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Original article

Red clover isoflavones are safe and well tolerated in women with a family history of breast cancer

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Abstract

Objective. To assess the safety and tolerability of a standardized 40 mg red clover isoflavone dietary supplement (Promensil®, Novogen) in women with a family history of breast cancer to evaluate the feasibility of using the supplement for prevention of breast cancer in healthy women.

Study design. Healthy women aged 35–70 years ($n = 401$) with at least one first-degree relative with breast cancer received red clover isoflavones or placebo for three years in a randomized, double-blind, placebo-controlled pilot trial. Participants were assessed clinically and blood samples taken for biochemical analysis every six months. In addition, study participants underwent mammography, bone density and transvaginal ultrasound (postmenopausal women only) once per year.

Results. No significant differences in breast density, endometrial thickness, serum cholesterol, follicle stimulating hormone levels and bone mineral density were detected between those taking red clover isoflavones and placebo. In postmenopausal women, some significant differences in bone marker levels were seen between active and placebo groups, at six months and at 12 months. The adverse event profile was similar across all red clover isoflavone and placebo groups.

Conclusion. This three-year study supports the growing body of evidence that treatment with red clover isoflavones is safe and well tolerated in healthy women. Supplements containing red clover isoflavones did not adversely affect breast density, skeletal strength or cardiovascular status. In postmenopausal women, endometrial status was not adversely affected. The adverse event profile was similar between red clover isoflavones, and placebo and endocrine status did not differ.

Keywords: Isoflavones, red clover, menopause, phytoestrogens, breast cancer

Introduction

Phytoestrogen isoflavones are biologically active agents, which occur naturally in the diet. Structurally similar to estrogens, they are weakly estrogenic showing at least 20 times higher affinity for the β than the α-subtype.[1] Therefore, isoflavones' estrogenic action is most active on tissues rich in estrogen receptor β, such as bone, capillary epithelium and the central nervous system.[2] However, isoflavones show antiestrogenic activity by competitively binding to estrogen receptors and by inhibiting 17ß-estrodiol[2] and can thereby inhibit the growth and proliferation of hormone-dependent cells including tumour growth[3] and exhibit antiangiogenic properties.[4]

These mechanisms may account for the lower incidence of breast malignancies and some other cancers reported in Asian populations that consume large quantities of soy.[5,6] High dietary intake of isoflavones has been reported to be associated with a substantial reduction in breast cancer risk.[6]

Epidemiological studies indicate that vasomotor menopausal symptoms, osteoporosis, estrogen-dependent tumours and other hormone-related diseases are less common and, when they occur, are less severe in Asian women compared with their counterparts in Europe and the USA. Dietary differences appear to account, at least partially, for this discordance.[2] In particular, Asian food is rich in isoflavones and other phytoestrogens, reflecting

the high intake of plants and especially soy (Glycine max) in their traditional diet.[2]

Hormone replacement therapy (HRT) remains the mainstay of treatment for menopausal vasomotor symptoms despite adverse publicity from recent studies, in particular, the Heart and Estrogen/progestin Replacement Study (HERS),[7] Women's Health Initiative (WHI)[8] study and the Million Women Study.[9] These studies changed clinicians' and patients' perceptions of the risks and benefits associated with HRT and prescription rates declined markedly in many countries. In Germany, for example, the number of current HRT users declined by 16% in the seven months after publication of HERS II and WHI.[10] In the United States, millions of older women stopped using HRT during 2002.

