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Dexlansoprazole and Esomeprazole Do Not Affect Bone Homeostasis in Healthy Postmenopausal Women

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BACKGROUND & AIMS: Epidemiological studies have associated proton pump inhibitor (PPI) therapy with osteoporotic fractures, but it is not clear if PPIs directly cause osteoporosis. We evaluated the effect of dexlansoprazole and esomeprazole on bone turnover, bone mineral density (BMD), true fractional calcium absorption (TFCA), serum and urine levels of minerals, and levels of parathyroid hormone (PTH) in healthy postmenopausal women. **METHODS:** We performed a prospective, multicenter, double-blind study of 115 healthy, postmenopausal women (45 to 75 years of age) from November 4, 2010, through August 7, 2014. Women were randomly assigned to groups given dexlansoprazole (60 mg), esomeprazole (40 mg), or placebo daily for 26 weeks. We measured plasma levels of procollagen type 1 N-terminal propeptide (P1NP) and C-terminal telopeptide of type 1 collagen (CTX) at 0 (baseline), 13, and 26 weeks. Primary outcomes were percent change in P1NP and CTX between weeks 0 and 26. We also measured changes in serum and urine levels of mineral, BMD, PTH (all subjects), and TFCA (n = 30). **RESULTS:** Between baseline and week 26, there were no significant within-group differences in markers of bone turnover; there was a nonsignificant increase in CTX levels in the dexlansoprazole group (0.12 ng/mL). The esomeprazole and dexlansoprazole groups had significantly increased levels of P1NP (18.2% and 19.2%, respectively) and CTX (22.0% and 27.4%, respectively) at week 26 compared with the placebo group, although these values remained within normal ranges. There were no statistically significant differences between groups in serum or urine levels of minerals, BMD, or PTH at week 26. PPI therapy did not reduce TFCA. **CONCLUSIONS:** In a prospective study of postmenopausal women, we found significant increases in markers of bone turnover in women given PPI therapy compared with women given placebo, but levels remained within the normal reference range. We found no significant differences among groups in changes in BMD, PTH, serum or urine levels of minerals, or TFCA. Our findings indicate that 26 weeks of treatment with a PPI has no clinically meaningful effects on bone homeostasis. Clinicaltrials.gov no: NCT01216293

Keywords: Clinical Trial; Proton Pump Inhibitor; Osteoporosis; Intestinal Calcium Absorption.

Proton pump inhibitors (PPIs) have been widely used for decades to treat acid-related diseases.[1] PPIs exhibit a well-established safety profile and reduce gastric acid more effectively than histamine-2 receptor antagonists or antacids.[2] As a result, several PPIs are available over the counter. However, observational studies suggest that long-term PPI therapy is associated with osteoporotic fractures, hypomagnesemia, and vitamin B_{12} deficiency.[2,3]

A large body of epidemiological data has detected an association between PPI use and fracture risk.[4,5] The clinical importance of this association is unclear because odds ratios are low, there is no consistent dose-response relationship, and confounding factors exist in many studies.[4] Although a plausible mechanism explaining a causal relationship between PPI use and fracture has not been definitively established,[4] hypotheses include decreased calcium absorption due to hypochlorhydria,[6,7] osteoclast V-ATPase inhibition, increased activity of parathyroid hormone (PTH) induced by hypergastrinemia,[8,9] and decreased magnesium absorption.[4,10–13] Mixed results also have been reported for bone mineral density (BMD) in relation to PPI use.[14–17] Some studies evaluating PPI effects on vitamin B_{12} and calcium absorption found a decrease associated with PPI use,[18–20] whereas others showed no effect.[7,21–23]

We evaluated the effects of PPIs on bone homeostasis in postmenopausal women, a population with the highest prevalence of osteoporosis and related fractures.[24,25] We measured bone turnover, BMD, true fractional calcium absorption (TFCA), PTH, and serum and urine mineral levels in women at baseline and 26 weeks after randomization to placebo or 2 potent PPIs, dexlansoprazole and esomeprazole. Bone turnover markers were the primary study outcome, as they are consensus standard markers for assessment of bone resorption (C-terminal telopeptide of type 1 collagen [CTX]) and formation (procollagen type 1 N-terminal propeptide [P1NP]) related to both fracture risk and monitoring osteoporosis medications.[26,27] We also measured bone-specific alkaline phosphatase (BsAP) and

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Abbreviations used in this paper: **AE, adverse event; BMD, bone mineral density; BsAP, bone-specific alkaline phosphatase; CI, confidence interval; CTX, C-terminal telopeptide of type 1 collagen; NTx, N-terminal telopeptide; P1NP, procollagen type 1 N-terminal propeptide; PPI, proton pump inhibitor; PTH, parathyroid hormone; TFCA, true fractional calcium absorption; 25(OH)D, 25-hydroxyvitamin D.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Epidemiologic studies have linked use of proton pump inhibitors (PPIs) to a higher risk of osteoporotic fracture. However, a mechanism by which PPI therapy might cause osteoporosis remains elusive.

NEW FINDINGS

In a randomized, placebo-controlled trial of 115 postmenopausal women with uniform vitamin D repletion and adequate calcium intake, PPI therapy increased bone turnover, but bone resorption and formation remained coupled and within the normal range.

LIMITATIONS

Short duration of exposure to PPI therapy, recruitment of healthy postmenopausal women, and small sample size.

IMPACT

The mechanism by which PPI therapy might cause skeletal harm remains unclear. In this study, 26 weeks of PPI therapy had no harmful effects on skeletal health.

urinary N-terminal telopeptide (NTx), which have shown significant associations with BMD response to osteoporosis therapies.[28]

Methods

Study Design

We conducted a phase 4, randomized, double-blind, placebo-controlled study in healthy postmenopausal women from November 4, 2010 (first participant in) to August 7, 2014 (last participant out), at 12 US centers. Institutional review board approval and participant consent were obtained before the study. This study was funded by Takeda Pharmaceuticals International, Inc. Takeda Pharmaceuticals International, Inc., was responsible for and sponsored the study design, data collection, data interpretation, and writing of this article. All authors had access to the study data and reviewed and approved the final manuscript.

