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Effects of genistein aglycone in glucocorticoid induced osteoporosis: A randomized clinical trial in comparison with alendronate

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ABSTRACT

Glucocorticoid-induced osteoporosis (GIO) complicates the clinical management of patients subjected to long-term glucocorticoid use. This study explored the effects of genistein on bone loss in a randomized double-blind alendronate-controlled trial in postmenopausal women with GIO. 200 postmenopausal women (taking at least 5 mg of prednisone equivalents) since 3 months, or more, and expected to continue for at least other 12 months, were randomized to receive genistein (54 mg/day daily) or alendronate (70 mg once a week) for 24 months. Both groups received also Calcium and Vitamin D3 supplementation. Median bone mineral density (BMD) at the antero-posterior lumbar spine significantly increased from 0.75 g/cm^2 at baseline to 0.77 g/cm^2 at 1 year and 0.79 g/cm^2 at 2 years in alendronate-treated patients and from 0.77 g/cm^2 at baseline to 0.79 g/cm^2 at 12 months and to 0.80 g/cm^2 at 24 months in genistein recipients. No difference was observed between the two treatments. Median BMD at the femoral neck increased from 0.67 g/cm^2 at baseline to 0.68 g/cm^2 at 1 year and 0.69 g/cm^2 at 2 years in alendronate-treated patients and from 0.68 g/cm^2 at baseline to 0.70 g/cm^2 at 12 months and to 0.71 g/cm^2 at 24 months in genistein recipients. No difference was observed between alendronate and genistein groups in BMD. Regarding bone markers genistein and alendronate statistically decreased c-terminal telopeptide, while osteocalcin, bone-ALP, and sclerostin showed greater changes in genistein treated patients. This randomized clinical trial suggests that genistein aglycone represents an additional therapeutic option for patients with GIO.

1. Introduction

Glucocorticoids are routinely prescribed for the management of several pathological conditions. It has been estimated that 1–2% of the global population is currently undergoing long-term glucocorticoid therapy [1–3]. The cumulative negative effects of glucocorticoid on the bone are well recognized and define what has been defined, glucocorticoid-induced osteoporosis (GIO). The clinical correlates of this pathological condition are often underestimated because they are not easily recognized at an early stage. As a result, appropriate treatment may be delayed [3]. The resulting fractures have dramatic consequences in terms of increasing individual morbidity and cause an enhanced economic burden on the national healthcare systems.

Long lasting therapy with oral glucocorticoids is accompanied by a quick and sudden rise in the risk of fracture that is dose-dependent and frequently evidenced after 3–6 months from the onset of therapy [4,5]. The rapid rise of fracture risk is explained, at least in part, by the high dosage regimen and by the disease severity at the earlier stages. Thereafter, the fracture risk remains elevated throughout the treatment and finally diminishes, but not completely, after the treatment is stopped

Abbreviation: BMD, bone mineral density; B-ALP, bone specific alkaline phosphatase; H, chi square value; CV, coefficient of variation; CTX, c-terminal telopeptide; df, degree of freedom; DXA, dual-energy X-ray absorptiometry; DKK-1, Dickkopf-related protein 1; SERMS, estrogen receptor modulators; GIO, glucocorticoid-induced osteoporosis; HDL, high density lipoproteins; LDL, low density lipoproteins; HRT, hormone replacement therapy; pQCT, peripheral quantitative computed tomography; PTH, parathyroid hormone; RANKL, NF-κB Ligand receptor activator; Vit. D3, Vitamin D3.

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[6]. The anatomical site subjected to fractures mostly associated with the long-term glucocorticoid use is the lumbar spine, but there is also a high probability of non-vertebral fractures, particularly at the hip [7].

Oral therapy with bisphosphonates represents the first line choice for the management of patients undergoing long-term glucocorticoids use, but its real efficacy has been recently questioned [8]. Besides bisphosphonates, teriparatide and denosumab have been also proposed for the treatment of the increased fracture risk in GIO, but they tend to be recommended for patients at very high probability of developing bone fractures. Indeed, the management of GIO patients is still not satisfactory and there is a need to identify treatments able to maximize bone formation and minimize the systemic side-effects.

Genistein aglycone is a botanically-derived nutrient belonging to a class of isoflavones that prevents bone loss in postmenopausal osteopenic women through estrogen receptors modulation [9–11]. A post-hoc analysis of a randomized controlled trial suggested that genistein may be also effective in patients with osteoporosis by rebalancing bone turnover towards bone formation [12,13]; thus, the aim of this study was to explore the effects of genistein on bone loss in a randomized double-blind alendronate-controlled trial in postmenopausal women

