# Changes in bone turnover markers in adolescents with gastroesophageal reflux disease treated with lansoprazole

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## Abstract

***Background:*** **Proton pump inhibitors (PPIs) have been suggested to lead to bone resorption, while the effects of PPIs on the bone mineral metabolism in children has received only limited attention in literature to date. The present study investigates whether lansoprazole alters bone turnover markers in adolescents with gastroesophageal reflux disease (GERD).**

***Patients and methods:*** **Included in the study were adolescents aged 16–18 with GERD and a healthy volunteers group. The GERD patient group was treated with lansoprazole 30 mg once daily for eight weeks. The serum calcium, phosphorus, magnesium, alkaline phosphatase (ALP), parathormone (PTH), 25 (OH) vitamin D, osteocalcin and urinary calcium, creatinine, deoxypyridinoline (DPD), collagen type-1 crosslinked C-telopeptide (CTX) and collagen type-1 crosslinked N-telopeptide (NTX) of both groups were studied before and after the end of the treatment.**

***Results:*** **A comparison of the 30 patients with GERD and the 30 volunteers revealed no significant difference in the serum calcium, phosphorus, magnesium, ALP, urinary calcium/creatinine ratio, 25 (OH) vitamin D and PTH levels measured before and after the lansoprazole treatment, while the osteocalcin, DPD, CTX and NTX values were found to be higher after treatment when compared to those at pre- treatment.**

***Conclusions:*** **The results of this study reveal that eight weeks of treatment with 30 mg lansoprazole daily increased the bone turnover markers of CTX, NTX, DPD and osteocalcin in adolescents aged 16-18.** (Acta gastroenterol. belg., **2022,** 85, **565-571**).

**Keywords:** Bone turnover, collagen type-1 cross linked C-telopeptide, collagen type-1 crosslinked N-telopeptide, deoxypyridinoline, osteocalcin, proton pump inhibitor

## Introduction

Gastroesophageal reflux disease (GERD) is generally treated with lifestyle changes and pharmacotherapy, while surgical treatments are rarely required. Proton pump inhibitors (PPIs) are the most potent options available for the treatment of GERD (1), although it has been suggested that PPIs can have undesirable effects on the bone mineral metabolism (2-4). These effects can be explained by several mechanisms: a) PPIs increase the pH of the small intestine, and thus decrease the intestinal calcium absorption; b) Hypergastrinemia and hyperparathyroidism secondary to increased gastric pH result in bone resorption; and c) PPIs inhibit bone-specific phosphatase (PHOSPHO1) activity, and thus matrix mineralization (5-8).

Studies to date have mainly evaluated whether PPIs reduce bone density or increase bone fracture risk, especially in the femoral neck or vertebra, and these studies have generally involved older adults with comorbidities, and have yielded conflicting results. Some of these studies have reported a relationship between PPIs and femoral neck/vertebral fractures, while no such relationship has been noted in others (9-11). Similarly, contradictory results have been obtained in studies of bone turnover markers in adults (12-14). Bone tissue remodeling is a dynamic process that involves osteoclastic and osteoblastic activities. Osteocalcin allows calcium to be included within the hydroxyapatite structure of the bone matrix, and a high serum osteocalcin level is an indicator of osteoblastic activity (15). Some 90% of the organic bone matrix consists of type I collagen, which has a triple helix structure. Bone resorption increases the urinary levels of type-I collagen fragments; collagen type-1 crosslinked C-telopeptide (CTX), collagen type-1 crosslinked N-telopeptide (NTX) and deoxypyridinoline (DPD), which can be used as highly specific bone turnover markers (16). The effects of PPIs on bone metabolism in children yet to be studied in any detail; thus the goal of this study is to evaluate the effects of eight weeks of lansoprazole treatment on bone turnover markers in adolescents with GERD.