For some years, there has been an increasing interest in the use of alternatives to HRT. Red clover and soy isoflavones are one such alternative used by women for the management of menopausal vasomotor symptoms.[11–13]

The evidence from randomized, placebo-controlled trials in Europe and the USA and from meta-analyses of the effects of isoflavones on the health of perimenopausal and postmenopausal women is conflicting. A recent systematic review and meta-analysis concluded that isoflavone supplementation, with either soy or red clover isoflavones, produces a 'slight to modest' reduction in the number of daily menopausal flushes especially in women, who experience a high number of flushes each day. Regression analysis indicated that women experiencing 10 flushes a day could expect a reduction of 22% with a range from 1 to 59%.[11] This reduction is sufficient to potentially improve quality of life for many menopausal women. A further meta-analysis of red clover isoflavone extracts trials demonstrated that hot flush frequency was not reduced.[14] Use of soy isoflavones for alleviating hot flushes in women surviving breast cancer, who experience significantly more frequent and severe hot flushes, is also not supported by randomized studies.[15]

In healthy postmenopausal women, there is some evidence that dietary supplementation with isoflavones reduces vaginal dryness,[16] loss of lumbar spine bone mineral density (BMD)[17] and low density lipoprotein (LDL) cholesterol.[18] Further well-designed randomized trials are needed.

Red clover (*Trifolium pratense*) is a rich source of the isoflavones genistein, daidzein, biochanin A and formononetin. The latter two undergo partial demethylation in the gastrointestinal tract yielding genistein and daidzein, the same isoflavones found in soy.[2]

Few prospective data are available on the effects of isoflavone extracts on breast cancer risk, in the breast tissue and on the endometrium, and on the long-term safety of isoflavone extract supplementation. The present study aimed to assess the safety and tolerability of red clover isoflavones at biologically effective doses in healthy women with a family history of breast cancer. The trial size was based on secondary objectives of assessing the impact of a 40 mg red clover isoflavone supplement on BMD and total cholesterol.

The primary objective of the study was to evaluate the feasibility of using the supplement for prevention of breast cancer. Its conclusions will have relevance also to perimenopausal and menopausal women among whom supplementation with red clover isoflavones for hot flush reduction is commonplace.

Methods

This randomized, double-blind trial was performed at the Royal Marsden Hospital (RMH) and the University Hospital of South Manchester (UHSM). Healthy women aged between 35 and 70 years inclusive of at least one first-degree relative with breast cancer were randomized (using a variable size permuted block design stratified by centre and menopausal status) to receive red clover isoflavones (40 mg daily) or placebo. Local pharmacies held the randomization lists.

The primary objective of the study was to assess the feasibility of undertaking a large double-blind randomized multicentre trial giving a 40 mg red clover isoflavone supplement or placebo to healthy women at a increased risk of breast cancer. Secondary objectives were to evaluate the effects of a 40 mg red clover isoflavone supplement or placebo on BMD, bone markers, serum lipids, uterine ultrasound (postmenopausal women only) and mammographic breast density. The size of the trial was based on the secondary objectives of BMD and total cholesterol. Any adverse events were monitored and recorded.

Red clover isoflavones were provided as a standardized dietary supplement containing defined amounts of genistein, daidzein, formononetin and biochanin extracted from red clover. One 40 mg red clover tablet produces isoflavone levels similar to that found in populations consuming high isoflavone diets.[19]

Healthy pre- and postmenopausal patients with at least one first-degree relative with breast cancer received 40 mg red clover isoflavones or an identical placebo for three years. Participants were assessed clinically and blood samples taken for biochemical analysis every six months. Mammography, bone density and transvaginal ultrasound (postmenopausal women only) measurements were performed once in a year.

The study excluded pregnant or lactating women; those taking oral contraceptives or HRT and women with a previous history of non-invasive or invasive breast cancer or other malignancy, except basal cell carcinoma or cervical cancer *in situ.* Women with a significant vasomotor symptoms were not included because of the possible need for HRT.