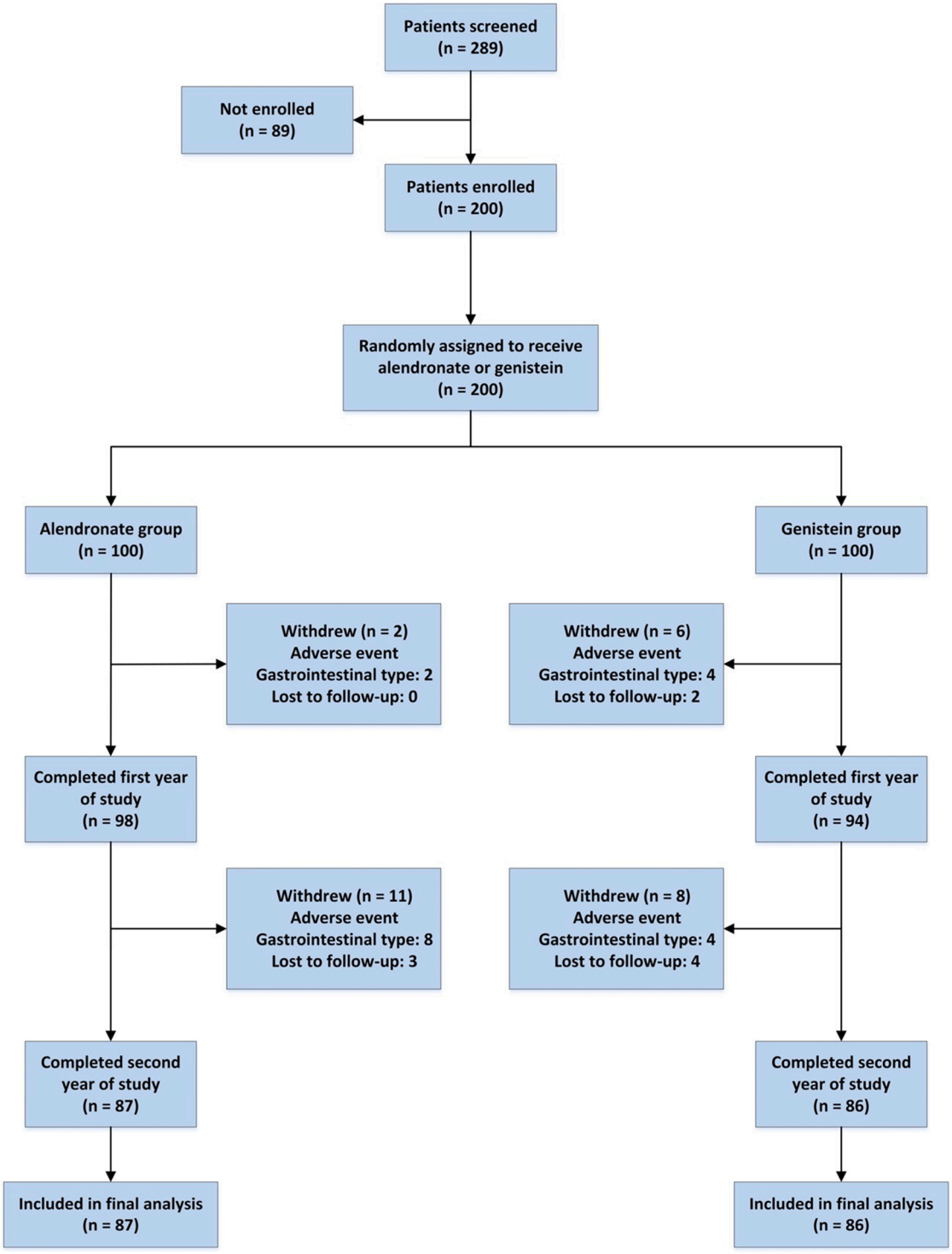

Fig. 1. Diagram for participant flow.

with GIO.

2. Material and methods

2.1. Design and Setting

The protocol of the study (ClinicalTrials.gov Identifier: NCT03040531) was approved from the local ethics committee (Comitato Etico Scientifico, Azienda Ospedaliera Universitaria Policlinico "G. Martino", Messina, Italy, Prot. 7/2016). Participants were recruited from patients reporting to the Department of Medical Sciences of the Policlinico Universitario "G. Martino" in Messina.

2.2. Participants

Women (n = 289), aged 50–75, were included in the trial after giving their informed consent. The study was carried out between January 2017 and February 2021. At the beginning of the study a full clinical visit and full laboratory evaluation were performed. The major inclusion criteria were to be postmenopausal, being treated with glucocorticoids (at least 5 mg of prednisone equivalents) for the preceding 3 months, and expected to continue the therapy for at least 12 months. The major exclusion criteria were: use of anti-osteoporotic drugs at any time, diagnosis of metabolic bone disease different from glucocorticoid induced osteoporosis, previous (up to 1 year) or concomitant treatment with hormone replacement therapy (HRT), other diseases affecting participation to the study (e.g. mental illness). A total of 200 women met the inclusion criteria and were assigned to randomization (Fig. 1).

2.3. Randomization and intervention

A computer-generated random number sequence has been used for a simple randomization of patients assigned to receive genistein plus calcium and vitamin D or alendronate plus calcium and vitamin D. Groups were labelled as A and B and received tablets labelled as A, B, C, and D. The tablet A contained 27 mg of 98% pure genistein + 500 mg Calcium + 200 IU Vit. D3. The tablet B contained 500 mg Calcium + 200 IU Vitamin D3. An additional tablet containing 500 mg Calcium + 200 IU Vitamin D3 was labelled "C" and the tablet containing alendronate (70 mg) was labeled "D".

The study group "A" received tablet A: each tablet contained 27 mg of 98% pure genistein + 500 mg Calcium + 200 IU Vit. D3. Subjects were instructed to take 2 tablets per day 6 days/week and once a week to take tablet "C" containing only 500 mg Calcium + 200 IU Vit. D3 for all the duration of the study.

The study group "B" received tablet B: patients took 2 tablets per day 6 days/week with 500 mg Calcium + 200 IU Vit. D3 and once a week took one tablet "D" containing 70 mg alendronate, for all the duration of the study.

In this way subjects were taking tablets termed as A and C or B and D and the masking was maintained until the end of the study. Tablets were prepared by Hering srl (Ragusa, Italy) and the single components were provided by Primus Pharmaceuticals Inc. (Scottsdale, AZ, USA). More specifically the capsules were only locally loaded by Hering srl in order to keep the blinding of the study, but the main ingredient (i.e. alendronate) provided by Primus Pharmaceuticals Inc. was obtained and purchased from the drug company that sells and distributes Fosamax, one of the most known brand formulations of alendronate. In this way, we had the same capsule appearance for alendronate, genistein plus calcium and Vit. D3 or calcium plus Vit. D3. Patients, doctors and investigators involved in the study were not aware of the treatment. Furthermore, patients were treated in agreement with the American College of Rheumatology guidelines [14].

