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Clinical Research Article

Sex-specific Association of Chronic Proton Pump Inhibitor Use With Reduced Bone Density and Quality

Fabio Bioletto,[1] Alessia Pusterla,[1] Federica Fraire,[1] Lorenzo Sauro,[1] Michela Presti,[2] Emanuela Arvat,[2] Ezio Ghigo,[1] Massimo Procopio,[1] and Marco Barale[2]

[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, Turin, 10126, Italy
[2]Division of Oncological Endocrinology, Department of Medical Sciences, University of Turin, Turin, 10126, Italy

Correspondence: Fabio Bioletto, MD, Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, Corso Dogliotti 14, Turin, 10126, Italy. Email: fabio.bioletto@unito.it.

Abstract

Context: Chronic use of proton pump inhibitors (PPIs) has been associated with an increase in bone fragility. However, evidence on the effect of chronic PPI use on bone density is conflicting, and data on bone microarchitectural quality are scarce.

Objective: The primary aim of this study was to evaluate whether trabecular bone microarchitecture, assessed by trabecular bone score (TBS), is altered in chronic PPI users. The association between PPI use and bone density was also evaluated as a secondary endpoint.

Methods: We extracted individual patient data from the 2005 to 2008 cycles of the population-based National Health and Nutrition Examination Survey (NHANES), in which lumbar spine dual-energy X-ray absorptiometry scans were acquired. TBS values were calculated from dual-energy X-ray absorptiometry images using a dedicated software. Multivariable linear regression analyses stratified by sex were performed to evaluate the association of chronic PPI use with TBS and bone mineral density (BMD), adjusting for relevant confounders.

Results: A total of 7478 subjects were included (3961 men, 3517 women). After adjustment for relevant confounders, chronic PPI use was associated with a worse bone health profile in men, with lower TBS (−0.039; 95% CI, −0.058 to −0.020; $P < .001$), lumbar spine T-score (−0.27; 95% CI, −0.51 to −0.04; $P = .023$), total hip T-score (−0.21; 95% CI, −0.41 to −0.01; $P = .041$), and femoral neck T-score (−0.22; 95% CI, −0.44 to −0.00; $P = .047$). Notably, the association between chronic PPI use and degraded TBS remained statistically significant even after further adjustment for BMD at lumbar spine and femoral neck (−0.026; 95% CI, −0.039 to −0.012; $P = .001$). In contrast, no significant association was observed between chronic PPI use and either TBS or BMD in women.

Conclusion: Chronic PPI use is associated with degraded trabecular bone quality in men, even after adjustment for BMD. No association was observed in women.

Key Words: proton pump inhibitors, PPI, osteoporosis, bone density, bone quality, trabecular bone score

Abbreviations: BMD, bone mineral density; BMI, body mass index; CKD, chronic kidney disease; DXA, dual-energy X-ray absorptiometry; NHANES, National Health and Nutrition Examination Survey; PPI, proton pump inhibitor; TBS, trabecular bone score.

Proton pump inhibitors (PPIs) represent a class of drugs widely used in clinical practice for their antacid action (1). Their use has increased rapidly worldwide because they generally are well tolerated and have few side effects (2, 3). However, recent investigations have raised concerns regarding the potential long-term adverse effects associated with chronic PPI use, such as an increased risk of enteric infections, community-acquired pneumonia, and possibly some forms of gastric cancer (4).

Another possible side effect of chronic PPI intake is an increase in bone fragility (5). In 2010, the U.S. Food and Drug Administration updated the list of potential side effects for PPIs to include an increased risk of hip, wrist, and vertebral fractures (6) following reports from multiple epidemiological studies conducted in preceding years (7-12). Subsequently, this association between PPI use and fracture risk has been further corroborated by numerous additional studies and meta-analyses (13-15).

Although the evidence regarding increased fracture risk is relatively concordant (13-15), studies regarding the effect of PPI use on bone mineral density (BMD) have reported conflicting results, with most of them showing either modest or no effect on BMD during PPI use (13, 14, 16). A frequent limitation of these studies is that, despite the well-known differences in the pathophysiology of bone metabolism between males and females (17, 18), the possible presence of sex-specific differences in the association between PPI use and BMD has only rarely been explored (12, 19). Moreover, although bone density is the gold standard for the diagnosis of osteoporosis and the most reliable noninvasive predictor of fracture risk (20), skeletal fragility can also be determined by alterations in bone quality, with subtler changes in bone microarchitecture that are not captured by BMD (20).

