

The Summer Meeting of the Nutrition Society, hosted by the Irish Section, was held at the University of Ulster, Coleraine on 16–19 July 2007

Symposium on ‘Diet and bone health’

Phyto-oestrogens and bone health

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As oestrogen deficiency is the main cause in the pathogenesis of osteoporosis hormone-replacement therapy remains the mainstay for prevention. However, prophylaxis by hormone-replacement therapy is limited. Phyto-oestrogens, which are weakly-oestrogenic compounds present in plants, deserve particular mention because emerging data support the suggestion that they may prevent bone loss associated with the menopause. In the past few years extensive research using animal models has provided convincing data to indicate a significant improvement in bone mass or other end points following feeding with soyabean. Moreover, observational studies relate the lower incidence of osteoporosis among women in the Eastern world to a diet rich in phyto-oestrogens. However, it is not valid to extrapolate to the Western situation. The varied clinical trials that have been published suggest that isoflavones reduce bone loss in women in the early period post menopause, but a definitive result requires more investigations of the effect of phyto-oestrogens on bone health that have substantial sample size and are of long duration. In addition, the clinical efficacy of soya foods in preventing osteopenia depends on their intestinal metabolism. Thus, phyto-oestrogens are a source for putative innovative dietary health intervention for post-menopausal women. However, more data are necessary, particularly in relation to their effect on the risk of fracture.

Phyto-oestrogens: Osteoporosis: Post-menopausal women

Osteoporosis, a systemic skeletal disease characterized by a low bone mass and microarchitectural deterioration, is one of society's most debilitating and costly diseases, particularly in elderly women and increasingly in men. It has become a worldwide problem that is expected to worsen in many countries with ageing populations; the lifetime risk for osteoporotic fracture is 40% for women and approximately 15% for men⁽¹⁾. This condition is characterized by an imbalance between the deposition of matrix and mineralization and the resorption activity resulting from an impairment in the remodelling process as a result of changes in the functional lifespan of osteoclasts and osteoblasts⁽²⁾.

Hormonal changes during the menopausal transition, which ultimately result in a decline in oestrogen, play a pivotal role in the development of this chronic disease⁽³⁾. Indeed, oestrogens exhibit a broad range of physiological activities that are important in the homeostatic regulation of many cells, including bone cells. In women the

menopause initiates an accelerated phase of predominantly cancellous bone loss that declines rapidly over 5–10 years to become asymptotic, with a subsequent slow phase that continues indefinitely. Oestrogen deficiency affects remodelling in several ways: it increases the activation frequency of bone modelling units, which leads to higher bone turnover; it induces a remodelling imbalance by prolonging the resorption phase and shortening the formation phase. As a consequence, the volume of the resorption cavity increases beyond the capacity of the osteoblasts to refill it⁽⁴⁾. Thus, hormone-replacement therapies have been the first line of treatment for osteoporosis⁽⁵⁾. However, available evidence appears to suggest that the long-term use of hormone-replacement therapy has numerous side effects (e.g. risk for developing breast and uterine cancers and CVD)^(6–8) that have led to a drastic reduction in the percentage of post-menopausal women on hormone-replacement therapy in the Western world.

Abbreviation: ER, oestrogen receptor.

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Currently, natural alternatives with oestrogen-like activities are being investigated as possible new strategies for the treatment of osteoporosis. As it is evident that the effects of oestrogen on bone to a large extent are mediated via activation of its oestrogen receptors (ER; oestrogen signalling in mammalian cells is primarily mediated at the molecular level by two members of the nuclear receptor superfamily, ER α and ER β)⁽⁹⁾, there is a great need for new activators of ER that selectively reproduce only the beneficial effects of oestrogen. Moreover, in addition to existing drug therapies, certain lifestyle and nutritional factors are known to reduce the risk of osteoporosis. Indeed, research into human nutrition has led to an awareness of the health benefits that diet can offer by providing several naturally-occurring bioactive molecules such as the phytochemicals, which include the phyto-oestrogens (whose common biological roles are to protect plants from stress or to act as part of a plant's defence mechanism) that can act as oestrogen mimics. While these natural secondary metabolites are widely occurring, being ubiquitous in higher plants, the major classes of current interest from a nutritional perspective are the isoflavones and the lignans, which are mainly found in soyabean and flaxseed respectively⁽¹⁰⁾ (although the lignans are much more widespread in plant foods, their investigation has been limited because of the complexity of measurement techniques). These compounds are capable of interacting with ER, showing both agonist and antagonist properties. A conspicuous feature of their non-steroidal chemical structure is the phenolic ring that is theoretically a prerequisite for an oestrogenic activity (binding to the ER). They also share a pair of hydroxyl groups, one being a substituent of an aromatic A ring, while the second lies at the opposite end^(11,12). These phyto-oestrogens thus have potential as a putative and innovative dietary health intervention for post-menopausal women, and are currently being studied for the prevention of sex hormone-related diseases such as breast cancer and prostate cancer⁽¹³⁾, as well as post-menopausal osteoporosis^(14–16).

