Current Author Addresses: Drs. Marini and Adamo: Department of Biochemical, Physiological and Nutritional Sciences, Section of Physiology and Human Nutrition, Azienda Ospedaliera Universitaria Policlinico "G. Martino," Via C. Valeria, 98125 Messina, Italy.
Drs. Minutoli, Polito, Bitto, Altavilla, and Squadrito: Department of Clinical and Experimental Medicine and Pharmacology, Section of Pharmacology, Azienda Ospedaliera Universitaria Policlinico "G. Martino," Via C. Valeria, 98125 Messina, Italy.
Drs. Atteritano, Gaudio, Mazzaferro, A. Frisina, N. Frisina, and Bonaiuto: Department of Internal Medicine, Azienda Ospedaliera Universitaria Policlinico "G. Martino," Via C. Valeria, 98125 Messina, Italy.
Dr. Lubrano: Department of Medical Physiopathology, "La Sapienza" University, Rome, Italy.
Drs. D'Anna, Cannata, and Corrado: Department of Obstetrical and Gynecological Sciences, Azienda Ospedaliera Universitaria Policlinico "G. Martino," Via C. Valeria, 98125 Messina, Italy.
Dr. Wilson: Department of Health Initiatives, National Jewish Medical and Research Center, Denver, CO 80206.

Author Contributions: Conception and design: H. Marini, R. D'Anna, F. Squadrito.
Analysis and interpretation of the data: H. Marini, L. Minutoli, F. Polito, R. D'Anna, S. Wilson, F. Squadrito.
Drafting of the article: H. Marini, A. Bitto, D. Altavilla, R. D'Anna, F. Squadrito.
Critical revision of the article for important intellectual content: H. Marini, R. D'Anna, F. Squadrito.
Provision of study materials or patients: M. Atteritano, A. Gaudio, S. Mazzaferro, A. Frisina, N. Frisina, C. Lubrano, M. Bonaiuto, R. D'Anna, M.L. Cannata, F. Corrado, E.B. Adamo.
Obtaining of funding: N. Frisina, C. Lubrano, R. D'Anna.
Administrative, technical or logistic support: N. Frisina, R. D'Anna, F. Squadrito.
Collection and assembly of data: H. Marini, L. Minutoli, F. Polito.

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Effects of the Phytoestrogen Genistein on Bone Metabolism in Osteopenic Postmenopausal Women

A Randomized Trial

Herbert Marini, MD; Letteria Minutoli, MD; Francesca Polito, PhD; Alessandra Bitto, MD; Domenica Altavilla, PhD; Marco Atteritano, MD; Agostino Gaudio, MD; Susanna Mazzaferro, MD; Alessia Frisina, MD; Nicola Frisina, MD; Carla Lubrano, MD; Michele Bonaiuto, MD; Rosario D'Anna, MD; Maria Letizia Cannata, MD; Francesco Corrado, MD; Elena Bianca Adamo, MD; Steven Wilson, PhD; and Francesco Squadrito, MD

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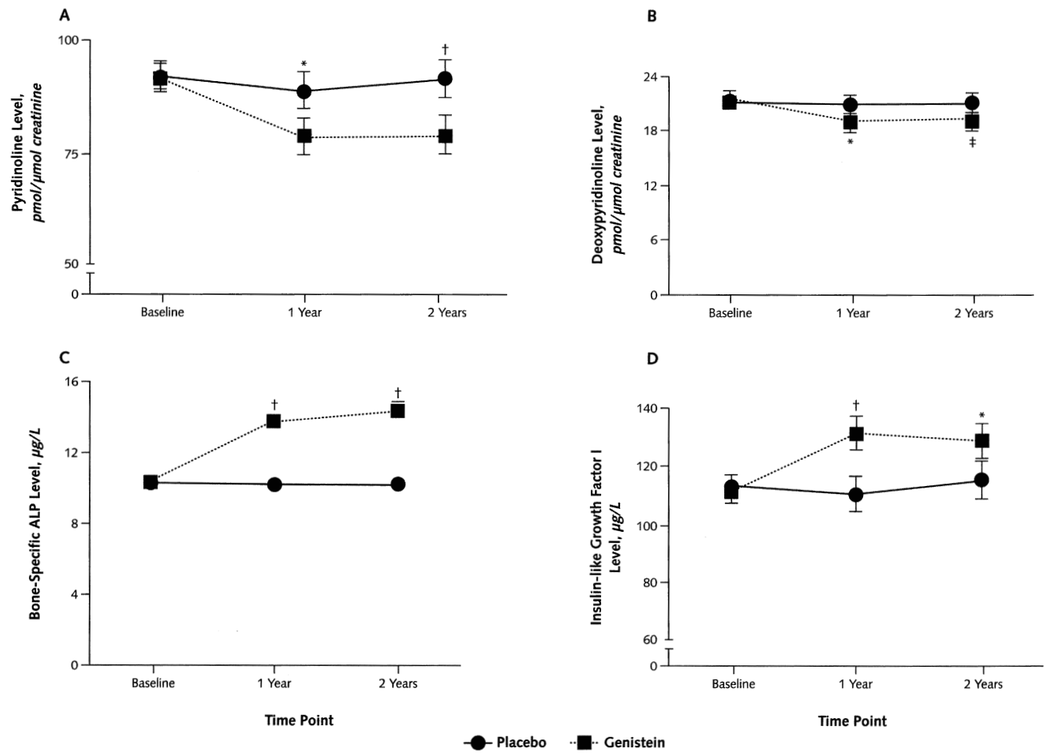
Figure 3. **Changes in biochemical variables over time.**



Estimates are expected means from mixed-effects models. ALP = alkaline phosphatase. *$P = 0.001$ vs. placebo. †$P < 0.001$ vs. placebo. ‡$P = 0.002$ vs. placebo.