## Method

The study was carried out in the pediatric gastroenterology department of a tertiary university hospital between 12.30.2019 and 12.31.2020. Local institutional review board approval was obtained for the study (No. B.30.2.ATA.0.01.00/500, dated 11.07.2019). The study was designed prospectively and registered on ClinicalTrials.gov (NCT04814316). The study group included patients who had been diagnosed with GERD, and a control group of individuals with no organic disease who were admitted for general health care. GERD was diagnosed based on a clinical evaluation of patients with symptoms such as retrosternal burning, regurgitation, burning in the throat and hoarseness, in accordance with the guidelines of the North American Society for

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Pediatric Gastroenterology, Hepatology and Nutrition, and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (17). Blood and urine samples were obtained from the patients and healthy volunteers, all of whom gave written and verbal consent at baseline and at the end of the 8th week.

*Inclusion Criteria*

1. Adolescents aged 16-18 years with Tanner stage 5 and
2. Healthy volunteers who were checked-up in the pediatric outpatient clinic.

*Exclusion Criteria*

1. People with acute or chronic diarrhea.
2. People with any chronic disease (hypothyroidism, hyperthyroidism, diabetes mellitus, celiac disease, liver diseases, etc.).
3. People with hyperparathyroidism.
4. People who were malnourished, overweight or obese (anthropometric measurements were evaluated according to our national data (18).
5. People who had taken medication (e.g. contraceptives, vitamin D) in the past 6 months that could affect calcium and bone metabolism.
6. People who did not use lansoprazole every day during the study.
7. People from whom blood and urine samples could not be taken in the eighth week.

Patients diagnosed with GERD who did not benefit from non-pharmacological treatment options such as lifestyle changes and dietary modifications were treated with lansoprazole. The patients were advised to take lansoprazole (Lansor, Sanovel Pharmaceuticals, Turkey) 30 mg half an hour before breakfast with some water, while the control group did not receive anything (no placebo was given for ethical reasons). Serum calcium, phosphorus, magnesium, bone-specific alkaline phosphatase (ALP), parathormone (PTH), 25 (OH) vitamin D, osteocalcin and urine calcium, creatinine, deoxypyridinoline (DPD), collagen type-1 crosslinked C-telopeptide (CTX) and collagen type-1 crosslinked N-telopeptide (NTX) levels were investigated.

*Follow-up charts*

A follow-up chart was established for each participant on which anthropometric measurements and clinical and laboratory features were recorded. The daily calcium intake was calculated from a 7-day diet list provided by the participants in both groups. Whether the patients used lansoprazole regularly and whether their complaints improved was recorded.

*Samples and analysis*

Blood samples were taken from the antecubital region using a vacutainer following an overnight fast, after which the participants were allowed to rest in a sitting position between 08:30 and 10:30 am. Blood samples taken into biochemistry tubes (Becton Dickinson, Tamse, Switzerland) were centrifuged for 30 minutes at room temperature after coagulation was completed. Urine samples were collected in urine tubes (Becton Dickinson, Tamse, Switzerland), and the supernatant was separated by centrifugation at 1000 rpm for 10 minutes, and then aliquoted and frozen at -80 degrees and stored until analysis.

Serum calcium, phosphorus, magnesium, ALP, urinary creatinine and urinary calcium were determined using the photometric method in a Beckman Coulter AU 5800 (Beckman Coulter, CA, USA) clinical chemistry analyzer. Serum PTH and 25 (OH) vitamin D were studied using the chemiluminescence method in a Beckman Coulter DXI 800 (Beckman Coulter, CA, USA). Serum osteocalcin was measured using the electrochemiluminescence method in a Cobas e601 immunoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany). Urine DPD was measured using the chemiluminescence method in an Immullite 2000 XPi (Siemens Healthcare Diagnostics Inc., Muenchen, Germany) immunoanalyzer. NTX and CTX were analyzed using SunRed ELISA kits (Cat No: 201-12-1384,201-12-1350, Shanghai, China) in a Dynex automated ELISA reader device (Dynex Technologies Headquarters, Chantilly, USA) in accordance with the manufacturer's recommended standard protocol. The measurement range of the kit for NTX was 1 to 200 nmol/L and 0.5 to 150 ng/ml units for CTX. Studies were carried out in accordance with quality procedures to ensure analytical errors did not exceed the national and international permissible error limits. The intra-assay coefficients of variation (CV) for ALP, PTH, 25 (OH) vitamin D, osteocalcin, DPD, CTX and NTX were 4%, 9.01%, 14.6%, 10%, <10%, <10% and <10%, respectively; and the inter-assay CV for ALP, PTH, 25 (OH) Vitamin D, osteocalcin, DPD, CTX and NTX were 5%, 9.9%, 17.5%, 11.3%, <10.06%, <12% and <12%, respectively.

