

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

# Metabolism

[www.metabolismjournal.com](http://www.metabolismjournal.com)

## Clinical Science

# Proton pump inhibitors have no measurable effect on calcium and bone metabolism in healthy young males: A prospective matched controlled study

Ala I. Sharara<sup>a,\*</sup>, Mustapha M. El-Halabi<sup>a,1</sup>, Ola A. Ghaith<sup>a</sup>, Robert H. Habib<sup>b</sup>, Nabil M. Mansour<sup>a</sup>, Ahmad Malli<sup>a</sup>, Ghada El Hajj-Fuleihan<sup>c</sup>

<sup>a</sup> Division of Gastroenterology, American University of Beirut Medical Center, Beirut, Lebanon

<sup>b</sup> Outcomes Research Unit, American University of Beirut Medical Center, Beirut, Lebanon

<sup>c</sup> Division of Endocrinology, Calcium Metabolism and Osteoporosis Program and WHO Collaborating Center for Metabolic Bone Disorders, American University of Beirut Medical Center, Beirut, Lebanon

## ARTICLE INFO

### Article history:

Received 18 June 2012

Accepted 19 September 2012

### Keywords:

Acid

Fracture

Osteoporosis

Calcitropic hormones

Bone turnover

## ABSTRACT

**Objectives.** Proton pump inhibitors (PPIs) are associated with an increased risk of bone fractures. This study sought to evaluate the effect of PPIs on biochemical markers of calcium and bone metabolism.

**Methods.** Prospective matched controlled study involving healthy adult males (age 18–50 years) suffering from frequent heartburn. Patients received standard-dose PPI for 12 weeks and were matched by age with healthy controls. Blood studies were taken at 0, 1 and 3 months for biochemical markers of mineral and bone metabolism. Two-way (time and PPI treatment) repeated measures analysis of variance (RM-ANOVA) and multiple linear regression were used for analysis.

**Results.** A total of 58 participants (29 per group) completed the study. Mean age of participants was 33.2±7.5 years. Baseline characteristics and biomarkers were similar for both groups except for higher BMI (28.6 vs. 25.6 kg/m<sup>2</sup>, p=0.008) and serum C-terminal cross linked telopeptides of type I collagen [CrossLaps, (300 vs. 228 pg/ml, p=0.028)] in the PPI group. There was no difference in parathormone (PTH), ionized calcium, vitamin D, osteocalcin and CrossLaps between the PPI and control subjects (all non-significant; 2-way RM-ANOVA). Multiple linear regression modeling showed no effect of PPIs on any of the studied calcium or bone metabolism biomarkers.

**Conclusion.** PPI intake for 12 weeks has no measurable effect on calcium or bone metabolism in healthy young males.

© 2013 Elsevier Inc. All rights reserved.

**Abbreviations:** PPI, Proton Pump Inhibitor; PTH, Parathyroid Hormone; CrossLaps, C-terminal cross-linked telopeptides of type I collagen; CV, Coefficient of Variance; RM, repeated measures; ANOVA, Analysis of Variance; NNH, Number Needed to Harm; BMD, Bone Mineral Density.

ClinicalTrial.gov identifier: NCT01139645.

\* Corresponding author. Division of Gastroenterology, American University of Beirut Medical Center, P.O. Box 11-0236/16-B, Beirut, Lebanon. Tel.: +961 1 350000x5351; fax: +961 1 366098.

E-mail address: [as08@aub.edu.lb](mailto:as08@aub.edu.lb) (A.I. Sharara).

<sup>1</sup> Contributed equally to the manuscript.

## 1. Introduction

Proton pump inhibitors (PPIs) are amongst the most widely used drugs worldwide. Because of a favorable side-effect profile and proven efficacy in the treatment of common gastric acid-related problems, PPIs are widely prescribed and purchased over the counter with estimated global sales of \$26.5 billion in 2008 [1]. However, the increasing prevalence of their chronic use has raised several concerns about potential drug-related long term adverse events as a result of prolonged inhibition of acid production by gastric parietal cells [2]. Recently, many observational case-control and cohort studies have shown a modest increase in spine, hip, and total fractures with chronic PPI intake [3,4]. Other studies have, however, failed to substantiate this association [5,6]. As a result, the US Food and Drug Administration issued in May 2010 an alert that PPIs may enhance the rate of bone fractures without adding a black box warning [7]. Given the heterogeneous nature of available studies and the diverging results, several meta-analyses were implemented that again led to conflicting conclusions [8,9]. While some estimated that as many as 4.7% of hip fractures might be attributable to PPI use, others found no or only a modest association without any evidence for an effect of duration, thus raising doubts whether the observed epidemiologic association is causal or the result of unmeasured confounders [8,9].

Importantly, the pathophysiologic mechanisms underlying this putative increased risk in fractures remain unclear. Possible explanations include hypochlorhydria-induced decreased calcium absorption [10], and/or a direct effect of PPIs on  $H^+K^+$  pumps in osteoclasts [11]. Clinical trials have described conflicting results regarding the effect of acid suppression on biochemical measurements of bone resorption in humans as well as discordant effects on bone mineral density. However, any of the aforementioned mechanisms should, in principle, result in perturbation of calcium homeostasis with changes in serum ionized calcium and consequently in parathyroid hormone and markers of bone turnover. To address this question, we designed a prospective matched controlled study to evaluate the effect of PPI use on serum markers of calcium and bone homeostasis.

