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The effect of isoflavones extracted from red clover (Rimostil) on lipid and bone metabolism

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ABSTRACT

Objective: This study was undertaken to evaluate the effects of varying doses of phytoestrogens on lipid and bone metabolism in postmenopausal women.

Design: A novel red clover isoflavone preparation (Rimostil) containing genistein, daidzein, formononetin, and biochanin was administered to 46 postmenopausal women in a double-blind protocol after a single-blind placebo phase and followed by a single-blind washout phase. Patients were randomized to receive either 28.5 mg, 57 mg, or 85.5 mg of phytoestrogens daily for a 6-month period.

Results: At 6 months, the serum high-density lipoprotein cholesterol had risen significantly by 15.7–28.6% with different doses ($p = 0.007$, $p = 0.002$, $p = 0.027$), although the magnitude of the response was independent of the dose used. The serum apolipoprotein B fell significantly by 11.5–17.0% with different doses ($p = 0.005$, $p = 0.043$, $p = 0.007$) and the magnitude of the response was independent of the dose used. The bone mineral density of the proximal radius and ulna rose significantly by 4.1% over 6 months with 57 mg/day ($p = 0.002$) and by 3.0% with 85.5 mg/day ($p = 0.023$) of isoflavones. The response with 28.5 mg/day of isoflavones was not significant. There was no significant increase in endometrial thickness with any of the doses of isoflavone used.

Conclusion: These results show that the administration of an isoflavone combination extracted from red clover was associated with a significant increase in high-density lipoprotein cholesterol, a significant fall in apolipoprotein B, and a significant increase in the predominantly cortical bone of the proximal radius and ulna after 6 months of treatment. Interpretation of the results is undertaken cautiously because of the absence of a simultaneously studied control group.

Key Words: Menopause – Phytoestrogens – HDL cholesterol – Apolipoprotein B – Bone mineral density – 25OH-vitamin D.

Isoflavone phytoestrogens are not steroids but mimic some actions of steroidal estrogens. The phenolic ring in the isoflavone class of phytoestrogens enables the binding of these molecules to the intracellular estrogen receptors.[1] It is now well documented that at least two forms of estrogen receptor exist, ER-α and ER-β. The more recently discovered second form of estrogen receptor, ER-β, binds isoflavones with greater affinity than ER-α.[2] Since steroidal estrogens are known to modify cholesterol metabolism and bone density, foodstuffs that contain isoflavones, such as soy protein and extracts of red clover, have been studied for their ability to influence cholesterol and bone metabolism. For example when 45 mg of isoflavone-containing soy protein was administered daily to premenopausal women, the serum cholesterol was significantly reduced,[3] but the high-density lipoprotein (HDL) cholesterol concentrations did not change. In a different study, postmenopausal women given 40 g/day of isoflavone-containing protein showed a reduction in serum non-HDL cholesterol and an increase in serum HDL cholesterol.[4] Baum et al.[5] also showed an increase in the serum HDL cholesterol in postmeno-

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pausal women given isoflavone-containing soy flour for 24 weeks. However, in another study, 58 postmenopausal women given isoflavone-containing soy flour for 14 weeks experienced no change in serum total cholesterol, HDL cholesterol, and triglycerides.[6] Anderson et al.,[7] in a meta-analysis of published studies, showed that 31–47 g/day of soy protein produced a fall in serum cholesterol and in serum low-density lipoprotein (LDL) cholesterol especially in those with high baseline serum cholesterol levels.

Both genistein and daidzein, isoflavones found in soy and in red clover, stimulated an increase in calcium content of rat femoral diaphyseal bone in culture.[8] In 2-month old rats subjected to ovariectomy, injections of genistein ameliorated bone loss, while histomorphometry showed increased rates of bone formation. Interestingly, genistein did not affect markers of bone resorption.[9] Genistein enhanced the proliferation and differentiation of human bone cells in culture.[10] Furthermore, female mice subjected to ovariectomy had markedly reduced trabecular bone in the distal femoral metaphysis that was prevented by genistein.[11] Daily administration for 6 months of soy protein containing 90 mg of isoflavones increased the bone mineral content and bone mineral density (BMD) of the lumbar spine in postmenopausal women, but no significant changes were seen at other skeletal sites. By contrast, daily administration of soy protein containing 55.6 mg of isoflavones for 6 months did not preserve bone mass.[4]

