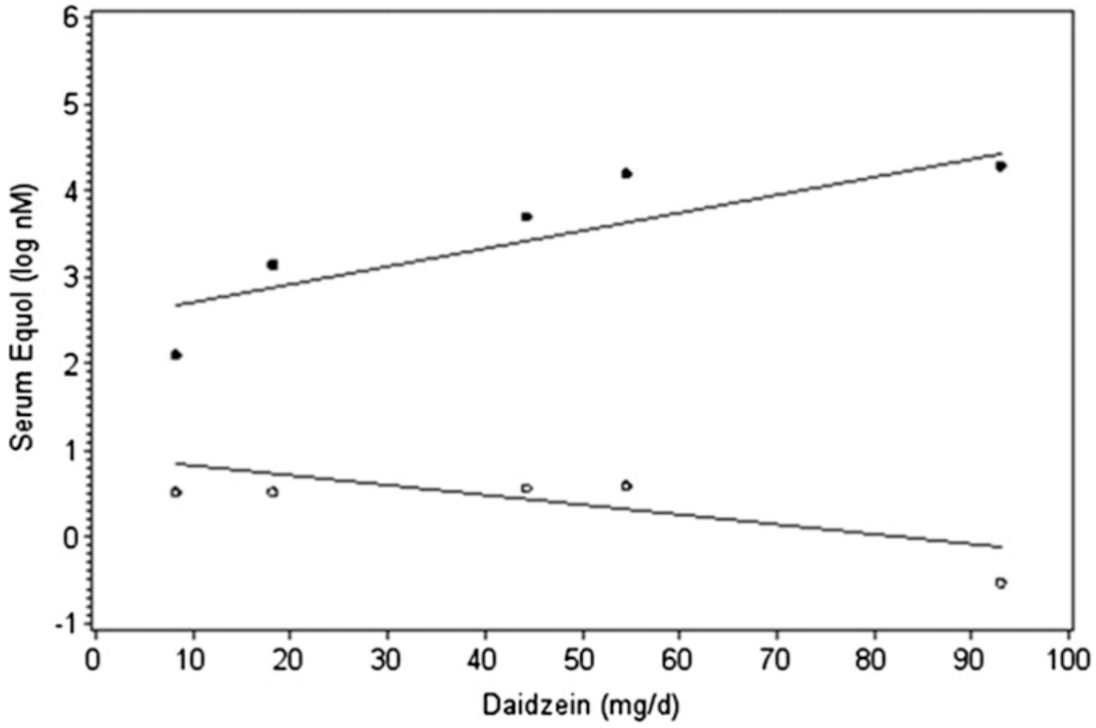

Figure *from the paper illustrating the increase in serum equol levels [**left Y-axis**] and levels of daidzein in the soy supplements [**bottom X-axis**]. Each dot represents a participant; solid circles represent equol-producers while open circles represent non-producers. As you can clearly see, increasing levels of daidzein did not have any impact on serum equol levels in equol non-producers. The effect of increasing daidzein intake in equol-producers was to increase serum equol levels. However, the changes in serum equol had no influence on the effectiveness of the soy isoflavone supplements on bone calcium retention.*

Interesting Finding

Most studies simply compare two different doses of supplements, or maybe modify a third variable. The present study took a really novel approach to determine effects of soy isoflavones, both total amount and composition. And perhaps the most interesting finding is that the greatest effect was observed in the low-dose mixed isoflavone supplement, rather than isolated higher dose genistein, for which there was no significant effect.

When comparing the composition differences, both the low-dose mixed isoflavones [46mg genistein, 44mg daidzein, 14mg glycitein] and the low-dose genistein [44mg genistein, 8mg daidzein, 0.5mg glycitein] contained the same level of genistein. The low-dose genistein yielded a 3.5% increase in bone calcium retention. Conversely, the significant 7.3% increase in calcium retention in the low-dose mixed isoflavone supplement indicates that addition of daidzein and glycitein to genistein, i.e., soy isoflavones in their natural ratios, is important for the effects of soy isoflavones.

Then let's think about the difference between both high-dose total isoflavones and high-genistein with total isoflavones, which both had near identical calcium retention increases of 5.8% and 5.5%, respectively. The former contained 93mg genistein, 18mg daidzein, 1.5mg glycitein; the latter contained 91mg genistein, 54mg daidzein, 15mg glycitein. Thus, the genistein was matched, but the higher total isoflavone content was achieved with higher daidzein and glycitein, but those levels were similar to the low-dose mixed isoflavone supplement.

Collectively, it was clear in this data that there was no additional benefit to higher genistein levels, and neither did higher-dose total isoflavones. Another fundamental principle of nutrition: more does not necessarily = better.

Relevance

In terms of context, it is important to bear in mind that this study investigated the impact of soy on calcium retention in bone. Thus, we must look to the wider research for the question of whether soy isoflavones would influence other bone endpoints, i.e., bone mineral density [BMD] or actual fracture incidence.

The research on BMD is mixed. In an intervention in postmenopausal Hong Kong Chinese women, supplementation with 80mg/d soy isoflavones increased bone mineral content [BMC] over 1yr, however, there was no effect of a 40mg/d dose [(11)]. However, a recent 2yr intervention in Taiwanese postmenopausal women showed no effect of 300mg/d soy isoflavones on BMD, despite significant increases in circulating soy isoflavone levels [(12)]. The Taiwanese study also appears to one of the few to assess fracture risk, and there was no significant reduction in risk for fractures from soy supplementation.

However, neither the two above-mentioned studies considered equol-producing status. One study that did was an intervention in postmenopausal Caucasian women supplementing 76mg total soy isoflavones from soy milk over 2yrs [(13)]. Their main analysis showed no change in BMD or BMC from soy supplementation, i.e., a protective effect against BMD and BMC loss. However, when stratifying participants by equol-producing status, they showed that isoflavone supplementation led to 2.4 % and 2.8 % increases in BMD and BMC, respectively, in the equol-producers, compared to just 0.6 % and 0.3 %, respectively, in the non-producers.

Thus, several research gaps exist in this area. Was the dose in the Taiwanese study too high? The other studies used doses more in range with the natural ratios of soy isoflavones in the diet. And what is the role of equol-producing status, which appears to modify effects of soy isoflavones on bone markers? Another study in menopausal Japanese women showed a significant reduction in urinary markers of bone resorption in equol-producers [14].

Although the present study indicates a benefit on bone calcium retention independent of equol status, it is a small trial. Larger, and longer, interventions that specifically consider equol-producing status with a range of relevant endpoints, fracture risk in particular, will be required to settle some of the open questions in this area.

Application to Practice

There are too many incomplete aspects of the evidential picture to make specific recommendations [e.g., do you know your equol status?!] regarding bone health in the postmenopausal period. However, the general overall assessment of the evidence is positive [6]. The fact that higher did not = better in the present study highlights that, for non-nutritive bioactive compounds like soy isoflavones, their physiological activity is primarily exerted at lower levels: this is consistent with everything we know to date about (poly)phenol compounds [15].

The other clear finding of this study, albeit non-nutritional, is that the drug had the greatest effect on bone calcium retention. In nutrition, it can often be tempting to want to take the slant that food is preferable to drugs because, you know, the whole natural thing. Sometimes drugs are just better. This doesn't mean there cannot be adjuvant benefit to diet; unfortunately, a combination drug+isoflavone intervention was not examined in the present study. But on the isoflavone side, it appears that the natural ratios consumed through isoflavone rich foods – soy milk, tofu – may be sufficient for any benefit accruing from isoflavones.

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