In recent years, various noninvasive methods for assessing bone quality have been developed, with the trabecular bone score (TBS) emerging as one of the simplest and most

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accessible (21, 22). TBS is a texture-based metric that measures the rate of gray-level changes in lumbar spine dual-energy x-ray absorptiometry (DXA) images, providing an indirect estimation of bone microarchitectural health (21, 22). Numerous studies have shown that TBS can predict osteoporotic fractures in postmenopausal women independently of bone density (23, 24); in addition, the evaluation of TBS is of particular relevance in various settings of secondary osteoporosis, in which the risk of fractures is often less associated with BMD, but rather related to changes in bone microstructure and quality (25-29).

To date, data about the possible effects of PPIs on TBS values are scarce and are essentially limited to 2 studies conducted on relatively small patient cohorts (30, 31).The primary aim of this study was thus to evaluate the possible association between the chronic PPI use and trabecular bone quality, as measured by TBS, in a larger unselected cohort of subjects extracted from the general U.S. population. The association between PPI treatment and bone density was also evaluated as a secondary endpoint. In both analyses, data were stratified by sex to account for the possible presence of gender-specific differences in the relationship between PPI use and bone outcomes.

Methods

Survey Design and Data Collection

This study analyzes data from the 2005 to 2008 cycles of the National Health and Nutrition Examination Survey (NHANES), a cross-sectional survey program conducted in the United States by the National Center for Health Statistics. The NHANES program is designed to include representative samples of the general, noninstitutionalized U.S. population of all age groups; to accomplish this objective, it employs a stratified, multistage, clustered probability sampling design, with oversampling of minorities such as non-Hispanic Black, Hispanic, and Asian persons, as well as people with low income and older adults. The survey involves a structured interview conducted in the homes of the participants, followed by a standardized health evaluation performed at a mobile examination center, which includes various laboratory tests and other examinations. A comprehensive description of the data collection methodology can be found elsewhere (32). The original survey received approval from the Centers for Disease Control and Prevention Research Ethics Review Board; written informed consent was obtained from all adult participants.

Clinical Data and Laboratory Tests

Body measurements, including weight (kg), height (cm), and body mass index (BMI, kg/m^2), were obtained during the mobile examination center visit. Information regarding menopausal status (in women), annual household income, current cigarette smoking, history of any liver disease, recent hospitalization (within 1 year), and history of chronic glucocorticoid treatment for ≥3 months was based on self-report. Habitual levels of physical activity were assessed on a weekly/monthly basis using specific questionnaires; moderate activities were defined as those causing light sweating and/or a slight-to-moderate increase in breathing or heart rate for at least 10 minutes; vigorous activities were defined as those causing heavy sweating and/or a large increase in breathing or heart rate for at least 10 minutes. Dietary calcium intake was estimated based on a structured dietary interview. Ongoing pharmacological therapies were reported by each subject during household interview, with direct verification of medication containers by the interviewer whenever possible; data on treatment duration and adherence were based on self-report; information on drug dosages and on previously discontinued prescriptions were not available. Laboratory methods for the measurement of all performed blood and urine tests are reported in detail on the NHANES website (32).

Diabetes mellitus was defined if any of the following conditions were met: (1) a fasting plasma glucose ≥126 mg/dL; (2) a glycated hemoglobin level ≥6.5% (48 mmol/mol); (3) a self-reported diagnosis of diabetes; and (4) a self-reported use of antidiabetic drugs; subjects not meeting any of these criteria were considered nondiabetic, unless data were missing in all 4. Estimated glomerular filtration rate was computed according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; in accordance with Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (33), severe chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate <30 mL/min/1.73 m^2 (stage G4-G5).

BMD and TBS Analysis

BMD was measured at the lumbar and femoral sites by DXA. All DXA examinations were performed by trained and certified radiology technologists. Scans were acquired on Hologic QDR-4500A fan-beam densitometers (Hologic, Inc., Bedford, Massachusetts) using software version Apex 3.0. Quality control phantoms were scanned daily to ensure accurate calibration of the densitometer. Further details of the DXA examination are documented on the NHANES website (32).

For each patient and at each skeletal site, we calculated T scores as $(BMD_{patient} - \mu_{reference})/\sigma_{reference}$, where $\mu_{reference}$ and $\sigma_{reference}$ are the mean and SD of BMD in the reference patient group. As recommended by the World Health Organization (34), the reference group for the calculation of T scores at the total hip and femoral neck consisted of non-Hispanic White females aged 20 to 29 years from the NHANES III report (35). For the calculation of T score at the lumbar spine, the reference curves of the DXA manufacturer for 30-year-old White females were considered, taking into account the specific subset of vertebrae for which a valid BMD measurement was available.