2.4. Primary outcomes

2.4.1. Bone mineral density

Bone mineral density of the anterior-posterior spine and femoral neck was measured with dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500 W, Techonologic Srl, Turin, Italy), at the start of treatment, after 1 year and 2 years, as previously reported [9]. The instrument was calibrated daily, reproducibility was calculated as coefficient of variation (CV) obtained with weekly measurements of a standard phantom of the instrument and with repeated measurements obtained on three patients of different ages. The CV of our instrument was 0.5% with the standard phantom; with a coefficient of variation of 1.1% for the lumbar spine and 1.5% for the femoral neck. The data were expressed in grams per square centimeter. Change in bone quality with pQCT (peripheral quantitative computed tomography) was not assessed because of a lack of funding.

2.5. Secondary outcomes

Bone fractures were also evaluated. X-rays were performed to assess the presence of bone fractures during the same follow-up visit. The fractures were also classified for their origin in traumatic or atraumatic.

Markers of bone turnover and other variables were evaluated on peripheral blood at baseline, at 1 year and 2 years as previously shown [9,10]. After a night of fasting, venous blood samples were taken, serum was separated by centrifugation and kept at − 20 °C for the determination of bone specific alkaline phosphatase (Bone-ALP), parathyroid hormone (PTH), Vitamin D3 (Vit. D3), c-terminal telopeptide (CTX), sclerostin and osteocalcin, using specific ELISA kits available on the market (PANTEC SRL, Turin, Italy).

Finally, total cholesterol, high density lipoproteins (HDL), triglycerides, low density lipoproteins (LDL) and glycemia were assessed by routine enzymatic methods.

2.6. Statistical analysis

Sample size was determined considering an expected difference of 2.0% in the increase of bone mineral density (BMD) in femoral neck or lumbar spine between the two treatments after 12 months (2 tailed p value of 0.05); the results indicated that 84 subjects per group would provide an 80% of power, also based on previous observations in a similar setting [9,15]. However, to avoid any possible bias due to drop-out, we also considered 15% more patients to be enrolled with at least a total number of 97 patients per group.

Descriptive and comparative analysis of primary and secondary outcomes were performed between the alendronate and genistein groups. In addition, descriptive and comparative analysis of outcomes were carried out between completers and non-completers.

The Kolmogorov–Smirnov test was performed to estimate the normal distribution of all outcomes. Since the variables were not normally distributed, a non-parametric approach was applied. Absolute and relative frequencies of categorical variables were calculated, while median values with interquartile ranges (IQ1-IQ3) were evaluated for the continuous variables except for the BMD percentage change that was reported as mean an SD. Mann Whitney's test (Z) was applied to compare continuous variables between different groups. For each group, the Wilcoxon and Friedman test were applied to compare all values of the continuous outcomes at the different time points reporting also the degrees of freedom (df) and Chi-square values (H). A p-value of < 0.05 was considered statistically significant. The statistical analysis was performed with SPSS version 23.0 (IBM Corp., SPSS Statistics, Armonk, NY, USA).

3. Results

3.1. Patient characteristics

Table 1 describes baseline characteristics of the randomized patients; no significant difference was observed in the primary and secondary outcomes between the two groups (Table 1). A post-hoc analysis was performed on the basal characteristics of the completers, no significant difference between the two groups was observed. Moreover, no significant differences were observed between participants who dropped out before ending the study (N = 27; 13 alendronate and 14 genistein) and the completers Dropouts were due to adverse events (all of gastrointestinal type)10 alendronate and 8 genistein, while 3 and 6 subjects in each group simply did not get back for the follow-up visit and when interviewed they reported personal reasons.

3.2. Primary outcomes: BMD

BMD at the antero-posterior lumbar spine and femoral neck were evaluated at 1 and 2 years. Median BMD at the antero-posterior lumbar spine significantly increased from 0.75 g/cm^2 at baseline to 0.77 g/cm^2 at 1 year and 0.79 g/cm^2 at 2 years in alendronate-treated patients (H=174.0; df=2; $p < 0.01$) (Table 2); and from 0.77 g/cm^2 at baseline to 0.79 g/cm^2 at 12 months and to 0.80 g/cm^2 at 24 months in genistein recipients (H=172.0; df=2; $p < 0.01$) (Table 2). No difference was observed between the two treatments.

An overlapping pattern was observed in the femoral neck. Median BMD at the femoral neck increased from 0.67 g/cm^2 at baseline to 0.68 g/cm^2 at 1 year and 0.69 g/cm^2 at 2 years in alendronate-treated patients (H=119.6; df=2; $p < 0.01$) (Table 2) and from 0.68 g/cm^2 at baseline to 0.70 g/cm^2 at 12 months and to 0.71 g/cm^2 at 24 months in genistein recipients (H=172.0; df=2; $p < 0.01$) (Table 2). No difference was observed between alendronate and genistein groups. Total hip median BMD (Table 2) also was positively affected by alendronate (from 0.79 g/cm^2 at baseline to 0.80 g/cm^2 at 1 year and to 0.82 g/cm^2 at 2 years) (H=162.8; df=2; $p < 0.01$) and genistein (from 0.80 g/cm^2 at baseline to 0.82 g/cm^2 at 1 year and to 0.84 g/cm^2 at 2 years) (H=172.0; df=2; $p < 0.01$). Likewise, BMD markedly improved in both arms of the study with no significant difference between the two treatments (Table 2).

We also evaluated the increase in BMD as percentage at the several time points. No significant difference in BMD increase in the lumbar spine was observed in patients treated with alendronate compared to patients taking genistein both after one year mean % change (± SD): 2.69 (1.47) vs 2.36 (1.08) and two years mean % change (± SD): 4.86 (2.56) vs 4.45 (2.27) after the start of the study (Fig. 2).