Rimostil is a recently developed red clover isoflavone mix containing daidzein and genistein. In addition, and in contrast to soy protein, Rimostil contains the isoflavones formononetin and biochanin. The effects of this extract on bone density and serum lipids have not been previously investigated. For this reason, the present study was undertaken to evaluate the effects of varying doses of isoflavones extracted from red clover on lipid and bone metabolism in postmenopausal women. The study consisted of a single-blind placebo baseline, a double-blind treatment phase, followed by a single-blind placebo washout phase.

PATIENTS AND METHODS

Fifty women, at least 1 year past their last menstrual period, aged less than 65 years and who had a serum follicle-stimulating hormone level of at least 40 mIU/mL, were recruited into the study. Women were excluded from the study if they had received hormonal treatment within 3 months of study entry, had a history of estrogen-dependent neoplasm, including breast cancer, acute or chronic liver disease, diabetes mellitus, hypertension (blood pressure > 160/90), or a body mass index (BMI) of >33 kg/m^2. All subjects were randomly assigned to one of three groups designated to receive 28.5 mg/day, 57 mg/day, or 85.5 mg/day of total isoflavones. Each patient also received 1000 mg of calcium daily. At the point of recruitment into the study and at every visit thereafter, patients were instructed to minimize the intake of isoflavone-containing food. The diet of participants was not otherwise modified. At time −1 month, all patients were administered three 500-mg placebo tablets. One month later, the double-blind treatment phase commenced in which subjects were randomized to receive one of the three isoflavone doses. Active tablets each contained 28.5 mg total isoflavone content and were presented in identical 500-mg tablet form. At this time, patients in the 28.5 mg/day group received one active and two placebo tablets per day, those in the 57 mg/day group received two active and one placebo tablet per day, and those in the 85.5 mg/day group received three active tablets per day. These combinations of tablets were administered daily for 6 months. This was followed by a 2-month washout period during which all patients were switched back to three placebo tablets per day. Two participants from the 28.5 mg/day group, one from the 57 mg/day group, and one from the 85.5 mg/day group withdrew from the trial after initial assessment, leaving 46 women who completed the trial. The isoflavones contained in the active tablets were obtained from red clover by a standardized extraction process and contained daidzein, genistein, formononetin, and biochanin in a novel proprietary ratio (Rimostil; Novogen Ltd., North Ryde, Australia). Twenty-four hour urine isoflavone analysis was undertaken at −1, 0, 3, 6, and 8 months. Daidzein, genistein, equol, biochanin, formononetin, and O-desmethylangolensin were analyzed in the urine using a modification of previously published methods.[12,13] Aliquots (10 ml) of urine were mixed with 100 ml of glucuronidase. The mixture was incubated for 24 h at 37°C after which it was extracted on a C-18 solid phase extraction column (Waters Pty. Ltd., Sydney, Australia). Isoflavones were eluted with 3 ml of methanol, and 10 ml of the extract was injected into the high-performance liquid chromatography system. The high-performance liquid chromatography system consisted of a 25-cm, 5 nM, C-18 stationary phase column (Symmetry, Waters Pty. Ltd.) and a gradient acetonitrile/water mobile phase. The limit of detection of the assay for each of the isoflavones measured was 5 ng/mL. The interassay coefficient of variation (CV) was <15%.

At 0, 3, and 6 months, the urine was assayed for deoxypyridinoline as a marker for osteoclast activity and corrected for creatinine excretion. Deoxypyridinoline was assayed by the Immulite Pyrilinks-D method (Diagnostic Products Corporation, Los Angeles, CA, USA). The interassay CV was 13.3% at 25 nmol/L and 3.3% at 100 nmol/L.