The rationale for phyto-oestrogens

Foods based on soyabean have generated much interest recently as a result of evidence that populations consuming large amounts of soyabeans have a lower risk of some chronic diseases, notably osteoporosis. Indeed, the marked differences in the incidence of clinically-diagnosed osteoporotic fractures within Europe and even worldwide (the lowest rate occurring in countries in the East and to the South) could be related to specific nutritional practices. However, identifying the individual or multiple components of the diet involved in the pathogenesis of osteoporosis is extremely difficult because of the complex interplay between lifestyle factors, genetics and many confounding variables. Soyabean, a legume that has been grown for thousands of years, is traditionally used to prepare both fermented and non-fermented foods and is a staple among Asian populations. Soya foods contain an array of biologically-active phytochemicals that may confer important health benefits. These compounds include

the isoflavones, which have received considerable attention because of their oestrogen-like properties in certain tissues, including bone. Thus, some investigators have referred to them as naturally-occurring selective ER modulators^(17,18).

The biological effects of phyto-oestrogens have been characterized using DNA microarrays⁽¹⁹⁾. Gene expression profiling has demonstrated that 17 β -oestradiol, genistein (one of the two major isoflavones) and the synthetic oestrogen diethylstilbestrol alter the expression of the same 179 genes in the intact immature mouse uterus (under conditions in which each compound produces an equivalent gravimetric and histological uterotrophic effect), including lactotransferrin, complement component 3 and c-fos⁽²⁰⁾.

Phyto-oestrogens may therefore have potential for maintaining or modestly improving the bone mass of human subjects. A substantial amount of research carried out in recent years using animal models has provided convincing data to indicate that feeding soyabeans results in a significant improvement in bone mass or other end points⁽²¹⁾. These studies strongly indicate that this bone-sparing effect of soyabean is attributable to its isoflavone component. However, because of the constraints associated with the systems used to study the ageing skeleton, the information gained is necessarily limited and the focus must be on clinical trials.

Phyto-oestrogens and bone health trials

Observational studies

Several studies have indicated a relationship between the lower incidence of oestrogen-deficiency-related diseases (i.e. osteoporosis) among women in Eastern countries and a diet rich in phyto-oestrogens. Moreover, a clear relationship has been demonstrated between the level of soyabean consumption and bone mineral density (see Table 1). However, it has been shown that extrapolation to the Western situation is not valid; a single component of a total lifestyle of such communities, in which several other significant lifestyle factors are operative, cannot be expected to show a definitive correlation with disease. Moreover, consumption of soyabean is very low within the Western population and phyto-oestrogen exposure varies substantially across human populations and individuals. Furthermore, Asian diets, which are particularly high in soyabean, result in isoflavone consumption as high as 1 mg/g body weight per d, with plasma isoflavone concentrations reaching 1 μ M in Japanese subjects. In Europe circulating levels are usually <0.07 μ M for omnivores, and 0.4 μ M-isoflavones and 0.8 μ M-lignans among vegetarians^(22,23). Another limitation is the window of exposure⁽²⁴⁾.

Intervention trials

In adolescents isoflavone supplementation (Novasoy; Archer Daniels Midland Company, Decatur, IL, USA; 50 mg isoflavone equivalents/d) for 6 weeks has been shown to have no measurable effect on bone turnover in adolescent boys⁽²⁵⁾, even though results from a study of