Eligible women were between 45 and 75 years of age, with body mass index between 18 and 30 kg/m^2 and with no menses for at least 5 years. Eligibility requirements included bone turnover markers and clinical laboratory evaluations within normal postmenopausal ranges (postmenopausal follicle-stimulating hormone levels $\geq$23 IU/L; creatinine clearance $\geq$50 mL/min; BMD T-score >-2.0 at the total hip, femoral neck, and lumbar spine by dual-energy x-ray absorptiometry); and willingness to take daily supplements of vitamin D and calcium carbonate ($CaCO_3$). Participants could not use medications and substances with a potential effect on bone homeostasis during the study (Supplementary Table 1). Further eligibility criteria and BMD screening methods are reported in the Supplementary Methods.

The study consisted of a 12-week screening period, a 26-week treatment period, and a follow-up visit at week 52 for BMD assessment. Dietary assessment was performed between the screening period and week 13 to assess calcium and vitamin D_3 intake. Participants were given vitamin D_3 to maintain 25-hydroxyvitamin D (25[OH]D) levels $\geq$32 ng/mL and calcium carbonate 600 mg/d to maintain a total (diet plus supplement) intake of 1200 mg/d.

Participants were randomly assigned to receive placebo, dexlansoprazole 60 mg once daily, or esomeprazole 40 mg once daily in a 1:1:1 ratio for 26 weeks, with study medication dispensed in bottles in a double-blind fashion. P1NP, CTX, urine NTx, and BsAP were measured at 0, 13, and 26 weeks. Serum samples were analyzed for P1NP using an electrochemiluminescence immunoassay on the Elecsys 2010 automated analyzer (Roche Diagnostics, Indianapolis, IN) and for BsAP using an enzyme immunoassay with the Metra BsAP enzyme immunoassay kit (Quidel, San Diego, CA). Plasma samples were analyzed for CTX using an electrochemiluminescence immunoassay with the beta-crosslaps immunoassay kit on the Elecsys 2010 automated analyzer. Urine samples were analyzed for NTx using an enzyme-linked immunosorbent assay with the Osteomark kit (Wampole Laboratories, Waltham, MA) and read on a SpectraMax microplate spectrophotometer. Subjects were instructed to fast without taking study medication, calcium, or vitamin D_3 supplements for a minimum of 8 hours before all laboratory tests (excluding first screening visit). BMD was measured in each subject using the same Hologic bone densitometer at baseline (−12 weeks), 26, and 52 weeks. Calcium and vitamin D_3 supplements were to be taken with the first meal of the day, approximately 1 hour after study drug administration. A comprehensive schedule of study procedures is reported in Supplementary Table 2.

Our primary outcomes were changes in bone formation and bone resorption. Changes in bone turnover precede changes in BMD and predict harmful (or beneficial) effects of an intervention on BMD.[29–31] We selected P1NP and CTX as our primary measures of bone formation and resorption, as they change more quickly with pharmacologic interventions.[29–32] We had no pilot data on PPI-mediated changes in bone turnover by which to calculate a potential sample size for the study. However, within-subject changes in the bone resorption marker CTX can be as high as 36%.[33] We therefore selected a sample size of 240 subjects so that the width of the 95% confidence interval (CI) for the difference in the percent change from baseline in each of the markers between PPI and placebo was restricted to no more than 30%. Finally, we chose a study duration of 6 months, as this is the duration of therapy for dexlansoprazole approved by the Food and Drug Administration. In addition, a 6-month exposure would minimize risks to participants while still providing sensitive information about bone turnover and alterations in mineral homeostasis resulting from PPI therapy.

The primary outcome was percent change from baseline to week 26 in the bone formation marker P1NP and the bone resorption marker CTX. The secondary endpoint was percent change from baseline to week 26 in urine NTx (resorption marker) and BsAP (formation marker). Additional endpoints included the 13-week percent change in P1NP and CTX; the 26- and 52-week percent change in femoral neck, total hip, and lumbar spine BMD, and the incidence of fractures, including clinical vertebral fractures. We also measured 26-week changes in 24-hour urinary calcium and magnesium excretion, serum PTH, calcium, phosphorus, magnesium, and 25(OH)D. A TFCA study was performed at baseline and 6 months in a subset of participants using dual stable ^{44}Ca (oral) and ^{42}Ca

(intravenous) isotopes[34] and an inpatient 24-hour urine collection. The inpatient diet provided during the 24-hour TFCA study period matched the participants' outpatient diet based on 4-day food diaries (additional information regarding the TFCA substudy is provided in the Supplementary Methods section). Adverse events (AEs) were classified according to Medical Dictionary for Regulatory Activities and by event severity.

Statistical Analyses

The safety data set included all participants who received at least 1 dose of the study drug. The pharmacodynamic data set included all participants who had baseline and post-baseline values for any primary or secondary endpoint. Assuming a standard deviation of percent change from baseline levels to be no more than 40% for each bone turnover marker, a sample size of 80 participants per arm provided a 95% CI of the estimated difference between PPI and placebo that extended no more than 15% in each direction, with an allowance for up to 30% dropouts.

After all study visits were completed, one of the trial sites (site 6019) was disqualified by the Food and Drug Administration, and all data collected at site 6019 were excluded from these analyses. Even without this site, the 95% CI for the between-arm percent change in all bone turnover markers was $\leq$33% wide, which is still comparable to the original assumption (95% CI to have a width not to exceed 30%). All differences in percent change from baseline endpoints between dexlansoprazole or esomeprazole and placebo were estimated using the nonparametric Hodges-Lehmann estimator and the Moses method for the 95% CI. Comparisons were considered statistically significant if the 95% CI excluded zero.

Results

Subjects

Of the 556 women screened at 11 centers, 115 were enrolled, and 93 participants completed the study (Supplementary Figure 1). No substantial differences in baseline demographics were observed between treatment groups (Table 1). Most participants (>90%) were white, with mean age of 62 ± 6 years, and mean body mass index of 25.2 ± 2.8 kg/m^2. Subjects' serum 25(OH)D levels were 39 ± 10 ng/mL at randomization. The calcium absorption substudy enrolled 34 participants (30 completing) with demographics and baseline characteristics representative of the overall study population. Mean study drug compliance was >95% in each treatment group. The incidence of the most common concurrent medical conditions relevant to study outcomes (osteoarthritis, osteopenia, and back pain) was comparable across treatment groups. The proportion of subjects with protocol deviations was also comparable across treatment groups (placebo 76%, dexlansoprazole 68%, and esomeprazole 74%) and primarily attributed to deviations in visit windows, which did not affect the overall outcome of the study.