TBS was extracted in adults aged 20 years or older from lumbar spine DXA images using a dedicated software (Med-Imap SA TBS Calculator version 2.1.0.2). In the calculation of TBS, BMI was used as an indirect measure of body thickness to improve the accuracy and comparability of TBS measurements across participants. Further details on TBS calculation are available on the NHANES website (32).

Sample Selection

A total of 8670 subjects aged 20 years or older participated to the 2005 to 2008 NHANES survey cycles and had DXA spine scan performed. Among these, 988 individuals were excluded because of no valid/reliable TBS data (237 because of insufficient number of valid vertebrae for TBS estimation, 45 from missing BMI data, and 706 from BMI outside the range of 15 to 37 kg/m^2, which represents the range within which TBS estimation is considered valid (22)). Of the remaining

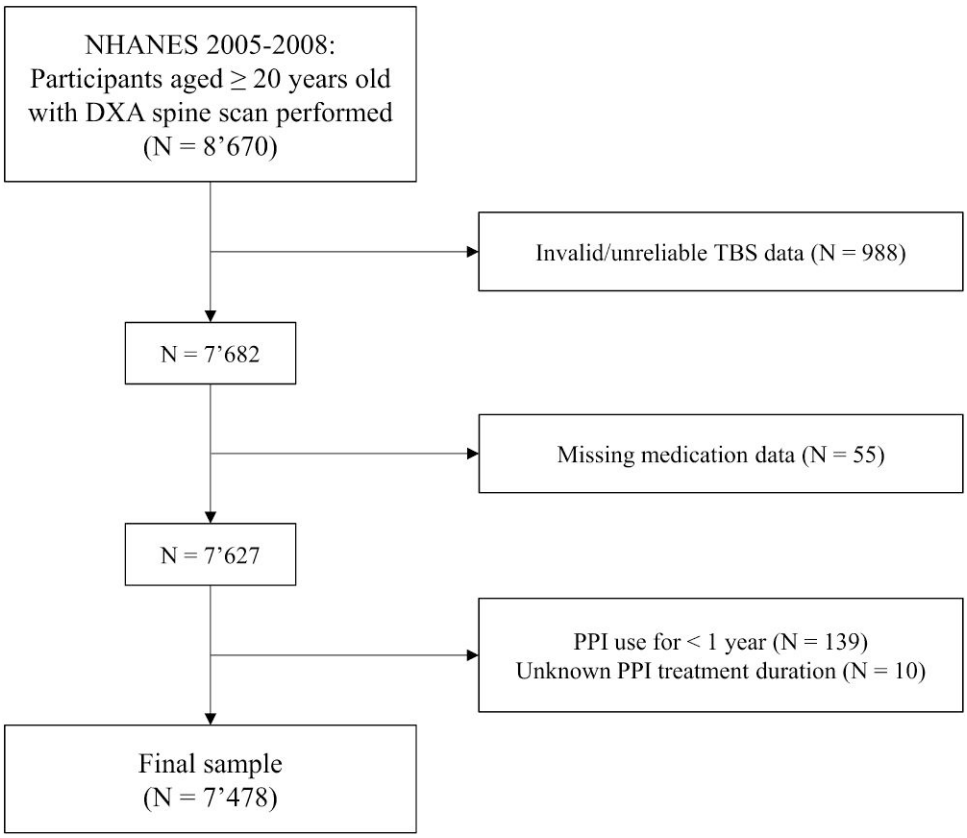

Figure 1. Flow-chart of patient inclusion. Abbreviations: DXA, dual-energy X-ray absorptiometry; NHANES, National Health and Nutrition Examination Survey; PPI, proton pump inhibitor; TBS, trabecular bone score.

7682 patients, 55 were excluded because of missing medication data. Finally, 139 patients were excluded because of current reported PPI use for <1 year, and 10 patients were excluded because of unknown ongoing PPI treatment duration, resulting in a final sample of 7478 patients. The full process of sample selection is summarized graphically in Fig. 1.

Statistical Analysis

All analyses were conducted accounting for the complex survey design of NHANES, using appropriate weighting as suggested by the National Center for Health Statistics. Data were summarized as weighted means and SD for continuous variables, and as weighted proportions for categorical data. Multivariable linear regression analyses were performed to evaluate the effect of chronic PPI use on TBS and BMD, accounting for relevant confounders; the choice of covariates for adjustment was based on clinical relevance, considering factors that could most likely influence the relationship between the exposure and the outcome. Missing values were imputed by multiple imputations with chained equation, stratified by gender. To avoid bias, the imputation model included all covariates and outcomes used to fit the analysis models, as recommended (36, 37). Fifty imputed datasets were created, and estimates were combined using Rubin's rules (36). In each analysis, patients with missing outcome were excluded. A cutoff of 0.05 was adopted for the definition of statistical significance. Statistical analysis was performed using STATA 18 (StataCorp, College Station, Texas, USA).