Table 1. Epidemiological studies in women

Reference	Population	Evaluation of soyabean or isoflavone consumption	Bone health evaluation	Main results
Kaardinaal <i>et al.</i> ⁽⁶⁴⁾	Caucasian women, <i>n</i> 67	Urinary excretion GC–MS	Radial BMD	No difference in urinary excretion of isoflavones for women losing bone at a yearly rate of 0.5% v. 2.5% Urinary concentrations of enterolactone higher in those having the highest rate of osteopenia
Tsuchida <i>et al.</i> ⁽⁶⁵⁾	Japanese middle-aged women, <i>n</i> 995, age 40–49 years	Self-reporting FFQ		Independent gradient of non-adjusted and adjusted BMD for age and weekly Ca intake through soyabean-intake frequency (<i>P</i> = 0.03)
Horiuchi <i>et al.</i> ⁽⁶⁶⁾	Japanese women, <i>n</i> 85, mean age 66.9 (SD 7.4) years	Food weighing over 3 days	Spine BMD	Positive association between soyabean-protein intake (12.6 g/d) and spine BMD
Mei <i>et al.</i> ⁽⁶⁷⁾	Chinese women, <i>n</i> 357, mean age 63 (SD 8.3) years	FFQ and interview	Spine and hip BMD Bone turnover biomarkers	Decrease in bone resorption Higher BMD in women with the highest isoflavone intake (tertile 47 mg/d) (<i>P</i> < 0.05) Decrease in both formation and resorption biomarkers (<i>P</i> < 0.05)
Somekawa <i>et al.</i> ⁽⁶⁸⁾	Japanese women, <i>n</i> 478	FFQ Urinary isoflavone assessment (HPLC)	Spine BMD	Mean consumption 54.3 mg Higher BMD in the highest quartile (<i>P</i> < 0.01)
Kim <i>et al.</i> ⁽⁶⁹⁾	Korean women, <i>n</i> 75, age 52–65 years	Urinary isoflavone excretion over 24 h (GC–MS)	Spine and femoral BMD	Positive association between enterolactone excretion and spine (<i>P</i> < 0.01) or hip (<i>P</i> < 0.05) BMD
Kritz-Silverstein & Goodman-Gruen ⁽⁷⁰⁾	Californian women, multiple ethnicity, <i>n</i> 208, age 47–74 years	FFQ over the previous year	Spine BMD Biomarkers for bone resorption (NTX)	Genistein consumption 0–13.9 mg/d Urinary NTX 18% lower in those having the highest intake, compared with those eating less isoflavones (<i>P</i> = 0.09)
Greendale <i>et al.</i> ⁽⁷¹⁾	Baseline data from the Study of Women’s Health Across the Nation, a US community-based cohort study of women, age 42–52 years: African-American, <i>n</i> 497; Caucasian, <i>n</i> 1003; Chinese, <i>n</i> 200; Japanese, <i>n</i> 227		Spine and femoral BMD	Trend toward a higher BMD at the spine (<i>P</i> = 0.07) Chinese women: no association between genistein and BMD Premenopausal, but not peri-menopausal, Japanese women whose intakes were greater had higher spine and femoral neck BMD Adjusted mean spinal BMD in the highest tertile of intake was 7.7% greater than that of women in the lowest tertile (<i>P</i> = 0.02); femoral neck BMD was 12% greater in the highest tertile v. the lowest tertile (<i>P</i> < 0.0001)
Nagata <i>et al.</i> ⁽⁷²⁾	Japanese women, <i>n</i> 87		Calcaneum BMD bALP Plasma genistein and daidzein (HPLC)	No relationship with isoflavone consumption (62 g soya foods/d, i.e. 32 mg isoflavones/d)
Hanna <i>et al.</i> ⁽⁷³⁾	Australian women, <i>n</i> 500, age 40–80 years	Phytoestrogen-frequency questionnaire (assessment of the intake over the previous month)	Total spine and hip BMD bALP	bALP higher in the lowest quartile compared with the higher three quartiles No difference in BMD

Zhang <i>et al.</i> ⁽³⁴⁾	Post-menopausal Chinese women in the Shanghai Women's Health Study, <i>n</i> 24 403, age 40–70 years	FFQ Mean follow-up of 4.5 years (110243 person-years)	Risk of fracture	The relative risks of fracture were 1.00, 0.72 (95% CI 0.62, 0.83), 0.69 (95% CI 0.59, 0.80), 0.64 (95% CI 0.55, 0.76), and 0.63 (95% CI 0.53, 0.76) across quintiles of soyabean-protein intake ($P < 0.001$ for trend) The multivariate relative risks of fracture comparing the extreme quintiles of soyabean-protein intake were 0.52 (95% CI 0.38, 0.70) for women within 10 years of menopause v. 0.71 (95% CI 0.56, 0.89) for women in later stage of menopause Similar results for isoflavone intake Natto intake helps to prevent post-menopausal bone loss No association with tofu or other soya foods
Ikedo <i>et al.</i> ⁽⁷⁴⁾	Japanese women, <i>n</i> 944, age 20–79 years	Habitual natto* intake evaluated by FFQ	Total hip BMD Forearm BMD	

BMD, bone mineral density; NTX, N-terminal cross-linked telopeptides; bALP, bone-specific alkaline phosphatase.