Bone Biomarkers

In all treatment arms, bone turnover remained within the normal reference range for postmenopausal women at baseline, 13, and 26 weeks. Levels of P1NP, BsAP, CTX, and NTx showed little change from baseline, and final values remained within the normal range for this population (Figure 1).

Bone formation, as assessed by P1NP, showed a statistically significant increase in the dexlansoprazole group at week 26 (19.2%; 95% CI 7.0%–30.2%) and the esomeprazole group at week 26 (18.2%; 95% CI 6.7%–30.4%) relative to placebo (Table 2). Levels of the formation marker BsAP also increased significantly in the dexlansoprazole group at week 26 (6.8%; 95% CI 0.4%–12.8%) and at week 26 in the esomeprazole group (7.2%; 95% CI 0.6%–12.9%) relative to placebo. Week 13 changes in P1NP and BsAP values in the esomeprazole group were not statistically significant.

Table 1. Baseline Demographics

	Placebo, n = 41	Dexlansoprazole, 60 mg QD, n = 38	Esomeprazole, 40 mg QD, n = 35	All subjects, N = 114
Mean age (SD), y[a]	61.2 (5.6)	62.4 (6.7)	63.2 (5.1)	62.2 (5.9)
Range	51–73	52–75	54–74	51–75
Race, n (%)				
White	37 (90)	34 (90)	33 (94)	104 (91)
Black/African American	2 (5)	2 (5)	2 (6)	6 (5)
Other[b]	2 (5)	2 (5)	0	4 (4)
Mean BMI (SD), kg/m^2	25.6 (2.6)	24.5 (3.0)	25.7 (2.6)	25.2 (2.8)
Range	21.3–30.9	18.2–31.3	19.6–30.7	18.2–31.3
Current drinker, n (%)	30 (73)	31 (82)	27 (77)	88 (77)
Serum calcium at randomization, mean (SD), mg/dL	9.5 (0.4)	9.5 (0.3)	9.6 (0.3)	9.5 (0.3)
Range	8.6–10.1	9.0–10.1	9.1–10.2	8.6–10.3
Serum vitamin D at randomization, mean (SD), ng/mL	40 (9)	40 (12)	36 (9)	39 (10)
Range	29–69	26–87	19–55	19–87

BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); QD, once daily; SD, standard deviation.
[a]n = 40 for placebo for age, because age was calculated at first dose and 1 participant was not dosed.
[b]Other races included American Indian/Alaska Native, Asian, or multiracial.

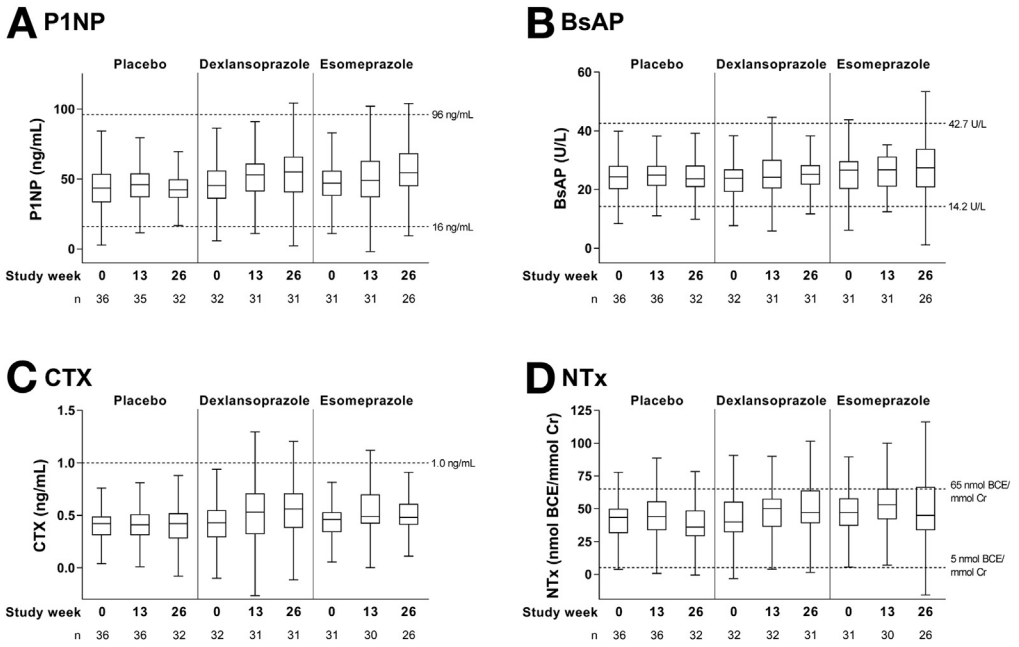

Figure 1. Bone turnover markers. Top and bottom of boxes represent first and third quartiles; the interior line represents the median. Whiskers terminate at 1.5 times (interquartile range) below the first quartile or above the third quartile. Horizontal dotted lines indicate upper and lower bound of normal range for women aged 45 to 75 years (*A*); upper and lower bound of normal range for women aged 45 to 110 years (*B*); upper bound of normal range for all women (lower bound = 0) (*C*); and upper and lower bound of normal range for all women (*D*). BCE, bone collagen equivalent; Cr, creatinine.

Resorption (CTX levels) significantly increased in the dexlansoprazole group at weeks 13 (15.9%; 95% CI 4.9%–29.6%) and 26 (27.4%; 95% CI 12.7%–43.0%) and in the esomeprazole group at weeks 13 (16.1%; 95% CI 5.0%–27.0%) and 26 (22.0%; 95% CI 8.4%–35.7%) relative to placebo. For NTx, the only statistically significant difference from placebo occurred at week 26 in the dexlansoprazole group (20.1%; 95% CI 4.0%–34.4%; Table 2). Within-group 26-week changes in bone turnover markers did not show statistically significant differences from baseline, except for a small increase in CTX levels in the dexlansoprazole group (0.12 ng/mL; 95% CI 0.03–0.23 ng/mL).

BMD and Fracture

We detected no statistically significant between-group changes in lumbar spine, total hip, or femoral neck BMD at 26 and 52 weeks (Table 3). No subject experienced a 26-week decrease in lumbar spine, femoral neck, or total hip BMD >0.021 g/cm^2. A 73-year-old woman randomized to dexlansoprazole experienced a 3% decrease in spine BMD, but her T-score at the spine was +0.6 even after the decrease. No fractures occurred during the treatment period. One woman randomized to dexlansoprazole sustained a traumatic foot fracture at week 52 because of a car accident. A humerus fracture (circumstance unknown) occurred at week 23 in one woman randomized to esomeprazole; she withdrew from the study after 17 weeks of treatment because of worsening fatigue that was considered to be drug related.

