

Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women¹⁻³

Susan M Potter, Jo Ann Baum, Hongyu Teng, Rachel J Stillman, Neil F Shay, and John W Erdman Jr

ABSTRACT The effects of soy protein (40 g/d) containing moderate and higher concentrations of isoflavones on blood lipid profiles, mononuclear cell LDL receptor messenger RNA, and bone mineral density and content were investigated in 66 free-living, hypercholesterolemic, postmenopausal women during a 6-mo, parallel-group, double-blind trial with 3 interventions. After a control period of 14 d, during which subjects followed a National Cholesterol Education Program Step I low-fat, low-cholesterol diet, all subjects were randomly assigned to 1 of 3 dietary groups: Step I diet with 40 g protein/d obtained from casein and nonfat dry milk (CNFDM), Step I diet with 40 g protein/d from isolated soy protein containing 1.39 mg isoflavones/g protein (ISP56), or Step I diet with 40 g protein/d from isolated soy protein containing 2.25 mg isoflavones/g protein (ISP90). Total and regional bone mineral content and density were assessed. Non-HDL cholesterol for both ISP56 and ISP90 groups was reduced compared with the CNFDM group ($P < 0.05$). HDL cholesterol increased in both ISP56 and ISP90 groups ($P < 0.05$). Mononuclear cell LDL receptor mRNA was increased in subjects consuming ISP56 or ISP90 compared with those consuming CNFDM ($P < 0.05$). Significant increases occurred in both bone mineral content and density in the lumbar spine but not elsewhere for the ISP90 group compared with the control group ($P < 0.05$). Intake of soy protein at both isoflavone concentrations for 6 mo may decrease the risk factors associated with cardiovascular disease in postmenopausal women. However, only the higher isoflavone-containing product protected against spinal bone loss. *Am J Clin Nutr* 1998; 68(suppl):1375S-9S.

KEY WORDS Soy, isoflavones, genistein, cholesterol, bone density, postmenopausal women, HDL cholesterol, LDL receptor mRNA, bone mineral content

INTRODUCTION

The reduction of blood total and LDL cholesterol concentrations with the consumption of products containing soy protein has been shown repeatedly in humans and various animal models (1-3). The component of the soy-protein products responsible for these changes has yet to be defined. Recently, some have postulated that isoflavones may be responsible.

Isoflavones are present in relatively large amounts in virtually all products containing soy protein with the exception of soy-

protein concentrates and isolates that have undergone alcohol extractions during processing (4). Isoflavones are known to be estrogen analogues and bind to estrogen receptors, eliciting an affinity of 7×10^{-6} to 8×10^{-4} that of estradiol (5). Thus, it is logical that if the isoflavones possess actions similar to estrogens, they could influence several biological processes including lipid and bone metabolism. Because postmenopausal women are at risk for health problems related to estrogen deficiency, such as cardiovascular disease and osteoporosis, consumption of soy products containing isoflavones might affect risk factors for these diseases. We designed the present study to examine the effect of soy protein containing different concentrations of isoflavones on blood lipid profiles, mononuclear cell LDL receptor messenger RNA (mRNA), and bone density in postmenopausal women.

SUBJECTS AND METHODS

We are reporting observations from a human intervention trial in which a number of variables were assessed (6). A brief description of methods will be given here.

Subjects

Sixty-six hypercholesterolemic, postmenopausal women completed the study and were included in the statistical analyses. Subjects were screened for initial total cholesterol concentrations (between 6.21 and 7.76 mmol/L), were interviewed, and completed health surveys to assess their appropriateness for inclusion as subjects in the study. Subject selection excluded those receiving any medications known to alter lipid, bone, or calcium metabolism, including hormone replacement therapy within the past 6 mo; those who had a menstrual period < 12 mo before initiation of the study protocol; and those with any systemic or endocrine disease known to affect lipid, mineral, or bone metabolism. Subjects were asked not to take any vitamin or

¹From the Department of Food Science and Human Nutrition, Division of Nutritional Sciences, and the Department of Kinesiology, University of Illinois at Urbana-Champaign.

²Supported by the Illinois Soybean Program Operating Board and Protein Technologies International, St Louis.

³Address reprint requests to SM Potter, Protein Technologies International, Inc, PO Box 88940, St Louis, MO 63188. Email: spotter@protein.com.

mineral supplements for the duration of the investigation. This study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign.