## 2. Materials and methods

### 2.1. Population

The study population consisted of 2 groups, the first included healthy men aged 18 to 50 years complaining of frequent heartburn and who had not received any prior PPI therapy in the preceding year, while the second consisted of age-matched ( $\pm 2$  years) healthy men without heartburn or dyspepsia. Patients were recruited by advertisement and all efforts were made to complete recruitment in the fall and winter period to minimize the effect of sun exposure on serum 25-hydroxy vitamin D levels. Exclusion criteria included female gender to avoid confounding effect of various phases of menstrual cycle on bone metabolism [12]. Men more than 50 years of age were also excluded in order to avoid impact of

confounders that are usually present in the elderly population and that can affect mineral metabolism like drugs, age, and concomitant diseases. Lastly, subjects were excluded in case of known allergy to PPIs, regular beach seekers (more than once a week), known small intestinal disease, recent fracture (within the past six months), history of kidney stones, and ongoing or recent (within 1 year) intake of vitamin D or calcium supplements, anticonvulsants, glucocorticoids, and PPIs or  $H_2$  receptor antagonists. Patients were also not allowed to have any of these supplements and medications once they were enrolled in the study and compliance was checked on every study visit. Once a patient with heartburn was enrolled in the study, he was directly matched by age ( $\pm 2$  years) with a healthy volunteer control that underwent blood studies within 2 weeks from the enrollment of the patient he was matched to. All subjects completed a baseline semi-quantitative food frequency dietary questionnaire, modified from a previously validated food frequency questionnaire developed by our group, at the time of recruitment [13]. The study was approved by the Institutional Review Board and was registered as a clinical trial, ClinicalTrials.gov identifier: NCT01139645. All patients and matched controls gave written informed consent.

### 2.2. Intervention

Participants with heartburn were randomized to receive a standard dose of one of three commercially available PPIs, taken once daily 30 min before breakfast for a period of 3 months. The PPI used was either esomeprazole at a 40 mg dose (Nexium®, AstraZeneca, London, England), rabeprazole at a 20 mg dose (Pariet®, Janssen-Cilag, Beerse, Belgium), or lansoprazole at a 30 mg dose (Lanzor®, Sanofi-Aventis, Paris, France). The medications were provided free and at monthly installments, with return of medication packages at each visit for pill count, to assess and ensure proper adherence and compliance. The matched volunteers in the control group were not given PPI or placebo. Participants in both groups had non-fasting blood studies at the time of recruitment and after 1 month, and 3 months. All blood studies were drawn before noon, between 8 am and 12 pm. These included total serum calcium, ionized calcium, phosphorus, albumin, parathormone (PTH), 25-OH-vitamin D, C-terminal cross-linked telopeptides of type I collagen (CrossLaps), and osteocalcin. In addition, serum creatinine was measured once at baseline. Serum calcium and phosphorus were measured by standard calorimetric methods, using the Hitachi 912 analyzer (Mannheim, Germany). Serum 25-OH-vitamin D level was measured by RIA using the IDS (Immunodiagnostic System Limited, UK) and serum PTH by ELISA-PTH immuno-radiometric assay (CisBio International, Gif-Sur-Yvette, Cedex, France). For PTH, the detection limit of the assay was 0.7 pg/ml and the normal range of the kit was 8–76 pg/ml. For 25-OH-vitamin D, the normal range of the kit was 20–60 ng/ml. The intra-assay and inter-assay variability for 25-OH-vitamin D and PTH is below 10%. Our institution has been a participant of the Vitamin D External Quality Assessment Scheme (DEQAS), which evaluates the performance of participating laboratories quarterly, for several years ([www.deqas.org](http://www.deqas.org)). Serum CrossLaps were measured by ECLIA (electro-chemi-luminescence immuno-

assay) with inter- and intra-assay coefficient of variance (CVs) between 1% and 5.5% for values between 140 and 359 pg/ml (Roche Diagnostics), and serum osteocalcin by IRMA (immuno-radiometric assay) (CIS Bio International, Gif-Sur-Yvette, France), with inter- and intra-assay CVs between 1.2% and 5.2% for values between 20.4 and 220 ng/ml.

### 2.3. Study endpoint

The study hypothesis considered the following: (1) if PPIs negatively influence calcium absorption, they would be expected to lower serum ionized calcium levels and raise serum levels of parathyroid hormone, and may also subsequently raise serum levels of biochemical markers of bone remodeling, i.e. osteocalcin and crosslaps and/or (2) if PPIs had a direct effect on bone remodeling via inhibition of osteoclastic  $H^+-K^+$  pumps, markers of bone remodeling would decrease. The study primary endpoint was therefore the change in PTH, and changes in the level of the biochemical markers of bone remodeling were considered as secondary endpoints. Sample size calculation was conducted based on an anticipated rise in PTH as a result of a possible decrease in calcium absorption in subjects taking PPIs. Based on a previous study conducted in young individuals[14] and assuming a rise in PTH of 7.5 pg/ml in the PPI group and a standard deviation of 10 pg/ml, a sample of 28 patients per arm would be needed to detect such a difference as significant, with a power of 80% and an  $\alpha=0.05$ .

### 2.4. Statistical analysis

Continuous variables were presented as mean $\pm$ SD. Baseline Values were compared by t-test or Mann Whitney rank sum test as appropriate based on normality of data. Changes in biochemical parameters with time – within each treatment group (PPI and Control) – were assessed using one-way repeated measures (RM) Analysis of Variance (ANOVA). Next, we tested for significant differences in post-intervention (1 and 3 months relative to baseline) levels of all biochemical markers between the two treatment groups by consideration of the interaction term (Treatment  $\times$  Time) derived from the covariate adjusted two-way RM ANOVA analysis. The Treatment  $\times$  Time association was also analyzed via multiple linear regression for each biomarker at 3 months, and these models considered all potential predictors of calcium metabolism including: baseline levels of the same biomarker, treatment group (PPI or control), age (years), BMI ( $kg/m^2$ ), average daily calcium intake (mg/day), creatinine (mg/dl), PTH (pg/ml) at baseline, and Vitamin D (ng/ml) at baseline. A  $p<0.05$  was used for significance. All analyses were done using SPSS version 19.0 (SPSS Inc., Chicago, Illinois, USA).