*Fermented soyabean.

104 Chinese adolescent girls aged 14–16 years indicate that supplementation with 375 ml Ca-fortified soya milk, or an equivalent of about two glasses, can be considered among the effective strategies for bone acquisition and the optimization of peak bone mass in adolescent girls (because the Chinese diet is low in Ca; although this study did not establish which component(s) of the Ca-fortified soya milk (or their combined effect) resulted in the beneficial effect on bone health)⁽²⁶⁾. In young adult females with normal menses (21–25 years of age) an isoflavone-rich soya preparation (approximately 90 mg total isoflavones/d) has been shown to have no effects on bone mineral content and bone mineral density over a 12-month period⁽²⁷⁾.

Intervention trials carried out in post-menopausal women are reported in Tables 2 and 3. These studies used either biochemical indices of bone turnover (Table 2) or direct measurements of bone mineral density (Table 3) to examine the effect of soyabean isoflavones ranging from 54 mg to 300 mg/d (most studies used 80–110 mg/d). Evidence from several studies suggests that soyabean proteins and/or their isoflavones may have beneficial effects on bone in post-menopausal women, whereas other trials do not show a benefit over the same or a longer period. Nevertheless, both studies that also targeted Ca have failed to show any significant effect on Ca retention^(28,29), despite a 15–20% lower renal acid excretion with the soyabean diet compared with meat⁽²⁹⁾. Moreover, there is also inconsistency among the studies that show favourable effects, with some finding a benefit in the spine but not the hip and others a benefit in the hip but not the spine.

One study has identified fifteen clinical trials that have examined the effects of isoflavones or isoflavone-rich soyabean protein on bone mineral density⁽³⁰⁾. Most trials were conducted for ≤ 1 year and involved relatively few (less than thirty) participants per group. Although the findings from these studies are inconsistent, in general they suggest that isoflavones reduce bone loss in post-menopausal women in the early years following the menopause (< 5 years post menopause). Furthermore, two consensus views of soyabean phyto-oestrogens in clinical interventions in post-menopausal women have been published recently; one by the French Food Safety Agency⁽²⁴⁾ and one from the EU-funded project Phytohealth⁽³¹⁾. The comprehensive literature search with well-defined inclusion or exclusion criteria suggests, although there is no conclusive evidence, that isoflavones from the sources studied so far have a beneficial effect on bone health. The health benefits of soyabean phyto-oestrogens in healthy post-menopausal women are subtle and even some well-designed studies do not show protective effects. Both papers conclude that until more definite data are available, soya foods and isoflavones cannot be viewed as substitutes for established anti-osteoporotic medication.

The varied results of the clinical studies that have been published suggest the need for investigations of the effect of phyto-oestrogens on bone health that have substantial sample size and long duration to provide a definitive result. It has been suggested that the differences may be a result of synergies or antagonisms among the flavones, threshold

Table 2. Intervention trials: effect of soyabean and isoflavones (IF) consumption on bone turnover in women