Results

Of the 7478 participants included in the analysis, 3961 were men and 3517 were women. Chronic continuous treatment with PPIs for ≥1 year was reported by 252 men and 232 women, with a weighted prevalence of PPI use of 6.1% (95% CI, 5.1-7.3) and 6.2% (95% CI, 5.0-7.6), respectively. The most frequently prescribed PPI was omeprazole (n = 161), followed by esomeprazole (n = 141), lansoprazole (n = 83), pantoprazole (n = 59), and rabeprazole (n = 40). Table 1 shows the clinical and biochemical characteristics of the patients, stratified by sex and PPI use. Compared to nonusers, PPI users were older, were more frequently of non-Hispanic White ethnicity, had lower levels of habitual physical activity, and were less likely to be current smokers; moreover, they were characterized by a generally more relevant burden of comorbidities, with higher BMI, higher prevalence of diabetes mellitus and CKD, more frequent self-reported history of liver disease (in females), and higher hospitalization rate in the previous year; of note, they were also more likely to be treated with other relevant drug classes that could possibly influence the relationship between PPI exposure and bone outcome, such as glucocorticoids (in males), loop diuretics, thiazide diuretics, nonsteroidal anti-inflammatory drugs, and H2 antagonists (in females).

As described in the Methods section, multivariable linear regression analyses were performed to evaluate the association between chronic PPI use and bone outcomes (Tables 2 and 3). After full adjustment for demographic, clinical, laboratory, and medication data, PPI use was associated with

Table 1. Descriptive characteristics of the study population, stratified by sex and PPI use

Parameter	Men (N = 3961)			Women (N = 3517)		
	PPI nonusers (N = 3709)	PPI users (N = 252)	*P* value	PPI nonusers (N = 3285)	PPI users (N = 232)	*P* value
Age (y)	44.3 ± 16.4	58.3 ± 13.7	<.001	46.5 ± 16.5	59.0 ± 15.0	<.001
Menopausal status (%)[a]			N/A			<.001
Premenopausal	N/A	N/A		57.3	27.7	
Postmenopausal	N/A	N/A		42.7	72.3	
Race/ethnicity (%)			<.001			<.001
Non-Hispanic White	69.9	88.3		71.7	84.8	
Non-Hispanic Black	10.3	5.3		10.6	7.8	
Hispanic	14.1	3.9		11.7	4.5	
Other	5.7	2.5		6.0	2.9	
Annual household income (%)[b]			.976			.109
≥$75 000	35.0	35.0		31.9	29.3	
$45 000–$74 999	24.6	23.3		24.0	18.4	
$20 000–$44 999	28.3	29.1		29.3	29.9	
<$20 000	12.1	12.6		14.8	22.4	
Habitual physical activity (%)			.004			.010
None	25.0	31.7		30.0	40.0	
Moderate	25.8	34.3		35.5	38.5	
Vigorous	49.2	24.0		34.5	21.5	
Current smoking (%)[c]	28.2	18.1	.006	21.8	11.0	<.001
BMI (kg/m^2)	27.3 ± 4.1	29.0 ± 4.2	<.001	26.5 ± 4.9	27.7 ± 4.5	.001
Diabetes mellitus (%)	8.9	21.0	<.001	7.2	18.7	<.001
CKD G4-G5 (%)[d]	0.3	1.6	.015	0.4	3.3	<.001
History of liver disease (%)[e]	3.5	4.9	.136	2.4	8.7	<.001
Recent hospitalization (≤1 year) (%)[f]	6.3	17.7	<.001	10.4	20.0	<.001
Dietary calcium intake (g/day)[g]	1.07 ± 0.70	0.97 ± 0.60	.100	0.84 ± 0.49	0.80 ± 0.45	.256
25OH-VitD (ng/mL)[h]	25.5 ± 8.4	25.1 ± 8.4	.594	26.9 ± 10.3	27.6 ± 10.0	.299
History of chronic GC treatment (%)[i]	1.8	6.1	<.001	3.4	4.7	.367
Hormone-blocking therapy (%)	0.1	0.0	.661	0.8	1.0	.672
Loop diuretics (%)	1.4	6.4	<.001	1.4	8.3	<.001
Thiazide diuretics (%)	5.2	12.4	<.001	8.3	20.8	<.001
NSAIDs (%)	3.6	9.3	<.001	4.4	12.2	<.001
H2 antagonists (%)	1.8	0.0	.138	1.5	5.1	.002