Calcium Absorption, Mineral, and Hormone Levels

Serum calcium and phosphorus concentrations remained stable and normal, with no between-arm changes. Likewise, we detected no statistically significant between-group changes in serum magnesium, 25(OH)D, or 24-hour urinary calcium or magnesium levels (Supplementary Table 3), and all values remained stable and normal. As expected, serum gastrin levels increased in participants randomized to PPI therapy, with median week 26 differences of 80 ng/mL (dexlansoprazole) and 85 ng/mL (esomeprazole) relative to placebo (Supplementary Table 3).[10,35] No statistically significant between-group changes in PTH were found (Supplementary Table 3). Likewise, there was no correlation between PPI-related changes in gastrin and PTH during the study.

In the substudy population, there were no decreases in TFCA in participants randomized to PPI therapy (Supplementary Table 4). The only statistically significant increase in TFCA relative to placebo occurred in the esomeprazole group (+0.06; 95% CI 0.02–0.11).

Safety

The median duration of treatment was comparable among groups. AEs were reported for 39% (16/41), 45% (17/38), and 37% (13/35) of participants in the placebo, dexlansoprazole, and esomeprazole groups, respectively. Most AEs (≥93%) were classified as being mild or moderate. The most frequently reported AEs were diarrhea and upper respiratory tract infection with placebo; diarrhea with dexlansoprazole; and urinary tract infection, dyspepsia, and upper respiratory tract infection with esomeprazole. The frequency of drug-related AEs within treatment groups was 9% (3/34; placebo), 30% (9/30; dexlansoprazole), and 48% (13/27; esomeprazole). AEs resulting in discontinuation were reported for 1 individual in the placebo group (diarrhea), 4 individuals in the dexlansoprazole group (diarrhea, 3; leukopenia, 1), and 2 individuals in the esomeprazole group (nausea and fatigue).

The only drug-related serious AE, nephrolithiasis, occurred in a dexlansoprazole participant at day 142 and

Table 2. Percent Change From Baseline to Weeks 13 and 26 for Biomarkers of Bone Homeostasis

	Median (IQR) of percent change						Difference vs placebo (95% CI)[a]			
	Placebo		Dexlansoprazole 60 mg QD		Esomeprazole 40 mg QD		Dexlansoprazole vs placebo		Esomeprazole vs placebo	
	Week 13, n = 38	Week 26, n = 32	Week 13, n = 36	Week 26, n = 31	Week 13, n = 34	Week 26, n = 26	Week 13	Week 26	Week 13	Week 26
Formation										
P1NP, ng/mL	7.2 (21.9)	–0.4 (24.1)	21.4 (31.1)	19.3 (41.0)	11.5 (28.8)	16.9 (37.4)	**12.3** (3.1 to 22.8)	**19.2** (7.0 to 30.2)	5.8 (–4.0 to 14.7)	**18.2** (6.7 to 30.4)
BsAP, U/L	–1.44 (15.0)	0.5 (14.1)	7.7 (12.0)	8.7 (20.1)	4.4 (15.0)	6.2 (17.8)	**6.5** (0.9 to 11.4)	**6.8** (0.4 to 12.8)	3.1 (–1.7 to 8.7)	**7.2** (0.6 to 12.9)
Resorption										
CTX, ng/mL	2.7 (20.0)	2.4 (29.2)	18.8 (48.8)	29.5 (39.8)	18.7 (32.7)	24.0 (30.3)	**15.9** (4.9 to 29.6)	**27.4** (12.7 to 43.0)	**16.1** (5.0 to 27.0)	**22.0** (8.4 to 35.7)
NTx (corrected)	5.5 (27.3)	–6.1 (36.2)	14.6 (36.7)	16.9 (39.7)	13.4 (26.9)	6.2 (40.3)	6.6 (–6.6 to 19.2)	**20.1** (4.0 to 34.4)	7.6 (–2.4 to 18.2)	11.6 (–2.7 to 28.3)

IQR, interquartile range; QD, once daily.
[a]Difference estimated with Hodges-Lehmann estimate; 95% CIs of the differences calculated by the Moses method. Percent change values were considered statistically significant if the 95% CI excluded zero (reported in bold text).

Table 3. Changes From Baseline in BMD

	Placebo, n = 38[a]		Dexlansoprazole 60 mg QD, n = 36[a]		Esomeprazole 40 mg QD, n = 34[a]		Difference in percent change vs placebo (95% CI[b])	
	n	Median percent change (IQR)[b]	n	Median percent change (IQR)[b]	n	Median percent change (IQR)[c]	Dexlansoprazole vs placebo	Esomeprazole vs placebo
Lumbar spine, g/cm^2								
Week 26	19	0.104 (5.029)	19	–2.236 (4.345)	20	–1.182 (3.024)	–0.888 (–3.263 to 1.084)	–0.840 (–2.755 to 1.061)
Week 52	17	–0.392 (2.362)	18	–0.537 (3.469)	18	0.793 (2.913)	–0.112 (–1.657 to 1.846)	1.344 (–0.202 to 3.171)
Total hip, g/cm^2								
Week 26	26	–0.181 (1.707)	27	–1.045 (2.402)	24	–0.774 (2.336)	–0.664 (–1.482 to 0.240)	–0.358 (–1.272 to 0.501)
Week 52	23	0.109 (1.849)	25	–0.441 (1.983)	23	–0.109 (1.721)	–0.344 (–1.168 to 0.587)	–0.004 (–0.682 to 0.972)
Femoral neck, g/cm^2								
Week 26	26	–0.688 (2.823)	27	–1.861 (3.002)	24	–1.515 (3.802)	–0.966 (–2.360 to 0.482)	–0.434 (–1.879 to 1.056)
Week 52	23	0.000 (3.892)	25	–0.437 (2.598)	23	0.000 (4.647)	–0.237 (–1.565 to 1.221)	0.285 (–1.250 to 2.165)

IQR, interquartile range; QD, once daily.
[a]Some scans were not evaluable and were excluded from the BMD analyses, which led to fewer participants than for other parameters.
[b]Difference estimated with Hodges-Lehmann estimate. 95% CIs of the differences calculated by the Moses method.
[c]Percent change calculated either from baseline to week 26 or from week 26 to week 52.

lasted for 37 days and then resolved. Analysis of the passed stone indicated it was 91% calcium oxalate, and although classified as drug related, it could also have been caused by concomitant calcium or vitamin D supplementation. Three serious AEs deemed unrelated to study drugs were breast cancer (n = 1, dexlansoprazole), aortic dissection (n = 1, dexlansoprazole), and hip arthroplasty (n = 1, placebo).