At −1, 0, 3, 6, and 8 months, nonfasting blood was drawn between 8:00 a.m. and 10:00 a.m. for the analysis of serum cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein B, and triglycerides. Measurement of total cholesterol and HDL cholesterol was performed on a Hitachi 747 analyser using a commercially available enzymatic colorimetric assay, and measurement of apolipoprotein B was performed on a Hitachi 902 analyser using an immunoturbidimetric test (Roche Diagnostics, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald equation. The CV for total cholesterol was 1.92% at 6.87 mmol/L, the CV for HDL cholesterol was 2.97% at 2.31 mmol/L, and the CV for triglycerides was 3.55% at 1.55 mmol/L. The CV for apolipoprotein B was 3.80% at 1.83 mmol/L.

At 0, 3, and 6 months, the BMD of the right forearm was measured at three sites: proximal radius and ulna at a point one third of the distance between the ulnar stylus and the olecranon; radius and ulna at a point in the distal forearm where the radius and ulna are 8 mm apart and where scanning proceeds from this point 1 cm proximally; and distal radius and ulna. BMD was quantified using a Norland pDEXA bone densitometer (Norland Corporation, Fort Atkinson, WI, USA). The precision of the method was 0.65% in the proximal radius and ulna and 0.66% for the distal radius and ulna.

At 0 and 6 months, the endometrial thickness was measured by transvaginal ultrasound (Aloka Echo Camera, model SSD-500; Aloka Co. Ltd, Tokyo, Japan). At 0, 3, 6, and 8 months, the serum 25OH-vitamin D concentrations were measured. The 25OH-vitamin D assay used in the study was a commercially available ^{125}I RIA kit (DiaSorin, Stillwater, MN, USA).

The protocol for the study was approved by the Royal North Shore Hospital Human Research Ethics Committee before individual patients entered the study.

Statistical analysis

The statistical software package SPSS for Windows was used to analyze the data. One-way analysis of variance was used to test for homogeneity of continuous variables such as age, weight, height, and years since menopause across treatment groups. When heterogeneity was detected, the least significant difference method of multiple comparisons was used to test for pairwise differences between groups.

TABLE 1. *Characteristics of the study group*

	Age (ys)	BMI Wt/Ht^2 kg/m^2	Years since LMP[a]
Group 1			
28.5 mg/d isoflavones	55 ± 3.3	26.1 ± 2.8	5 ± 2.3
Group 2			
57 mg/d isoflavones	59 ± 4.1	25.3 ± 6.2	9 ± 4.2
Group 3			
85.5 mg/d isoflavones	56 ± 6.4	25.1 ± 4.5	6 ± 4.0

Values given are mean ± 1 SD.
[a]LMP, last menstrual period.

The effect of treatment was assessed by the within patient change over time using the Wilcoxon signed rank test. Nonparametric two-way analysis of variance (Kruskal-Wallis) with repeated measures was used to test for effects of treatment, time, and their interaction on the continuous outcome variables such as blood lipid levels and BMDs.

Spearman rank correlation coefficients were used to quantify the degree of linear association between continuous variables. A significance level of 5% was considered to be statistically significant throughout.

RESULTS

The age, BMI, and years since menopause for the study groups are shown in Table 1. The mean ages of the three groups were not significantly different. There were no significant differences between groups for BMI. The number of years since menopause was significantly greater for group 2 when compared with groups 1 and 3 ($p = 0.035$).

The changes in the serum lipids are shown in Table 2. The total serum cholesterol did not change significantly in any treatment group. The serum HDL cholesterol rose significantly after 6 months of treatment with each of the doses given. With 28.5 mg of isoflavone daily, the serum HDL rose by 15.8% ($p = 0.007$), with 57 mg by 28.6% ($p = 0.002$), and with 85.5 mg by 15.7% ($p = 0.027$). There was no significant difference in the HDL response between different doses of isoflavones.

There was no significant change in the calculated serum LDL cholesterol concentrations over the 6-month treatment interval in any treatment group ($p = 0.874$), and the serum triglyceride concentrations did not change significantly in any treatment group ($p = 0.879$).