References	Subjects	Type of study	Length of the study (months)	Main results
Scambia <i>et al.</i> ⁽⁷⁵⁾	<i>n</i> 39	IF consumption, 50 mg/d	1.5	No change in osteocalcin
Wangen <i>et al.</i> ⁽⁷⁶⁾	Pre-menopausal women, <i>n</i> 14; post-menopausal women, <i>n</i> 17	Soyabean proteins containing 8 (controls), 65 or 130 mg IF/d	3	No change in bone turnover
Upmalis <i>et al.</i> ⁽⁷⁷⁾		Genistein, 50 mg/d	3	No change in osteocalcin and urinary NTX
Scheiber <i>et al.</i> ⁽⁷⁸⁾	<i>n</i> 42	Soya food consumption, i.e. 60 mg IF/d	3	A 13.9% decrease in urinary DPD A 10.2% increase in plasma osteocalcin
Yamori <i>et al.</i> ⁽⁷⁹⁾	Japanese women, <i>n</i> 20	Daily consumption of soyabean germ, i.e. 37.3 mg IF	2.5	Decrease in urinary DPD and pyridinoline compared with the placebo group (<i>n</i> 20)
Uesugi <i>et al.</i> ⁽⁸⁰⁾	Peri-menopausal women, <i>n</i> 23	61.8 mg IF/d	1	Excretion of bone resorption markers was reduced significantly in the IF group
Lucas <i>et al.</i> ⁽⁸¹⁾	Post-menopausal women	40 g ground flaxseed or wheat-based comparative control regimen daily	3	Markers of bone formation and resorption not affected by either of the treatments
Arjmandi <i>et al.</i> ⁽⁸²⁾	<i>n</i> 71	40 g soyabean protein/d	3	Decrease in urinary DPD
Dalais <i>et al.</i> ⁽⁸³⁾	Post-menopausal women, <i>n</i> 106		3	No effect on markers for bone resorption (urinary excretion of pyridinoline or deoxypyridinoline)
Harkness <i>et al.</i> ⁽⁸⁴⁾	Post-menopausal women, <i>n</i> 19, mean age 70.6 (SD 6.3) years	110 mg soyabean IF/d	6	A 37% decrease in urinary concentration of type 1 collagen α 1-chain helical peptide (a marker for bone resorption)
Zittermann <i>et al.</i> ⁽⁸⁵⁾	Young Caucasian women, <i>n</i> 70, mean age 24.0 (SD 0.9) years	Cross-over design Cookies, 52 mg IF/d	One menstrual cycle	IF affect physiological fluctuations in bone turnover (CTX (bone resorption):osteocalcin (bone formation)) was slightly higher during the soyabean period
Roudsari <i>et al.</i> ⁽⁸⁶⁾	Women, <i>n</i> 15, age 45–64 years	35 g soyabean protein/d	3	Reduction in urinary DPD and increased total ALP No change in osteocalcin, CTX, type 1 collagen telopeptide and IGFBP3

NTX, N-terminal cross-linked telopeptides; DPD, deoxypyridinoline; CTX, C-terminal cross-linked telopeptides; ALP, alkaline phosphatase; IGFBP3, insulin-like growth factor-binding protein.

or biphasic dose effects, life-stage oestrogen status or environmental interactions, including the ability to produce metabolites on ingestion of isoflavones⁽³²⁾. Perimenopausal women and women in early menopause may therefore be more receptive to the therapeutic effects of isoflavones before the decrease in ER that occurs in the post-menopausal years⁽³³⁾. Moreover, the major weakness of previous clinical trials is that the risk of fracture, which is actually the ultimate hallmark of bone quality, was not targeted. However, the relationship between usual soyabean food consumption and fracture incidence has been examined in 24 403 post-menopausal Chinese women (recruited in the Shanghai Women's Health Study, a cohort study of approximately 75 000 women aged 40–70 years)⁽³⁴⁾. Evidence was found that soya food consumption may reduce the risk of fracture in these women, particularly among those in the early years following menopause, (after adjustment for confounding factors, the relative risks of fracture were 1.00, 0.72 (95% CI 0.62, 0.83), 0.69 (95% CI 0.59, 0.80), 0.64 (95% CI 0.55, 0.76) and 0.63 (95% CI 0.53, 0.76) across quintiles of soyabean protein intake ($P < 0.001$ for trend). However, it is questionable whether these data can be extrapolated to the Caucasian population.

Phyto-oestrogen bioavailability and bone health

It has been found that the intestinal metabolism of isoflavones could be the more important clue to the clinical efficacy of soya foods in preventing osteopenia⁽³⁵⁾. Indeed, the bioavailability of isoflavones requires an initial hydrolysis of the sugar moiety by intestinal bacterial (*Lactobacilli*, *Bacteroides* and *Bifidobacteria*) β -glucosidases to allow subsequent uptake by enterocytes. Moreover, the final exposure depends on the microbial potential to activate all different groups of phyto-oestrogens within each individual subject. Indeed, phyto-oestrogens (and more especially daidzein) can undergo a further metabolism and be converted into a more potent molecule, equol⁽³⁵⁾. However, this bacterial metabolite has been found, on average, in only 45% of the post-menopausal women studied⁽³⁶⁾. It has been suggested that individuals can be separated into high, moderate and low O-desmethylangolensin, equol, enterodiol, enterolactone or 8-prenylnaringenin producers, even though the metabolism of isoflavones, lignans and prenylflavonoids follows separate independent pathways⁽³⁷⁾. Moreover, enterodiol production correlates negatively with *Clostridium coccooides*–*Eubacterium rectale* counts, while O-desmethylangolensin production is associated with the abundance of methanogens, whereas equol production is correlated with sulfate-reducing bacteria.