Finally, 2 years of genistein therapy at 54 mg/d was not associated with clinically significant adverse effects on the uterus. This isoflavone dosage is similar to that in vegetarian Asian diets. However, caution is needed when administering genistein, especially in patients at high risk for endometrial or breast cancer. Moreover, whether the safety profile demonstrated in our study extends to isoflavones as a class remains unclear and warrants further study.

We observed a moderate number of gastrointestinal side effects in women who received genistein. Some placebo recipients also experienced gastrointestinal adverse effects, which may have been related to calcium carbonate. However, significantly more gastrointestinal adverse effects occurred in the genistein group than in the placebo group; these effects were most likely attributable to the isoflavone (44, 45).

In conclusion, we found that 2 years of treatment with genistein improved BMD and markers of bone turnover in a cohort of osteopenic postmenopausal women. On the basis of these data, future studies in osteoporotic women are warranted to determine whether genistein also significantly decreases fracture risk in this group. In addition, studies are needed to determine whether genistein positively affects bone loss not related to postmenopausal ovarian hormone loss, such as glucocorticoid-induced osteoporosis.

From Azienda Ospedaliera Universitaria Policlinico "G. Martino," University of Messina, Messina, Italy.

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Requests for Single Reprints: Francesco Squadrito, MD, Department of Experimental and Clinical Medicine and Pharmacology, Section of Pharmacology, Torre Biologica, 5th Floor, Azienda Ospedaliera Universitaria Policlinico "G. Martino," Via C. Valeria, 98125 Messina, Italy; e-mail, Francesco.Squadrito@unime.it.

Current author addresses and author contributions are available at www.annals.org.

Annals of Internal Medicine | ARTICLE

Effects of the Phytoestrogen Genistein on Bone Metabolism in Osteopenic Postmenopausal Women

A Randomized Trial

Herbert Marini, MD; Letteria Minutoli, MD; Francesca Polito, PhD; Alessandra Bitto, MD; Domenica Altavilla, PhD; Marco Atteritano, MD; Agostino Gaudio, MD; Susanna Mazzaferro, MD; Alessia Frisina, MD; Nicola Frisina, MD; Carla Lubrano, MD; Michele Bonaiuto, MD; Rosario D'Anna, MD; Maria Letizia Cannata, MD; Francesco Corrado, MD; Elena Bianca Adamo, MD; Steven Wilson, PhD; and Francesco Squadrito, MD

Background: Observational studies and small trials of short duration suggest that the isoflavone phytoestrogen genistein reduces bone loss, but the evidence is not definitive.

Objective: To assess the effects of genistein on bone metabolism in osteopenic postmenopausal women.

Design: Randomized, double-blind, placebo-controlled trial.

Setting: 3 university medical centers in Italy.

Patients: 389 postmenopausal women with a bone mineral density (BMD) less than 0.795 g/cm^2 at the femoral neck and no significant comorbid conditions.

Intervention: After a 4-week stabilization period during which participants received a low-soy, reduced-fat diet, participants were randomly assigned to receive placebo (n = 191) or 54 mg of genistein (n = 198) daily for 24 months. Both the genistein and placebo tablets contained calcium and vitamin D.

Measurements: The primary outcome was BMD at the anteroposterior lumbar spine and femoral neck at 24 months. Secondary outcomes were serum levels of bone-specific alkaline phosphatase and insulin-like growth factor I, urinary excretion of pyridinoline and deoxypyridinoline, and endometrial thickness. Data on adverse events were also collected.

Results: At 24 months, BMD had increased in genistein recipients and decreased in placebo recipients at the anteroposterior lumbar spine (change, 0.049 g/cm^2 [95% CI, 0.035 to 0.059] vs. −0.053 g/cm^2 [CI, −0.058 to −0.035]; difference, 0.10 g/cm^2 [CI, 0.08 to 0.12]; $P < 0.001$) and the femoral neck (change, 0.035 g/cm^2 [CI, 0.025 to 0.042] vs. −0.037 g/cm^2 [CI, −0.044 to −0.027]; difference, 0.062 g/cm^2 [CI, 0.049 to 0.073]; $P < 0.001$). Genistein statistically significantly decreased urinary excretion of pyridinoline and deoxypyridinoline, increased levels of bone-specific alkaline phosphatase and insulin-like growth factor I, and did not change endometrial thickness compared with placebo. More genistein recipients than placebo recipients experienced gastrointestinal side effects (19% vs. 8%; $P = 0.002$) and discontinued the study.

Limitations: The study did not measure fractures and had limited power to evaluate adverse effects.

Conclusion: Twenty-four months of treatment with genistein has positive effects on BMD in osteopenic postmenopausal women.