*Statistical Analysis*

A licensed copy of IBM SPSS Statistics (Version 24.0. Armonk, NY: IBM Corp.) and Microsoft Excel programs were used for the statistical analysis, with the results presented as mean, median and standard deviation. The suitability of the parameters to normal distribution was evaluated with a Kolmogorov-Smirnov test, and as the parameters showed a normal distribution, a two way ANOVA was carried out, while in cases where the p value was <0.05, a Tukey's Multiple Comparisons test.

*Results*

Included in the study were 45 patients diagnosed with GERD were included in the study along with and 30 volunteers as a control group. In the patient group, two patients with hyperparathyroidism and vitamin D

Table 1. — **Characteristics of the participants**

| Parameter | GERD N=30 | Control N=30 | p value |
|---|---|---|---|
| **Age (year)** | 16.42 ± 0.45 | 16.39 ± 0.24 | 0.84 |
| **Gender (M/F)** | 15/15 | 15/15 | 0.60 |
| **Height Z score** | -0.26 ± 0.07 (-0.29) | -0.24 ± 0.11 (-0.41) | 0.62 |
| **Weight Z score** | -0.15 ± 0.13 (-0.12) | -0.13 ± 0.10 (-0.16) | 0.37 |
| **Calcium intake (mg/day)** | 814.45 ± 217.39 (781.7) | 796.45 ± 231.20 (746.4) | 0.72 |

**GERD** Gastroesophageal reflux disease. Parameters are given as the mean ± standard deviation and the median (in parentheses), Student's T-test was performed**.**

Table 2. — **Symptoms of the patients with gastroesophageal reflux**

| Symptom | N (%) |
|---|---|
| Heartburn | 21 (70.0) |
| Regurgitation | 8 (26.6) |
| Belching | 8 (26.6) |
| Burning in the throat | 7 (23.3) |
| Postprandial cough | 3 (10.0) |
| Hoarseness | 2 (6.6) |

deficiency, five patients who were obese or overweight, five patients who could not be tested after treatment and three patients who did not take the lansoprazole regularly were excluded from the study. As a result, 30 patients were included in the study group and 30 volunteers in the control group.

There were no statistically significant differences in the descriptive characteristics of the two groups in terms of age, weight-for-height Z-score and mean daily calcium consumption (Table 1).

Heartburn (21/30, 70.0%), belching (8/30, 26.6%), regurgitation (8/30, 26.6%), burning in the throat (7/30, 23.3%), postprandial cough (3/30, 10.0%) and hoarseness (2/30, 6.6%) were the patient symptoms recorded at the time of admission (Table 2).

At the end of the study, the symptoms of 21 of the patients treated with lansoprazole had fully resolved, while the remaining nine patients had significant improvement.

No statistically significant differences were observed in the baseline parameters of the groups (Table 3). Serum calcium, phosphorus, magnesium, ALP, 25 (OH) vitamin D, PTH, osteocalcin, urinary calcium creatinine ratio, DPD, CTX and NTX were no different in the females and males in the control group at baseline and at week eight (Table 4). While there were no significant differences in the serum calcium, phosphorus, magnesium, ALP, urinary calcium creatinine ratio, 25 (OH) vitamin D and PTH levels in the female and male patients pre- and post-lansoprazole treatment, osteocalcin (53.82±13.09 vs. 43.87±13.63, in females and 56.34±23.00 vs. 45.91±15.67, in males, $p<0.01$), DPD (17.39±4.31 vs. 12.85±4.26, in females and 19.14±6.21 vs. 11.76 ± 3.57, in males, $p<0.01$), CTX (60.29±17.07 vs. 44.34±18.38, in females and 63.44±23.26 vs. 46.87±13.34, in males, $p<0.01$) and NTX (84.26±22.94 vs. 66.81±21.73, in females and 87.78±17.58 vs. 69.76±26.98, in males, $p<0.01$) were higher post-treatment than in the pre-treatment phase in the patient group (Table 5).