Study design and diet

Before initiation of the study protocol, subjects were placed on a low-fat (<30% of energy), low-cholesterol (<300 mg/d) diet (National Cholesterol Education Program Step I diet) as instructed by a registered dietitian (7). All subjects followed this basal diet for ≥ 2 wk, at which time they were randomly assigned to 1 of the 3 dietary treatment groups that provided 40 g protein/d from one of the following: isolated soy protein containing moderate concentrations of isoflavones (ISP56; Supro 675, Protein Technologies International, St Louis), isolated soy protein containing higher concentrations of isoflavones (ISP90; Protein Technologies International), or casein and nonfat dry milk (CNFDM; New Zealand Milk Products, Wellington, New Zealand). Both isolated soy proteins were fortified with calcium (calcium phosphate) to amounts comparable with those found in casein. The study lasted 26 wk: 2 wk for the basal lead-in period and 24 wk for the intervention period.

Test proteins were incorporated into a variety of food items including breads, muffins, drinks, milks, and soups. Breakfast was provided 3 d/wk and subjects were instructed to consume food items totaling 40 g test protein/d. Food intake and activity diaries were obtained every 4 wk and consumption diaries for food items containing test proteins were obtained daily. Body weight was measured weekly throughout the study and nutrient intake was analyzed by using a nutrient database (NUTRITIONIST IV, version 3.0; N-Squared Computing, Salem Park, OR).

Blood lipid analyses

On 2 separate days at the end of the 2-wk adaptation period (baseline) and every 6 wk for the duration of the 24-wk study, fasting blood samples were collected into tubes containing either heparin or EDTA. HDL was separated immediately by heparin-manganese precipitation (8) and plasma samples were stored at -70°C in separate portions for subsequent analyses. Total plasma cholesterol, HDL, and total triacylglycerols were quantified by using automated techniques (Hitachi 704 Auto Analyzer; Boehringer Mannheim Corporation, Indianapolis) and commercially available kits (Boehringer Mannheim; Sigma Diagnostics, St Louis; and Raichem, San Diego). The accuracy of plasma measurements was verified by use of either Centers for Disease Control and Prevention or International Federation of Cereal Chemists quality-control plasma samples of known concentrations (Northwest Lipid Research Laboratories, Seattle). Non-HDL concentrations were calculated by subtracting HDL from total cholesterol.

LDL receptor mRNA analysis

Mononuclear cells were isolated as described by Lovati et al (9). Because of the labor required for the isolation process, mononuclear cell LDL receptor mRNA was analyzed for 38 of the 66 subjects ($n = 15$ for ISP56, $n = 12$ for ISP90, and $n = 11$ for CNFDM) at baseline and after 12 and 24 wk of the study. Selection of subjects for this analysis was random and based on subjects' willingness to provide additional blood. Total cellular RNA was prepared from mononuclear cells by using the single-step acid guanidinium thiocyanate-phenol-chloroform method

TABLE 1
Characteristics of subjects¹

Variable	Baseline
Age (y)	
ISP56	59.8 \pm 9.1 (49–73)
ISP90	61.2 \pm 10.3 (39–83)
CNFDM	61.3 \pm 6.3 (51–74)
Body mass index (in kg/m ²)	
ISP56	28.2 \pm 6.0 (16.6–40.4)
ISP90	26.2 \pm 4.6 (20.5–40.6)
CNFDM	29.1 \pm 5.2 (22.6–40.8)
Body weight (kg)	
ISP56	74.1 \pm 15.6 (43.5–109)
ISP90	68.5 \pm 14.4 (49.4–110)
CNFDM	78.0 \pm 13.2 (59.6–111)
Years since menopause (y)	
ISP56	12.2 \pm 9.7 (0.6–32.6)
ISP90	13.7 \pm 8.3 (1.9–31.0)
CNFDM	12.6 \pm 8.5 (1.3–30.1)
Total cholesterol (mmol/L)	
ISP56	6.6 \pm 0.9 (5.5–8.6)
ISP90	6.5 \pm 0.9 (5.6–9.1)
CNFDM	6.3 \pm 0.7 (5.4–7.5)
HDL cholesterol (mmol/L)	
ISP56	1.3 \pm 0.3 (0.7–2.0)
ISP90	1.4 \pm 0.3 (0.9–2.0)
CNFDM	1.4 \pm 0.3 (0.8–2.2)
Non-HDL cholesterol (mmol/L)	
ISP56	5.2 \pm 0.9 (4.1–7.5)
ISP90	5.1 \pm 1.0 (3.9–7.7)
CNFDM	4.9 \pm 0.8 (3.2–6.4)
Triacylglycerol (mmol/L)	
ISP56	1.9 \pm 1.0 (0.8–4.6)
ISP90	1.7 \pm 0.7 (0.8–3.4)
CNFDM	1.8 \pm 1.1 (0.7–5.7)

¹ $\bar{x} \pm \text{SD}$; range in parentheses. $n = 66$. ISP56, isolated soy protein with moderate isoflavones; ISP90, isolated soy protein with higher isoflavones; CNFDM, casein and nonfat dry milk.

