# Phytoestrogens and osteoporosis

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## INTRODUCTION

### Osteoporosis and fracture risk

Osteoporosis is a silent condition, which may have severe consequences due to low energy fractures, with typical localisation in the wrist, spine and hip. Lifetime risk of hip fracture for caucasian women and men are17,5% and 6%, respectively (Dennison 2000). Fracture rates of the (lumbar) spine are difficult to estimate as large numbers remain undiagnosed, but lifetime risk of a diagnosed fracture for women in the US and Europe is estimated to 15,6% and 11%, respectively (Dennison 2000).

### Individual impact of osteoporosis

Hip fractures are a serious problem regarding *mortality*, with a 20% death rate <1y for women and 30% for men, 50% due to co-morbidity (Johnell 1995); *morbidity*, where 40% are not able to walk alone, 60% are not able to carry out at least one activity of daily living and 80% are not able to carry out at least one independent activity of daily living (driving or shopping), one year post-fracture ( Dennison 2000).

### Economic impact of osteoporosis

The health economic impact of osteoporosis is considerable. As part of costs for the physical inactivity related diseases a canadian paper has estimated that 2,5% of total direct health care costs are attributable to physical inactivity, with osteoporosis second in rank, exceeded only by coronary artery disease (Katzmarzyk, 2000). Direct medical costs are available for the US and Europe: Immediate medical costs are estimated to USD 3300 for at hip fracture and USD1300 for a non-hip fracture( Martin 2001). Total one year costs are estimated to a mean of 17.027 USD / person, ranging from 13.726 USD if returning to community, to 28.343 USD if transferred to long-term-care (Wiktorowicz 2001).
Translating these numbers to the populations of Asia and Latin America, sets the stage for both a social and a health care system challenge.

### Contributing causes

Established contributing causes such as low physical activity, high alcohol intake, very low calcium intake, vitamin D levels, low body weight and smoking are important risk factors, yet knowledge about these does not explain the variation in hip fracture incidence worldwide, and the study of other contributing factors warrants further investigation. Apart from calcium, vitamin D and alcohol, nutritional intake, nutritional patterns and specific dietary factors has not been well studied for their risk- or protective capacity (WHO 2003).
Both the balance between animal and vegetable foods in general (Frassetto 1999), protein type (Abelow 1992) and specific factors such as tea ( Wu 2000), soy ( Somekawa 2001, Setchell 2003), refined carbohydrates, fatty acids , fruit and vegetables are likely to be of potential importance for bone health. Specific foods or food intake patterns may carry the potential of being ready-available, low-tech, low-cost nutritional candidates for a contribution to the prevention of osteoporosis worldwide. Among these are phytoestrogen rich foods such as soy.

## ABSTRACT

*Osteoporosis and the associated low- energy fractures is a major public health problem, affecting 30% of the postmenopausal caucasian population in the US, rising to 70% in the +80 year old. There is a large global variation in the age-adjusted incidence of hip fractures (HFI), ranging from <10 pr 100.000 py in China, New Guinea, Nigeria , Thailand and South Africa to +100 pr 100.000 py in North America, Northwest Europe and Oceania. With increasing life-expectancy world wide, the current number of +65 years old will increase from 323 mio representing 1,66 mio annual hip fractures in 1990, to 1555 mio and the estimated corresponding 6,26 mio hip fractures by year 2050; a 4-5 fold increase. Phytoestrogens, specially from soy foods with isoflavones, carry the potential of being a nutritional candidate for adding a contribution to the prevention of osteoporosis worldwide. Several studies have been performed to determine the role of soy intake in preventing bone loss in postmenopausal women. Studies with fracture endpoint is not yet available, but several other lines of evidence have been investigated: epidemiological and clinical studies with either markers of bone turnover or measurement of bone mineral density- and content (BMD /BMC).*

*Keywords: Soy, isoflavones, genistein, daidzein, equol, bone health, osteoporosis.*

## RESULTS

A number of studies have been performed to determine the role of soy intake in preventing bone loss in postmenopausal women. Studies with fracture endpoint is not yet available, but several other lines of evidence have been investigated: epidemiological and clinical studies with either markers of bone turnover or measurement of bone mineral density- and content (BMD /BMC), used to assess the relation between various risk factors and bone health.