## Discussion

PPIs are recommended as the first-choice drug for the treatment of GERD for 4 to 8 weeks in children and adolescents. Diseases other than GERD, such as eosinophilic esophagitis and the long-term use of corticosteroids may necessitate long-term PPI therapy. There are concerns about the overuse of PPIs without exact indications (19). In the present study we hypothesized that lansoprazole would have negative effects on bone turnover, and the effect on patients requiring long-term PPI therapy may be severe. To minimize external factors that could affect the results of the study, we excluded patients undergoing long-term PPI therapy with underlying chronic diseases other than GERD, and so only the short-mid term results of lansoprazole on bone health were evaluated.

It has been reported that hypergastrinemia and secondary hyperparathyroidism associated with the use of PPIs may lead to bone resorption in adults (7-13), while their effects on PTH in children have not previously been evaluated. In the present study, no statistically significant difference was noted in PTH after treatment. The recommended daily calcium consumption of a healthy adolescent is around 1300 mg, although this age group is known to often consume less than the recommended amount (20). In theory, calcium intake affects bone formation, although not only the consumed calcium, but also the quality of the proteins in foods and the source of the calcium can affect bone formation. Perhaps with the such factors, the effects of calcium intake on bone turnover markers have not been clearly demonstrated (21). The average daily calcium

Table 3. — **Comparison of the parameters between the patient and control groups**

| Parameter | GERD N=30 | | Control N=30 | | p value |
|---|---|---|---|---|---|
| | Female | Male | Female | Male | |
| Serum calcium (mg/dL) | 9.56 ± 0.40 (9.4) (9.0-10.5) | 9.34 ± 0.56 (9.3) (9.1-10.8) | 9.21 ± 0.52 (9.3) (9.0-10.64) | 9.42 ± 0.60 (9.4) (9.5-10.8) | 0.51 |
| Serum phosphorus (mg/dL) | 4.11 ±0.51 (3.52-5.36) | 4.07 ±0.39 (3.60-5.40) | 4.28±0.45 (3.68-5.44) | 4.16±0.49 (3.81-5.10) | 0.76 |
| Serum magnesium (mg/dL) | 2.00 ± 0.14 (1.99) (1.83-2.40) | 2.02 ± 0.19 (1.97) (1.80-2.33) | 1.99 ± 0.10 (1.96) (1.84-2.38) | 2.06 ± 0.31 (1.98) (1.94-2.60) | 0.12 |
| ALP (iu/L) | 76.23 ± 21.44 (74) (42-114) | 80.12 ± 31.92 (76) (45-128) | 74.18 ± 19.21 (73) (54-150) | 77.32±26.84 (75) (49-120) | 0.27 |
| Urine calcium/creatinine | 0.10 ± 0.08 (0.08) (0.06-0.16) | 0.12 ± 0.07 (0.09) (0.04-0.15) | 0.11± 0.06 (0.10) (0.07-0.18) | 0.12 ± 0.03 (0.10) (0.05-0.17) | 0.48 |
| Vitamin D (ng/mL) | 18.70 ± 5.28 (16.2) (11.9-30.1) | 22.12 ± 6.76 (20.3) (13.6-32.5) | 17.14 ± 4.12 (15.5) (12.1-30.0) | 24.52 ± 6.54 (22.3) (13.1-33.5) | 0.14 |
| PTH (pg/mL) | 30.61 ± 14.20 (24.4) (11.3-54.6) | 27.39±10.69 (23.2) (14.6-51.5) | 34.52 ± 16.28 (30.2) (12.2-48.6) | 35.12±19.43 (32.6) (15.6-60.1) | 0.31 |
| Osteocalcin (ng/mL) | 43.87 ± 13.63 (42.2) (26.5-60.0) | 45.91 ± 15.67 (43.1) (28.5-87.5) | 42.12 ± 15.32 (40.5) (20.5-70.5) | 44.65±17.45 (44.1) (24.5-76.5) | 0.81 |
| DPD (nM/mM) | 12.85 ± 4.26 (11.3) (8.2-28.6) | 11.76 ± 3.57 (10.4) (7.4-30.2) | 13.50± 6.24 (12.3) (7.2-32.1) | 12.40±5.12 (11.2) (8.4-29.2) | 0.65 |
| CTX (ng/mL) | 44.34 ± 18.38 (41.1) (30.4-80.2) | 46.87 ± 13.34 (43.6) (31.5-80.5) | 45.74± 15.23 (42.3) (32.6-76.3) | 46.97±12.21 (44.7) (33.3-87.4) | 0.72 |
| NTX (nM/mL) | 66.81± 21.73 (64.2) (52.7-88.6) | 69.76 ± 26.98 (64.5) (50.6-90.4) | 67.12 ± 24.26 (63.3) (47.3-96.2) | 68.65±30.11 (65.1) (50.2-91.1) | 0.30 |