Abbreviations: 25OH-VitD, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index; CKD G4-G5, chronic kidney disease stage G4-G5; GC, glucocorticoid; N/A, not applicable; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor; TBS, trabecular bone score.
[a]Missing data in 223 (6.3%) women.
[b]Missing data in 240 (3.2%) subjects.
[c]Missing data in 5 (<0.1%) subjects.
[d]Missing data in 321 (4.3%) subjects.
[e]Missing data in 14 (0.2%) subjects.
[f]Missing data in 3 (<0.1%) subjects.
[g]Missing data in 174 (2.3%) subjects.
[h]Missing data in 813 (10.9%) subjects.
[i]Missing data in 58 (0.8%) subjects.

worse bone health profile in men, with lower TBS values (−0.039; 95% CI, −0.058 to −0.020; $P < .001$), lumbar spine T score (−0.27; 95% CI, −0.51 to −0.04; $P = .023$), total hip T score (−0.21; 95% CI, −0.41 to −0.01; $P = .041$), and femoral neck T score (−0.22; 95% CI, −0.44 to −0.00; $P = .047$) (Table 2, model 2). Notably, the association between PPI use and TBS remained statistically significant even after further adjusting the multivariable regression analysis for bone density at lumbar spine and femoral neck (−0.026; 95% CI, −0.039 to −0.012; $P = .001$) (Table 2, model 3).

In contrast, no significant association was observed between chronic PPI use and bone outcomes in women. In fact, in the fully adjusted analysis encompassing demographic, clinical, laboratory, and medication data, female PPI users showed similar TBS (−0.005; 95% CI, −0.017 to 0.007; $P = .427$), lumbar spine T score (−0.09, 95% CI, −0.30 to 0.11; $P = .345$), total hip T score (+0.04; 95% CI, −0.09 to 0.18; $P = .511$), and femoral neck T score (−0.01; 95% CI, −0.15 to 0.12; $P = .845$) compared to nonusers (Table 3, model 2). The lack of a significant association between PPI

Table 2. Multivariable linear regression models evaluating the effect of chronic PPI use on TBS and on BMD T-scores at lumbar spine, total hip, and femoral neck in men

Model	Lumbar spine TBS (N = 3961)			Lumbar spine T-score (N = 3961)			Total hip T-score (N = 3839)[a]			Femoral neck T-score (N = 3839)[a]		
	β-coeff	95% CI	*P* value	β-coeff	95% CI	*P* value	β-coeff	95% CI	*P* value	β-coeff	95% CI	*P* value
Model 1[b]	−0.039	(−0.059, −0.020)	<.001	−0.25	(−0.47, −0.04)	.024	−0.21	(−0.41, −0.01)	.039	−0.22	(−0.43, −0.01)	.044
Model 2[c]	−0.039	(−0.058, −0.020)	<.001	−0.27	(−0.51, −0.04)	.023	−0.21	(−0.41, −0.01)	.041	−0.22	(−0.44, −0.00)	.047
Model 3[d]	−0.026	(−0.039, −0.012)	.001	—	—	—	—	—	—	—	—	—

Abbreviations: 25OH-VitD, 25-hydroxyvitamin D; BMI, body mass index; CKD G4-G5, chronic kidney disease stage G4-G5; GC, glucocorticoid; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor; TBS, trabecular bone score; β-coeff, β-coefficient.
[a]Missing outcome data in 122 (3.1%) subjects.
[b]Adjusted for age, race/ethnicity, income, habitual physical activity, current smoking, BMI, diabetes mellitus, CKD G4-G5, history of liver disease, recent hospitalization (≤1 year), dietary calcium intake, and 25OH-VitD levels.
[c]Adjusted for the same covariates as model 1, plus current use of hormone-blocking therapy, loop diuretics, thiazide diuretics, NSAIDs, H2 antagonists, and history of chronic GC therapy.
[d]Adjusted for the same covariates as model 2, plus lumbar spine T score and femoral neck T score.