Similarly, no significant difference was observed in total hip BMD mean percentage changes in the alendronate group compared to patients receiving genistein at both 12 months mean % change (± SD): 1.55 (0.28) vs 1.68 (1.66) and 24 months mean % change (± SD): 4.34 (1.85) vs 3.97 (2.17) from baseline (Fig. 2).

Furthermore, median femoral neck BMD percentage increase was similar between the two treatments at either one mean % change (± SD): 2.25 (0.42) vs 2.21 (0.45) or two years mean % change (± SD): 3.75 (0.71) vs 3.66 (0.72) (Fig. 2).

In addition, no difference in the BMD values (femoral neck, lumbar spine and total hip) between the two intervention groups in both patients receiving glucocorticoids for less than 1 year and for more than 1 year was observed, as well as in patients receiving glucocorticoids for less than 12 mg/day and for more than 12 mg/day. Finally, no difference in the BMD values (femoral neck, lumbar spine and total hip) between the two intervention groups in both patients affected by rheumatoid arthritis and systemic lupus erythematosus was detected.

Table 1
Characteristics of patients at baseline.

Variable: [Median (IQ1-IQ3)]	Alendronate Group (100)	Genistein Group (100)	P value
Age	62.0 (58.0–67.8)	60.0 (57.0–66.0)	0.185
Prednisone equivalent dose (mg/Day), n (%)			
5.0 to < 7.5	4 (4.0)	3 (3)	0.922
7.5 to < 12	72 (72.0)	72 (72)	
> 12	24 (24)	25 (25)	
Months of glucocorticoid therapy	12 (10.0–15.0)	12 (10.0–16.0)	0.563
Medical Conditions n (%)			
RA	92 (93)	93 (93)	0.788
SLE	8 (8)	7 (7)	
Body mass index kg/m^2	24.6 (22.7–26.3)	23.8 (22.7–25.9)	0.288
Bone mineral density g/cm^2			
Anteroposterior lumbar spine	0.75 (0.72–0.81)	0.78 (0.74–0.80)	0.178
Femoral neck	0.67 (0.64–0.72)	0.68 (0.66–0.74)	0.204
Total hip	0.80 (0.75–0.85)	0.81 (0.77–0.87)	0.304
PTH mg/dL	35.3 (27.8–41.2)	35.4 (28.4–38.2)	0.694
Vit. D3 UI/L	27.6 (22.4–32.8)	27.8 (21.5–33.4)	0.741
CTx ng/mL	0.17 (0.12–0.22)	0.18 (0.12–0.22)	0.806
Osteocalcin ng/mL	8.8 (7.2–11.5)	9.0 (7.2–11.3)	0.732
Sclerostin pmol/L	40.9 (34.8–48.4)	43.0 (37.0–49.0)	0.554
Bone-ALP µg/L	10.1 (8.0–12.3)	10.3 (8.2–12.4)	0.716
Glucose mg/dL	107.0 (102.0–120.0)	110.0 (101.3–120.0)	0.752
Cholesterol mg/dL	203.0 (184.1–217.3)	205.0 (188.3–226.0)	0.350
HDL mg/dL	59.0 (51.5–67.7)	61.0 (52.3–67.8)	0.627
Triglycerides mg/dL	137.7 (115.5–156.0)	140.0 (120.0–158.3)	0.657
LDL md/dL	118.6 (98.8–132.6)	117.7 (100.8–136.9)	0.705

CTX, c-terminal telopeptide; Vit. D3, vitamin D3; PTH, parathyroid hormone; B-ALP, bone specific alkaline phosphatase; HDL, high density lipoproteins; LDL, low density lipoproteins.

3.3. Secondary outcomes: bone remodeling markers

Levels of Bone-ALP and osteocalcin increased in both genistein and alendronate arms as the years progressed and reached a statistically significant difference from baseline at 2 years (median values Bone-ALP µg/L from baseline to 2 years; genistein group: 10.3–14.4 H=172.0; df=2; $p < 0.01$ and alendronate group: 10.0–11.6 H=170.1; df=2; $p < 0.01$) (median values osteocalcin ng/mL from baseline to 2 years; genistein group: 9.0–13.2, H=172.0; df=2; $p < 0.01$ and alendronate group: 8.7–10.3 H=170.1; df=2; $p < 0.01$)(Table 2). Between group analyses showed that genistein use caused a statistically greater increase at both time points than alendronate (genistein vs alendronate group; median absolute change Bone-ALP µg/L at 1 year: 2.58 vs 0.91 $Z = -11{,}04$ $p < 0.01$ and at 2 years 4.12 vs 1.54 $Z = -11{,}05$ $p < 0.01$) (genistein vs alendronate group; median absolute change osteocalcin ng/mL at 1 year: 1.80 vs 0.82, $Z = -9{,}75$; $p < 0.01$ and at 2 years 4.18 vs 1.47 $Z = -11{,}10$; $p < 0.01$) (Table 3).

The bone resorption marker CTX started to decrease at 1 year in both genistein and alendronate treated patients and the levels were statistically significant lower at 2 years in both arms (median values CTX ng/mL from baseline to 2 years; genistein group: 0.18–0.13, H=86.5; df=2; $p < 0.01$ and alendronate group: 0.17–0.13, H=162.3; df=2; $p < 0.01$) (Table 2). The between-group analyses showed no statistically significant differences in the decrease of the bone resorption marker CTX values at both time points (Table 3).

Sclerostin levels were studied to explore the ability of the two treatments to modulate the Wnt-beta catenin pathway that boosts bone formation: in fact sclerostin is an endogenous inhibitor of the pathway. Genistein administration caused a significant reduction in the levels of sclerostin at both 1 and 2 years from baseline (median values sclerostin pmol/L from baseline 43.0–1 year 38.7 and to 2 years 22.0 H=172.0; df=2; $p < 0.01$) (Table 2). In contrast, a significant increase in sclerostin

Table 2
Characteristics of alendronate and genistein treated patients by time points.