**ALP** Alkaline phosphatase, **CTX** Collagen type-1 crosslinked C-telopeptide, **DPD** Deoxypyridinoline, **GERD** Gastroesophageal reflux disease, **NTX** Collagen type-1 crosslinked N-telopeptide, **PTH** parathormone. Parameters are given as the mean ± standard deviation and the median and ranges (in parentheses), two way ANOVA was performed.

consumption of the participants in the present study was approximately 800 mg/day in both groups. Given the similarity of the calcium consumption and the normal PTH levels at baseline in both groups, we can ignore the potential effect of the lower than recommended calcium intake on our results.

The majority of studies investigating the effect of acid-suppressing treatments on bone health have evaluated whether PPIs alter bone density or increase the risk of bone fracture in adults. While some of these studies have reported a relationship between PPIs and femoral neck/vertebral fractures, no such relationship was found in the others (22-25). There have been a number of studies of adult samples investigating the effects of PPIs on various bone turnover markers (12-14), although the majority of these were not randomized or controlled, and most included participants of advanced age, and these limitations make it difficult to determine a definite cause-effect relationship. Our review of literature revealed only one study evaluating the effect of PPI on osteocalcin and CTX in children. The study, in which 34 prepubertal and pubertal pediatric patients of different ages who had been diagnosed with Helicobacter pylori or GERD were involved, determined that two weeks of omeprazole treatment had no effect on osteocalcin or CTX excretion. In addition, being pre- or post-pubertal had no effect on the examined bone turnover markers, although the relevant results were not provided separately (26). In the present study, CTX, NTX and DPD, as highly sensitive markers of bone resorption, and osteocalcin, as a marker of bone formation, increased after eight weeks of lansoprazole treatment. Osteocalcin is also increased in metabolic bone diseases, such as osteoporosis and hyperparathyroidism (27), and consequently the increase in osteocalcin was considered to be related to the increase in bone turnover. Osteocalcin, DPD, CTX and NTX levels differ according to the age, sex and pubertal stage. During periods of rapid linear growth, the levels of all these markers have been recorded as high, while more stable levels are observed after puberty (28). In the present study, only adolescents of the same age and at the same stage of puberty were included to prevent the need to take into account growth-related differences. In addition, the absence of any difference between the baseline and week eight bone turnover markers in the control group proved that there were no skeletal development-related variables. The participants' sex was taken into account during the evaluation of the parameters. Overweight and obese cases were excluded from the study as the measured parameters have been shown to be affected by such conditions.