Table 3. Multivariable linear regression models evaluating the effect of chronic PPI use on TBS and on BMD T scores at lumbar spine, total hip, and femoral neck in women

Model	Lumbar spine TBS (N = 3517)			Lumbar spine T score (N = 3517)			Total hip T score (N = 3344)[a]			Femoral neck T score (N = 3344)[a]		
	β-coeff	95% CI	*P* value	β-coeff	95% CI	*P* value	β-coeff	95% CI	*P* value	β-coeff	95% CI	*P* value
Model 1[b]	−0.004	(−0.016, +0.007)	.441	−0.08	(−0.29, +0.14)	.475	+0.03	(−0.10, +0.16)	.611	−0.01	(−0.14, +0.12)	.872
Model 2[c]	−0.005	(−0.017, +0.007)	.427	−0.09	(−0.30, +0.11)	.345	+0.04	(−0.09, +0.18)	.511	−0.01	(−0.15, +0.12)	.845
Model 3[d]	−0.001	(−0.015, +0.013)	.875	—	—	—	—	—	—	—	—	—

Abbreviations: 25OH-VitD, 25-hydroxyvitamin D; BMI, body mass index; CKD G4-G5, chronic kidney disease stage G4-G5; GC, glucocorticoid; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor; TBS, trabecular bone score; β-coeff, β-coefficient.
[a]Missing outcome data in 173 (4.9%) subjects.
[b]Adjusted for age, menopausal status, race/ethnicity, income, habitual physical activity, current smoking, BMI, diabetes mellitus, CKD G4-G5, history of liver disease, recent hospitalization (≤1 year), dietary calcium intake, and 25OH-VitD levels.
[c]Adjusted for the same covariates as model 1, plus current use of hormone-blocking therapy, loop diuretics, thiazide diuretics, NSAIDs, H2 antagonists, and history of chronic GC therapy.
[d]Adjusted for the same covariates as model 2, plus lumbar spine T score and femoral neck T score.

use and bone quality was confirmed also when further adjusting the regression model using bone density data (−0.001; 95% CI, −0.015 to 0.013; $P = .875$) (Table 3, model 3). No significant effect modification was observed according to menopausal status (P for interaction >.5 in all analyses).

The possible presence of differences between different PPIs was explored, without finding any significant heterogeneity (Bonferroni-corrected P-value >.2 for all pairwise comparisons in all analyses). A sensitivity analysis was performed after excluding patients actively treated with antiosteoporotic drugs (n = 223; 12 males and 211 females; specifically, 185 patients on bisphosphonates, 37 on raloxifene, 1 on teriparatide, none on other treatments), with no relevant changes in the results (Supplementary Tables S1 and S2) (38).

As ancillary findings, the role of other known parameters as predictors of bone outcomes was confirmed. The full results regarding the relationships between the covariates used in the regression models and bone outcomes are reported in Supplementary Tables S3 and S4 (38).

Discussion

In the present study, we evaluated the possible association between chronic PPI use and TBS in a community-based sample extracted from the general U.S. population. Our findings revealed a significant relationship between chronic PPI use and lower TBS values in male patients, whereas no significant association was found in females. The same pattern was observed for BMD T scores at all sites, with a statistically significant reduction observed in males, but not in females. Notably, in males, the association between chronic PPI use and lower TBS values persisted even after adjustment for BMD, suggesting a specific impairment of bone quality in addition to the extent of bone mass loss.

Over the past 2 decades, a consistent body of evidence has been published supporting the association of chronic PPI use with an increased risk of fragility fractures, including hip, wrist, spine, and any-site fractures (13-15). The mechanisms underlying the observed increase in fracture risk, however, are not fully elucidated, and several potential pathways have been suggested.

From a pathophysiological point of view, the acidic environment in the stomach helps to convert insoluble calcium salts to ionized soluble calcium, facilitating calcium absorption in the intestine (5, 39, 40); chronic PPI use may thus induce a reduction in calcium absorption and bioavailability, promoting PTH oversecretion, with consequent increase of mineral bone resorption and bone microstructure deterioration (5, 39, 40). Furthermore, the altered gastric pH leads to a decreased intestinal absorption of magnesium; magnesium deficiency could exert as well negative effects on bone health through a reduction in osteoblastic activity and the formation of large hydroxyapatite crystals, which decrease bone stiffness (41).

Other authors also suggested a possible path mediated by a reduced intestinal absorption of vitamin B6, vitamin B9 (folate), and vitamin B12. The lack of adequate absorption and bioavailability of these vitamins, in fact, may lead to a sustained increase in homocysteine levels, which is known to impair bone quality through the inhibition of the enzyme lysyl-oxidase and an altered crosslink formation in collagen fibers (5, 42-44).

Another possible mechanism is the one mediated by hypergastrinemia. In fact, gastrin oversecretion from G cells results in enterochromaffin-like cells hyperplasia, which consequently leads to an increased release of histamine (45). It has been hypothesized that histamine hypersecretion could be detrimental on bone health because it increases the differentiation of osteoclast precursors (46). Moreover, hypergastrinemia has been described as a possible cause of parathyroid hypertrophy or hyperplasia, with consequent increase in PTH secretion (47, 48).