	Alendronate group [Median (IQ1-IQ3)]			Genistein group [Median (IQ1-IQ3)]		
	Baseline (87)	1 year (87)	2 years (87)	Baseline (86)	1 year (86)	2 years (86)
Body mass index kg/m2	24.6 (22.9–26.4)	25.1 (23.4–26.9)	25.1 (23.4–26.9) *	23.8 (22.5–25.9)	23.8 (22.3–25.7)	23.8 (22.0–25.4) *
Bone mineral density g/cm^2						
Anteroposterior lumbar spine	0.75 (0.72–0.79)	0.77 (0.73–0.82)	0.79 (0.74–0.85) *	0.77 (0.74–0.79)	0.79 (0.75–0.82)	0.80 (0.76–0.85) *
Femoral neck	0.67 (0.63–0.72)	0.68 (0.65–0.73)	0.69 (0.66–0.74) *	0.68 (0.66–0.74)	0.70 (0.67–0.74)	0.71 (0.68–0.75) *
Total hip	0.79 (0.75–0.85)	0.80 (0.76–0.86)	0.82 (0.78–0.90) *	0.80 (0.77–0.84)	0.82 (0.78–0.86)	0.84 (0.80–0.88) *
PTH mg/dL	34.9 (27.5–41.2)	34.3 (27.6–40.2)	34.8 (27.5–39.3) *	35.9 (24.8–40.0)	35.5 (28.7–39.2)	34.6 (27.9–38.0) *
Vit. D3 UI/L	27.6 (22.2–32.9)	26.9 (22.0–32.6)	27.4 (21.8–33.1) *	27.8 (21.4–32.9)	27.5 (21.3–32.9)	27.5 (20.9–33.9)
CTx ng/mL	0.17 (0.12–0.21)	0.16 (0.11–0.20)	0.13 (0.09–0.16) *	0.18 (0.12–0.22)	0.17 (0.11–0.21)	0.13 (0.09–0.16) *
Osteocalcin ng/mL	8.7 (7.2–11.5)	9.6 (7.8–12.8)	10.3 (8.4–13.1) *	9.0 (7.4–11.4)	10.8 (8.9–13.6)	13.2 (10.8–16.6) *
Sclerostin pmol/L	40.9 (35.3–48.4)	44.6 (39.2–53.3)*	47.6 (41.1–56.1) *	43.0 (37.0–49.0)	38.7 (33.3–44.1)*	22.0 (19.1–25.4) *
Bone-ALP µg/L	10.0 (7.9–12.0)	10.8 (8.7–13.3)	11.6 (9.3–13.9) *	10.3 (8.0–12.4)	12.9 (10.0–15.5)	14.4 (11.2–17.4) *
Glucose mg/dL	107.0 (102.0–120.0)	116.6 (111.3–132.1) *	124.8 (117.6–138.7) *	108.0 (100.0–120.0)	100.4 (93.0–111.6)*	104.9 (95.4–114.5) *
Cholesterol mg/dL	203.0 (183.4–217.1)	223.2 (199.9–237.8) *	221.6 (199.7–245.0) *	205.0 (190.0–226.5)	184.5 (171.0–203.9) *	192.3 (179.1–208.7) *
HDL mg/dL	58.3 (51.5–67.0)	58.1 (49.9–66.4)	58.6 (48.9–65.2)	60.5 (52.0–67.0)	63.9 (55.9–71.5)*	65.0 (58.2–74.3) *
Triglycerides mg/dL	142.3 (117.5–157.5)	156.5 (128.5–173.8) *	155.9 (131.0–174.8) *	141.5 (121.5–159.3)	123.1 (105.7–138.5) *	125.2 (110.7–144.1) *
LDL mg/dL	117.9 (98.7–132.7)	134.8 (115.0–153.5) *	138.6 (112.1–153.9) *	117.7 (102.2–139.7)	97.3 (82.7–115.1)*	101.7 (84.9–115.9) *

CTX, c-terminal telopeptide; Vit. D3, vitamin D3; PTH, parathyroid hormone; B-ALP, bone specific alkaline phosphatase; HDL, high density lipoproteins; LDL, low density lipoproteins. * $p < 0.01$

at both time points was observed in the alendronate group (median values sclerostin pmol/L from baseline 40.9–1 year 44.6, and to 2 years 47.6, H=170.1; df=2; $p < 0.01$) (Table 2).

Vit. D3 and PTH levels were also monitored throughout the 2 years of the study. In both groups the levels of PTH decreased significantly (median values PTH mg/dL from baseline to 2 years; genistein group: 35.9–34.6, H=12.4; df=2; $p < 0.01$ and alendronate group: 34.9–34.8 H=33.5; df=2; $p < 0.01$), while Vit. D3 significantly reduced only in the alendronate group at two years (median values Vit. D3 UI/L from baseline to 2 years; alendronate group: 27.6–27.4 H=35.2; df=2; $p < 0.01$) (Table 2). The between-group analysis showed a significantly greater decrease in Vit. D3 levels in the alendronate group compared to genistein at 1 year (genistein vs alendronate group; median absolute change Vit. D3 UI/L at 1 year: 0.57 vs 0.55, $Z = -2.47$ $p < 0.05$). No significant differences were observed in PTH variations between groups at all time points (Table 3).

3.4. Secondary outcomes: glycemia, and lipid profile

Genistein administration caused a significant reduction in the levels of glucose at both 1 year and 2 years from baseline (median values glucose mg/dL from baseline 108.0–1 year 100.4, and to 2 years 104.9, H=76.3; df=2; $p < 0.01$) (Table 2). In contrast, a significant increase in glucose at both time points were observed in the alendronate group (median values glucose mg/dL from baseline 107.0–1 year 116.6, and to 2 years 124.8, H=170.1; df =2; $p < 0.01$) (Table 2).