Menopausal status was defined as follows:

- Participants were considered premenopausal if: their last menstrual period (LMP) was within three months of randomization; or LMP >3 months before randomization, but follicle stimulating hormone (FSH) <35 mLU/mL, luteinizing hormone (LH) ≤40 mLU/mL or both; or hysterectomy and FSH <35 mLU/mL or any period after randomization.
- Women were considered postmenopausal, if their LMP was >1 year before randomization; LMP >1 year before randomization and FSH <45 mLU/mL, LH >40 mLU/mL or both; post hysterectomy and bi-lateral oophorectomy or hysterectomy and FSH >35 mLU/mL, LH >40 mLU/mL or both.
- Participants who did not fit into either of the category were considered perimenopausal.

The number of patients enrolled was based on a RMH breast cancer prevention trial,[20] in which postmenopausal women taking tamoxifen showed an increase in spinal BMD of 0.91% per annum (standard deviation [SD] 2.44). This was compared with a decrease of 0.35% annually

(SD 2.65) in the placebo group. In premenopausal women, tamoxifen reduced spinal BMD by 1.88% annually (SD 2.32) compared with a 0.21% annual increase (SD 1.61) in women on placebo. If red clover isoflavones had a similar effect on BMD, then 134 post- and 36 premenopausal women would be sufficient to detect a difference between the active and placebo arms ($\alpha = 5\%$; power = 80%).

In the above trial,[20] postmenopausal women taking tamoxifen showed a reduction in total cholesterol of 14.6% at two years (SD 11.6) compared with a 4.9% reduction (SD 15.7) among the placebo-treated women. In premenopausal women, the reductions were 10.1% (SD 13.4) and 1.6% (SD 12.6), respectively. If red clover isoflavones had a similar effect, then 72 postmenopausal and 80 premenopausal women would be sufficient to detect this difference ($\alpha = 5\%$; power 80%).

The accrual target was initially set at 300 patients; it was estimated that this should be adequate to demonstrate a treatment effect on BMD and cholesterol, at least in premenopausal women. Postmenopausal women were included in this pilot feasibility study as they would be the candidates for any future randomized trial. Recruitment of postmenopausal women was slower than expected and the study was extended in order to increase the numbers in this group. Despite this extension, the study did not have the power to detect differences in postmenopausal bone density or cholesterol.

Increased breast density measured on mammography has consistently been associated with risk for breast cancer.[21]

Most women in the trial had baseline mammograms on film. The RMH introduced digital mammography during follow-up; the UHSM has used film mammography throughout the trial. Mammograms were conducted on both breasts. All film images were digitalized and breast density was determined from the digital or digitalized images. Breast density was measured on a scale of 0–100 with higher figures representing more dense breasts.[22]

BMD were measured in standard units and a conversion factor applied to adjust for differences between the density machines. The percentage change in lumbar spine and total femur BMD was calculated as:

$$\frac{100(\text{Follow-up BMD} - \text{baseline BMD})}{\text{baseline BMD}}$$

The analysis percentage combined changes in BMD in the peri- and postmenopausal patients and assessed differences using the Mann-Whitney non-parametric test.

Blood for markers of bone metabolism were taken in postmenopausal women at the RMH at baseline, six and 12 months. The bone markers were measured at the Academic Unit of Bone Metabolism, University of Sheffield. The ratio of type I collagen-cross-linked *N* telopeptide (NTx) to creatinine was measured in urine. Levels of osteocalcin, bone alkaline phosphatase (BAP) and serum beta C-terminal collagen I telopeptide (CTx) were measured in blood. Each marker was expressed as a percentage of the baseline level. Changes from baseline measurements were assessed using the Wilcoxon's test and differences between the treatment arms by the Mann-Whitney test.

Total cholesterol was measured at baseline and every six months for three years. The percentage change in total cholesterol, measured from the blood samples, was calculated as follows:

$$\frac{100(\text{Follow-up cholesterol} - \text{baseline cholesterol})}{\text{baseline cholesterol}}$$

Pre- and postmenopausal patients were analysed separately and treatment differences assessed using the Mann-Whitney test.