## 3. Results

Sixty two individuals signed the informed consent and were originally enrolled in the study (31 in the PPI group, and 31 in the matched control group), and 58 subjects (29 in each group) completed the study (Fig. 1). Two patients in the PPI group were excluded because of a diagnosis of hyperparathyroidism

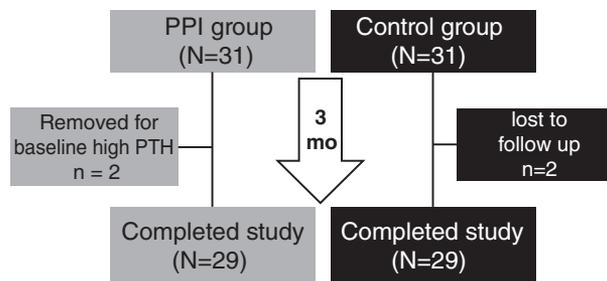


Fig. 1 – Study flow chart.

at baseline. Two subjects in the control group self-prescribed vitamin D at high doses and could not be included in the analyses. The PPI medications used were as follows: 11 patients used esomeprazole (40mg), 9 used rabeprazole (20mg), and 9 used lansoprazole (30mg). Adherence to the prescribed treatment was excellent (>90% adherence to prescribed daily dosing). Estimated calcium intake at study entry was  $410\pm 259$  mg/day in the PPI group, and  $291\pm 156$  mg/day in the control group ( $p=0.04$ ). Table 1 summarizes the baseline characteristics of the two study groups. The mean age of subjects in the PPI and control groups was similar at  $32.8\pm 7.5$  and  $33.6\pm 7.6$  years. There were no statistical differences in any of the baseline characteristics between the 2 groups at study entry, except for BMI [ $28.6\pm 4.4$  vs.  $25.6\pm 3.8$   $kg/m^2$  ( $p=0.008$ )] and serum CrossLaps [ $300\pm 140$  vs.  $228\pm 100$  pg/ml ( $p=0.028$ )] which were significantly higher in the PPI group. Mean serum PTH levels were similar at  $36.2\pm 13.3$  and  $38.6\pm 19.1$  pg/ml (Fig. 2). All variables measured were within the healthy age-matched reference range in both study groups. The only exception was vitamin D levels, where 50 out of 58 participants (86.2%) had hypovitaminosis D with 25-OH-vitamin D levels below 20 ng/ml: 60.3% had insufficient levels between 10 and 20 ng/ml and 25.9% were vitamin D deficient with levels below 10 ng/ml. Mean serum 25-OH vitamin D levels were  $16.0\pm 7.8$  and  $13.9\pm 5.1$  ng/ml in the PPI and control groups, respectively.

Separate consideration of individual treatment group data at baseline, 1 and 3 months (Time Effect via one way RM-ANOVA) revealed that mean levels of 25-OH vitamin D and

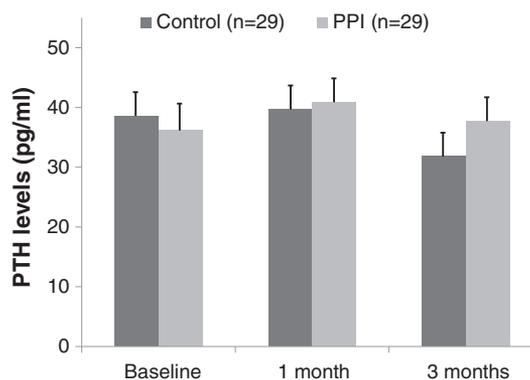


Fig. 2 – Comparison of parathyroid hormone (PTH) levels for the two groups at baseline ( $p=0.583$ ), 1 month ( $p=0.804$ ) and 3 months ( $p=0.186$ ). Error bars=standard error.

**Table 1 – Baseline characteristics.**

Biomarker/ characteristic	Mean±SD		p- value <sup>a</sup>
	Control group N=29	PPI group N=29	
Age (years)	33.6±7.6	32.8±7.5	0.691
BMI (kg/m <sup>2</sup> )	25.6±3.8	28.6±4.4	0.008
Creatinine (mg/dl)	0.85±0.15	0.84±0.14	0.653
Albumin (g/l)	47.8±2.3	47.9±2.3	0.822
Phosphate (mg/dl)	3.70±0.59	3.83±0.53	0.377
Calcium (mg/dl)	9.55±0.34	9.52±0.40	0.750
Ionized Calcium (mmol/l)	1.33±0.04	1.33±0.04	0.793
PTH (pg/ml)	38.6±19.1	36.2±13.3	0.583
25-OH-Vitamin D (ng/ml)	13.9±5.1	16.0±7.8	0.238
Osteocalcin (ng/ml)	23.5±6.3	25.7±7.3	0.215
CrossLaps (pg/ml)	228±100	300±140	0.028

<sup>a</sup> p values derived using two tailed independent t-test.

osteocalcin increased significantly in the control group; while mean 25-OH vitamin D and serum crosslaps levels increased, and phosphate levels decreased significantly in the PPI group (Table 2).

Two-way RM-ANOVA was also performed with adjustment for age, BMI, serum creatinine, average daily calcium intake, and vitamin D at baseline, and did not show a significant Time × Treatment interaction term on any of the parameters of interest (Table 2).

Multiple linear regressions on the biomarker data at 3months were largely consistent with the adjusted 2-way RM ANOVA analysis (Table 3). Briefly, PPI use was again shown not to affect any of these biomarkers. Our primary endpoint,

PTH at 3 months, was predicted by baseline PTH (P<0.001) and BMI (p<0.001) but not by PPI use (Table 3). Consistent multiple linear regression results were found when “change in biomarker”, defined as values on 3months minus baseline values, was used instead of actual values at 3months (Table S1 of online supplement).