An improvement in the lipid profile was observed in patients treated with genistein. A significant reduction in total cholesterol (median values total cholesterol mg/dL from baseline 205.0–1 year 184.5, and to 2 years 192.3 H=125.2; df=2; $p < 0.01$), triglyceride (median values triglyceride mg/dL from baseline 141.5–1 year 123.1, and to 2 years 125.2 H=132.9; df=2; $p < 0.01$) and LDL cholesterol values (median values LDL cholesterol mg/dL from baseline 117.7–1 year 97.3, and to 2 years 101.7 H=131.5; df=2; $p < 0.01$) and a significant increase in HDL values were observed both at one year and two years from baseline (median values HDL cholesterol mg/dL from baseline 60.5–1 year 63.9, and to 2 years 65.0, H=87.2; df=2; $p < 0.01$) (Table 2). In contrast, the alendronate group showed a worsening of the lipid profile. A significant increase in the values of total cholesterol (median values total cholesterol mg/dL from baseline 203.0–1 year 223.2 and to 2 years 221.6, H=87.2; df=2; $p < 0.01$), triglycerides (median values triglyceride mg/dL from baseline 142.3–1 year 156.5 and to 2 years 155.9 H=132.2; df=2; $p < 0.01$) and LDL (median values LDL cholesterol mg/dL from baseline 117.9–1 year 134.8 and to 2 years 138.6 H=132.2; df=2; $p < 0.01$) at different time points was observed (Table 2).

3.5. Secondary outcomes: bone fractures

Fig. 3 reports the number of non-vertebral and vertebral fractures throughout the observational period. All the observed fractures were of atraumatic origin and were confirmed by X-rays examination. The percentage of fractures was overlapping in the two arms of the study although there was a tendency towards a lower number of femoral fractures in the genistein group, but the small number of fractures precluded meaningful statistical analysis.

3.6. Adverse events

Eight alendronate treated patients and ten genistein recipients dropped out from the study because of adverse events. More specifically 8% of the alendronate and 10% of the genistein patients discontinued treatment. No significant difference was observed between the two arms of treatments. All adverse events were of gastrointestinal type.

4. Discussion and conclusion

Our results point out that genistein treatment succeeded in increasing BMD in glucocorticoid treated patients and positively modified bone turnover markers. In addition, genistein counteracted the metabolic changes caused by the chronic administration of glucocorticoids.

A vast array of pharmacological interventions is nowadays used for the treatment of osteoporosis. From a mechanistic point of view, the anti-osteoporotic medicines are classically divided into drugs that inhibit bone resorption and agents that prompt bone formation [16,17]. Bisphosphonates are recommended as the first-line in primary

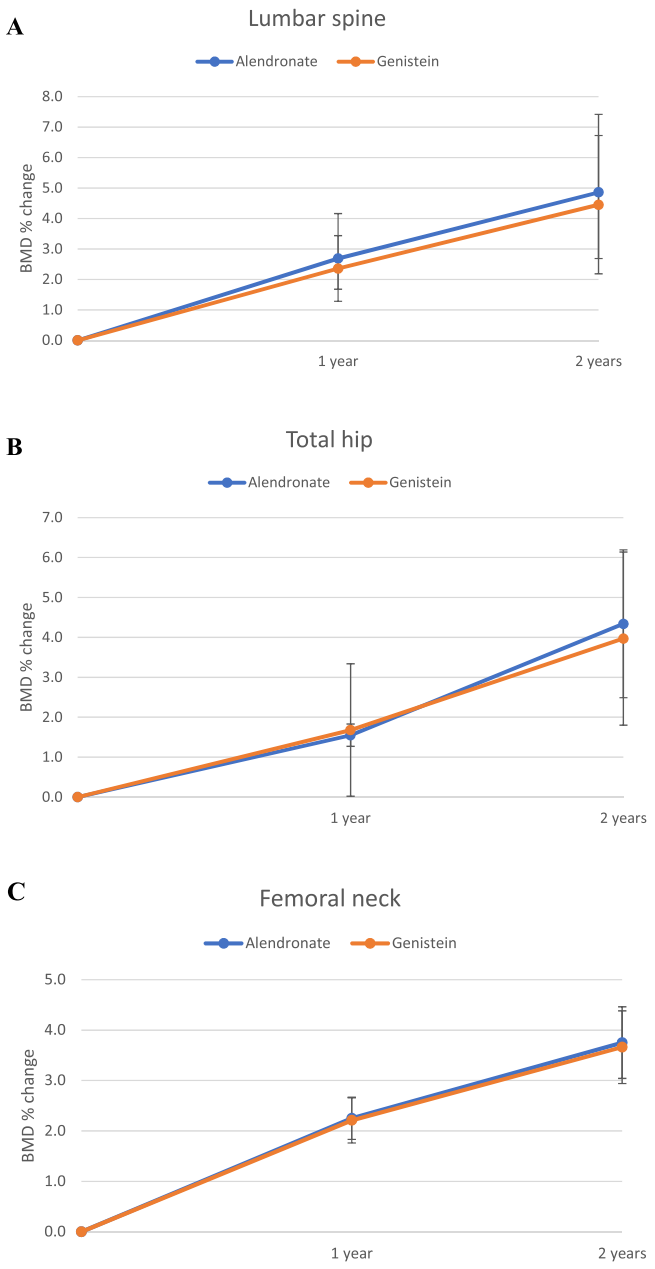

Fig. 2. Comparison in the Lumbar spine (A), Total hip (B), Femoral neck (C) BMD mean % change ± SD from baseline to 2 years; n = 86 Genistein group and n = 87 Alendronate group.

prevention at the start of glucocorticoid therapy (grade A recommendation). By contrast, daily subcutaneous administration of parathyroid hormone and in particular of its active fragment 1–34 (teriparatide) stimulates both neoformation and bone resorption. In a 36-month comparison study with alendronate, teriparatide was more effective in terms of both densitometric changes and the incidence of new vertebral fractures [18]. More recently, denosumab, a monoclonal antibody which prevents the binding of RANKL (NF-κB Ligand receptor activator) to its receptor, has been proposed for the treatment of glucocorticoid induced osteoporosis. RANKL is involved in the regulation of bone remodeling processes and induces the genesis and differentiation of osteoclasts. In the post-hoc analysis of the results on changes in BMD and bone resorption markers from a randomized controlled trial in patients with rheumatoid arthritis (some on glucocorticoid therapy) denosumab has been shown to significantly increase lumbar and hip BMD compared to placebo [18]. Indeed, the efficacy of these therapeutics

Table 3
Comparison in the absolute change between the two groups of all variables studied.