Table 4. — **Comparison of the admission and eighth week parameters in the control group**

| Parameter | On admission N=30 | | 8th week N=30 | | p value |
|---|---|---|---|---|---|
| | Female | Male | Female | Male | |
| Serum calcium (mg/dL) | 9.21 ± 0.52 (9.3) (9.0-10.64) | 9.42 ± 0.60 (9.4) (9.5-10.8) | 9.28 ± 0.31 (9.4) (9.0-10.11) | 9.31 ± 0.40 (9.3) (9.3-10.6) | 0.40 |
| Serum phosphorus (mg/dL) | 4.28±0.45 (3.68-5.44) | 4.16±0.49 (3.81-5.10) | 4.35±0.27 (3.70-5.55) | 4.23±0.34 (3.76-5.14) | 0.71 |
| Serum magnesium (mg/dL) | 1.99 ± 0.10 (1.96) (1.84-2.38) | 2.06 ± 0.31 (1.98) (1.94-2.60) | 2.03 ± 0.24 (1.91) (1.91-2.20) | 2.05 ± 0.22 (1.96) (1.90-2.60) | 0.22 |
| ALP (ıu/L) | 74.18 ± 19.21 (73) (54-150) | 77.32±26.84 (75) (49-120) | 67.40 ± 15.40 (76) (51-117) | 73.87±21.544 (79) (60-106) | 0.87 |
| Urine calcium/creatinine | 0.11± 0.06 (0.10) (0.07-0.18) | 0.12 ± 0.03 (0.10) (0.05-0.17) | 0.10± 0.04 (0.11) (0.04-0.13) | 0.11 ± 0.02 (0.12) (0.05-0.16) | 0.58 |
| Vitamin D (ng/mL) | 17.14 ± 4.12 (15.5) (12.1-30.0) | 24.52 ± 6.54 (22.3) (13.1-33.5) | 18.72 ± 5.50 (16.2) (13.2-31.40) | 23.12 ± 7.55 (20.5) (13.6-31.3) | 0.36 |
| PTH (pg/mL) | 34.52 ± 16.28 (30.2) (12.2-48.6) | 35.12±19.43 (32.6) (15.6-60.1) | 33.88 ± 12.63 (29.7) (14.4-47.1) | 35.20±27.81 (34.2) (17.7-51.8) | 0.49 |
| Osteocalcin (ng/mL) | 42.12 ± 15.32 (40.5) (20.5-70.5) | 44.65±17.45 (44.1) (24.5-76.5) | 40.52 ± 17.63 (46.5) (22.5-72.5) | 43.41±21.30 (41.2) (20.5-66.5) | 0.69 |
| DPD (nM/mM) | 13.50± 6.24 (12.3) (7.2-32.1) | 12.40±5.12 (11.2) (8.4-29.2) | 14.47± 7.60 (11.4) (7.9-30.2) | 10.97±8.79 (9.9) (8.8-31.2) | 0.14 |
| CTX (ng/mL) | 45.74± 15.23 (42.3) (32.6-76.3) | 46.97±12.21 (44.7) (33.3-87.4) | 42.11± 18.22 (43.4) (30.6-78.2) | 48.52±15.10 (45.4) (32.1-82.7) | 0.92 |
| NTX (nM/mL) | 67.12 ± 24.26 (63.3) (47.3-96.2) | 68.65±30.11 (65.1) (50.2-91.1) | 64.97 ± 21.52 (64.8) (33.5-96.2) | 66.40±22.63 (66.2) (44.2-82.1) | 0.66 |

**ALP** Alkaline phosphatase, **CTX** Collagen type-1 crosslinked C-telopeptide, **DPD** Deoxypyridinoline, **NTX** Collagen type-1 crosslinked N telopeptide, **PTH** parathormone. Parameters are given as the mean ± standard deviation and the median and ranges (in parentheses), two way ANOVA was performed.