TABLE 2. *Serum lipids (mmol/L)*

	Time (mo)			
	0	3	6	8
28.5 mg				
$n = 15$				
TC[a]	5.92 ± 0.52	5.76 ± 1.11	6.22 ± 0.93	5.91 ± 0.44
HDL	1.75 ± 0.37	1.77 ± 0.37	2.01 ± 0.41	1.85 ± 0.41
LDL	3.69 ± 0.61	3.39 ± 1.07	3.76 ± 0.84	3.48 ± 0.49
ApoB	1.30 ± 0.27	1.20 ± 0.25	1.15 ± 0.23	1.04 ± 0.12
Tg	1.05 ± 0.38	1.20 ± 0.54	1.12 ± 0.41	1.37 ± 0.76
57 mg				
$n = 16$				
TC	5.94 ± 1.04	5.57 ± 1.01	6.15 ± 1.05	6.17 ± 1.15
HDL	1.60 ± 0.48	1.70 ± 0.53	1.95 ± 0.64	1.85 ± 0.41
LDL	3.51 ± 1.06	3.10 ± 1.06	3.41 ± 1.01	3.62 ± 1.18
ApoB	1.35 ± 0.28	1.25 ± 0.29	1.12 ± 0.28	1.17 ± 0.34
Tg	1.63 ± 0.68	1.34 ± 0.61	1.56 ± 0.70	1.61 ± 0.74
85.5 mg				
$n = 15$				
TC	5.50 ± 0.79	5.58 ± 0.97	5.83 ± 1.12	5.77 ± 0.98
HDL	1.61 ± 0.41	1.73 ± 0.42	1.82 ± 0.39	1.79 ± 0.45
LDL	3.38 ± 0.93	3.27 ± 0.93	3.35 ± 0.89	3.46 ± 1.02
ApoB	1.26 ± 0.30	1.23 ± 0.33	1.09 ± 0.29	1.12 ± 0.29
Tg	1.14 ± 0.52	1.06 ± 0.56	1.05 ± 0.51	1.13 ± 0.61

Values given are mean ± 1 SD.
[a]TC, total cholesterol; HDL, HDL cholesterol; LDL, LDL cholesterol; ApoB, apolipoprotein B; Tg, triglyceride.

The serum apolipoprotein B concentration fell significantly after 6 months of treatment with each of the doses given. With 28.5 mg of isoflavone daily, the serum apolipoprotein B fell by 11.5% ($p = 0.005$), with 57 mg by 17.0% ($p = 0.043$), and with 85.5 mg by 11.5% ($p = 0.007$). There was no significant difference in the apolipoprotein B response between different doses of isoflavones.

By 8 months, 2 months after cessation of the isoflavone active treatment phase, the serum HDL cholesterol had fallen by 9.4% in the 28.5 mg/day isoflavone treatment group, by 7.1% in the 57 mg/day treatment group, and by 1% in the 85.5 mg/day isoflavone treatment group.

The data for the changes in BMD in the forearm are shown in Table 3. The BMD in the proximal radius and ulna rose by 2.9% over the 6-month period with 28.5 mg of isoflavones daily ($p = 0.118$ NS), by 4.1% with 57 mg of isoflavones daily ($p = 0.002$), and by 3.0% with 85.5 mg of isoflavones daily ($p = 0.023$) (Wilcoxon signed rank test). The average increase in BMD for the whole group was 3.3%.

The BMD of the distal radius and ulna showed no significant change in relation to the doses of isoflavones used. The BMD of the radius and ulna at the 8-mm gap between radius and ulna showed a significant decline with time ($p = 0.037$, paired t test; $p = 0.057$, Wilcoxon signed rank test) independent of the dose of isoflavones given. In the combined group of 45 patients, this fall was 1.5% over 6 months.

The data for the changes in urine deoxypyridinoline are shown in Table 4. There were no significant changes in urine deoxypyridinoline over time between 0 and 6 months and no differences between the doses of isoflavones used adjusted for times.