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For author affiliations, see end of text.
ClinicalTrials.gov registration number: NCT00355953.

Postmenopausal osteoporosis is caused by a sharp decrease in estrogen levels that leads to an increased rate of bone remodeling (1–3). Currently available treatments for postmenopausal osteoporosis include hormone replacement therapy; calcitonin; bisphosphonates; and selective estrogen receptor modulators, such as raloxifene (4, 5).

Although hormone replacement therapy is effective in reducing postmenopausal bone loss (6–8), it is associated with a higher risk for breast, endometrial, and ovarian cancer; cardiovascular disease; venous thromboembolism; and stroke (8–10). Epidemiologic data indicate that women who ingest high amounts of phytoestrogens, particularly isoflavones in soy products, have less risk for osteoporosis than do those who consume a typical Western diet (11–13). Consequently, many women use phytoestrogens to maintain bone density.

Genistein, an isoflavone phytoestrogen that is abundant in soybean products, structurally resembles 17β-estradiol (14). As a natural selective estrogen receptor modulator, genistein may positively regulate bone cell metabolism without harmful estrogenic activity in the breast and uterus. This safe profile results from the greater affinity of genistein for estrogen receptor-β, which is more abundant in bone, than for estrogen receptor-α, which is abundant in reproductive tissue. Observational studies suggest that postmenopausal Asian women who consume diets high in isoflavones have a lower rate of fracture than that in other groups (15, 16). However, the mechanism of action of genistein on bone is not yet fully understood.

In postmenopausal women, treatment with genistein (54 mg/d) increased bone mineral density (BMD) at the lumbar spine and femoral neck with no clinically significant adverse effects on the breast and uterus (17). In the same cohort, genistein decreased the ratio of soluble recep-

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Context

Women seeking alternative treatments to preserve bone often use phytoestrogens, but evidence of their effectiveness is lacking. Phytoestrogens are found in soy products. Genistein is a phytoestrogen with a structure similar to that of 17β-estradiol.

Contribution

This randomized trial compared genistein, 54 mg/d, with placebo for 24 months in 389 osteopenic postmenopausal women. Increases in bone mineral density were greater with genistein than with placebo. Genistein also had favorable effects on markers of bone metabolism. Genistein did not increase endometrial thickness, but it did cause gastrointestinal side effects.

Implications

Genistein appears to have a favorable effect on markers of bone health in osteopenic postmenopausal women. Studies of its effect on fractures are needed.

—The Editors

tor activator of nuclear factor-κB ligand to osteoprotegerin, which may partly account for its positive effects on BMD (18). These investigations were the first to evaluate the effects of purified, standardized genistein on bone health, but they were short in duration and included only 90 patients. One published trial assessed the effect of isoflavones on BMD in postmenopausal women, but it compared isoflavone-rich soy milk (containing only a small amount of genistein) with natural transdermal progesterone (19). Other studies of pure genistein from our research group have focused on cardiovascular outcomes or menopausal symptoms rather than on bone health (20–22).

We performed a randomized, placebo-controlled trial of the effects of pure genistein on bone density and bone metabolism over 24 months in a larger cohort of osteopenic postmenopausal women.

METHODS

Design and Setting

The study protocol is consistent with the principles of the Declaration of Helsinki, and participants gave written informed consent. Participants were recruited from women reporting to the Center for Osteoporosis in the Department of Internal Medicine and the Center for Menopause in the Department of Obstetrical and Gynecological Sciences, University of Messina (Messina, Italy), and to the Department of Medical Physiopathology, University "La Sapienza" (Rome, Italy). Three hundred eighty-nine women met the inclusion criteria and agreed to participate (**Figure 1**).

Participants

Participants were women 49 to 67 years of age who had been postmenopausal for at least 12 months at baseline, were in good general health, had not had a menstrual period in the preceding year, had not undergone surgically induced menopause, and had a follicle-stimulating hormone level greater than 50 IU/L and a serum 17β-estradiol level of 100 pmol/L or less ($\leq$27 pg/mL). At the start of the study, a complete family history was obtained, physical examination and laboratory evaluation (chemical analytes and hematologic measurements) were performed, and BMD was measured at the lumbar spine and femoral neck. Exclusion criteria were clinical or laboratory evidence of confounding systemic diseases, such as cardiovascular, hepatic, or renal disorders; coagulopathy; use of oral or transdermal estrogen, progestin, androgens, selective estrogen receptor modulators, or other steroids; use of biphosphonates, cholesterol-lowering therapy, or cardiovascular medications (including antihypertensive drugs) in the preceding 6 months; smoking habit of more than 2 cigarettes daily; treatment in the preceding year with any drug that could have affected the skeleton; family history of estrogen-dependent cancer; and BMD at the femoral neck greater than 0.795 g/cm^2 (which corresponds to a T-score of -1.0 SD).

Randomization and Intervention

We assigned patients to groups by using a computer-generated randomization sequence with a permuted block size of 4, stratified by center. After a 4-week stabilization period during which participants received a standard low-soy, reduced-fat diet, participants were assigned to receive genistein (n = 198), 54 mg/d in 2 tablets (Laboratori Plants, Messina, Italy), or placebo (n = 191) (**Figure 1**). The biological effects of phytoestrogen intake are described elsewhere (23). No patient withdrew from the study during the stabilization period. The purity of genistein was 98%. Placebo and genistein tablets were identical in appearance and taste. Both genistein and placebo tablets contained calcium carbonate (500 mg) and vitamin D (400 IU).