PPIs might also have a direct effect on bone cells, as some preclinical studies suggest. In cell models, in fact, it has been shown that PPIs can upregulate osteoclastic genes and increase osteoclastic activity (49). In addition, an effect of increased osteoblast apoptosis and decreased osteoblast activity was observed both in vitro (50) and in a murine model (51).

Finally, various studies suggested that the observed increase in fracture risk in PPI users could be due, at least in part, to an increase in the risk of falls (52-54). Although the reasons of this effect are likely multifactorial, a role may be played by the already-mentioned PPI-induced deficiencies of vitamins of the B group, which may increase the propensity to develop muscle weakness and various neurological disturbances (55, 56).

Overall, these potential direct and indirect effects may theoretically have an impact on bone mass and mineralization, which in turn could account for the higher fracture incidence observed in PPI users. However, although the evidence regarding increased fracture risk is relatively concordant (13-15), previous studies reported discrepant results regarding the potential association between PPI use and bone density, with most of them showing either modest or no effect on BMD (13, 14, 16). This suggests that the increased fracture risk associated with PPI use may not be entirely from loss of bone mass; consequently, other factors related to bone health may also contribute to the bone fragility observed in PPI users.

In recent years, the field of osteoporosis research has undergone significant evolution, with growing emphasis on indices of bone quality as a complementary measure in the assessment of fracture risk (21, 22). The assessment of bone quality, in fact, has emerged as a powerful tool to better evaluate skeletal health in various settings of primary and secondary osteoporosis (23-29). However, to date, data on how chronic PPI use affects bone microarchitecture in humans are scarce. In particular, to the best of our knowledge, only 2 studies have yet investigated the association between PPI use and TBS (30, 31).

In the first (30), conducted by Shin et al in a cohort of Korean women followed for clinical purposes in a tertiary referral center, the authors reported lower TBS and BMD in those treated with PPIs when compared to the control group. This study pioneered the topic related to the evaluation of TBS among PPI users but, as the authors themselves acknowledged, had some inherent limitations. In fact, apart from being limited to female patients of Korean ethnicity, it was based on a relatively limited sample size, comprising 446 patients equally divided in the PPI exposure group and in the control group. Moreover, the study cohort was retrospectively extracted from patients who underwent DXA scans for clinical purposes in a tertiary referral center, thus leading to possible selection bias and to an uncertain degree of generalizability of the results to a community-based general population. Finally, but not less importantly, the reported results are based on minimally-adjusted analysis, which accounted only for age and BMI as possible confounders. More recently, another evaluation comparing TBS between PPI users and nonusers

was published by Kondapalli et al (31), based on data from a multiethnic mixed-gender community-based cohort study conducted on U.S. elderly (aged ≥65 years old) residents. In this study, the authors did not find any significant differences in TBS values based on PPI use in either sex. Nevertheless, although some of the limitations of the study by Shin et al were overcome, the conclusions were still hampered by a relatively limited sample size (402 females and 199 males).

In our study, significant sex differences were observed in both TBS and BMD analyses. Specifically, the association of PPI use with lower BMD and TBS was only evident in male patients, with no significant effect noted in females. This gender-specific association is not well-established in the literature, but this could be in part possibly because of the methodological approaches adopted by previous studies. In fact, most of the studies that evaluated the association between PPI use and BMD on mixed-gender cohorts have handled sex as a confounder, applying statistical controls like regression adjustment rather than testing for possible interactions and applying stratification (57-61). This methodological approach, by its inherent characteristics, may have masked any sex-related effect modification (62-64), and possible gender differences may have gone undetected. Indeed, when studies analyzed male and female cohorts separately, evidence of a possible sexual dimorphism in the impact of chronic PPI use on bone parameters frequently emerged.

In a study by Gao et al (19), conducted on a previous NHANES cohort, a significant gender difference was observed in the relationship between PPIs and BMD; chronic PPI use, in fact, was associated with lower lumbar spine BMD in male patients, whereas no significant effect was found in females. Similar results were found by Yu et al (12), who reported findings from 2 large cohorts segmented by gender: the Osteoporotic Fractures in Men Study (MrOS), which exclusively included male participants, and the Study of Osteoporotic Fractures (SOF), composed solely of female participants. In fact, after adjusting for multiple covariates, the results revealed that men in the MrOS cohort who used PPIs had slightly lower total hip and femoral neck BMD compared to nonusers. Conversely, in the SOF cohort of women, no significant association was found between PPI use and BMD. Also consistent with these data were some findings of the aforementioned study by Kondapalli et al (31); in fact, although no clear gender differences were observed in terms of BMD and TBS, likely because of the limited sample size, the evaluation of high-resolution peripheral computed tomography (HR-pQCT) parameters highlighted a decrease in cortical volumetric bone density at the tibia in male PPI users, with no differences observed in females.