[Median (IQ1-IQ3)]	Alendronate Group Change value at 1 Year (87)	Genistein Group Change value at 1 Year (86)	Alendronate Group Change value at 2 Years (87)	Genistein Group Change value at 2 Year (86)
Body mass index kg/m2	0.49 (0.46/0.53)	0.0 (0.0/0.0) * *	0.49 (0.46/0.53)	-0.50 (−0.76/−0.09) * *
PTH mg/dL	-0.80 (−1.02/0.70)	0.77 (−1.28/1.25)	-0.03 (−0.38/−0.02)	-0.34 (−2.86/0.69)
Vit. D3 UI/L	-0.57 (−0.86/0.52)	0.55 (−0.97/1.06) *	-0.03 (−0.29/−0.01)	-0.02 (−3.03/4.30)
CTx ng/mL	-0.01 (−0.02/−0.01)	-0.01 (−0.05/0.04)	-0.04 (−0.06/−0.03)	-0.05 (−0.06/−0.03)
Osteocalcin ng/mL	0.82 (0.67/1.12)	1.80 (1.48/2.27) * *	1.47 (1.04/1.81)	4.18 (3.42/5.27) * *
Sclerostin pmol/L	3.87 (3.20/4.81)	-4.30 (−4.90/−3.70) * *	6.65 (5.06/7.95)	-20.22 (−23.36/−17.24) * *
Bone-ALP μg/L	0.91 (0.72/1.16)	2.58 (2.01/3.10) * *	1.54 (1.21/1.79)	4.12 (3.21/4.96) * *
Glucose mg/dL	10.5 (8.7/12.8)	-7.6 (−8.4/−7.0) * *	18.0 (15.3/19.7)	-0.5 (−13.5/2.5) * *
Cholesterol mg/dL	19.5 (15.7/22.8)	-20.5 (−22.7/−19.0) * *	19.8 (11.7/27.8)	-7.3 (−34.5/−2.0) * *
HDL mg/dL	-1.3 (−1.8/1.2)	4.5 (3.9/5.0) * *	-0.6 (−3.3/3.0)	8.8 (0.3/10.3) * *
Triglycerides mg/dL	13.4 (11.3/15.5)	-18.4 (−20.7/−15.8) * *	13.1 (8.5/19.3)	-9.6 (−25.6/−6.8) * *
LDL md/dL	16.7 (14.6/20.5)	-21.5 (−23.5/−18.9) * *	17.9 (13.0/21.9)	-13.7 (−25.5/−10.3) * *

CTX, c-terminal telopeptide; Vit. D3, vitamin D3; PTH, parathyroid hormone; B-ALP, bone specific alkaline phosphatase; HDL, high density lipoproteins; LDL, low density lipoproteins. * $p < 0.05$; * * $p < 0.01$

(bisphosphonates, teriparatide and denosumab) may be hampered by the occurrence of several side effects and, very often, they encounter low adherence in patients, especially in the elderly ones. Both occurrence of adverse events and low adherence to the therapy impair long-term use of these agents [18]. Indeed, there is a lack of data on the efficacy and safety of complementary and alternative regimens which may be more acceptable to patients.

Chronic use of glucocorticoid can increase the apoptosis of osteoblasts and osteocytes [19], but the estrogen presence reduces the translocation of glucocorticoid receptor into the nucleus and thus counteracts their effects [19]. However very few reports have been published on the use of estrogen receptor modulators (SERMS) in ameliorating the negative glucocorticoid effects on osteoblasts. As previously shown genistein (a natural SERM) is able to increase bone mass in rats with glucocorticoid-induced osteoporosis [20], and in women has been also useful in the management of menopausal symptomology [21–23] and related metabolic changes [24–26]. Furthermore, the preclinical data have unmasked a higher translational potential for treatment of osteoporosis, due to an anti-resorption mechanism in analogy to the classical estrogen replacement therapy [25,26]. The results of these preclinical animal models of osteoporosis combined with the recent post-hoc analysis of a randomized clinical trial strongly suggest that this molecule has also the ability to exert anti-osteoporotic activity [27]. In our study both therapeutic regimens caused an increase in BMD at the femoral neck, lumbar spine and total hip. However, the number of incidental fractures in both arms was not different. Since concerns have

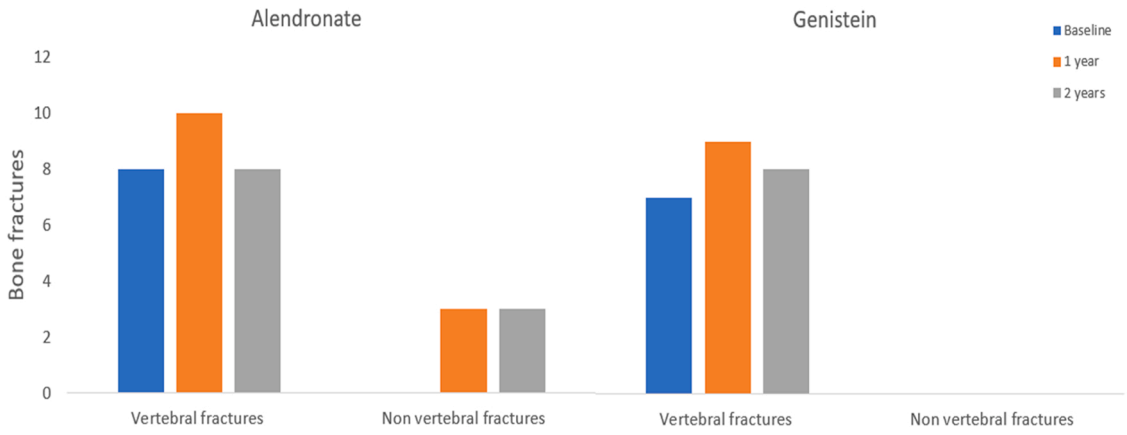

Fig. 3. The graphs represent the fracture incidence in the alendronate and genistein group at different time points; n = 86 Genistein group and n = 87 Alendronate group.