All participants were counseled on an isocaloric, reduced-fat diet composed of 25% to 30% energy from fat, less than 10% energy from saturated fatty acids, 55% to 60% energy from carbohydrates, and 15% energy from protein, with a cholesterol intake less than 300 mg/d and fiber intake of 35 g/d or greater. Recommended daily caloric intake was based on body size and was calculated by using the Harris–Benedict equation. We used this diet during the stabilization period to ensure that all participants had the same energy intake and to avoid interference with the lipid profile. The intake of soy products, legumes, or other nutrient supplements was prohibited. The isoflavone intake before randomization, as assessed by using a food-frequency questionnaire, was 1 to 2 mg/d. This intake has been shown to be typical in Western populations (24). Participants used this diet throughout the study, and ad-

Table 2—**Continued**

Placebo Group		Genistein Group vs. Placebo Group			
Change in Value at 1 Year (95% CI)	Change in Value at 2 Years (95% CI)	Adjusted Mean Difference in Change at 1 Year (95% CI)	*P* Value	Adjusted Mean Difference in Change at 2 Years (95% CI)	*P* Value
−0.016 (−0.023 to 0.007)	−0.037 (−0.044 to −0.027)	0.023 (0.011 to 0.034)	<0.001	0.062 (0.049 to 0.073)	<0.001
−0.027 (−0.038 to 0.015)	−0.053 (−0.058 to −0.035)	0.055 (0.036 to 0.073)	<0.001	0.10 (0.08 to 0.12)	<0.001
−0.003 (−0.011 to 0.004)	−0.004 (−0.011 to 0.004)	0.586 (0.576 to 0.596)	<0.001	0.61 (0.60 to 0.62)	<0.001
0.04 (0.023 to 0.058)	0.043 (0.023 to 0.06)	0.013 (−0.01 to 0.033)		0.016 (0.015 to 0.046)	
0.16 (0.09 to 0.23)	0.17 (0.09 to 0.24)	0.05 (−0.04 to 0.13)	0.01	0.062 (−0.059 to 0.183)	0.31
0.003 (−0.023 to 0.029)	0.04 (0.01 to 0.064)	0.039 (0.01 to 0.068)		0.013 (−0.019 to 0.042)	
0.01 (−0.07 to 0.09)	0.12 (0.03 to 0.20)	0.12 (0.03 to 0.21)	0.01	0.04 (−0.06 to 0.13)	0.45
0.05 (−3.8 to 3.9)	−3.5 (−7.5 to 0.4)	2.7 (−1.4 to 6.8)	0.20	4.9 (0.5 to 9.3)	0.03
12.1 (10.0 to 14.3)	12.7 (10.5 to 14.9)	0.33 (−1.95 to 2.61)	0.77	0.14 (−2.31 to 2.59)	0.91
−0.10 (−0.3 to 0.1)	−0.11 (−0.3 to 0.1)	3.7 (3.2 to 4.0)	<0.001	4.2 (3.8 to 4.6)	<0.001
−2.8 (−8.9 to 3.2)	1.8 (−4.5 to 8.0)	20.7 (13.8 to 27.6)	<0.001	13.1 (5.7 to 20.5)	<0.001
−2.8 (−6.8 to 1.2)	−0.4 (−4.5 to 3.8)	−10.0 (−14.5 to 5.6)	<0.001	−12.2 (−17.0 to 7.5)	<0.001
−0.28 (−1.2 to 0.67)	−0.17 (−1.1 to 0.81)	−1.8 (−2.9 to 0.8)	0.001	−1.7 (−2.8 to −0.6)	0.002

suppress osteoclast activity through several mechanisms (26–31). Furthermore, in vivo studies indicate that genistein prevents estrogen-deficiency bone loss in ovariectomized animals (32, 33).

In a 6-month study, isolated soy isoflavones decreased bone resorption in postmenopausal women (34). Moreover, our previous study (18) showed that 1 year of genistein therapy (54 mg/d) had antiresorptive action in postmenopausal women. This action was probably mediated through direct effects on the soluble nuclear factor-κB ligand–osteoprotegerin system (18).

We observed increases in the bone-specific alkaline phosphatase level in the genistein group, which may be caused by a direct genomic estrogen receptor–mediated effect or a nongenomic action in target cells. Genistein may act on de novo protein synthesis (35, 36) and on amplification of the interaction between the estrogen receptor complex and nuclear DNA in osteoblasts. These cells express both estrogen receptor-α and estrogen receptor-β (37), and genistein may act on bone by a mechanism involving estrogen receptor-β (14). Moreover, genistein may stimulate osteoblast proliferation, although this phytoestrogen has been shown to inhibit both basal- and growth factor–induced proliferation of several normal and cancer cell lines (38–40).