The total urine isoflavones increased from a baseline value of 1.820 ± 2.473 mg/24 h to 5.786 ± 5.555 mg/24 h after 6 months in the group treated with 28.5 mg of isoflavones ($p = 0.018$), from 0.271 ± 0.423 mg/24 h to 9.497 ± 5.687 mg/24 h in the group treated with 57 mg isoflavones ($p = 0.002$), and from 1.529 ± 3.508 mg/24 h to 18.767 ± 12.054 mg/24 h in the group treated with 85.5 mg isoflavones ($p = 0.0001$). There was a significant difference between doses ($p = 0.001$) in that 85.5 mg/day of isoflavones produced a larger urinary output than either 28.5 mg or 57 mg daily, which were comparable.

There was no correlation between the change in either the serum HDL cholesterol or the serum apolipoprotein B between 0 and 6 months of treatment and the change in total urine isoflavones over the same time interval. However, the change in the BMD of distal radius and ulna between 0 and 6 months was directly correlated with the increase in daidzein ($p = 0.017$). There was no correlation between the changes in BMD at other sites and the changes in individual urine isoflavones.

A puzzling and unexpected finding was that the serum 25OH-vitamin D declined significantly between 0 and 6 months of treatment and this decline was independent of the dose of isoflavones. The mean serum 25OH-vitamin D was 94.5 ± 28.2 nmol/L at time 0, and 75.3 ± 24.5 nmol/L after 6 months of treatment with isoflavones ($p < 0.0001$). At the eighth month, 2 months after treatment with isoflavones was ceased, the serum 25OH-vitamin D had risen to 79.6 ± 24.0 nmol/L. In comparison to the 6 month value, this rise was significant ($p < 0.001$).

The endometrial thickness, as assessed by transvaginal ultrasound, did not change significantly during the 6 months of treatment with isoflavones. Endometrial thickness at time 0 was 2.9 ± 1.1 mm, and after 6 months of isoflavones it was 3.1 ± 1.1 mm (NS). The endometrial thickness did not exceed 5 mm after 6 months of treatment with isoflavones in any of the study patients.

DISCUSSION

This study showed that the administration of an isoflavone combination extracted from red clover, Rimos-

TABLE 3. *BMD-forearm (g/cm^2)*

Dose[a]		Time (mo) 0	3	6
28.5 mg	Distal radius and ulna	0.313 ± 0.062	0.312 ± 0.071	0.311 ± 0.061[b]
n = 15	Radius and ulna, 8 mm	0.418 ± 0.060	0.425 ± 0.071	0.424 ± 0.059
	Proximal radius and ulna	0.723 ± 0.071	0.739 ± 0.066	0.743 ± 0.066
57 mg	Distal radius and ulna	0.326 ± 0.044	0.323 ± 0.041	0.317 ± 0.039[b]
n = 15	Radius and ulna, 8 mm	0.438 ± 0.049	0.434 ± 0.049	0.428 ± 0.048
	Proximal radius and ulna	0.746 ± 0.059	0.768 ± 0.060	0.779 ± 0.062[c]
85.5 mg	Distal radius and ulna	0.318 ± 0.061	0.309 ± 0.053	0.316 ± 0.054[b]
n = 15	Radius and ulna, 8 mm	0.439 ± 0.066	0.426 ± 0.073	0.427 ± 0.074
	Proximal radius and ulna	0.732 ± 0.070	0.752 ± 0.084	0.754 ± 0.076[c]

Values given are mean ± 1 SD.
[a]Column one shows the daily dose of isoflavones given to each group.
[b]The change in BMD in the distal radius and ulna between 0 and 6 months is not significant.
[c]The change in the bone mineral of the proximal radius and ulna between 0 and 6 months is significant for the 57 mg and 85.5 mg groups but not for the 28.5 mg group.