### Epidemiological studies

Observational studies have been performed in both asian and caucasian populations.
For postmenopausal women 12 studies exists (Setchell 2003, Greendale 2002, Ho 2003) of which eight report a positive association between soy intake and bone mass (Setchell 2003, Ho 2003), with BMD levels approx. 6-14% higher in the high- intake groups, regardless of race and place of residence. In populations with neligible intake, no such association is found (Kaardinal 1998, Greendale 2002).
For premenopausal women five studies have reported variable outcomes, thus no conclusion can be drawn (Setchell 2003, Greendale 2003).
The risk of fractures increases as BMD declines, with a 1,5–3 fold increase in fracture rate for

each standard deviation fall in BMD. One SD is approximately 10%, depending on population, thus it is likely that a habitual high soy intake contributes to the lower current fracture rates in Asia.

### Studies with bone- marker endpoint

Thus far, both 9 observational studies and 10 dietary intervention studies ( Setchell 2003, Yamori 2002) have shown significant relationships between soy with phytoestrogens or purified phytoestrogen intake and surrogate markers for bone turnover that are indirectly consistent with reduced bone turnover.
Markers indicative of osteoblast and osteoclast activity that have been measured include urinary calcium, magnesium and phosphorous, hydroxyproline, and collagen cross-links, while serum measures have included bone specific alkaline phosphatase, tartarate resistant acid phosphatase, osteocalcin, IGF-1 and IL-6. An advantage of utilizing these sensitive markers is that biochemical events occurring in bone can be detected long before significant changes in BMD or BMC can be measured, or fractures occur.
Most of the studies, both observational and experimental, have found that when soy foods containing substantial levels of isoflavones are included in the diet of postmenopausal women, the effect is to downregulate resorption markers (Setchell 2003), consistent with reduced bone resorption.

### Clinical trials with DEXA endpoint

Seven intervention studies of at least 6 months duration with postmenopausal women, are completed:
Three 6-month studies, two with soy protein including isoflavones in the 90 mg/d range and one with a clover- derived isoflavone supplement in the 57 – 85 mg/d range, have demonstrated a preventive effect on bone loss, the soy studies in the lumbar spine and the clover study in the forearm (Potter1998, Alekel 2000, Clifton-Bligh 2001).
One 12 month study comparing purified genistein at 54 mg/d with estradiol and placebo demonstrated a similar protective effect of genistein and estradiol, with gains in BMD ≈ 3% in both lumbar spine and hip for the two active groups (Morabitu 2002).
One of the two 2y studies completed has demonstrated a preventive effect on lumbar spine bone loss with isoflavone-rich soy milk, 500ml / 80 mg isoflavones/d , with only minimal changes occurring in the hip in the control group, having soy milk without isoflavones (Lydeking-Olsen, 2004). The other 2y study found less than 1% bone loss in the whole body over two years regardless of isoflavone content (5, 42 and 58 mg respectively), indicating a calcium sparing effect in the whole body bone mass. Specific- site DXA measurements were not performed in this study, making it difficult to compare with the other 2y study (Vitolins, 2001). A 9 mo study did not show protective effect on bone loss (Gallagher 1998).