Genistein also positively affected levels of insulin-like growth factor I, a marker of bone growth in postmenopausal women, in a pattern similar to that observed for bone-specific alkaline phosphatase. Isoflavones have been shown to upregulate insulin-like growth factor I and transforming growth factor-β and inhibit osteoclastogenesis in bone marrow cells of rats (41). Furthermore, several phytochemicals and synthetic environmental chemicals display estrogenic properties and mimic estrogens by activating insulin-like growth factor I–signaling events through estrogen receptor-α binding (42). Klotz and coworkers (42) observed variability in the lowest dose of genistein that increased insulin-like growth factor I receptor tyrosine phosphorylation. This effect may be due to tyrosine kinase inhibitory activity of genistein (43) or, alternatively, to a greater affinity for estrogen receptor-β (14).

Overall, genistein has multiple effects on bone turnover and produces a significant increase in lumbar spine and femoral neck BMD compared with placebo after 24 months of treatment. This effect was time-dependent, suggesting that long-term intake produces ongoing effects on bone metabolism.

Table 3. **Uterine Safety and Main Adverse Effects***

Variable	Placebo Group (n = 191)	Genistein Group (n = 198)
Mean endometrial thickness (SD), *mm*		
Baseline	3.2 (1.8)†	3.1 (1.5)‡
Year 1	3.0 (1.5)§	3.0 (1.4)‖
Year 2	3.0 (1.1)¶	3.2 (1.4)**
Adverse events, *n (%)*		
Abdominal pain	3 (2)	6 (3)
Epigastric pain	0 (0)	5 (3)
Dyspepsia	3 (2)	9 (5)
Vomiting	3 (2)	4 (2)
Constipation	6 (3)	13 (7)
Participants who discontinued treatment because of adverse events, *n (%)*	15 (8)	37 (19)

* Values are based on participants for whom no data were missing. Women with endometrial thickness greater than 8 mm at baseline, polyps, or hyperplasia and those who had had a hysterectomy were excluded from this analysis.
† Based on 183 measurements.
‡ Based on 186 measurements.
§ Based on 164 measurements.
‖ Based on 166 measurements.
¶ Based on 154 measurements.
** Based on 150 measurements.

Table 2. **Changes in Biochemical Variables***

Outcome Measure	Genistein Group			
	Mean Value at Baseline (SD)	Change in Value at 1 Year (95% CI)	Change in Value at 2 Years (95% CI)	Mean Value at Baseline (SD)
Bone mineral density, g/cm^2				
Femoral neck	0.667 (0.055)	0.016 (0.007 to 0.023)	0.035 (0.025 to 0.042)	0.674 (0.056)
Lumbar spine	0.842 (0.080)	0.024 (0.012 to 0.034)	0.049 (0.035 to 0.059)	0.837 (0.099)
Genistein level, *μmol/L*	0.149 (0.020)	0.580 (0.572 to 0.587)	0.603 (0.595 to 0.611)	0.146 (0.018)
Calcium level				
mmol/L	2.39 (0.09)	0.063 (0.043 to 0.08)	0.06 (0.04 to 0.078)	2.40 (0.12)
mg/dL	9.54 (0.36)	0.25 (0.17 to 0.32)	0.24 (0.16 to 0.31)	9.58 (0.48)
Phosphorus level				
mmol/L	1.17 (0.15)	0.03 (0.006 to 0.061)	0.04 (0.013 to 0.068)	1.16 (0.18)
mg/dL	3.63 (0.46)	0.10 (0.02 to 0.19)	0.13 (0.04 to 0.21)	3.60 (0.55)
Parathyroid hormone level, *pg/mL*	48.8 (16.0)	2.1 (−1.6 to 5.9)	0.75 (−3.2 to 4.7)	48.2 (16.7)
25-Hydroxyvitamin D_3 level, *IU/dL*	29.5 (10.2)	13.5 (11.4 to 15.6)	13.9 (11.7 to 16.1)	30.6 (10.9)
Bone-specific alkaline phosphatase level, *μg/L*	10.4 (2.1)	3.4 (3.2 to 3.6)	3.9 (3.7 to 4.2)	10.3 (1.9)
Insulin-like growth factor I level, *μg/L*	111 (27)	20.2 (14.3 to 26.1)	17.2 (10.9 to 23.4)	113 (31)
Pyridinoline level, *pmol/μmol creatinine*	92.0 (21.7)	−13.3 (−17.2 to 9.3)	−13.0 (17.2 to 8.7)	91.6 (31.9)
Deoxypyridinoline level, *pmol/μmol creatinine*	21.7 (5.5)	−2.7 (−3.6 to 1.8)	−2.5 (−3.5 to 1.5)	21.1 (5.8)

* Values were obtained from mixed-model analyses.

Secondary Outcomes

Mixed-model analyses were performed for secondary measures (**Table 2**). At 1 year and 2 years, genistein levels were increased from baseline values in the genistein group but remained unchanged or decreased slightly in the placebo group. The between-group difference was statistically significant at both time points.

Urinary excretion of pyridinoline and deoxypyridinoline had statistically significantly decreased at 1 year and 2 years in the genistein group but did not significantly change in the placebo group (**Figure 3**). Between-group analyses showed that genistein use statistically significantly decreased urinary excretion of pyridinoline and deoxypyridinoline at both time points compared with placebo (**Table 2**). Likewise, levels of bone-specific alkaline phosphatase and insulin-like growth factor I statistically significantly increased from baseline in the genistein group but did not significantly change for the placebo group. Between-group differences were statistically significant for each of these variables at 1 year and 2 years (**Figure 3**).