Bone turnover markers have circadian variations and the results are affected by food intake (26). In the present study, therefore, samples were taken in the morning after overnight fasting. Patients with dyspeptic symptoms that could be associated with Helicobacter pylori, which has been suggested to be a risk factor for the development of osteoporosis (29), were also excluded, which meant that the external factors capable of influencing the study results were reduced. It may be questioned whether eight weeks of lansoprazole treatment is sufficient for the assessment of changes in bone turnover markers. With the use of anabolic and antiresorptive agents such as recombinant PTH and bisphosphonates, rapid changes in bone turnover markers within a few weeks have been demonstrated (30,31).

We believe, therefore, that the results observed after the eight weeks of lansoprazole treatment – which can be considered short–mid term treatment – accurately reflects the changes in bone metabolism.

The prevalence of vitamin D deficiency has reported as high in Turkey (32), and the mean 25 (OH) vitamin D levels in the patient and control groups revealed vitamin D deficiency among the females and insufficiency among the males, although these low levels had not led to hyperparathyroidism. The fact that females have lower 25 (OH) vitamin D levels than males may be associated with their clothing style. Although it is theoretically assumed that vitamin D deficiencies increase bone turnover markers, randomized controlled trials have not yielded conclusive results (33). In a study conducted with adolescents, a positive correlation was noted between 25 (OH) vitamin D levels and osteocalcin, CTX (34). Since the serum PTH and calcium levels of all participants were normal at the time of admission and at the end of the eighth week, we can strongly argue that the results of the study were not affected by the participants' 25 (OH) vitamin D status.

It must be acknowledged that the present study had a number of limitations. First, the effects of lansoprazole on the bone turnover markers were investigated in adolescents within a specific age range to eliminate the likelihood of growth-related differences. The study had thus a non-randomized design due to the limited number of participants. Similar assessments could be made involving a wider population within any age range. Second, CTX levels may differ between follicular and luteal phases, and may even differ during the follicular phase and luteal phase (35). It was not possible to take samples from the female participants on the same menstrual days. Third, the study evaluates only the short–mid term

Table 5. — **Comparison of the of the admission and eighth week parameters in the patient group**

| Parameter | On admission N=30 | | 8th week N=30 | | p value |
|---|---|---|---|---|---|
| | Female | Male | Female | Male | |
| Serum calcium (mg/dL) | 9.56 ± 0.40 (9.4) (9.0-10.5) | 9.34 ± 0.56 (9.3) (9.1-10.8) | 9.62 ± 0.32 (9.5) (9.1-10.3) | 9.43± 0.73 (9.5) (9.3-10.8) | 0.86 |
| Serum phosphorus (mg/dL) | 4.11 ±0.51 (3.52-5.36) | 4.07 ±0.39 (3.60-5.40) | 3.94 ±0.37 (3.31-5.24) | 3.91 ±0.42 (3.45-5.28) | 0.43 |
| Serum magnesium(mg/dL) | 2.00 ± 0.14 (1.99) (1.83-2.40) | 2.02 ± 0.19 (1.97) (1.80-2.33) | 2.01 ± 0.19 (1.96) (1.84-2.22) | 2.03± 0.21 (1.97) (1.85-2.35) | 0.68 |
| ALP (ıu/L) | 76.23 ± 21.44 (74) (42-114) | 80.12 ± 31.92 (76) (45-128) | 83.47 ± 28.52 (80) (40-126) | 89.46 ± 30.16 (84) (55-119) | 0.24 |
| Urine calcium/creatinine | 0.10 ± 0.08 (0.08) (0.06-0.16) | 0.12 ± 0.07 (0.09) (0.04-0.15) | 0.11± 0.05 (0.09) (0.05-0.18) | 0.13± 0.04 (0.10) (0.03-0.19) | 0.37 |
| Vitamin D (ng/mL) | 18.70 ± 5.28 (16.2) (11.9-30.1) | 22.12 ± 6.76 (20.3) (13.6-32.5) | 17.87 ± 5.83 (15.8) (12.2-32.2) | 23.30 ± 8.47 (20.5) (14.10-33.4) | 0.34 |
| PTH (pg/mL) | 30.61 ± 14.20 (24.4) (11.3-54.6) | 27.39±10.69 (23.2) (14.6-51.5) | 35.40 ± 20.76 (30.2) (13.2-62.4) | 32.18±14.89 (28.3) (11.3-64.1) | 0.11 |
| Osteocalcin (ng/mL) | 43.87 ± 13.63 (42.2) (26.5-60.0) | 45.91 ± 15.67 (43.1) (28.5-87.5) | 53.82 ± 13.09 (51.1) (36.5-83.2) | 56.34±23.00 (53.3) (37.4-133.0) | **<0.01*** |
| DPD (nM/mM) | 12.85 ± 4.26 (11.3) (8.2-28.6) | 11.76 ± 3.57 (10.4) (7.4-30.2) | 17.39±4.31 (16.3) (8.7-40.4) | 19.14±6.21 (17.1) (9.4-44.6) | **<0.01*** |
| CTX (ng/mL) | 44.34 ± 18.38 (41.1) (30.4-80.2) | 46.87 ± 13.34 (43.6) (31.5-80.5) | 60.29 ± 17.07 (61.3) (28.9-92.6) | 63.44±23.26 (62.2) (40.2-115.5) | **<0.01*** |
| NTX (nM/mL) | 66.81± 21.73 (64.2) (52.7-88.6) | 69.76 ± 26.98 (64.5) (50.6-90.4) | 84.26 ± 22.94 (81.3) (56.9-144.9) | 87.78±17.58 (80.3) (46.2±110.2) | **<0.01*** |