#### 4. Discussion

The evidence incriminating PPIs in bone fracture is based primarily on observational case-control or prospective cohort studies [4,15,16]. Most such epidemiologic studies have identified a modest increase in risk of hip, wrist, and vertebral fractures while others have failed to substantiate this association particularly in those without other risk factors for fractures [5,6]. Many of these studies, however, had important limitations, including retrospective design, inability to control for important potential confounders, small sample size, heterogeneous population at risk (older than 18years of age vs. post-menopausal females vs. males), retrospective outcome (fracture) ascertainment and limited information on PPI exposure [4,8,9]. Two recent meta-analyses [8,9] have supported this modest risk association but reached different conclusions regarding the attributable risk. The meta-analysis by Yu et al. [8] observed an increased risk of fracture even with PPI exposure of less than 1year and estimated that 4.7% of hip fractures might be attributable to PPI use. The meta-analysis by Ngamruengphong et al. [9] failed to demonstrate an increased risk of fracture with longer duration of exposure, and estimated the effect size, if true, to

**Table 2 – Summary of One-Way (Time-Effect) and Two-Way (Time-, Group- and Interaction Effects) Repeated Measure ANOVA Results.**

Biomarker	Group N=29 each	Repeated Measurements (Mean±SD)			Analysis of Variance (p Values)			
		Time 1	Time 2	Time 3	1W-RM		2W-RM with Covariate Adjustment <sup>a,b</sup>	
		Baseline	1 mo	3 mo	w/in group (Time Effect)	w/in subj (Time Effect)	Between Subj (Group Effect)	Interaction
Albumin	Control	47.8±2.3	47.3±2.6	47.9±2.6	0.294	0.611	0.908	0.568
	PPI	47.9±2.3	47.1±2.6	46.9±2.4	0.057			
Phosphate	Control	3.70±0.59	3.53±0.53	3.66±0.51	0.322	0.445	0.760	0.634
	PPI	3.83±0.53	3.48±0.47	3.60±0.50	0.009			
Calcium	Control	9.55±0.34	9.50±0.44	9.53±0.38	0.828	0.414	0.241	0.888
	PPI	9.52±0.40	9.38±0.32	9.39±0.29	0.142			
Ionized Calcium	Control	1.33±0.04	1.34±0.04	1.35±0.05	0.163	0.107	0.251	0.307
	PPI	1.33±0.04	1.32±0.04	1.33±0.05	0.748			
PTH	Control	38.6±19.1	39.7±18.3	31.8±15.6	0.072	0.886	0.554	0.449
	PPI	36.2±13.3	40.9±19.2	37.7±17.5	0.323			
25-OH-vitamin D	Control	13.9±5.1	16.8±6.8	19.4±8.0	<0.001	0.045	0.971	0.210
	PPI	16.0±7.8	15.0±4.7	18.9±7.4	0.017			
Osteocalcin	Control	23.5±6.3	25.5±6.4	26.1±8.2	0.026	0.339	0.562	0.681
	PPI	25.7±7.3	26.8±8.5	26.4±7.8	0.600			
CrossLaps <sup>c</sup>	Control	228±100	243±128	266±157	0.351	0.575	0.050	0.650
	PPI	300±140	311±124	366±151	0.015			

<sup>a</sup> Covariates are: age, BMI, creatinine at baseline, daily calcium intake, and 25-OH-vitamin D at baseline.

<sup>b</sup> 25-OH-vitamin D level at baseline was not adjusted for as a covariate when calculating the 2 way RM ANOVA for 25-OH-vitamin D.

<sup>c</sup> “CrossLaps” stands for C-terminal cross-linked telopeptides of type I collagen.

**Table 3 – Multiple linear regression models for each of calcium and bone metabolism biomarkers.<sup>a</sup>**

Biomarker	Group (PPI effect)	Age	BMI	Daily calcium intake	Creatinine	PTH at baseline	Vitamin D at baseline	Osteocalcin at baseline	Crosslaps at baseline	Ionized calcium at baseline	Calcium at baseline	R <sup>2</sup>
PTH at 3 months	NS	NS	0.677 (<0.001)	NS	NS	0.438 (<0.001)	NS					0.859
Vitamin D at 3 months	NS	NS	NS	NS	12.690 (<0.001)	NS	0.546 (<0.001)					0.884
Osteocalcin at 3 months	NS	NS	NS	NS	NS	NS	0.186 (0.059) <sup>b</sup>	0.945 (<0.001)				0.957
CrossLaps at 3 months	NS	NS	NS	0.166 (0.035)	91.508 (0.088) <sup>b</sup>	NS	NS		0.681 (<0.001)			0.859
Ionized Calcium at 3 months	NS	NS	NS	NS	NS	NS	-0.002 (0.067) <sup>b</sup>			1.029 (<0.001)		0.999
Calcium at 3 months	NS	NS	NS	NS	1.030 (0.003)	0.006 (0.049)	NS				0.878 (<0.001)	0.999

Factors considered in multiple linear regression for each biomarker at 3 months were as follows: baseline levels of the same biomarker, treatment group (PPI or control), age (years), BMI (kg/m<sup>2</sup>), average daily calcium intake (mg/day), creatinine (mg/dl), PTH (pg/ml) at baseline, and Vitamin D (ng/ml) at baseline. Shaded areas represent variables that were not included in the biomarker-specific regression model.

<sup>a</sup> Values in table represent the unstandardized coefficients of the model predictor variables with corresponding p-values in parentheses.

<sup>b</sup> Nearly significant.

be very small with an estimated number-needed-to-harm (NNH) of 2672 for hip fracture and 337 for vertebral fracture per year, depending on baseline risk of patients, and cautioned against the potential of confounding by indication [9]. In the most recent report from the Nurses' Health Study [3], the risk of hip fracture was increased with PPI use after adjustment for body mass index, physical activity, and intake of calcium. However, the relation with PPI use differed by smoking history where current and past smokers had a 50% increase in fracture risk, while no increased risk was detected among women who never smoked.