TABLE 4. *Urinary deoxypyridinoline (nmol/mmol Cr)*

Dose	Time (mo) 0	3	6
28.5 mg	6.576 ± 2.671	6.831 ± 1.771	6.186 ± 1.934
57 mg	5.369 ± 1.711	7.056 ± 1.687	7.191 ± 2.800
85.5 mg	7.079 ± 3.415	7.233 ± 2.481	7.131 ± 2.676

Values are mean ± 1 SD.

til, was associated with a marked and significant increase in serum HDL cholesterol. This effect was seen at each dose level of isoflavones (28.5 mg/day, 57 mg/day, and 85.5 mg/day) and the magnitude of the response was not correlated with the dose of isoflavones. This study was designed to assess the possible effects of different doses of isoflavones on lipid parameters described and did not include a placebo arm. This is a major weakness of the study because it is therefore not possible to ascertain whether the observed changes in serum HDL cholesterol and serum apolipoprotein B are due to the effects of phytoestrogens administered or whether they are due to factors operating independent of any phytoestrogen effect. However, the fall in serum HDL cholesterol in the 2 months after isoflavones were ceased was partial evidence that the effects observed were due to the administration of isoflavones. It is also possible that a maximum effect on serum HDL cholesterol and serum apolipoprotein B had already been exerted by the lowest dose of phytoestrogen used, 28.5 mg/day, and that the use of higher doses did not exert any additional effects.

There is strong evidence in women that higher levels of serum HDL cholesterol are associated with a reduced risk of coronary heart disease. It has been calculated that a hypothetical 0.026 mmol/L increase in serum HDL cholesterol could translate into a 3.2% reduction in cardiovascular mortality in women.[14] The results reported here were equivalent to a 0.34 mmol/L increase in serum HDL cholesterol, which could imply, if the effect is linear, that a proportionately greater clinical benefit could be achieved. A sustained increase in serum HDL cholesterol brought about by the consumption of red clover-derived isoflavones might favor an environment in which deposition of cholesterol into the walls of arteries is reduced. However, in another study in which postmenopausal women with preexisting coronary atherosclerosis, whose average age was 66 years, were given Premarin 0.625 mg/day, the mean serum HDL cholesterol rose by 18.8%; but the rate of progression of coronary atherosclerosis, as assessed by angiography, was not different from placebo-treated women.[15]

An interesting aspect of the changes in serum HDL cholesterol observed in the present study was that since the 6-month response was greater than the 3-month response, the full expression of the response may not have been reached even at 6 months. The finding in the present study of no significant dose response effect with the different doses of isoflavones was similar to the finding of Baum et al.[5] who used isoflavone-containing soy protein at different doses. In that study, the increase in the serum HDL cholesterol was 5.2% for 50 mg of isoflavone and 3.6% for 90 mg of isoflavone. In our study, the response was substantially greater. One possible explanation for this is that extracts of red clover contain the additional isoflavones formononetin and biochanin, which may have more pronounced effects on the HDL metabolism than soy isoflavones. The HDL cholesterol response between individuals showed wide variability even with the same dose of isoflavones

and this response was independent of the amount of isoflavones secreted in the urine.

The significant fall in the plasma apolipoprotein B at each dose of isoflavone given is also an interesting finding. Previous studies have shown a direct relationship between plasma apolipoprotein B levels and the risk of premature coronary artery disease particularly in women,[16,17] so that a fall in the plasma apolipoprotein B levels might be associated with a reduced risk of premature coronary artery disease. The use of isoflavones in this study did not bring about a significant fall in plasma LDL cholesterol. Because the plasma apolipoprotein B did fall significantly, this would lead to a rise in the LDL cholesterol/apolipoprotein B ratio. A lower LDL cholesterol/apolipoprotein B ratio has been associated with the formation of smaller, dense LDL particles and increased numbers of these particles has been linked with an increased risk of premature coronary heart disease.[18,19] A rise in the LDL cholesterol/apolipoprotein B ratio would be expected to lead to an increased LDL particle size, which may be less atherogenic. It should be noted, however, that in the present study, apolipoprotein B was measured in whole plasma and not in the LDL fraction of plasma, so that true LDL cholesterol/apolipoprotein B ratios were not obtained. Alternatively, since LDL cholesterol is a calculated value based on HDL and triglyceride levels, the fact that triglyceride levels were measured nonfasting may have influenced the calculation of LDL cholesterol levels. However, there was no significant change in serum triglycerides with time or between different doses of phytoestrogens such that it might affect the derived LDL and HDL cholesterol calculations. Since apolipoprotein B is not affected by food, apolipoprotein B may be a more robust indicator of LDL changes in this study.