Results

Of the 401 women randomized, 320 were premenopausal, 20 perimenopausal or of uncertain menopausal status and 61 postmenopausal (Table 1). The demographics of the placebo and red clover isoflavones groups were well matched (Table 1). A total of 71% (284) of the women completed three-year's treatment and 18% (73) withdrew. Concordance was assessed by patient enquiry.

Sixteen women stopped because they required HRT, 14 because of toxicity, eight because they developed cancer and six for other reasons (Figure 1). There was no significant difference in the number of women developing cancer between treatment groups (three in the active group, compared with five in the placebo group). The women in this study population are at higher risk of breast cancer because of their family history, and assuming a doubling of risk for each first-degree relative, 4.6 breast cancers would be expected during the study period. Hence, there are more cancers than would be expected (eight observed, compared with 4.6 expected), but this is not a significant increase ($P = 0.8$). There was no difference in completion rates between the red clover isoflavones and placebo arms ($P = 0.7$). Table 2 summarizes the adverse events reported by the patients.

Changes in breast density from baseline were calculated at 1–3 years from mammograms conducted in most cases on both breasts. In a few premenopausal cases, only one side could be imaged or the density read. In order to ensure accuracy of density readings, it was necessary for the technician to read mammograms taken at 1–3 years, all at the same occasion. Mammograms conducted at three years for around one-half of the study participants were not available at the time of reading those from one year and two years, hence full three-year analysis of breast density changes was not possible for the whole population of women who completed three years of treatment. The number of evaluable patient mammograms at three years are shown in Table 3.

Table 1 Demographics of the red clover isoflavones and placebo groups

		Red clover isoflavones	Placebo
Patients (*n*)		199	202
Treatment centre	RMH	159	163
	UHSM	40	39
Stratification	Premenopausal	160	164
	Postmenopausal	39	38
Age	Median (range)	45 (35–69)	45 (35–69)
Body mass index	Median (range)	24.8 (17–44)	25.2 (17–51)
Number of affected first-degree relatives	One	175	179
	Two or more	24	23

RMH, Royal Marsden Hospital; UHSM, University Hospital of South Manchester

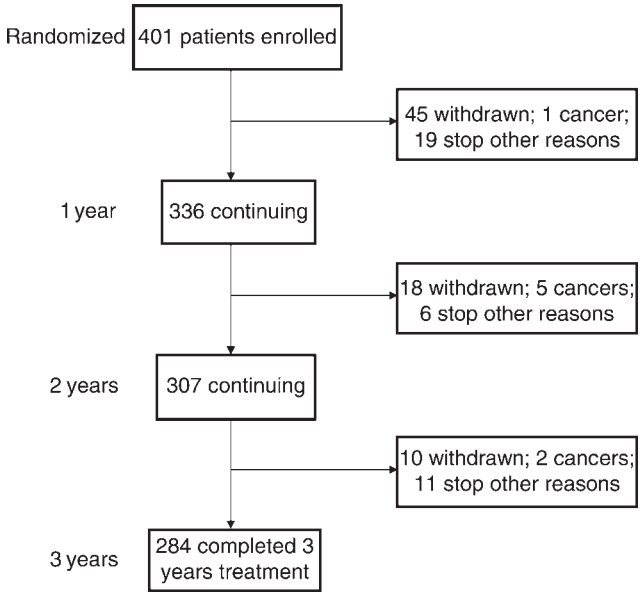

Figure 1 CONSORT diagram.

Separate analyses of premenopausal and postmenopausal women showed no significant differences between the placebo and active groups from baseline to each of the annual reviews as reported in Table 3. Although the trial was not designed to test for a treatment effect on breast density, the Atkinson study[21] indicates that this study would have 90% power to detect a difference of 5% density between treatments at three years.

Postmenopausal women ($n = 36$) underwent annual transvaginal ultrasound. There were no significant differences in endometrial thickness (Figure 2) between the red clover isoflavones and placebo groups. Although the numbers are small, the data suggest that an increase in endometrial thickness on treatment of 4 mm or greater can be excluded with 80% power.