Levels of calcium and 25-hydroxyvitamin D_3 increased statistically significantly from baseline in both groups, but the changes did not differ significantly between the groups. The phosphorus level had increased statistically significantly at 1 year and 2 years in the genistein group but not in the placebo group; between-group comparison of changes was not significant. Within-group and between-group changes in parathyroid hormone level were not statistically significant (**Table 2**). Finally, body mass index did not change significantly in either group after 1 year and 2 years of treatment.

Adverse Events, Uterine Safety, and Climacteric Symptoms

Results on routine biochemical, liver function, and hematologic testing did not change over time in placebo or genistein recipients. Eight placebo recipients and 16 genistein recipients withdrew because of adverse events in the first 12 months of treatment (**Figure 1**). During the second year, 7 placebo recipients and 21 genistein recipients had adverse events (**Figure 1**). In total, 37 (19%) genistein recipients and 15 (8%) placebo recipients discontinued therapy because of adverse events ($P = 0.002$), all of which were gastrointestinal (**Table 3**). No patient who remained in the study experienced an adverse event.

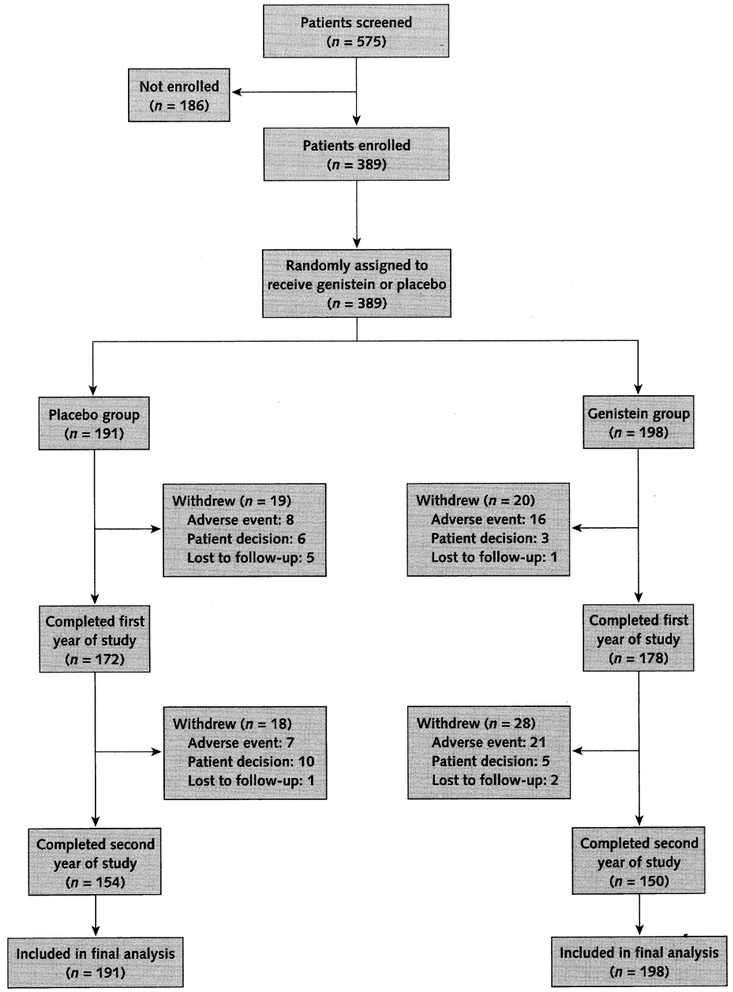
Genistein at a dosage of 54 mg/d did not cause a significant change in the endometrial thickness compared with placebo (**Table 3**). At 2 years, genistein treatment markedly reduced the mean number of hot flushes per day compared with placebo (1.7 [SD, 0.21] vs. 3.9 [SD, 0.26]; $P < 0.001$).

Discussion

We found that treatment with genistein, an abundant soy isoflavone, prevents bone loss caused by estrogen deficiency without affecting the uterus in osteopenic postmenopausal women. Genistein decreased levels of bone resorption markers and increased levels of markers of new bone formation, producing a net gain in bone mass after 1 year and 2 years of therapy. Nevertheless, although BMD and bone markers are considered good surrogates of bone strength and bone quality, they may not correlate perfectly with reduction in fracture risk.

Urinary excretion of pyridinoline and deoxypyridinoline was decreased in genistein recipients compared with placebo recipients. This finding confirms that the positive effects of genistein on bone loss reduction are caused, at least in part, by a constant decrease in bone resorption. Genistein-mediated effects on bone resorption markers are explained by in vitro studies showing that genistein can

Figure 1. **Study flow diagram.**



herence was reinforced by a nutritionist. Diet and body mass index were evaluated in all participants during follow-up.

Primary Outcome

The BMD at the anteroposterior lumbar spine and femoral neck was measured by using dual-energy x-ray absorptiometry (Hologic QDR 4500 W, Technologic, Turin, Italy) at baseline and after 12 and 24 months of treatment. The instrument was calibrated daily according to the manufacturer's instructions. Reproducibility was calculated as a coefficient of variation obtained by weekly measurements of a standard phantom on the instrument and by repeated measurements obtained in 3 patients of different ages. The coefficient of variation of our instrument is 0.5% with the standard phantom; in vivo, we calculated a coefficient of variation of 1.1% for the lumbar spine and 1.5% for the femoral neck.