To better elucidate the PPI–calcium metabolism association, it is essential to investigate its biologic plausibility by examining potential pathophysiologic mechanisms of PPI-associated bone fractures. These include decreased calcium absorption secondary to hypochlorhydria with or without secondary bacterial overgrowth [17], hypomagnesemia-associated inhibition of parathyroid function [18–20], and/or a direct effect of PPIs on H<sup>+</sup>-K<sup>+</sup> pumps in osteoclasts with possible abnormal repair of microfractures [21]. Whether one or more of these mechanisms are at play, the net effect should, in principle, lead to perturbation of calcium homeostasis and/or markers of bone turnover. Although conflicting, the bulk of evidence, including this study, does not support a direct relationship between PPI use and abnormal calcium homeostasis or bone metabolism. In this matched controlled study addressing the effect of daily intake of PPI on calcium and bone metabolism in healthy males, we could not identify any measurable effect on calcium or bone metabolism following 12 weeks of acid-suppressive therapy with PPIs. To date, there have been a total of 9 published clinical trials in the literature that assessed the direct effect of PPIs on calcium absorption or bone metabolism (Table 4). The studies are somewhat heterogeneous and have discor-

dant results. The duration of PPI therapy was a major limitation in most studies, with PPI use for less than 12 days in five studies [14,22–25]. In the remaining 4 studies, PPI use ranged from 2 weeks to 2 months [21,26–28]. Two studies had an intervention period of 2 months [21,26] with one restricted to dialysis patients [26]. Another limitation of the above trials was the relatively small number of participants ranging from 8 to 34 [23,28]. Interestingly, although some of these studies reported that PPIs decreased calcium absorption, the two that used the “gold standard” dual-isotope method to determine calcium absorption, one of which included 21 participants with one month PPI exposure, suggested that PPIs do not change calcium absorption [14,27]. Our study had the longest duration of PPI exposure and the largest number of participants of all published clinical trials investigating the effect of PPIs on calcium metabolism and is the only one to include a well-matched control arm.

Similarly, discordant results have been described in studies evaluating the effect of PPI on bone density and fracture risk. In the Women's Health Initiative, bone mineral density (BMD) changed only mildly at the hip after 3 years of PPI use without notable change in any other skeletal site. Of interest, the increase in fracture risk associated with PPI in that study was noted at the spine, wrist and forearm but not the hip [29]. A case–control study by Targownik et al. [15] reported a modest increase in risk of osteoporotic hip fractures after 5 years of PPI exposure, which oddly increased by another 3-fold after 2 or more additional years of exposure. This led the authors to state that the effect of exposure to PPI on osteoporosis-related fractures might be “similar in size to [the effect of] other established osteoporotic-fracture risk factors, such as smoking, low body mass index and excessive alcohol intake.” However, in cross-sectional and longitudinal analyses of the

**Table 4 – Clinical trials assessing the effect of PPIs on calcium absorption and bone metabolism (PPI=proton pump inhibitors).**

Study	Subjects	Intervention	Clinical trial	Methodology	Results
Serfaty-Lacrosniere et al. 1995 [22]	13 healthy adults: 8 omeprazole, 5 controls Median age 59years	Omeprazole 40mg daily for 7 days	Randomized controlled trial No placebo	After a standard test meal, calcium levels were measured using whole gut lavage	No difference in intestinal absorption of calcium → PPI had no effect on calcium absorption
Graziani et al. 1995 [23]	8 healthy men Mean age 38years	Omeprazole 60mg per day or placebo for 3 days	Crossover placebo-controlled trial	24h urinary calcium excretion & 5h postprandial increment in serum calcium on 3rd day of treatment	PPI group had significantly lower serum and urinary calcium levels than placebo → PPI decreased calcium absorption
Hardy et al. 1998 [26]	16 dialysis patients Mean age 61years	Omeprazole 20mg/d for 2months or 2months without PPI	Open crossover trial No placebo	Serum calcium levels measured weekly pre-dialysis	PPI group had significantly lower serum calcium levels → PPI decreased calcium absorption
Graziani et al. 2002 [24]	30 dialysis patients Mean age 57years	Omeprazole 60mg/day for 3 days	Open crossover trial No placebo	6h postprandial increment in serum total and ionized calcium during the third day of treatment	Disappearance of postprandial calcium peaks after PPI intake → PPI decreased calcium absorption
O'Connell et al. 2005 [25]	18 postmenopausal ♀ Mean age 76years	Omeprazole 20mg or placebo daily for 7 days	Randomized double-blind placebo controlled crossover	Fasting serum <sup>45</sup> Ca isotope level 5hours after consuming 500mg of <sup>45</sup> Ca carbonate	PPI significantly decreased the FCA over placebo → PPI decreased calcium absorption
Wright et al. 2010 [14]	12 young adults Age 18–45years	Esomeprazole 20mg or placebo twice weekly for 9 days	Randomized, double-blind placebo controlled crossover trial	1. Dual-stable calcium isotopes to assess absorption 2. PTH, vitamin D, total & ionized calcium; urinary calcium excretion	No change in FCA or in any other outcome → PPI had no effect on calcium absorption and metabolism
Hansen et al. 2010 [27]	21 ♀ at least 5 years past the onset of menopause Mean age 58years	Omeprazole 40mg daily for 30days	No control group	1. Dual-stable calcium isotope 2. serum PTH, 25 (OH) vitamin D, and 1,25 (OH) <sub>2</sub> vitamin D; and urinary levels of crosslaps	No change in FCA or in any other outcome → PPI had no effect on calcium absorption and metabolism nor on bone resorption
Mizunashi et al. 1993 [21]	32 patients with history of gastric ulcer: 19 in study group 13 in control group	Omeprazole 20mg daily for 8 weeks	Control group: no treatment	Urinary calcium & hydroxyproline levels; serum PTH, osteocalcin, ALP & TRAP levels at baseline & after 8 wks	PPI group: ↓ urinary calcium & hydroxyproline, ↑ serum PTH, osteocalcin, ALP & TRAP → PPI decreased bone resorption
Kocsis et al. 2002 [28]	34 children aged 2-18years	Omeprazole 20mg daily for 2 weeks	No control group	Serum ALP, osteocalcin, crosslaps urinary calcium excretion	No change in any outcome → PPI had no effect on bone metabolism

FCA=Fractional calcium absorption; ALP=Alkaline phosphatase; TRAP=tartrate-resistant acid phosphatase.

Manitoba BMD database involving a large number of patients, the same group reported that long-term use of PPI for up to 5 years was not associated with osteoporosis at the hip or the lumbar spine, nor with any significant decrease in bone density at either skeletal site. The latter observations led the authors to conclude that the association between PPI use and

hip fracture is probably related to factors independent of osteoporosis [30].