Equol has a longer half-life and a much higher affinity for the ER than its precursor (daidzein) and has the highest antioxidant capacity among isoflavones⁽³⁵⁾. It has been reported that isoflavone treatment in subjects who have the capacity to produce equol differentially affects gene expression as compared with non-producers⁽³⁸⁾, supporting the plausibility of the importance of equol production. In general, isoflavones have a stronger effect on some putative oestrogen-responsive genes in equol producers than in

non-producers. This finding could explain the results from a 2-year study carried out in post-menopausal women randomized to consume 500 ml soya milk either with or without isoflavones⁽³⁹⁾. In this study greater effects on bone health were elicited when volunteers were able to produce equol (a 2.4% increase in lumbar-spine bone mineral density compared with the control group), while no significant difference was shown in non-producers. Similarly, it has been reported that in 128 volunteers classified by equol status (producers or non-producers) the percentage changes in bone mineral density for equol producers are -0.53 and $+0.13$ in the sub-whole body and total hip respectively, which are significantly different from the corresponding changes of -1.35 and -1.77 in non-producers in the isoflavone group ($P = 0.049$ and $P = 0.040$ respectively)⁽⁴⁰⁾. However, in another study it was found that total leg and head bone mineral density are greater (6%) in O-desmethylangolensin producers ($n = 76$) compared with non-producers ($n = 16$), while total and site-specific bone mineral density are not different in equol producers ($n = 24$) and non-producers ($n = 68$)⁽⁴¹⁾. Furthermore, equol has been shown to inhibit bone loss in ovariectomized mice, apparently without oestrogenic activity in the reproductive organs⁽⁴²⁾. Hence, substances that can modulate the intestinal microflora could affect equol production. The prebiotic fructo-oligosaccharides have been reported to enhance the effects of soyabean isoflavones on bone mineral density⁽⁴³⁾ and to reverse the loss of certain microarchitectural variables such as tibial trabecular number, separation and thickness⁽⁴⁴⁾ in ovariectomized osteopenic female rats.

Mechanisms of action

Phyto-oestrogens could elicit their bone sparing effect by modulating Ca metabolism, as has been reported for oestrogens⁽⁴⁵⁾. There is evidence that intestinal Ca absorption is higher in ovariectomized rats given soya milk-containing diets than in those receiving a control diet⁽⁴⁶⁾. Nevertheless, it has been shown that ipriflavone, a synthetic phyto-oestrogen-like compound that is similar in structure to the soyabean isoflavone daidzein, increases total Ca transport under low oestrogen conditions, but not under oestrogen-free conditions (via an increased transcellular transport)⁽⁴⁷⁾. Moreover, coumestrol or apigenin have no effect on Ca transport.

Phyto-oestrogens can directly modulate bone metabolism. Using a radioactive cDNA microarray to investigate gene expression profiles (1152 genes), it has been shown that genistein treatment in ovariectomized mice modulates bone metabolism-related gene expression, including calcitropic receptor, cytokines, growth factors and bone matrix protein; thirty-eight genes being up regulated (e.g. mitogen-activated protein kinase 10), while eighteen are down regulated (e.g. matrix metalloproteinase 13)⁽⁴⁸⁾. Similarly, it has been demonstrated that in hFOB/ER α 9 cells (normal fetal osteoblasts) both genistein and enterodiol increase the endogenous gene expression of the progesterone receptor, the proteoglycan versican and alkaline

Table 3. Intervention trials: effect of soyabean and isoflavones (IF) consumption on bone mineral density in post-menopausal women