The percentage changes in BMD showed no significant differences between red clover isoflavones versus placebo in either of the premenopausal or the peri/postmenopausal participants (Figure 3).

The measurements of the bone marker levels at baseline, six months and 12 months were as follows. In postmenopausal women, the NTx/Cr ratio showed no statistically significant difference between the placebo and red clover isoflavones groups at baseline. Neither treatment arms showed significant changes from baseline either at six or 12 months (red clover isoflavones: six months, $P = 0.4$; 12 months, $P = 0.7$ and Placebo: six months, $P = 0.7$; 12 months, $P = 0.6$). There were also no significant differences between treatment arms (six months: $P = 0.5$; 12 months $P = 0.7$).

Similarly in this postmenopausal group, BAP did not differ significantly between treatment arms at baseline. When compared with baseline, BAP rose in the placebo group after six months (median 112% of baseline $P = 0.04$) and at 12 months (median 108% of baseline $P = 0.006$). No such increase emerged in postmenopausal women taking red clover isoflavones (six months median 102% of baseline $P = 0.9$; 12 months median 103% of baseline $P = 0.3$). There were no significant differences between treatment arms (six months, $P = 0.2$; 12 months, $P = 0.1$).

At baseline, osteocalcin levels were marginally higher in postmenopausal women randomized to red clover isoflavones than placebo (mean 22.5 and 18.1 units; $P = 0.04$). Osteocalcin levels did not significantly change compared with baseline in the red clover isoflavones (six months, $P = 0.2$; 12 months, $P = 0.3$) or placebo (six months, $P = 0.6$; 12 months, $P = 0.2$) groups. There were no significant differences between placebo and red clover isoflavones (six months, $P = 0.3$; 12 months, $P = 0.2$).

At baseline, serum beta CTx was higher in postmenopausal women randomized to red clover isoflavones than placebo (mean 0.20 and 0.15 units; $P = 0.03$). When compared with baseline, serum beta CTx levels increased for women in the placebo arm at six months (median 120% of baseline $P = 0.09$). The difference was non-significant at 12 months (median 124% of baseline $P = 0.1$). No such increase was seen for women on red clover isoflavones (six months mean 97% of baseline $P = 0.4$; 12 months mean 93% of baseline $P = 0.8$). At six months patients on red clover isoflavones had a lower level of serum beta CTx (as a percentage of baseline) then women on placebo ($P = 0.03$). The difference was non-significant at 12 months ($P = 0.2$).

At baseline, the levels of cholesterol were not significantly different for women on red clover isoflavones or placebo. Serum cholesterol levels in pre and postmenopausal women did not show any statistically significant differences for red clover isoflavones or placebo. There was a tendency for FSH to increase over the study. However, no statistically significant differences emerged between the red clover isoflavones and placebo groups.

Table 2 Adverse events reported by the red clover isoflavones and placebo groups

	Number of women reporting each side effect	
	Red clover isoflavones	Placebo
Nausea/vomiting	4	2
Headaches	3	7
Hot flushes	4	5
Weight gain	7	13
Period abnormality	2	3
Breast abnormality	20	20
Mood changes	6	7
Vaginal discharge	3	1
Eye problems	3	2
Fluid retention	5	6
Hair/nail problems	1	6
Skin	10	9
Sleep disturbance	2	1
Indigestion	4	1
Other abdominal problems	2	4
Aching joints	8	9
Dizzy	2	3
Bowel/constipation/diarrhoea	9	6
Bladder	3	1
Weight loss	2	0
Lethargy	7	8
Gynaecological	2	0
Other minor side-effects	43	41

Discussion

Although the results of this study were not sufficiently significant to support the investment in a longer term breast cancer prevention study in healthy women, the study offers some reassuring data about the use of the red clover isoflavone supplement studied.