Discussion

Recent population-based epidemiological studies have generated conflicting results about a relationship between chronic PPI therapy and fracture risk.[4,5,11–13,36] Low odds ratios (<2), lack of dose response, biological implausibility, and uncontrolled potential confounders limit any firm conclusions about the causal nature between PPI therapy and osteoporosis.[37,38] Although some studies have detected an association between PPI use and fractures in postmenopausal women, prospective studies have found no differences in BMD between PPI users and nonusers.[14,25,39]

We undertook this prospective, double-blind, randomized, placebo-controlled trial to evaluate the effects of PPIs on bone homeostasis in healthy postmenopausal women. Overall, we found no evidence of bone loss among healthy postmenopausal women who received dexlansoprazole or esomeprazole for 26 weeks compared with those who received placebo. PPI therapy was associated with increases in both bone formation (P1NP increased 17% to 19%; BsAP increased 6% to 9%) and bone resorption (CTX increased 24% to 30%; corrected NTx increased 6% to 17%) compared with placebo. However, absolute changes within treatment groups were small and largely remained within the range expected for postmenopausal women.

In adults, uncoupled bone resorption and formation with a net increase in resorption results in bone loss and decreased BMD, leading to osteoporosis.[40,41] In the current trial, resorption and formation appeared to remain coupled over 26 weeks, with increases in both formation and resorption. Furthermore, there were no significant between-arm differences in BMD during 26 weeks of PPI therapy or at 52 weeks from baseline. The very small within-subject changes in BMD observed in this study were consistent with expected changes in postmenopausal women (1% to 2% bone loss per year during the first 5 to 8 years after menopause).[42] Our data are consistent with a Canadian longitudinal study that found no association between PPI use and BMD loss over 10 years, and with the Study of Women's Health Across the Nation (SWAN), a longitudinal study of 2068 premenopausal or early premenopausal women, that found no difference in BMD change in PPI users compared with nonusers.[14,39]

Gastrin-induced PTH production leading to excess bone resorption has been proposed as a mechanism to explain PPI effects on bone homeostasis.[8,9,43] Gastrin levels were increased with PPI administration in this study (an increase from baseline of 80–85 pg/mL), but there were no corresponding increases in PTH levels in either PPI treatment group. Likewise, serum calcium, urine calcium, and serum phosphorus levels remained stable and normal during the study, with no between-arm changes. Together, these data suggest that hypergastrinemia had no effect on PTH, calcium, or phosphorus concentrations.

Another proposed mechanism by which PPIs might influence bone homeostasis is through reduced calcium absorption due to increased gastric pH.[7] TFCA, the proportion of a given dose of calcium actually absorbed, is approximately 25% in adult men and nonpregnant women across a wide age range[44] and declines on average by 0.2% per year after age 40.[45] Calcium is absorbed in the intestinal tract by passive transport and by active transport that requires adequate vitamin D status.[46] The pH of the small intestine is nearly neutral, which suggests that gastric pH would not affect calcium absorption.[7] Our results support this theory. Serum 25(OH)D levels were stable throughout the study, which suggests that vitamin D metabolism did not influence the TFCA results. Median TFCA values ranged from 16% to 20% across the 3 treatment groups at the baseline and week 26 assessments, as expected in this population.[44,45] Consistent with another study of 30-day administration of omeprazole,[7] 26-week PPI administration was not associated with a decreased TFCA in postmenopausal women.

Although rare, hypomagnesemia has been reported in patients treated with PPIs for at least 3 months (in most cases after a year of therapy).[47,48] In this study, neither PPI reduced serum or urine magnesium, and no clinically significant effects on 24-hour urine magnesium levels were observed for subjects in the TFCA substudy.

There were no significant safety concerns identified in this study. The proportion of subjects with at least 1 AE was comparable in the 3 treatment groups, and the incidence of individual AEs in each of the treatment groups was low and consistent with previously reported AEs in randomized, placebo-controlled PPI studies.[49–51]

No fractures were reported within 30 days after the last dose of study drug, although 1 traumatic foot fracture (dexlansoprazole) and 1 humerus fracture (esomeprazole) occurred at day 359 and day 161, respectively, both after the patients took the last dose of study drug. Most of the AEs that resulted in discontinuation of the active study drug were consistent with the known AE profiles for these drugs. The serious AE reported during the study (nephrolithiasis in the dexlansoprazole group) was possibly consistent with the supplementation of calcium and vitamin D throughout the study. No other clinically important findings were noted in the postdose clinical laboratory safety measures, physical examination, vital signs, or electrocardiogram results.

Strengths of this study include the prospective, randomized, placebo-controlled study design, use of multiple bone turnover markers, use of standard methods to measure BMD, and use of a gold standard method to measure TFCA. In addition, we standardized vitamin D status and calcium intake in all subjects, so that PPI or placebo therapy was the only intervention during the trial. We also acknowledge limitations of this study. The first limitation is a relatively small sample size. We initially recruited 235

women into the trial, only to learn after completion of the trial that one site's data were unreliable. Had all 235 women's data been available for analysis, we likely would have observed smaller CIs around the study outcomes of bone turnover, BMD, and TFCA. Even with a smaller sample size, ours is the only study in which participants were randomized to PPI or placebo with the specific objective of measuring multiple parameters of skeletal health. The ultimate goal was to identify a potential mechanism whereby PPI therapy might increase the risk of fracture. Thus, we feel that our study adds important information to the literature on PPI and fracture. The second limitation of our study is the short duration of exposure to PPI therapy. We designed this study to evaluate the direct effects of PPIs on skeletal health. We chose bone turnover as our primary outcome because changes in bone turnover precede changes in BMD and serve as an early marker of adverse (or beneficial) interventions. We had no pilot data regarding the effects of PPIs on bone turnover by which to calculate a potential sample size for the study. However, within-subject changes in the bone resorption marker CTX can be as high as 36%.[33] Thus, we selected a sample size of 240 subjects, so that the width of the 95% CI for the difference in the percent change from baseline in each of the markers between dexlansoprazole or esomeprazole and placebo was restricted to no more than 30%. Despite having only 115 subjects with valid data, the width of the 95% CI for the between-arm changes in bone turnover markers were still $\leq$33%. Finally, we studied healthy postmenopausal women and cannot state whether study outcomes might differ in other populations.