(10). Reverse transcription–polymerase chain reaction was used to quantitate concentrations of LDL receptor mRNA (11). Specific details are given by Baum et al (6).

Bone measurements

The Bone mineral content and density of the lumbar spine (L1–L4), the proximal femur (including the femoral neck and Ward's triangle), and the total body was measured by dual-energy X-ray absorptiometry (DXA; Hologic QDR-2000, Waltham, MA) at the end of the lead-in period and then again after the 24-wk intervention period. Regions of interest for the spine and femur were defined according to the manufacturer's guidelines. All measurements were made and analyzed by the same 2 experienced operators; the in vivo precision error in this laboratory for bone mineral density was 1% for the total body, 1.3% for the spine, and 1.8% for the hip.

Statistical analysis

The effects of dietary intervention on various outcomes were evaluated by using multiple linear regression analyses. Treatment effects were indicated by using 2 dummy-coded variables, one comparing the ISP90 and CNFDM diets and the other comparing the ISP56 and CNFDM diets. Treatment by covariate

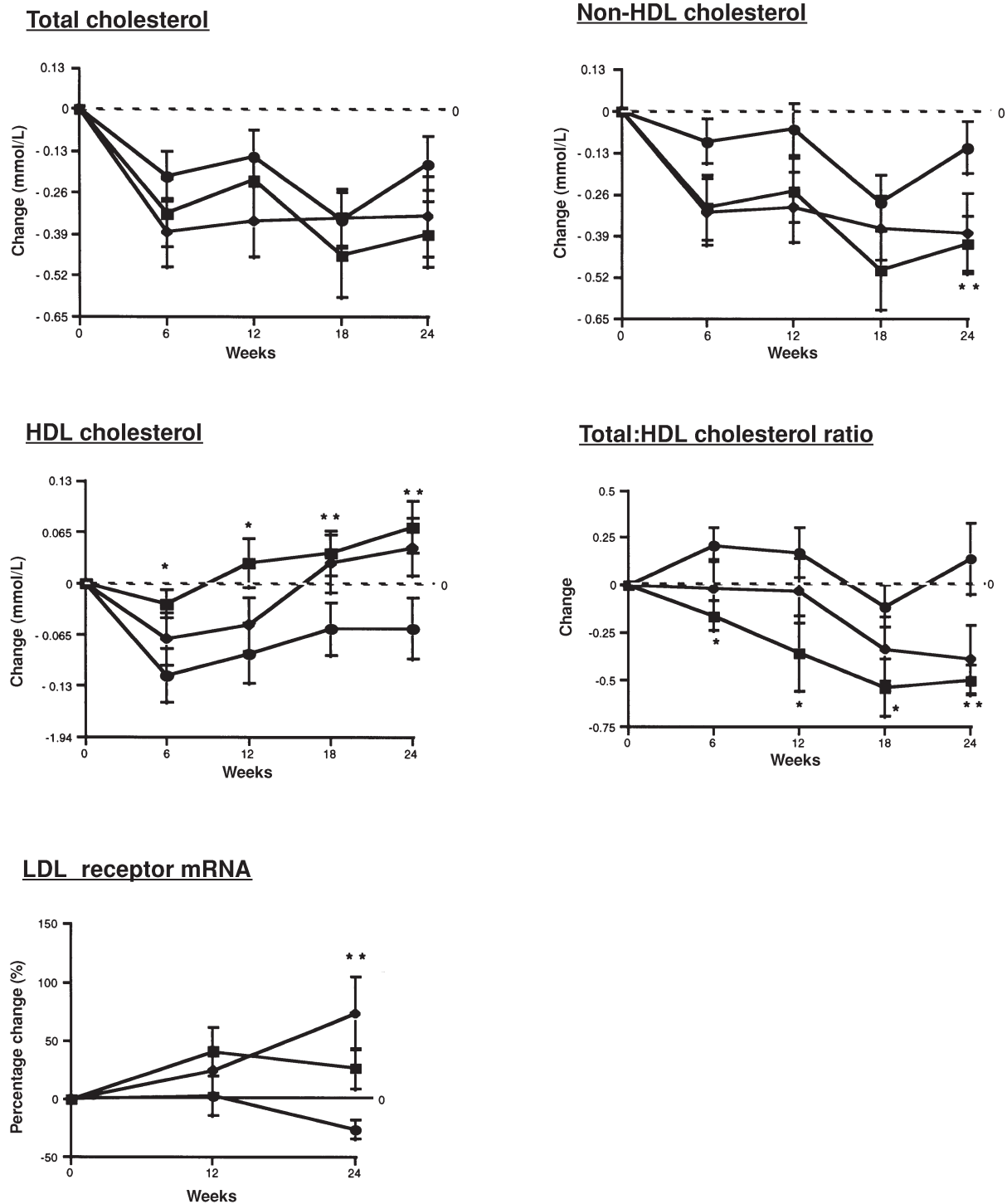


FIGURE 1. Mean (\pm SD) changes in blood lipids and LDL receptor messenger RNA (mRNA) expression over the 24-wk treatment period in subjects consuming isolated soy protein with moderate isoflavones (ISP56, ■), isolated soy protein with higher isoflavones (ISP90, ◆), and casein and nonfat dry milk (CNFDM, ●) ($n = 22$). ** ISP56 and ISP90 significantly different from CNFDM, $P < 0.05$. *ISP56 significantly different from CNFDM ($P < 0.05$).

interaction effects were tested as described by Weigel and Narvaez (12). Covariates for the bone analysis were used as baseline values of the outcome variables: body weight, age, body fat, and years since menopause. If no significant interaction effects were detected, the interaction terms were removed from the model. The temporal onset of effects for blood lipids was detected

sequentially by testing for the presence of significant treatments effects first at 24 wk posttreatment and then proceeding backwards to test for significant effects at 18, 12, and 6 wk, proceeding to the earlier time in sequence only when significant effects had been identified at each later time. Changes from baseline within each group were evaluated by using paired t tests. All sta-



TABLE 2
Lumbar spine (L1–L4) bone mineral content and density of subjects completing the study¹

Treatment group	Content g/cm	Density g/cm ²
ISP56		
Week 0	55.5 ± 11.8	0.971 ± 0.145
Week 24	55.1 ± 11.7	0.969 ± 0.143
ISP90		
Week 0	49.6 ± 8.3	0.892 ± 0.114