Fewer studies have examined the direct effect of PPIs on skeletal metabolism after earlier *in vitro* studies [31] suggested that omeprazole may inhibit bone resorption. An *in vivo* study involving 32 patients with a history of gastric ulcers on a

maintenance dose of H<sub>2</sub>RAs changed to either omeprazole or no treatment paradoxically found an increase in serum tartrate-resistant acid phosphatase, serum alkaline phosphatase, and osteocalcin suggesting an increase in bone turnover with omeprazole treatment [21]. The effect of long-term intake of very high dose PPI (omeprazole 30mg/kg × 8 weeks) on bone turnover was more recently reported in an experimental rat model with analysis of the signaling pathway involved in osteoclast differentiation, bone resorption, bone formation, BMD, and alteration of trabecular bone microstructure. In the absence of a calcium-deficient diet, PPI did not alter signaling pathway of osteoclast differentiation, bone formation or resorption, BMD or bone microstructure [32]. Combined, the above studies suggest that PPI use is not associated with osteoporosis or accelerated bone mineral loss, and that the association with bone fracture is related to factors independent of calcium absorption, bone turnover and osteoporosis.

The observation of an increased risk of fractures associated with PPI use remains therefore largely unproven. Some studies have suggested that hypergastrinemia caused by hypochlorhydria may lead to hyperparathyroidism [33,34] which will in turn increase bone resorption and decrease bone mineral density. Other suggested mechanisms include nutrient malabsorption due to secondary bacterial overgrowth [17], vitamin B12 malabsorption [35] leading to peripheral neuropathies – and increased risk of falls – or to an increase in blood homocysteine levels leading to deleterious effects on cross-linking of skeletal collagen and more fragile bone that is easier to fracture. Severe hypomagnesemia and concomitant hypoparathyroidism were recently described as very rare adverse effects of long-term PPI use, as reported in only 2 case studies [18,19]. Such metabolic abnormalities have been described to affect bone remodeling in animal models. Kenney et al. found that severe hypomagnesemia can cause a decrease in femur strength and fracture threshold without any change in bone weight, diameter and cross-sectional area in rats [20]. However, Rude et al. showed that reproducing less severe hypomagnesemia in rats, similar to that found in humans, leads to hypoparathyroidism, a decrease in osteoblasts and increase in osteoclasts causing osteoporosis [36]. All of the above suggested mechanisms should in principle result in changes in calcium homeostasis and/or bone metabolism, none of which were detected in this study. It has been suggested that the association of increased fractures with PPI use may simply be the result of confounding by indication of use as studies have shown that patients receiving chronic acid suppression therapy including PPIs have significantly more medical illnesses than those not on such therapy [37] and this association could simply be a marker of a general poorer health and multiple comorbidities.

Our study has some potential limitations. The study population was younger and healthier than populations where an increased risk of fractures was reported with PPI use. Therefore, our results cannot be applied to the general population, or to subjects who have experienced or are at risk for fractures. Only randomized controlled trials, including older subjects and females, can provide the ultimate evidence for generalization of our findings. Study subjects in both groups were largely vitamin D insufficient, and although 25-

OH vitamin D increased similarly in both groups at 3 months, most likely because of seasonal changes, they remained similarly low in both groups, and are not different from what is reported in the Lebanese population, including young adults [38]. Furthermore, the low calcium intake and hypovitaminosis D would have favored the unmasking of the deleterious effect of PPI on calcium absorption, and thus on ionized calcium and PTH levels, especially that calcium absorption is not yet at a minimum at the 25-OH vitamin D levels and calcium intakes of our patients [39–41]. Although there is some disagreement on serum 25-OH vitamin D levels above which calcium absorption is optimized, robust evidence by Need et al. reveals radio calcium absorption (fraction/hour) to plateau at serum 25-OH vitamin D concentrations above 20–25 nmol/l, that is 8–10 ng/ml, in elderly individuals [39]. This point was also confirmed by the IOM Committee in their 2011 report when they examined the relevant evidence to that effect [41]. Children may even do better. Indeed, two Nigerian children with rickets and serum 25-OHD of 5 ng/ml, were shown to have preserved fractional calcium absorption of 76% and 88% [40]. Therefore, we believe that calcium absorption was not diminished in our sample population at the start of the study since mean circulating 25-OH vitamin D concentrations in our study subjects, at study entry, were above these cut-offs. Despite low circulating 25-OH vitamin D levels, PTH levels were normal, an observation that is consistent with findings in other study groups from our population [42]. Indeed, our group has shown PTH levels to be higher in older compared to younger individuals, with comparable low vitamin D levels [43]. This reflects the multidimensional regulation of calcium absorption by many other variables, such as age, calcium intake, 1,25(OH)<sub>2</sub> vitamin D levels, and polymorphisms of specific receptors/transporters (like VDR, ER, TRPV6). Although the study used three different commercially-available PPIs, which were readily available for study use, the epidemiologic evidence and pathophysiologic mechanisms for a putative negative effect of PPI on bone are indeed those of a drug class effect. Moreover, approved standard dose PPIs, equipotent in terms of acid suppression, were used in our study. Serum levels of biochemical markers were also not always measured fasting, nor at the same exact time for all patients and at every visit. This is because study subjects were young working individuals who came in for their visits as allowed by their work schedule. We would however anticipate that the impact of fasting state, activity level, and time of the day has occurred randomly, be equally distributed amongst both study groups, and thus would not have biased study results substantially. In addition, all blood studies were taken before noon, between 8am and 12pm, and this should have minimized the potential bias or noise introduced by time. Finally, “Time of day” was not significantly different between the 2 study groups and when entered as a predictor in the multivariate model, it did not affect the results on markers, and was therefore removed from the final regression model (data not shown). We do not have any explanation for the higher serum CrossLaps at study entry in the PPI group, nor has it been described, to our knowledge, in subjects with heartburn. Although the study subjects were heavier than the control group, there are no recommendations to adjust for body size that we are aware of [44]. There was a small