Conclusions

Overall, this prospective, randomized, double-blind clinical trial detected minimal changes in bone homeostasis related to 26 weeks of PPI therapy. Increases in bone turnover were detected, but formation and resorption both increased and remained coupled. There were no PPI-associated declines in BMD, serum or urine mineral levels, PTH, or TFCA. Our study provides strong evidence against PPI-mediated alterations in calcium absorption or mineral homeostasis. If there is a causal relationship between PPI and fracture, that relationship is not mediated by the most common metabolic pathways predisposing to fracture.

The findings reported here suggest that PPI use alone may not be a reason for postmenopausal women to have additional bone density evaluations. The US Preventive Services Task Force recommends screening for osteoporosis in women aged 65 years and older, as well as in younger women with comparable or greater fracture risk.[52]

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2018.11.023.

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Author contributions: Drs Hansen, Nudurupati, Metz, and Perez contributed to the study design. Drs Hansen and Nieves contributed to the data collection. Drs Hansen, Nieves, Nudurupati, Metz, and Perez contributed to data interpretation and writing the manuscript.

Conflicts of interest
Dr Hansen was paid for her work as a consultant in the design and conduct of the study and received a grant to conduct the study. Dr Nieves received a research grant from Takeda to conduct this research. Dr Nudurupati was an employee of Takeda Pharmaceuticals at the time this study was conducted. Dr Metz received administrative and editorial support for study design and analysis of the data. Dr Perez is employed by Takeda Pharmaceuticals.

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Supplementary Methods

Study Randomization and Blinding

Randomization of patients in a 1:1:1 ratio to dexlansoprazole 60-mg capsules, esomeprazole 40-mg capsules, or placebo was scheduled and maintained using an interactive voice-activated response system. Patients were randomized using a blocked design stratified by vitamin D_3 supplementation (low or high) required by the subject at the screening visit (week −12) after the result of 25(OH)D was received, and the entire block of randomization numbers was assigned to each site. The investigational drug blind (double-blind) was maintained using the interactive voice-activated response system and was not broken except for reasons of medical necessity.

Additional Inclusion and Exclusion Criteria

Participants could not take over-the-counter PPIs within 9 months of study start or during the study, apart from study medication. Participants were ineligible if they had PTH or thyroid-stimulating hormone levels outside of the reference range for the testing laboratory at week −12; a 25(OH)D level <10 ng/mL at week −12 or <32 ng/mL at week −2; a disorder strongly associated with osteoporosis; a clinical history of fragility of wrist, hip, spine, or leg fractures; or a family history of genetic bone disorders. Participants who used any nicotine-containing products within 3 months of study start also were excluded. Potential candidates also were excluded if they had <2 evaluable vertebrae or a condition that interfered with dual-energy x-ray absorptiometry measurement (ie, hip replacement, vertebral deformity, or severe lumbar scoliosis).

Glucocorticoids, immunosuppressants, antiepileptics, selective serotonin reuptake inhibitors, lithium, bisphosphonates, loop and thiazide diuretics, PPIs, histamine-2 receptor antagonists, antacids, systemic fluoride, aromatase inhibitors, antidiabetic treatments, RANK ligand inhibitors, estrogen therapy, hormone replacement therapy, low-molecular-weight heparin, warfarin, clopidogrel, oral antifungals, and multivitamins could not be used for up to 1 year before the study.

Calcium Absorption Substudy Design

A 4-day food diary was completed by all substudy participants. On day −1 and week 26, participants arrived at the research ward at 7 AM while fasting. With breakfast, participants received a 600-mg calcium supplement, along with 2 stable calcium isotopes (8 mg of ^{44}Ca by mouth in calcium-fortified apple juice and 3 mg of ^{42}Ca intravenously), and consumed food for the next 24 hours that replicated the macronutrient and micronutrient content of their regular diet, including kilocalories, calcium, carbohydrates, protein, fat, fiber, vitamin D_3, sodium, magnesium, caffeine, and oxalate. Uneaten food was bagged and weighed to be assessed by a nutritionist for calcium intake. Research nurses collected all urine for 24 hours during an inpatient stay. Urine was frozen at −70° Celsius and analyzed in batches to determine the concentrations of ^{42}Ca and ^{44}Ca relative to ^{43}Ca. The inpatient diet provided during the 24-hour TFCA study period matched the participants' outpatient intake of kilocalories, calcium, carbohydrates, protein, fat, fiber, vitamin D_3, sodium, magnesium, caffeine (servings per day), and oxalate (servings per day). TFCA was calculated using the formula of Eastell and colleagues[1]:

$$\text{TFCA} = \frac{\%\ \text{excess 44Ca (oral)}}{\%\ \text{excess 42Ca (intravenous)}} \times \frac{\text{natural abundance 44Ca}}{\text{natural abundance 42Ca}} \times \frac{\text{dose 42Ca}}{\text{dose 44Ca}}$$

Quality Control

Quality control via 10 repeated scans of the Bona Fide Phantom (BioClinica, Inc, Newtown, PA) within each center resulted in a coefficient of variance <0.5% during the study. There were no changes in dual-energy x-ray absorptiometry machines and no equipment issues noted.

Monitoring visits were made to study sites periodically during the study to ensure that the protocol was followed. Protocol deviations were made only to eliminate immediate hazard to study participants and were documented in source documents. Quality assurance audits were done by the sponsor or regulatory agencies, such as the US Food and Drug Administration. The study was conducted according to ethical principles based on the Declaration of Helsinki and the international Guideline for Good Clinical Practice.