Week 24	50.8 ± 8.7 ²	0.912 ± 0.119 ²
CNFDM		
Week 0	56.0 ± 11.0	0.940 ± 0.159
Week 24	55.4 ± 10.3	0.934 ± 0.153

¹ $\bar{x} \pm SD$. $n = 66$. ISP56, isolated soy protein with moderate isoflavones; ISP90, isolated soy protein with higher isoflavones; CNFDM, casein and nonfat dry milk.

²Significantly different from CNFDM subjects, $P < 0.05$.

tistical procedures were conducted with the STATISTICAL ANALYSIS SYSTEM (version 2.1; SAS Institute, Cary, NC). An α level of 0.05 was used in all statistical tests.

RESULTS

Baseline characteristics of the subjects are shown in **Table 1**. Subjects consumed the products containing test proteins without difficulty. Mean changes in blood lipid as well as LDL receptor mRNA concentrations are shown in **Figure 1**. Although total cholesterol was not altered by dietary treatment, HDL cholesterol increased starting at week 6 in the ISP56 group and at week 18 in the ISP90 group ($P < 0.05$), and non-HDL cholesterol decreased at week 24 ($P < 0.05$) in both soy-protein groups compared with the CNFDM group. The ratio of total to HDL cholesterol was improved with respect to cardiovascular risk in both soy-protein groups at 18 and 24 weeks (**Figure 1**; $P < 0.05$). Dietary treatment did not influence total triacylglycerol concentrations (data not shown). In subjects consuming either ISP56 or ISP90, LDL receptor mRNA concentrations increased significantly at 24 wk compared with concentrations in control subjects (**Figure 1**; $P < 0.05$). Of the skeletal sites tested, lumbar-spine bone mineral content and density increased significantly at the end of the 24-wk treatment period in the ISP90 group (**Table 2**; $P < 0.05$). No significant changes were noted in bone mineral density or content in total-body or other skeletal sites.

DISCUSSION


Results from this study indicate that soy protein is effective in modulating the risks of both cardiovascular disease and osteoporosis in postmenopausal women. Interestingly, the amount of isoflavone consumed had little effect on blood lipid variables but was a factor in bone measurements. In fact, the ISP56 diet group, with the moderate concentration of isoflavones, had significantly improved blood lipid profiles before 24 wk whereas the ISP90 group, receiving higher concentrations of isoflavones, did not show significant improvement until later in the study (18–24 wk). The reason for this may be that the dietary concentration of isoflavones needed to affect lipid metabolism is different from

that needed to influence bone metabolism. The possibility also exists that the cholesterol-lowering component of soy is not or is only partially related to isoflavones.