Reference	Subjects	Type of study	Length of the study	Main results
Potter <i>et al.</i> ⁽⁸⁷⁾	Hypercholesterol, <i>n</i> 66, age 41–83 years	40 g soyabean-protein isolate (90 mg IF)/d	6 months	Increase in BMC and BMD at the spine
Dalais <i>et al.</i> ⁽⁸⁸⁾	<i>n</i> 52, age 45–65 years	Bread containing either 45 g soyabean, linseed or wheat/d	3 months	A 5.2% increase in BMC
Alekel <i>et al.</i> ⁽⁸⁹⁾	<i>n</i> 69, mean age 50.6 years	40 g soyabean protein (80.4 mg IF aglycones)/d	6 months	A 5.6% and 10.1% increase in BMD and BMC respectively at the spine
Clifton-Bligh <i>et al.</i> ⁽⁹⁰⁾	<i>n</i> 46, age <65 years	28.5, 57 or 85.5 mg IF/d	6 months	Consumption of the two highest doses is associated with a 4.1 and 3.0% increase in BMD at the radius and cubitus level No change in DPD
Hsu <i>et al.</i> ⁽⁹¹⁾	<i>n</i> 37	150 mg IF/d		No change in BMD measured on the calcaneum
Chiechi <i>et al.</i> ⁽⁹²⁾	<i>n</i> 187	47 mg IF/d	6 months	Prevention of osteopenia observed in the placebo group Increase in plasma osteocalcin
Lydeking-Olsen <i>et al.</i> ⁽³⁹⁾	<i>n</i> 108	500 ml soya milk (85 mg IF aglycones or depleted in IF (<1 mg))/d	2 years	A 1.1 and 2.2% increase in BMD and BMC respectively in the IF group <i>v.</i> a 4.2 and 4.3% decrease respectively in the placebo group
Vitolins <i>et al.</i> ⁽⁹³⁾	<i>n</i> 172	25 g soyabean protein (5, 42 or 58 mg IF)	2 years	Protective effect on total BMD, whatever the dose of IF
Morabito <i>et al.</i> ⁽⁹⁴⁾	<i>n</i> 90, age 47–57 years	54 mg genistein/d, HRT or placebo	1 year	Genistein supplementation associated with a decrease in urinary DPD, an increase in biomarkers for osteoblast activity and an increase in spine and hip BMD
Chen <i>et al.</i> ⁽⁹⁵⁾	Chinese population, <i>n</i> 203, age 48–62 years	40 or 80 mg IF/d, 500 mg Ca, 3.1 µg vitamin D	1 year	Protective effect on BMC at the hip and trochanter with IF
Gallagher <i>et al.</i> ⁽⁹⁶⁾	<i>n</i> 65, age 55–75 years	96 mg IF, 52 g IF or soyabean without IF (<4 mg)/d	9 months	No effect on BMD of the spine or femoral neck in any of the three groups BMD increased significantly in the trochanter at 9 months ($P = 0.02$) and at 15 months ($P < 0.05$) in the group given IF-free soyabean compared with the other two groups
Harkness <i>et al.</i> ⁽⁸⁴⁾	<i>n</i> 19, mean age 70.6 (sd 6.3) years	110 mg soyabean IF/d	6 months	A non-significant increase in total spine and hip BMD with the IF treatment
Lydeking-Olsen <i>et al.</i> ⁽⁹⁷⁾	Caucasian women with established osteoporosis or at least three risk-factors for osteoporosis, <i>n</i> 89	Soya milk, with or without IF (76 mg/d) with natural transdermal progesterone, a combination or placebo	2 years	Prevention of lumbar spine bone loss (BMD +1.1% <i>v.</i> -4.2% in the control group)
Kreijkamp-Kaspers <i>et al.</i> ⁽⁹⁸⁾	<i>n</i> 202, age 60–75 years	25.6 g soyabean protein containing 99 mg IF	1 year	No effect on BMD (lumbar spine and femur) Women who had the most recent onset of menopause had better results

Atkinson <i>et al.</i> ⁽⁹⁹⁾	<i>n</i> 177, age 49–65 years	A red clover (<i>Trifolium pratense</i>)-derived IF supplement (daily dose of 26 mg biochanin A, 16 mg formononetin, 1 mg genistein and 0.5 mg daidzein)	1 year	Lower loss of lumbar spine BMC and BMD ($P = 0.04$ and $P = 0.03$, respectively) in the IF group No significant treatment effects on hip BMC or BMD, markers of bone resorption Bone formation markers significantly increased in the intervention group
Arjmandi <i>et al.</i> ⁽¹⁰⁰⁾	<i>n</i> 62	Soyabean-containing foods providing 25 g protein and 60 mg IF/d	1 year	No significant change in total hip BMD and BMC irrespective of treatment Whole body and lumbar BMD decreased Increased alkaline phosphatase, osteocalcin
Huang <i>et al.</i> ⁽¹⁰¹⁾	<i>n</i> 43, age 45–67 years	100 or 200 mg IF/d	1 year	Protective effect of the lowest dose on bone loss Lack of benefit with 200 mg IF/d
Newton <i>et al.</i> ⁽¹⁰²⁾	Women, <i>n</i> 13 and men, <i>n</i> 98, age 50–80 years	83 mg IF/d	1 year	Not significant in men Mean percentage change in hip BMD in women from baseline 0.54 (SE 0.38) and -0.13 (SE 0.36) in controls
Wu <i>et al.</i> ⁽¹⁰³⁾	Japanese women (<5 years after the onset of menopause), <i>n</i> 136	75 mg IF conjugate/d with or without walking exercise (45 min/d, 3 d/week)	12 months	A significant effect on BMD only at Ward's triangle Combined intervention of IF and walking exercise showed a trend for a greater effect on BMD at total hip and Ward's triangle regions than either alone
Ye <i>et al.</i> ⁽¹⁰⁴⁾	Early post-menopausal Chinese women, <i>n</i> 90, age 45–60 years	0, 84 or 126 mg IF/d	6 months	Dose-dependent linear relationship between the supplemental IF and percentage changes in BMD at the spine ($P = 0.042$) and the hip ($P = 0.016$)