BMD was measured at three separate sites in the forearm. The forearm was chosen in this study because of the previously demonstrated high precision of measurements in the forearm and the expectation that any changes in the BMD brought about by increased consumption of isoflavones would be small over a 6-month period. In fact, the average increase with all doses in BMD in the proximal radius and ulna in a 6-month period was 3.3%. In the 57 mg/day group, a 4.1% increase was observed. Significant increases in BMD in the proximal radius and ulna were seen with 57 mg/day or 85.5 mg/day of isoflavones but not with 28.5 mg/day. Therefore, in the proximal radius and ulna, a threshold dose effect was seen with which a significant increase in bone mass occurred. The bone structure in the proximal radius and ulna is predominantly cortical bone, and increases in cortical bone have not been seen in other studies with isoflavones. The distal radius and ulna contain predominantly trabecular bone, and the BMD at this site did not change significantly in the present study. In other studies, the BMD of the lumbar spine, predominantly trabecular bone, did increase after ingestion of isoflavones[4] and in ovariectomized mice, trabecular bone was preserved by genistein. In the present study, there was no correlation between changes in BMD at any site and changes in urinary genistein. In the distal radius and ulna, there was a positive correlation between changes in BMD and changes in urinary daidzein. The potential for isoflavones to preserve or increase bone mass in postmenopausal women is being further explored in a double-blind, placebo-controlled trial to be continued over 2 years.

The finding in the present study of an increase in the BMD of predominantly cortical bone is of particular interest because studies using estrogen in postmenopausal women, also having at least 1000 mg of calcium/day, have shown only a very small increase in the BMD of the proximal radius of less than 1%.[20] It should be noted that our patients received 1000–1200 mg/day of supplemental calcium throughout the study, and it is possible that the increase in BMD could have been due to the effect of calcium supplementation. However, in a separate study conducted by us[21] in which 80 postmenopausal women, average age 56.1 years, were given 1000 mg calcium/day, the BMD of the proximal radius and ulna increased by 0.94% in 12 months, somewhat less than the 3.3% increase observed in the present study in a 6-month period.

It is not known whether the effects of phytoestrogens in Rimostil on bone are mediated by ERα or ERβ, or both, or whether the action is one of stimulation of ER-mediated events or inhibition of the ability of the receptor to bind estradiol. Interestingly, in adult ERβ knockout mice, the cortical bone density of the tibia and femur is increased, whereas the trabecular bone density of the tibia and femur is unchanged,[22] a similar pattern of response seen in the present study. It cannot, however, be implied that any of the phytoestrogens used in the present study inhibit the function of the ERβ in bone.

A further interesting and unexpected finding was that the administration of isoflavones was associated with a significant fall in the serum 25OH-vitamin D concentrations. The mean serum 25OH-vitamin D concentrations after 6 months of isoflavone administration were still above the lower limit of normal and it is not certain what effect a fall of this magnitude would have on the maintenance of bone mass, or whether giving

supplemental vitamin D would enhance the response to isoflavones. The mechanism of the fall in the serum 25OH-vitamin D has not been determined. Twenty-nine of the 50 subjects entered during the summer and therefore completed the 6 months of isoflavone treatment during winter. This may have explained the fall in 25OH-vitamin D observed. The fall in serum 25OH-vitamin D could also have been due to an increase in the rate of inactivation of 25OH-vitamin D in the liver.[23] However, an increase in the BMD of the proximal radius and ulna occurred despite this fall in serum 25OH-vitamin D.

Finally, the isoflavones in the form administered did not cause an increase in endometrial thickness over the 6-month period of observation. This is consistent with previous data indicating the lack of endometrial effects by isoflavones,[24] supporting the safety of this type of intervention.The data reported in this study provide some evidence for a beneficial effect on cholesterol profile and bone density in postmenopausal women receiving a unique red clover isoflavone supplement. The magnitude of the effects here are likely to be clinically relevant, and although the absence of a simultaneously studied placebo group prevents a more robust link between phytoestrogens and the parameters studied, based on previously published risk factor analysis they may be associated with reduced risk of cardiovascular disease and bone fracture rates.

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