Secondary Outcomes

Bone Resorption Markers

At baseline, 12 months, and 24 months, a 2-hour fasting morning urine sample was collected at the same time of day to assess urinary excretion of pyridinium crosslinks (pyridinoline and deoxypyridinoline). Pyridinoline (nor-

Table 1. **Baseline Characteristics**

Variable	All Participants (n = 389)		Genistein Group (n = 198)		Placebo Group (n = 191)	
	Placebo Group (n = 191)	Genistein Group (n = 198)	Women Who Completed the Study (n = 150)	Women Who Withdrew (n = 48)	Women Who Completed the Study (n = 154)	Women Who Withdrew (n = 37)
Mean age (SD), *y*	54.2 (2.7)	54.7 (3.5)	54.9 (3.7)	54.3 (2.9)	54.2 (2.8)	53.9 (2.1)
Mean body mass index (SD), kg/m^2	25.1 (4.2)	25.0 (3.3)	24.8 (3.5)	25.9 (2.6)	25.5 (4.3)	23.4 (3.6)
Mean time since menopause (SD), *mo*	59.1 (38.4)	66.8 (45.8)	69.5 (47.4)	58.3 (39.6)	59.8 (38.8)	56.1 (37.4)
Mean bone mineral density (SD), g/cm^2						
Anteroposterior lumbar spine	0.837 (0.099)	0.842 (0.08)	0.85 (0.08)	0.82 (0.06)	0.83 (0.10)	0.86 (0.07)
Femoral neck	0.674 (0.055)	0.667 (0.055)	0.67 (0.06)	0.66 (0.04)	0.67 (0.06)	0.68 (0.04)

mal range, 26 to 91 pmol/μmol creatinine) and deoxypyridinoline (normal range, 3 to 21 pmol/μmol creatinine) were measured by using high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, California).

Bone Formation and Bone Growth Markers and Other Variables

After an overnight fast, venous blood samples were collected between 8 a.m. and 9 a.m. through a polyethylene catheter inserted in a forearm vein. The serum was separated from the blood corpuscles by centrifugation and kept frozen at −70 °C until analysis for bone formation and bone growth markers, calcium, intact parathyroid hormone, 25-hydroxyvitamin D_3, 17β-estradiol, follicle-stimulating hormone, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides.

Serum bone-specific alkaline phosphatase (normal range, 8.5 to 17.9 μg/L) and insulin-like growth factor I (normal range, 9.6 to 21.2 nmol/L) were measured by using an immunoenzymatic assay (Pantec, Turin, Italy). Serum calcium (normal range, 2.25 to 2.75 mmol/L [9 to 11 mg/dL]), serum phosphorus (normal range, 1.13 to 1.45 mmol/L [3.5 to 4.5 mg/dL]), and urinary creatinine (130 to 220 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ [14.71 to 24.89 mg/kg of body weight per day]) were measured by using automated routine procedures. Parathyroid hormone (normal range, 12 to 100 pg/dL), 25-hydroxyvitamin D_3 (normal range, 1.25 to 7.5 nmol/L), and follicle-stimulating hormone (normal range, 21 to 153 IU/L in the postmenopausal phase) were measured by using high-performance liquid chromatography (Bio-Rad Laboratories). 17β-Estradiol (normal range, 37 to 110 pmol/L in the postmenopausal phase) was evaluated by using a solid-phase immunoassay (Roche Diagnostics, Monza, Italy). Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by using a routine enzymatic method, and the low-density lipoprotein cholesterol level was calculated by using the Friedewald formula: [total cholesterol (mg/dL) − high-density lipoprotein cholesterol (mg/dL) − triglycerides (mg/dL)/5].

Genistein was measured in serum samples by using a time-resolved fluorometric assay according to the manufacturer's instructions (Labmaster TR-FIA test, Labmaster, Turku, Finland) (25). The fluorescence was read by using a Victor 1420 Multilabel Counter (PerkinElmer Life and Analytical Sciences, Waltham, Massachusetts).

Adverse Events

Participants were asked about symptoms at clinic visits every 3 months. Standard clinical evaluations and laboratory analyses, including hematologic, renal, and liver function tests, were done every 6 months. Endometrial thickness was evaluated by using ultrasonography at baseline, 1 year, and 2 years. The endometrial thickness was measured in the sagittal plane from 1 basal layer to the other. If the endometrial thickness was 8 mm or greater or if uterine bleeding occurred, hysteroscopy and endometrial biopsy were performed. All unfavorable and unintended clinical effects were considered adverse effects and were evaluated for severity, duration, seriousness, and relation to the study drug and outcome. We specifically checked all participants for gastrointestinal symptoms, breast tenderness, vasomotor symptoms, depression, irritability, insomnia, and vaginal bleeding. This evaluation was conducted by using a checklist.

Statistical Analysis

We estimated that at least 97 participants in each group would provide 80% power to detect a significant expected absolute between-group difference in femoral neck or lumbar BMD of 20%, assuming a 2-tailed α level of 0.05. We estimated that genistein recipients would have a statistically significant increase in BMD at both sites, whereas BMD would continue to decrease in placebo recipients, and that the change in BMD between the 2 groups would differ by at least 20% by the end of 2 years of treatment.