**ALP** Alkaline phosphatase, **CTX** Collagen type-1 crosslinked C-telopeptide, **DPD** Deoxypyridinoline, **NTX** Collagen type-1 crosslinked N telopeptide, **PTH** parathormone. Parameters are given as the mean ± standard deviation and the median and ranges (in parentheses). Two way ANOVA followed by Tukey's Multiple Comparisons test was performed. $p < 0.01$*, results at week 8 for both girls and boys compared with admission.

effects of lansoprazole on bone turnover, and so we do not know whether the observed differences in markers are sufficient to reflect increased bone turnover, whether different compensatory mechanisms in bone formation will develop, or whether serum PTH, ALP, calcium and phosphorus will be deteriorated in long-term use. Fourth, no dual-energy X-ray absorptiometry was performed, as there are currently no standard reference values for children, and we considered such an examination would be unable to reveal changes over the eight-week period. If patients develop osteoporosis, clinicians should be alert to the such adverse effects of prolonged PPI therapy, and prophylactic treatments may be considered. Fifth, we were unable to calculate a sample size at the time of study planning. Previous studies have been conducted with heterogeneous patient groups of different ages. However, since the investigated parameters are affected by many external factors, including age, we preferred to conduct this study in a narrow age range. This clinical feasibility study was conducted under the definitions and protocols described in a published tutorial in a pilot study, (36) and lack of previous studies, a convenience sample size of 30 was used for each group.

In conclusion, this study has examined the effects of eight weeks of lansoprazole treatment on the highly sensitive bone turnover markers in patients with GERD, and found that such treatments increased bone turnover markers. This is the first study to evaluate various bone turnover markers in children treated with lansoprazole, however, further studies are required to examine also the mechanisms of increased bone turnover.

## Author contributions

A. Islek and H. Keskin substantially contributed to the conception and design of the study and the acquisition, analysis and interpretation of the data; A. Islek, H. Keskin, N. Erol Kizilelma and N. Ozturk drafted the article and made critical revisions related to the intellectual content of the manuscript and approved the final version of the article to be published.

## Institutional review board statement

Approval for the study was granted by Atatürk University's Clinical Research Ethical Committee (No. B.30.2.ATA.0.01.00/500, dated 11.07.2019).

**Grands**

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**Conflict-of-interest statement**

A. Islek, N. Kizilelma Erol, H. Keskin and N. Ozturk declare that they have no conflict of interest.

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