Supplementary Reference

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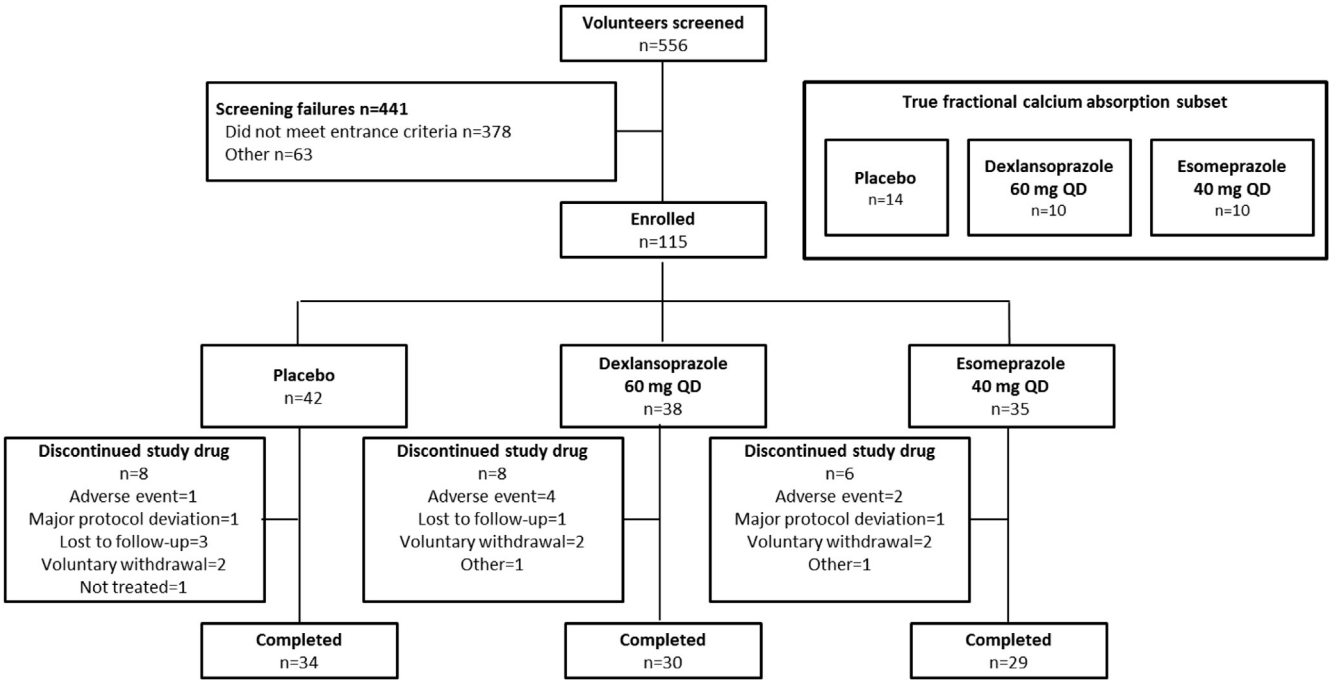

Supplementary Figure 1. Disposition of participants (CONSORT flowchart). QD, once daily. Volunteers screened: Numbers exclude the subjects from one site that was disqualified by the US Food and Drug Administration.

Supplementary Table 1. Excluded Medications and Substances

Excluded medications	Period of exclusion
Systemic glucocorticoids (eg, prednisone, cortisone, hydrocortisone, dexamethasone)	6 mo before screening (visit 1) and through last dose of study drug
Topical glucocorticoids	Topical glucocorticoids were allowed during the study as long as the use did not exceed more than the equivalent of 5 mg/d prednisone for more than 14 d
Inhaled glucocorticoids[a]	Inhaled glucocorticoids were allowed during the study as long as the use did not exceed 21 d in a 3-mo period or 42 d in 1 y through last dose of study drug
Immunosuppressors	2 mo before screening (visit 1) and through last dose of study drug
Antiepileptic agents, SSRIs, and lithium	3 mo before screening (visit 1) and through last dose of study drug
Oral bisphosphonates	Use within 1 y before screening (visit 1) and through last dose of study drug
Loop and thiazide diuretics	Use within 1 y before screening (visit 1) and through last dose of study drug
PPIs, over-the-counter PPIs (more than 3 doses per month)	6 mo before screening (visit 1) and through last dose of study drug
H2RAs, antacids	From screening (visit 1) through last dose of study drug
Intravenous bisphosphonates	Ever taken
Systemic fluoride treatment, PTH analog	Ever taken
Aromatase inhibitors, antidiabetic treatments	1 y before screening (visit 1) and through last dose of study drug
RANK ligand inhibitors	6 mo before screening (visit 1) and through last dose of study drug
Systemic estrogen therapy or hormone replacement therapy[b]	1 y before screening (visit 1) and through last dose of study drug
Selective estrogen-receptor modulator (tamoxifen and raloxifene)	3 mo before screening (visit 1) and through last dose of study drug
Low-molecular-weight heparin (dalteparin, enoxaparin, tinzaparin)	3 mo before screening (visit 1) and through last dose of study drug
Warfarin	3 mo before screening (visit 1) and through last dose of study drug
Clopidogrel	1 mo before screening (visit 1) and through last dose of study drug
Oral antifungals	2 mo before screening (visit 1) and through last dose of study drug
Multivitamins and other vitamins/supplements containing calcium or vitamin D_3 other than what was required per protocol	Screening (visit 1) and through last dose of study drug

H2RA, histamine 2-receptor antagonist; RANK, receptor activator of nuclear factor kappa-B ligand; SSRI, selective serotonin-reuptake inhibitor.
[a]Inhaled glucocorticoids were allowed during the study as long as the use did not exceed 21 consecutive days.
[b]Local vaginal estrogen was permitted.

Supplementary Table 2. Schedule of Study Procedures

	Screening		Randomization		Treatment		Follow-up BMD
	Visit 1	Visit 2	Visit 3		Visit 4	Visit 5	
Study procedure or assessment[a]	Wk –12	Wk –2	Day –1	Day 1	Wk 13	Wk 26 or early termination	Wk 52
Medical history, demographics, and concurrent medical conditions	X						
Physical examination, vital signs, ECG	X		X			X	
Height and BMI calculation[b]	X					X	X
Clinical laboratory tests (chemistry, hematology, and urinalysis)	X	X	X			X	
BMD by DXA	X					X	X
24-hour urine calcium excretion, magnesium excretion and 24-hour urine creatinine[c]			X			X	
Urine magnesium		X			X	X	
Estimated CrCl calculation	X	X				X	
Gastrin			X			X	
FSH	X						
P1NP, CTX, urine NTx,[c] BsAP		X			X	X	
Serum phosphorus, calcium, albumin, and magnesium		X			X	X	
25(OH)D	X	X	X		X	X	
PTH	X	X			X	X	
AE monitoring					X	X	
Dispense double-blind treatments (dexlansoprazole 60 mg, esomeprazole 40 mg, or placebo)			X		X		
Calcium absorption substudy			X			X	

BMI, body mass index; CrCl, creatinine clearance; DXA, dual-energy x-ray absorptiometry; ECG, electrocardiogram; FSH, follicle-stimulating hormone.
[a]All laboratory tests required an 8-hour fast except at week 12.
[b]BMI calculation performed at visit 1 (week –12) only.
[c]Urine NTx samples were collected in clinic with the second fasting void of the day during visit 2, visit 4, and visit 5. For visit 5, the second fasting void of the day was defined as the first void of the day after completion of the 24-hour urine collection. For visit 2 and visit 4, the second fasting void of the day was defined as the first void after 6 AM.