## DISCUSSION

Results from the studies of soy intake are promising, yet more studies are needed to draw definitive conclusions. Several aspects need further investigation in future studies: Study length, design and dosing of isoflavones, measurement, bioavailability and metabolism of isoflavones, choice of control product and the role of soy protein itself.
In the completed studies, duration have varied, but most have been small and of short duration, given the slow rate of bone- turnover. There is a need for larger and longer-term trials of at least 2 years duration, as well as fracture studies.
The landmark paper by Potter *et al.* which found a significant bone-sparing effect (BMD increased 2.2%) at the lumbar spine of a soy protein diet with an intake of 90 mg/d isoflavones over a 6-month period, but not with 45mg/d set the benchmark for the choice of isoflavone levels in subsequent studies (Potter 1998). Additionally it is not always clear how the isoflavone intake is calculated: The absolute level of isoflavone is considerably higher if expressed as total isoflavones (including the glycoside part of the molecule), compared to the aglycone part, ie 90 mg of total isoflavone is equivalent to 50-55 mg of isoflavones after removal of the glycoside moiety by intestinal bacteria ( Setchell 2002) and this is the maximal bioavailable fraction of the molecule.
The typical levels of isoflavone intake in asian women consuming a traditional diet is estimated to 15-50 mg/d (Nagata 1998, Chen 1999, Wakai 1999), certainly lower than the levels demonstrating a protective effect in the clinical trials.
The high- intake- level from the epidemiological studies, demonstrating an association btw soy/ isoflavone intake and higher bone mass is ~ 50 mg (Setchell 2003), whereas no detectable effect has been seen in the shorter term trials at this level. It remains to be established if the lower doses of isoflavones ~ 50 mg/d, need much longer time to be effective in preventing bone loss.
A potential confounder in human isoflavone research is the heterogeneity in bioavailability and metabolism of the isoflavones – thus the equol-status clearly separate subjects in two distinct groups who are likely to have markedly different responses to soy food feeding (30-50% being equol-producers in various populations) by a variety of factors determining the presence and activity of the so far little known gut flora bacteria responsible for the conversion of daidzein to equol: dietary factors and antibiotic history and use, beeing the two most likely contributors (Setchell 2002). Equol, a specific bacterial metabolite of daidzein (Axelson 1982) and an isoflavone not found in soy itself, was formed in only 45% of the postmenopausal women studied in our own study (Lydeking-Olsen 2004) but in those capable of making equol, referred to as *'equol producers'* lumbar spine BMD increased by 2.4% ($p < 0.001$ compared with control group), while there was no significant change in BMD in the 'non-equol producers'.
Equol has a much higher affinity for the estrogen receptor than daidzein, its precursor phytoestrogen, and of all the isoflavones it is has the highest antioxidant capacity (Setchell 2002) - factors that could account for the greater effects observed in *'equol producers'* in this bone study (Lydeking-Olsen 2004).

Soy is, however, more than isoflavones and other substances in soy can influence bone health as well. A recent calcium-balance study demonstrated equal calcium absorption from casein/whey and calcium-enriched soy protein (25g protein and 1100 mg Ca /d), but a significantly reduced calcium excretion with soy protein, regardless of isoflavone level. Net calcium retention was significantly increased to a positive balance with soy compared to a negative balance with casein/whey. The absolute difference of 50-60 mg/d, translates into ~20 g/year or approx. 1% of the total skeleton. This demonstrates a clinically important calcium-sparing effect of soy protein itself (Spence 2002) .
Adding this information to the result from the two year study by Vitolins, examining whole-body bone-mass, where all 3 groups received soy protein ±isoflavones, and lost less than 1% in two years suggests that it is the soy protein itself that has the calcium-sparing effect. To examine this further it is necessary to include casein/whey control groups when examining soy effects on cortical bone.
The 2y study by Vitolins examined whole body bone-mineral density with soy ÷ and different isoflavone levels. There was no difference between the groups – all 3 groups lost less than 1% over 2 years indicating that soy protein itself have a calcium sparing effect, which is likely to be measured best in the total skeleton or the hip where cortical bone is dominant (Vitolins 2002). Other constituents in certain soy foods, such as

vitamin K developed during fermentation processes or calcium added during manufacturing, may influence bone health positively as well ( Somekawa 2001).

## CONCLUSION

Data from different lines of evidence suggest that.
Soy protein itself in a 15 – 25 g/d range (regardless of isoflavone levels) may have a calcium sparing effect on the skeleton of 50-60 mg/d, translating to ≈ 20 g/year or 1% of the total bone mass.
Isoflavone-rich varieties of soy foods provide an endocrine effect (possibly most pronounced in equol-producers) that prevents spinal bone loss if an isoflavone level of 50 – 90 mg/d (aglycone equivalent) is reached on a long-term basis. Both genistein and daidzein have been shown to double the OPG/RANKL ratio in vitro, thus targeting the central regulatory mechanism of bone remodeling.Some soy foods are good calcium sources and fermented types also deliver vitamin K, factors contributing to healthy bones.

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