The cholesterol-lowering effect of soy protein was not as pronounced in women in our current study ($\approx 6\%$ reduction in total cholesterol and $\approx 7\%$ reduction in non-HDL cholesterol) as was reported previously. We (13) reported an 11–12% reduction in total- and LDL-cholesterol concentrations in mildly hypercholesterolemic men consuming 50 g soy protein/d. In a subsequent study, we found that 25 g soy protein/d produced a 5–6% reduction in total cholesterol in men (14). Our prior work is consistent with the findings from the meta-analysis performed by Anderson et al (2) of 38 clinical trials, in which most subjects were men.

Differences between our current findings in postmenopausal women and previous findings in men could be due to differences in responsiveness to soy between the sexes. The fact that we did observe a significant increase in HDL cholesterol, a finding typically not present in men consuming soy protein, may indicate that part of the response to soy protein in women is related to isoflavones and their interaction with estrogen receptors. However, we did not observe significant decreases in total cholesterol or significant increases in total triacylglycerols, which are common responses to estrogens given to postmenopausal women (15).

Our findings that ISP90 produced significant increases in bone mineral content and density in the spine was of interest for 2 reasons. First, of all skeletal sites measured, the spine is the area that is thought to be the most sensitive to estrogen because of its higher content of trabecular bone. The spine is remodeled more rapidly than is the hip, which contains a higher proportion of cortical bone (16, 17). Second, although we had hypothesized that the isoflavone-containing soy-protein diets would delay the decrease in bone density compared with that for the control diet, we found that there was a slight increase in bone density and mineral content (2%), an intriguing finding. However, this is a short study with respect to bone and these findings need to be confirmed by longer studies (eg, 2–3 y).

In conclusion, our data suggest that isolated soy protein at either concentration of isoflavones used in this study may be protective against cardiovascular diseases by altering lipoprotein profiles in postmenopausal women. Furthermore, there may be a possible protective role of isoflavones on bone maintenance. Unfortunately, many women in the United States either cannot or will not comply with standard hormone replacement therapy, which is the therapy of choice for prevention and treatment of cardiovascular disease and osteoporosis in this population. Thus, it is possible that the addition of soy products containing isoflavones to the diet may provide a viable alternative mode of therapy in improvement of health in postmenopausal women. 

REFERENCES

- Potter SM. Soy protein and serum lipids. *Curr Opin Lipidol* 1996;7:260–4.
- Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 1995;333:276–82.
- Carroll KK. Review of clinical studies on cholesterol-lowering response to soy protein. *J Am Diet Assoc* 1991;91:820–7.
- Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J Nutr* 1995;125:606S–11S.
- Klein KO. Isoflavones, soy-based infant formulas, and relevance to endocrine function. *Nutr Rev* 1998;56:193–204.

6. Baum J, Teng H, Erdman JW Jr, et al. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. *Am J Clin Nutr* 1998;68:545-51.
7. NIH Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease. Triglyceride, high-density lipoprotein, and coronary heart disease. *JAMA* 1993;269:505-10.
8. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res* 1982;23:1206-23.
9. Lovati MR, Manzoni C, Canavesi A, et al. Soybean protein diet increases low-density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. *J Clin Invest* 1987;80:1498-502.
10. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
11. Wang AM, Doyle MV, Mark DF. Quantitation of mRNA by the polymerase chain reaction. *Proc Natl Acad Sci U S A* 1989;86:9717-21.
12. Weigel RM, Narvaez M. Multiple regression analysis of differential response to treatment in randomized controlled clinical trials. *Control Clin Trials* 1991;12:378-94.
13. Potter SM, Bakhit RM, Essex-Sorlie D, et al. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *Am J Clin Nutr* 1993;58:501-6.
14. Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman JW Jr, Potter SM. Intake of 25 g soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. *J Nutr* 1994;124:213-22.
15. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Prev Med* 1991;20:47-63.
16. Ettinger B, Genant HK, Steiger P, Madvig P. Low-dosage micronized 17-estradiol prevents bone loss in postmenopausal women. *Am J Obstet Gynecol* 1992;166:479-88.
17. Odell WE, Heath H. Osteoporosis: pathophysiology, prevention, diagnosis, and treatment. *Dis Mon* 1993;19:789-98.