Supplementary Table 3. Baseline and Week 26 Median Urinary Calcium, Serum Calcium, Phosphorus, Magnesium, Urine Magnesium, PTH, Serum Vitamin D, and Gastrin Levels

	Placebo, n = 38			Dexlansoprazole 60 mg QD, n = 36			Esomeprazole 40 mg QD, n = 34			Difference vs placebo (95% CI)[a]	
	n	Median (range)	Difference from baseline (95% CI)[a]	n	Median (range)	Difference from baseline (95% CI)[a]	n	Median (range)	Difference from baseline (95% CI)[a]	Dexlansoprazole vs placebo	Esomeprazole vs placebo
Serum gastrin, *pg/mL*											
Baseline	28	27.5 (13.0–325.0)	–	30	28.5 (18.0–60.0)	–	25	29.0 (11.0–73.0)	–	–	–
Week 26	28	30.5 (15.0–703.0)	1.0 (–5.0 to 7.0)	30	105.5 (32.0–684.0)	**78.0** (56.0 to 99.0)	25	113.0 (30.0–892.0)	**84.0** (64.0 to 108.0)	**79.5** (55.0 to 106.0)	**85.0** (66.0 to 110.0)
PTH, *pg/mL*											
Baseline	36	28 (16- 53)	–	30	27 (15–77)	–	31	31 (15–48)	–	–	–
Week 26	32	30 (15–70)	1 (–4 to 7)	29	31 (16–82)	1 (–4 to 8)	26	33 (20–57)	3 (–2 to 9)	1 (–3 to 4)	2 (–2 to 7)
Urinary calcium, *mg/dL*											
Baseline	29	145 (42–325)	–	29	174 (59–346)	–	24	121 (57–355)	–	–	–
Week 26	29	119 (14–326)	–26 (–66 to 16)	29	112 (24–286)	–50 (–92 to –11)	24	103 (23–242)	–34 (–84 to 1)	–17 (–59 to 21)	–8 (–64 to 27)
Serum calcium, *mg/dL*											
Baseline	36	9.5 (8.6–10.1)	–	32	9.5 (9.0–10.1)	–	30	9.6 (9.1–10.3)	–	–	–
Week 26	32	9.5 (8.1–10.5)	0.0 (–0.2 to 0.2)	31	9.5 (8.8–10.0)	0.0 (–0.2 to 0.2)	26	9.6 (9.1–10.1)	–0.1 (–0.2 to 0.1)	0.0 (–0.2 to 0.2)	0.0 (–0.3 to 0.2)
Serum phosphorus, *mg/dL*											
Baseline	36	3.8 (2.8–4.2)	–	32	3.8 (3.2–4.9)	–	30	3.9 (3.1–4.5)	–	–	–
Week 26	32	3.8 (3.2–5.2)	0.0 (–0.2,0.2)	31	3.9 (3.0–5.1)	0.1 (–0.1 to 0.3)	26	4.0 (3.5–4.7)	0.0 (–0.1 to 0.2)	0.0 (–0.2 to 0.2)	–0.1 (–0.3 to 0.2)
Urine magnesium, *mEq/L*											
Baseline	35	3.7 (0–8)	–	32	3.6 (1–18)	–	31	3.6 (1–13)	–	–	–
Week 26	31	3.3 (1–8)	–0.2 (–1.4 to 1.0)	31	3.7 (1–8)	–0.3 (–1.5 to 1.0)	26	3.1 (1–12)	–0.1 (–1.5 to 0.9)	–0.3 (–1.6 to 0.9)	–0.3 (–1.6 to 1.0)
Serum magnesium, *mEq/L*											
Baseline	36	1.7 (1.5–2.0)	–	32	1.7 (1.4- 2.0)	–	30	1.8 (1.6–2.0)	–	–	–
Week 26	32	1.7 (1.4–2.0)	0.0 (–0.1 to 0.1)	31	1.7 (1.5–1.9)	0.0 (–0.1 to 0.0)	26	1.8 (1.5–2.1)	0.0 (–0.1 to 0.0)	0.0 (–0.1 to 0.0)	0.0 (–0.1 to 0.0)
25(OH)D, *ng/mL*											
Baseline	36	39 (29–69)	–	32	38 (26–87)	–	31	35 (19–55)	–	–	–
Week 26	32	36 (25–56)	–2 (–6 to 2)	30	40 (27–50)	1 (–3 to 5)	26	38 (22–49)	2 (–2 to 6)	3 (–2 to 8)	4 (–1 to 9)

NOTE. Information is presented as the change from baseline, so dashes were used for the baseline point.
QD, once daily.
[a]Difference from baseline or placebo estimated using Hodges-Lehmann estimate; 95% CIs of the differences in actual values calculated by the Moses method. Comparisons were considered statistically significant if the 95% CI excluded zero (reported in bold text).

Supplementary Table 4. True Fractional Calcium Absorption Change From Baseline to Week 26

	Placebo, n = 12	Dexlansoprazole 60 mg QD, n = 9	Esomeprazole 40 mg QD, n = 10
Baseline, median (range)	0.16 (0.10 to 0.29)	0.18 (0.11 to 0.24)	0.19 (0.10 to 0.23)
Week 26, median (range)	0.17 (0.08 to 0.27)	0.19 (0.11 to 0.29)	0.20 (0.14 to 0.30)
Change, median (range)	–0.02 (–0.08 to 0.07)	0.02 (–0.04 to 0.10)	0.07 (–0.07 to 0.08)
Difference between treatment vs placebo (95% CI)[a]	–	0.03 (–0.02 to 0.08)	**0.06** (0.02 to 0.11)

QD, once daily.
[a]Difference estimated with Hodges-Lehmann estimate. Change values were considered statistically significant if the 95% CI excluded zero (reported in bold text).