Table 3 Changes in breast density during treatment with red clover isoflavones or placebo

	Number of women	Number of breasts assessed	Mean change	95% CI	Significance
(a) *Premenopausal women*					
Change from Baseline → 1 year					
Red clover isoflavones	133	264	−0.63%	−1.91:0.66%	
Placebo	127	250	−0.82%	−1.85:0.21%	$P = 0.6$
Change from Baseline → 2 years					
Red clover isoflavones	123	246	−2.01%	−3.56:−0.47%	
Placebo	120	240	−2.02%	−3.24:−0.80%	$P = 1.0$
Change from Baseline → 3 years					
Red clover isoflavones	111	122	−3.03%	−5.53:−0.54%	
Placebo	111	119	−6.60%	−9.04:−4.16%	$P = 0.2$
(b) *Postmenopausal women*					
Change from Baseline → 1 year					
Red clover isoflavones	22	50	−1.5%	−4.0:1.0%	
Placebo	25	44	0.6%	−3.1:4.2%	$P = 0.8$
Change from Baseline → 2 years					
Red clover isoflavones	22	43	−5.8%	−9.1:−2.6%	
Placebo	22	44	−3.9%	−8.0:0.3%	$P = 0.9$
Change from Baseline → 3 years					
Red clover isoflavones	8	16	−6.9%	−11.6:−2.1%	
Placebo	11	22	−8.0%	−15.7:−0.2%	$P = 0.7$

The percentage changes in BMD showed no significant differences between red clover isoflavones versus placebo. A previous study found that isoflavone supplements significantly reduced the loss of lumbar spine bone mineral content ($P = 0.04$) and BMD ($P = 0.03$) compared with placebo in predominantly postmenopausal women.[17] In contrast, around 80% of women in our study were premenopausal and any effect on bone loss might be expected to be less than in postmenopausal patients. Adequately, powered studies need to determine whether isoflavone supplements benefit BMD. However, there is currently no evidence of a detrimental effect.

Previous studies have shown that isoflavone supplements significantly increased levels of bone-specific alkaline phosphatase ($P = 0.04$) and *N*-propeptide of collagen type I ($P = 0.01$), both markers of bone formation.

Regarding markers of bone resorption or body composition, the osteocalcin levels and the NTx/Cr ratio did not show statistically significant differences. Compared with baseline, BAP rose in the placebo arm after six months and at 12 months. No such increase emerged in postmenopausal women on red clover isoflavones. At baseline, serum beta CTx was higher in women randomized to red clover isoflavones than placebo. At six months, postmenopausal patients on red clover isoflavones had a lower level of serum beta CTx than women on placebo. However, isoflavone supplements did not alter bone mineral content or BMD. The changes in these markers may not be clinically significant, given the lack of effect on BMD, but again suggest that supplements containing red clover isoflavones do not adversely affect skeletal strength.

Serum cholesterol in pre- and postmenopausal women did not show any statistically significant differences for women taking red clover isoflavones or placebo. A previous study has shown 90 days treatment with red clover isoflavones, decreased total cholesterol, LDL-cholesterol and triglyceride levels. The difference in triglyceride levels