been raised regarding the long-term effects of bisphosphonates on bone quality through the interference with the survival of osteoclasts and their incorporation into the hydroxyapatite instead of calcium, they should be stopped after 5 years. Indeed, a prolonged use has been associated with atypical fractures of the femur due to excessive bone mineralization, with consequent loss of the elastic capacity of the bone itself. In agreement with the alendronate mode of action, our patients treated with alendronate had marked reduction in the blood levels of CTX, a bone resorption marker, while showed a significant reduced increase in Bone-ALP, a marker of bone formation, when compared to genistein. By contrast, genistein treatment caused not only a marked reduction in CTX levels but also a marked and higher increase in the Bone-ALP. This result confirms previously published preclinical and clinical evidences [9,10] suggesting that genistein may differ from the classical SERMs owing also ability of boosting bone formation. Indeed, it has been reported that bone resorption markers decrease within 3 months by at least 50% following alendronate treatment and are maintained at those decreased levels while on therapy [19]. That was not observed in our alendronate group: this could be ascribed to a different timing of sampling (3 months vs 12 or 24 months in our study), different length of glucocorticoid therapy, the gender of enrolled population and the underlined diseases for which patients received the corticosteroid therapy. However, the results are in close agreement as far as the tendency is concerned (reduction following alendronate treatment). In addition, an increase in bone formation markers has been documented in patients supplemented with calcium and vitamin D and therefore the augmented bone formation markers observed in the alendronate group can be likely due to the concomitant administration of these two factors that boost bone formation. Nevertheless, there is no significant change in bone formation markers from baseline to 1 year. Indeed, this is in agreement with the previously reported findings with alendonate. The bone forming markers raise only at the end of study, following two years of treatment. This could represent a bias of our investigation generated by several factors. First it should be underlined that our population lives in Sicily (South of Italy) and the high exposure to the sunlight could amplify the bone forming effects of Vitamin D. Secondly the enrolled patients consumed a typical mediterranean diet rich in vegetables fruit and tomatoes that are rich in lycopene that has well established effects on bone formation [25,28]. Lastly the age and the gender of our study population (they were all postmenopausal women) might have caused an overly homogenous group emphasising even small changes in bone formations and thus rendering them statistically different.

Osteoblasts also release Dickkopf-related protein 1 (DKK-1), that together with sclerostin, produced by the osteocyte, inhibit the Wnt/β-catenin signaling. Interestingly, the canonical Wnt/β-catenin pathway is deeply involved in the intracellular events leading to osteoblast maturation and bone formation [29]. Therefore, it can be speculated that modulation of osteoblast function might represent an interesting strategy not only to preserve bone strength and architecture, but also to treat osteoporosis. Within secondary outcomes the blood levels of sclerostin were evaluated and despite GIO patients had increased levels, genistein administration reduced sclerostin levels. Therefore, it is tempting to speculate that genistein bone formation effects might be mediated by a positive increased signaling of the Wnt/β-catenin pathway. However, it remains to be elucidated, whether this genistein effect may result in a better quality of the bone, thus conferring a more robust bone architecture. Only long-term observation (up to five years) will give this interesting information.

Among the other outcomes, metabolic profile was investigated and also in this peculiar population of postmenopausal women genistein treatment improved lipid and glucose levels, demonstrating a mode of action quite similar to the ideal SERM. Indeed, alendronate has been shown to ameliorate glycemic and lipid profile [30]. However, we did not observe any metabolic positive effect in the alendronate administered group. The reasons for this lack of alendronate effects under this peculiar clinical setting (GIO) could be that glucocorticoids worsen the metabolic profile of the patients and may abolish the alendronate effects. In agreement with this hypothesis it has been show that alendronate does not affect at least lipid profile in prediabetes [31], a clinical scenario that is overlapping to that induced by glucocorticoid treatment.

In conclusion the results of this randomized trial, suggest that genistein aglycone deserves scientific and clinical attention and interest in the management of GIO. Further additional randomized trials will be necessary to confirm this first clinical evidence and to obtain more insights into the genistein mode of action.

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CRediT authorship contribution statement

Francesco Squadrito: Conceptualization, Writing – original draft, Supervision. **Egidio Imbalzano:** Conceptualization. **Michelangelo Rottura:** Formal analysis. **Vincenzo Arcoraci:** Formal analysis. **Giovanni Pallio:** Investigation. **Antonino Catalano:** Investigation. **Marco Atteritano:** Investigation. **Natasha Irrera:** Investigation: **Federica Mannino:** Investigation. **Giovanni Squadrito:** Investigation. **Mario**

Vaccaro: Investigation. **Pierangela Irrera:** Investigation. **Igor Pirrotta:** Investigation. **Alessandra Bitto:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

None.

Data Availability

Data will be made available on request.

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None.

Conflict of interest

Francesco Squadrito and Alessandra Bitto received research grants from Primus Pharmaceuticals Inc and are listed as inventors in several patents related to Primus Pharmaceutical Inc. However, they do not retain economic benefits from the commercial use of these patents.

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