The primary efficacy data on femoral neck and lumbar spine BMD were analyzed according to the intention-to-treat principle. These analyses included all 389 postmenopausal women in whom BMD was measured at baseline. Characteristics of women who withdrew from the study were also investigated.

Descriptive data are given as the mean (SD), and 95% CIs are provided where appropriate. The significance of between-group differences was assessed by using a mixed-model, repeated-measures analysis that included fixed effects for treatment and visit and the interaction of these 2 terms, as well as random effects for intercept and slope. This analysis was repeated for secondary outcome measures.

Before analysis, all end points were tested for normality by using the Kolmogorov–Smirnov test, and normal probability plots, box-and-whiskers plots, and plots of raw scores against normal percentile plots were constructed. These analyses revealed no pronounced deviations from normality, and all analyses were therefore conducted on raw scores.

A P value of 0.05 or less was considered statistically significant, and 95% CIs were calculated wherever possible. Statistical analysis was performed by using SAS software, version 9.1 (SAS Institute, Cary, North Carolina).

Role of the Funding Sources

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RESULTS

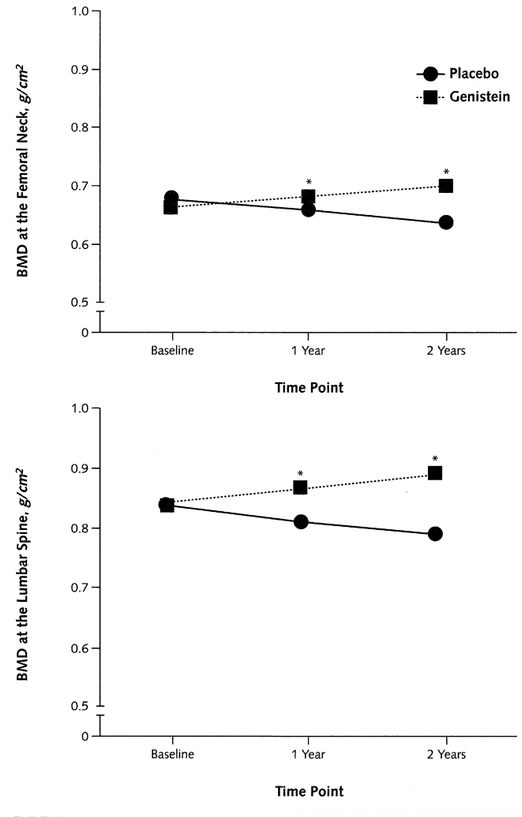
Patient Characteristics

Table 1 shows baseline characteristics of the participants. No differences were observed between groups. A post hoc comparison was done of the baseline characteristics of women who withdrew from the study before completion (n = 85) and those of women who completed the study and between the 2 treatment groups. Baseline BMD at the lumbar spine was statistically significantly lower in genistein recipients who withdrew, and body mass index was statistically significantly lower in placebo recipients who withdrew (**Table 1**). However, these findings were judged to be unlikely to affect outcomes of interest. Twenty-eight (58%) of 48 genistein recipients who withdrew and 18 (48%) of 37 placebo recipients who withdrew completed 1 year of follow-up. The BMD at 1 year did not differ between these women and those who completed the study.

Primary Outcome

Mean BMD at the femoral neck increased from 0.667 g/cm^2 at baseline to 0.683 g/cm^2 at 1 year and 0.702 g/cm^2 at 2 years in genistein recipients and decreased from 0.674 g/cm^2 at baseline to 0.659 g/cm^2 at 1 year and 0.638 g/cm^2 at 2 years in placebo recipients. Similarly, mean BMD at the lumbar spine was 0.842 g/cm^2, 0.866 g/cm^2, and 0.891 g/cm^2 at baseline, 1 year, and 2 years, respectively, in the genistein group and 0.837 g/cm^2, 0.807 g/cm^2, and 0.784 g/cm^2, respectively, in the placebo group.

Figure 2. **Change in bone mineral density (*BMD*) over time.**



Estimates are expected means from mixed-effects models. *$P < 0.001$ vs. placebo.

To further investigate the changes in BMD at the femoral neck and lumbar spine, we conducted a mixed-model analysis in which the intercepts were allowed to vary. This analysis treated missing data as missing at random and thus used all available information to determine the underlying covariance structure as well as mean and variance information from nonmissing data. The expected mean difference in change in femoral neck BMD (genistein values minus placebo values) was 0.023 g/cm^2 (95% CI, 0.011 to 0.034 g/cm^2) ($P < 0.001$) at 1 year and 0.062 g/cm^2 (CI, 0.049 to 0.073 g/cm^2) ($P < 0.001$) at 2 years (**Figure 2** and **Table 2**). The expected mean difference in change in lumbar spine BMD (genistein values minus placebo values) was 0.055 g/cm^2 (CI, 0.036 to 0.073 g/cm^2) ($P < 0.001$) at 1 year and 0.10 g/cm^2 (CI, 0.08 to 0.12 g/cm^2) ($P < 0.001$) at 2 years (**Figure 2** and **Table 2**).