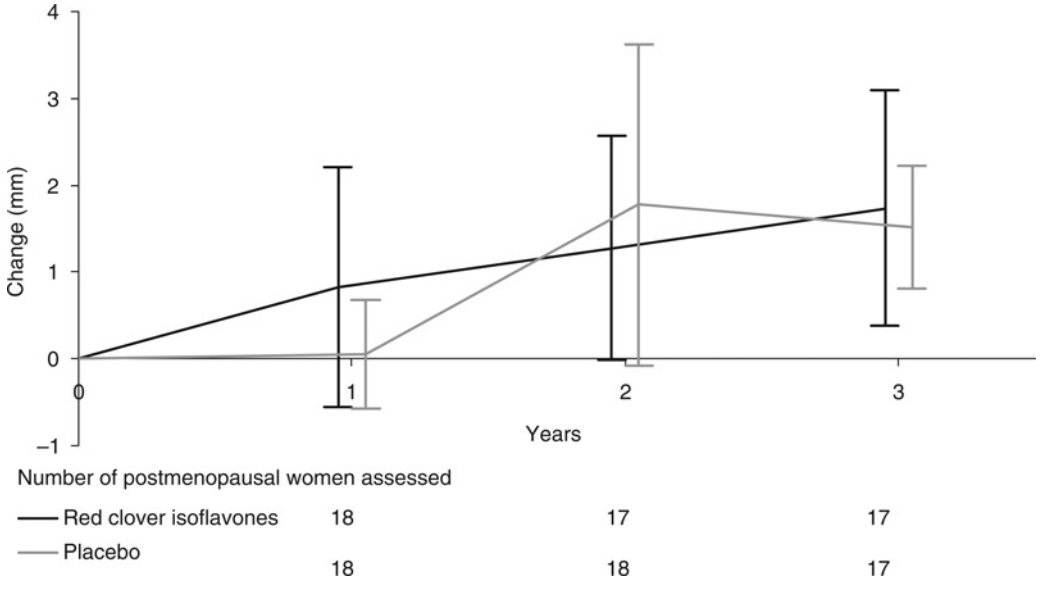

Figure 2 Changes in endometrial thickness during treatment with red clover isoflavones or placebo (mean and 95% CI).

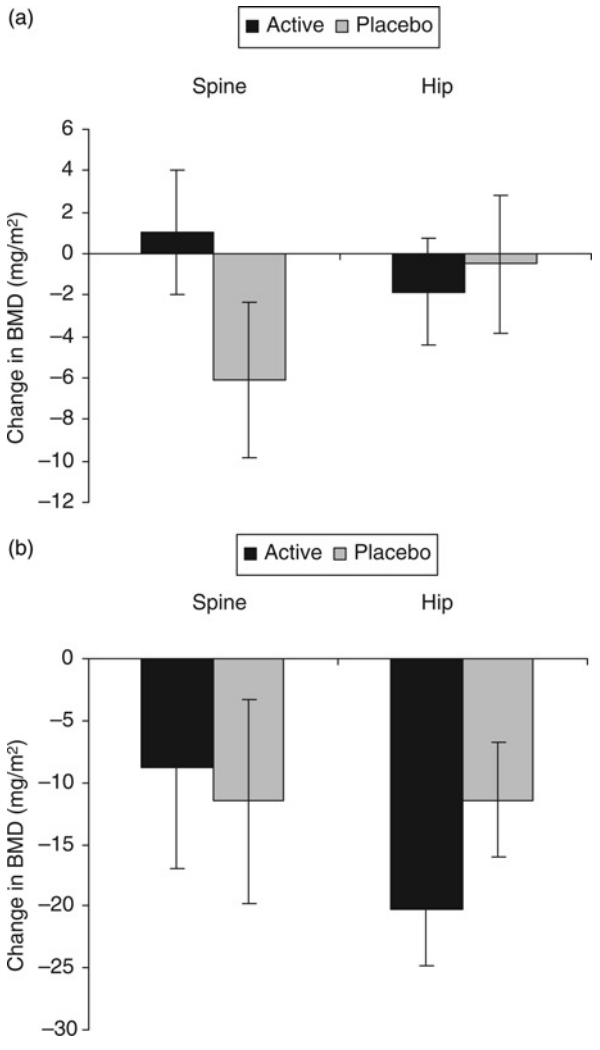

Figure 3 Changes in BMD during treatment with red clover isoflavones or placebo. (a) Premenopausal women; (b) Peri/postmenopausal women.

reached statistical significance.[23] Further studies should examine the effects of red clover isoflavones on lipid subfractions. The weight of evidence suggests that red clover isoflavones do not adversely affect cardiovascular status.