Unsurprisingly, if the pathophysiological mechanisms that cause skeletal fragility in PPI users have yet to be fully elucidated, the reasons for differences between the sexes remain even less clear. In a recent study, Zhang et al (65) investigated the influence of PPIs on bone density through changes in plasma metabolite levels, particularly focusing on the sex hormone pathways; the researchers identified a decrease in several plasma metabolites, particularly lipids and sulfated steroids related to androgen metabolic cascade, which were negatively impacted by PPI use. These findings suggest that PPIs may indirectly affect BMD through modulation of metabolites involved in sex hormone signaling, which might thus possibly explain the observed gender differences in the effects of PPI use on bone mineralization and microarchitecture.

Further supporting the hypothesis of a cross-talk between chronic PPI use and the gonadal axis function, long-term treatment with PPIs has been linked to endocrine disruptions, including hyperprolactinemia (66, 67), whose biological effect on the hypothalamus-pituitary-gonadal axis goes through the inherent gender-specific characteristics of the axis itself. An alternative hypothesis for the observed sexual dimorphism may relate to the impact of PPI use on micronutrient absorption. In fact, differences in dietary habits and micronutrient intake between males and females are well documented (68-70). Therefore, it is not implausible to hypothesize that gender-specific interactions between PPI use, dietary habits, and micronutrient absorption could also play a role in the observed sexual dimorphism.

Moving to a broader perspective, in any case, it is still essential to acknowledge that an increased risk of fractures has been documented in PPI users across both genders (8, 9, 11, 15). Consequently, the interplay between PPI use, bone mineralization and microarchitecture, and fracture risk is likely to be mediated by even more complex and multifaceted mechanisms, in which extra-skeletal factors also play a relevant role. As already discussed, for example, prior research has associated PPI use with an elevated risk of falls (52-54) and, interestingly, this effect appears to be comparable in both men and women, with no clear gender differences (53).

Our study has several strengths. One is that it is a study conducted on a large, unselected, community-based sample extracted from the general U.S. population; previous studies examining the relationship between PPI use and bone-related outcomes, even when conducted on large samples, have often been based on restricted populations, typically comprising individuals referred for DXA testing for clinical purposes. Our analyses, on the contrary, were based on a representative sample drawn through a stratified, multistage, clustered probability design, thus minimizing selection bias and enhancing the generalizability of the results, as they are less likely to be influenced by the specific characteristics of a more narrowly defined group. Another strength of our study is that we considered potential sex-specific effects on the relationship between PPI use and bone outcomes. This differs from the many of the previous studies, which did not include interaction terms to explore the interplay between sex and PPI use, nor did they stratify their analyses by sex, thus possibly overlooking possible gender differences in how PPI use affects bone microarchitecture and mineralization.

Our study has also some limitations. First, although multivariable analyses accounted for many key determinants of bone quality and mineralization, the possible presence of residual confounding cannot be ruled out, given the observational nature of the study. Second, its cross-sectional design allowed only the assessment of associations, preventing the identification of cause-effect relationships and temporal trends. Third, data on specific dosage or previously discontinued prescriptions were not available; therefore, the possible presence of a dose-response relationship between PPI use and bone outcomes could not be investigated, nor could the effect of previous PPI use.

In conclusion, this is the first study that evaluates the association between chronic PPI use and trabecular bone quality, as measured by TBS, in a large, unselected, community-based sample extracted from the general population. Our results suggest that chronic PPI use is associated with lower TBS values and lower BMD in males; interestingly, the association

between PPI use and lower TBS values persisted also after adjustment for BMD, suggesting a greater impairment of bone quality in these patients, independently to the extent of bone mass loss. On the other hand, no association between PPI use and either TBS or BMD was found among females, regardless of menopausal status. The lack of prospective data does not allow to assess the prognostic role of TBS in the prediction of incident fractures in this clinical setting and, in particular, whether it may play a specific role in the assessment of fracture risk among male PPI users; specifically designed longitudinal studies are needed to better address this point.

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Disclosures

The authors report no conflicts of interest in this work.

Data Availability

Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in the references. NHANES data are publicly available at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

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