HRT, hormone-replacement therapy; BMC, bone mineral content; BMD, bone mineral density; DPD, deoxypridinoline.

phosphatase, but inhibit osteopontin gene expression and IL-6 protein levels⁽⁴⁹⁾.

In fact, both genomic and non-genomic mechanisms have been proposed to explain their effect. They are able to interact with enzymes and receptors and, because of their stable structure and low molecular weight, they can pass through cell membranes⁽⁵⁰⁾. Mechanistically, phyto-oestrogens have been shown to bind to the two types of ER, their relative affinity for ER β being greater than that for ER α ⁽⁵¹⁾, and may thus produce distinct clinical effects from oestrogens by differentially triggering transcriptional activation or repression pathways via ER β ^(52,53). Moreover, they can act on both bone cell lineages. It has been reported that phyto-oestrogens such as coumestrol, genistein and daidzein increase alkaline phosphatase activity and enhance bone mineralization in MC3T3-E1 cells (osteoblast-like cell)⁽⁵⁴⁾. It has been demonstrated that daidzein stimulates osteoblast differentiation at various stages (from osteoprogenitors to terminally-differentiated osteoblasts)^(55,56). Daidzein induces changes in the actin cytoskeleton responsible for cell adhesion and motility and activates two transcription factors, cAMP-response element-binding protein and Elk1, which are linked to early genes controlling cell proliferation and differentiation via the mitogen-activated protein kinases extracellular signal-regulated kinases 1 and 2⁽⁵³⁾. It is also recognized that isoflavones inhibit *in vitro* bone resorption, via direct targeting of osteoclasts and/or osteoclast progenitors. Certainly, genistein can decrease osteoclast differentiation and increase osteoclast apoptosis or interfere with signalling pathways such as intracellular Ca²⁺, cAMP or protein kinase and protein tyrosine phosphatase or via inhibition of topoisomerase II activity^(57,58). Tyrosine kinase inhibition, in turn, has been reported to directly inhibit osteoclast membrane HCl transport⁽⁵⁹⁾. Furthermore, the maturation of osteoclast is dependent on two factors, macrophage colony-stimulating factor and receptor activator of NF- κ B-ligand. It has been shown that coumestrol has an inhibitory effect on the differentiation of osteoclasts, at least partially via decreased receptor activator of NF- κ B-ligand-induced phosphorylation of extracellular signal-regulated kinases/p44/42⁽⁵⁵⁾. These data are consistent with other published data⁽⁶⁰⁾. Furthermore, phyto-oestrogens such as genistein are able to enhance osteoblastic osteoprotegerin production through ER α -dependent mechanisms and concurrently suppress receptor activator of NF- κ B-ligand gene expression, which is associated with an inhibition of osteoclastogenesis; bone resorption during the remodelling cycle being coupled to bone formation through the receptor activator of NF- κ B/receptor activator of NF- κ B-ligand/osteoprotegerin system⁽⁶¹⁾.

Finally, PPAR have recently been identified as additional targets of phyto-oestrogen, which can dose dependently activate PPAR and induce divergent effects on adipogenesis and osteogenesis. Dominant ER-mediated effects (increased osteogenesis and decreased adipogenesis) can only be seen at low concentrations of phyto-oestrogens, whereas dominant PPAR-mediated effects are only evident at high concentrations^(62,63). Consequently, divergent actions can be produced in the same cell-tissue system.

Conclusion

Continued awareness and promotion of preventive health services relevant to issues of post-menopausal women will certainly contribute to achieving a healthier population. Thus, the use of phyto-oestrogens is receiving great scrutiny for the purpose of both enhancing the health of tissue and preventing several common diseases, including osteoporosis. However, the limits of the information obtained so far must be appreciated and more data is needed before health professionals can actively advocate the increased consumption of such compounds. Indeed, it will be important to further characterize their physiological effects and margins of safety. In targeting bone health the main issue that remains to be resolved is the effect of phyto-oestrogens in terms of bone strength (which is the ultimate hallmark of bone quality).

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