FSH showed a tendency to increase over the study. However, no statistically significant differences emerged between red clover isoflavones and placebo. In other studies, red clover isoflavones shows inconsistent effects on hormone levels. Atkinson *et al.*[21] reported that FSH, oestradiol and LH do not change during a year's treatment with red clover isoflavones. In another study, red clover isoflavones did not alter levels of 17ß-estrodiol, FSH or sex hormone-binding globulin compared with either baseline or placebo.[2] In contrast, one study[23] found a correlation between decrease in serum FSH and the improvement in hot flushes. The lack of effect on FSH in this and other studies may suggest that endocrine changes may not be solely responsible for the potential benefits on vasomotor symptoms. Further studies are needed to confirm the mode of action and hormonal effects.

The study shows that dietary supplementation with red clover isoflavones has no obvious toxicity and there does not appear to be any estrogenic effect on breast density. There were no significant differences detected in breast density from baseline at 1–3 years between treatment arms. There were no differences detected in endometrial thickness between postmenopausal women taking red clover isoflavones and those randomized to placebo.

Our study adds to a body of evidence that isoflavones do not appear to increase breast density.[21,24,25] In a study that enrolled 205 women aged between 49 and 65 years, taking red clover isoflavones for a year did not increase mammographic breast density, in contrast to conventional HRT.[21] In another study, 220 premenopausal women consumed either two daily servings of soy foods equivalent to 50 mg of isoflavones or their regular diet. Over two years, the total area of the breast increased and the size of the dense areas decreased in both groups. After two years, mean percentage density decreased by 2.8 and 4.1% in those eating soy and controls, respectively. Women whose lifetime diet included large amounts of soy had higher percentage densities than women whose diet included little soy. However, the difference reached statistical significance only in Caucasians.[25]

Overall, the data indicate that red clover isoflavones do not appear to increase radiological breast density, even in women who already show an increased density.[26] Further studies should assess the effect of higher doses of red clover isoflavones on healthy women, especially those who show increased breast density.[26]

The lack of effect on endometrial thickness with red clover isoflavone supplementation, albeit shown in small numbers of postmenopausal women, is consistent with the results from other studies.[27–29] Furthermore, the lack of effect on endometrial thickness supports the selective estrogen receptor modulator-like binding by isoflavones to ER-β in tissues, such as bone, arteries and the central nervous system, rather than the ER-α receptors expressed in uterine and breast tissue.[29]

This study has some limitations. First, the study enrolled women with at least one first-degree relative with breast cancer, the majority of whom were premenopausal. Eligibility criteria for this trial excluded postmenopausal women taking HRT which meant that fewer postmenopausal women enrolled than expected. Further studies need to assess the safety in a less selected cohort. Secondly, there are several formulations containing isoflavones from soy or red clover. These formulations may differ.[30] As a result, the results we obtained may not apply to formulations other than the red clover isoflavone supplement studied. Thirdly, our study was not powered to detect an effect on the bone health or cholesterol levels of postmenopausal women.

Overall, this study supports previous data indicating that red clover isoflavones at this dose are safe with little apparent toxicity or evidence of harmful effect on the breast, the endometrium and in premenopausal women, on bone health or blood cholesterol levels. Although the data supporting red clover isoflavone supplementation for vasomotor symptom control are inconsistent, the use of red clover isoflavones by menopausal women, as is increasingly common practice, would appear safe at this time.

Conclusion

In conclusion, red clover isoflavones are safe, well tolerated and show no evidence of an estrogenic effect on breast density when taken for three years by women with a first-degree relative with breast cancer. In particular,

supplements containing red clover isoflavones did not adversely affect breast density, skeletal strength or cardiovascular status. In postmenopausal women, no significant changes in endometrial thickness were detected. The lack of a stimulatory effect found in this study – the longest to date – is encouraging and supports the tolerability of red clover isoflavones based on epidemiological and tissue selectivity data.

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