difference in oral calcium intake between the 2 study groups, but that was corrected for in the 2W-RM-ANOVA and linear regression. We did not measure calcium absorption, but the lack of any change in PTH and serum ionized calcium levels serially within the PPI group and the lack of any difference in these variables between the two groups render the presence of any clinically significant difference in absorption unlikely. Our results are also consistent with our review of the effect of PPI on calcium absorption in various trials showing a significant effect in large part in a subset of patients who were usually older subjects and/or had other co-morbidities that affect mineral metabolism, as detailed in Table 4. Lastly, the fact that our study did not show an effect on serum ionized calcium, PTH and bone remodeling markers raises the question of a false-negative result due to a type 2 statistical error. This is however not the case because our sample size was projected from anticipated baseline mean PTH ( $\pm$ SDs) levels in young subjects, and their changes based on PPI absorption studies [14]. Furthermore, Wright et al. [14] projected a needed sample size of only 12 subjects per treatment arm to demonstrate a 7% decrease in calcium absorption (power 90%), in a population group with similar age and baseline PTH levels. Our sample size was twice that estimate, and should have unmasked differences in view of a significantly lower estimated calcium intake, the use of a higher PPI dose, and for a longer duration (3 months as opposed to 3 weeks).

In conclusion, use of standard doses of PPIs for 3 months – the longest duration for any clinical trial to date – is not associated with significant changes in serum markers of calcium homeostasis or bone turnover in healthy young males. The association of PPI use with bone fracture remains largely unexplained from a pathophysiologic aspect and could arguably be the result of confounding variables (including comorbidities that are unaccounted for) given the inconsistent and conflicting results. This conclusion is in line with the position statement of the Canadian Association of Gastroenterology which found no persuasive evidence that the association is causal and stated that the current data do not support a particular change in prescribing PPI therapy due to concerns about the risk of hip fractures [45].

### Author contributions

A. Sharara: Study idea, concept, design and supervision; interpretation of data; review of the literature; drafting of the manuscript. Guarantor of the study.

M. El-Halabi: patient recruitment, data collection and statistical analysis, interpretation and tabulation of data, review of literature, drafting of the manuscript.

O. Ghaith, N. Mansour, A. Malli: patient recruitment, interpretation and tabulation of data, critical review of the manuscript.

R. Habib: Statistical analysis, interpretation of data, critical review of the manuscript.

G. El-Hajj Fuleihan: Study design, review of data, interpretation of results, and critical review of the relevant literature and of manuscript.

All authors approved the submitted version of the manuscript.

### Funding

The study was funded in part by a grant from the University Research Board (URB) at the American University of Beirut and in part by a grant from Astra-Zeneca. URB and Astra-Zeneca were the only funding agencies of the study and their role was restricted solely to funding the study. They had nothing to do with the study idea, concept, design, recruitment, analysis, interpretation or write up of the manuscript.

### Conflict of interest

The authors have no personal conflicts of interests to declare.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.metabol.2012.09.011>.

### REFERENCES

- [1] Sandler RS, Everhart JE, Donowitz M, et al. The burden of selected digestive diseases in the United States. *Gastroenterology* 2002;122:1500–11.
- [2] Jensen RT. Consequences of long-term proton pump blockade: insights from studies of patients with gastrinomas. *Basic Clin Pharmacol Toxicol* 2006;98:4–19.
- [3] Khalili H, Huang ES, Jacobson BC, et al. Use of proton pump inhibitors and risk of hip fracture in relation to dietary and lifestyle factors: a prospective cohort study. *BMJ* 2012;344:e372.
- [4] Kwok CS, Yeong JK, Loke YK. Meta-analysis: risk of fractures with acid-suppressing medication. *Bone* 2011;48:768–76.
- [5] Kaye JA, Jick H. Proton pump inhibitor use and risk of hip fractures in patients without major risk factors. *Pharmacotherapy* 2008;28:951–9.
- [6] Yu EW, Blackwell T, Ensrud KE, et al. Acid-suppressive medications and risk of bone loss and fracture in older adults. *Calcif Tissue Int* 2008;83:251–9.
- [7] U.S. Food and Drug Administration. FDA drug safety communication: possible increased risk of fractures of the hip, wrist, and spine with the use of proton pump inhibitors.
- [8] Yu EW, Bauer SR, Bain PA, et al. Proton pump inhibitors and risk of fractures: a meta-analysis of 11 international studies. *Am J Med* 2011;124:519–26.
- [9] Ngamruengphong S, Leontiadis GI, Radhi S, et al. Proton pump inhibitors and risk of fracture: a systematic review and meta-analysis of observational studies. *Am J Gastroenterol* 2011;106:1209–18 [quiz 19].
- [10] Recker RR. Calcium absorption and achlorhydria. *N Engl J Med* 1985;313:70–3.
- [11] Sheraly AR, Lickorish D, Sarraf F, et al. Use of gastrointestinal proton pump inhibitors to regulate osteoclast-mediated resorption of calcium phosphate cements in vivo. *Curr Drug Deliv* 2009;6:192–8.
- [12] Gass ML, Kagan R, Kohles JD, et al. Bone turnover marker profile in relation to the menstrual cycle of premenopausal healthy women. *Menopause* 2008;15:667–75.
- [13] Salamoun MM, Kizirian AS, Tannous RI, et al. Low calcium and vitamin D intake in healthy children and adolescents and their correlates. *Eur J Clin Nutr* 2005;59:177–84.
- [14] Wright MJ, Sullivan RR, Gaffney-Stomberg E, et al. Inhibiting gastric acid production does not affect intestinal calcium

- absorption in young, healthy individuals: a randomized, crossover, controlled clinical trial. *J Bone Miner Res* 2010;25: 2205–11.
- [15] Targownik LE, Lix LM, Metge CJ, et al. Use of proton pump inhibitors and risk of osteoporosis-related fractures. *CMAJ* 2008;179:319–26.
- [16] Yang YX, Lewis JD, Epstein S, et al. Long-term proton pump inhibitor therapy and risk of hip fracture. *JAMA* 2006;296: 2947–53.
- [17] Lewis SJ, Franco S, Young G, et al. Altered bowel function and duodenal bacterial overgrowth in patients treated with omeprazole. *Aliment Pharmacol Ther* 1996;10:557–61.
- [18] Epstein M, McGrath S, Law F. Proton-pump inhibitors and hypomagnesemic hypoparathyroidism. *N Engl J Med* 2006;355:1834–6.
- [19] Broeren MA, Geerdink EA, Vader HL, et al. Hypomagnesemia induced by several proton-pump inhibitors. *Ann Intern Med* 2009;151:755–6.
- [20] Kenney MA, McCoy H, Williams L. Effects of magnesium deficiency on strength, mass, and composition of rat femur. *Calcif Tissue Int* 1994;54:44–9.
- [21] Mizunashi K, Furukawa Y, Katano K, et al. Effect of omeprazole, an inhibitor of H<sup>+</sup>, K<sup>(+)</sup>-ATPase, on bone resorption in humans. *Calcif Tissue Int* 1993;53:21–5.
- [22] Serfaty-Lacrosniere C, Wood RJ, Voytko D, et al. Hypochlorhydria from short-term omeprazole treatment does not inhibit intestinal absorption of calcium, phosphorus, magnesium or zinc from food in humans. *J Am Coll Nutr* 1995;14:364–8.
- [23] Graziani G, Como G, Badalamenti S, et al. Effect of gastric acid secretion on intestinal phosphate and calcium absorption in normal subjects. *Nephrol Dial Transplant* 1995;10:1376–80.
- [24] Graziani G, Badalamenti S, Como G, et al. Calcium and phosphate plasma levels in dialysis patients after dietary Ca-P overload. Role of gastric acid secretion. *Nephron* 2002;91: 474–9.
- [25] O'Connell MB, Madden DM, Murray AM, et al. Effects of proton pump inhibitors on calcium carbonate absorption in women: a randomized crossover trial. *Am J Med* 2005;118:778–81.
- [26] Hardy P, Sechet A, Hottelart C, et al. Inhibition of gastric secretion by omeprazole and efficiency of calcium carbonate on the control of hyperphosphatemia in patients on chronic hemodialysis. *Artif Organs* 1998;22:569–73.
- [27] Hansen KE, Jones AN, Lindstrom MJ, et al. Do proton pump inhibitors decrease calcium absorption? *J Bone Miner Res* 2010;25:2510–9.
- [28] Kocsis I, Arato A, Bodanszky H, et al. Short-term omeprazole treatment does not influence biochemical parameters of bone turnover in children. *Calcif Tissue Int* 2002;71:129–32.
- [29] Gray SL, LaCroix AZ, Larson J, et al. Proton pump inhibitor use, hip fracture, and change in bone mineral density in postmenopausal women: results from the Women's Health Initiative. *Arch Intern Med* 2010;170:765–71.
- [30] Targownik LE, Lix LM, Leung S, et al. Proton-pump inhibitor use is not associated with osteoporosis or accelerated bone mineral density loss. *Gastroenterology* 2010;138:896–904.
- [31] Tuukkanen J, Vaananen HK. Omeprazole, a specific inhibitor of H<sup>+</sup>-K<sup>+</sup>-ATPase, inhibits bone resorption in vitro. *Calcif Tissue Int* 1986;38:123–5.
- [32] Moon JM, Park JA, Lee BJ, et al. The effect of PPI (proton pump inhibitors) on the bone mineral density, bone microstructure and bone metabolism of rat. *Gut* 2010;59(Suppl. III):A110.
- [33] Gagnemo-Persson R, Samuelsson A, Hakanson R, et al. Chicken parathyroid hormone gene expression in response to gastrin, omeprazole, ergocalciferol, and restricted food intake. *Calcif Tissue Int* 1997;61:210–5.
- [34] Yang YX. Proton pump inhibitor therapy and osteoporosis. *Curr Drug Saf* 2008;3:204–9.
- [35] Valuck RJ, Ruscini JM. A case-control study on adverse effects: H<sub>2</sub> blocker or proton pump inhibitor use and risk of vitamin B12 deficiency in older adults. *J Clin Epidemiol* 2004;57:422–8.
- [36] Rude RK, Singer FR, Gruber HE. Skeletal and hormonal effects of magnesium deficiency. *J Am Coll Nutr* 2009;28:131–41.
- [37] Jacobson BC, Ferris TG, Shea TL, et al. Who is using chronic acid suppression therapy and why? *Am J Gastroenterol* 2003;98:51–8.
- [38] Arabi A, El Rassi R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. *Nat Rev Endocrinol* 2010;6:550–61.
- [39] Need AG, O'Loughlin PD, Morris HA, et al. Vitamin D metabolites and calcium absorption in severe vitamin D deficiency. *J Bone Miner Res* 2008;23:1859–63.
- [40] Thacher TD, Abrams SA. Relationship of calcium absorption with 25(OH)D and calcium intake in children with rickets. *Nutr Rev* 2010;68:682–8.
- [41] IoM Food and Nutrition Board (2011) Dietary reference intakes for calcium and vitamin D. pp. 263–7. The National Academies Press, Washington DC.
- [42] Arabi A, Mahfoud Z, Zahed L, El-Onsi L, El-Hajj Fuleihan G. Effect of age, gender and calciotropic hormones on the relationship between vitamin D receptor gene polymorphisms and bone mineral density. *Eur J Clin Nutr* 2010;64:383–91.
- [43] Arabi A, Baddoura R, El-Rassi R, El-Hajj Fuleihan G. Age but not gender modulates the relationship between PTH and vitamin D. *Bone* 2010;47:408–12.
- [44] Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int* 2011;22:391–420.
- [45] Moayyedi P, Cranney A. Hip fracture and proton pump inhibitor therapy: balancing the evidence for benefit and harm. *Am J Gastroenterol* 2008;103:2428–31.