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ORIGINAL ARTICLE

Higher plasma platelet-activating factor levels are associated with increased risk of vertebral fracture and lower bone mineral density in postmenopausal women

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Abstract Despite experimental and animal evidence showing the detrimental effects of platelet-activating factor (PAF) on bone metabolism, there are no clinical studies relating PAF to osteoporosis-related phenotypes. This case–control study investigates the association between plasma PAF, osteoporotic vertebral fracture (VF), and bone mineral density (BMD) in postmenopausal Korean women. Among 474 eligible women not taking any drug or having any disease that could affect bone metabolism, we identified 73 cases defined as subjects with radiological VF. The controls were randomly selected from the remaining 401 subjects and matched 1:1 to cases in terms of both age and body mass index (BMI). Lateral thoracolumbar radiographs, BMD, and plasma PAF levels were determined for all subjects. Postmenopausal women with VF demonstrated 34.6 % higher plasma PAF levels than subjects without VF after adjusting for age, BMI, smoking habits, alcohol intake, regular exercise, and parental history of osteoporotic fractures ($P = 0.021$). Multiple logistic regression analyses revealed that the odds ratio for VF linearly increased across increasing PAF quartiles (P for trend $= 0.040$) and the odds for VF were 2.88-fold higher in subjects in the highest quartile in comparison with those in the lowest quartile (95 % CI 1.04–8.01). Plasma PAF levels were inversely correlated with BMD at various sites ($\gamma = -0.253$ to -0.176, $P = 0.003$–0.041). These findings suggest that plasma PAF may be a potential biomarker for predicting poor bone health in postmenopausal women.

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Introduction

Bone is a highly dynamic tissue that constantly changes and regenerates throughout life in response to biochemical and mechanical signals. Under normal conditions, the balance between osteoclastic breakdown and osteoblastic rebuilding during bone remodeling is tightly controlled in a local, coordinated, and sequential manner [1, 2]. However, after menopause, excessive bone resorption that is not adequately balanced by bone formation leads to bone loss, which is mainly due to abrupt declines in estrogen levels [3]. The resulting postmenopausal osteoporosis and osteoporotic fracture are important medical problems worldwide [4–7].

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent phospholipid mediator that triggers various cellular functions by binding to PAF receptor (PAFR), which is a G protein-coupled receptor [8, 9]. This protein exerts diverse biological effects and has been implicated in several pathologic conditions including platelet activation, airway constriction, hypotension, and systemic anaphylaxis [10–12]. Evidence now indicates that PAF plays an important role in bone metabolism as well. PAF has been detected in inflamed human gingival tissue [13] and the arthritic joint fluids of rabbits with acute antigen-induced arthritis [14], suggesting its association with diseases that affect bone resorption. PAF directly enhances osteoclast motility and resorptive activity according to in vitro studies [15, 16]. Furthermore, an animal study that used PAFR-deficient mice in a postmenopausal osteoporosis model reported that estrogen depletion

enhances PAF production as a unique autocrine factor for osteoclast function, including bone resorption activity [17]. However, despite the clear implications of the effects of PAF on bone metabolism, there are no clinical studies relating PAF to osteoporosis-related phenotypes.

We performed this current case–control study to validate previous in vitro and in vivo data and investigate the associations between plasma PAF, osteoporotic vertebral fracture (VF), and bone mineral density (BMD) at various sites in postmenopausal Korean women.

Materials and methods

Study subjects and protocol

Study subjects were postmenopausal Korean women who were seen at the Asan Medical Center (AMC; Seoul, Korea) between January 2006 and June 2010. All women either visited the osteoporosis clinic for concerns regarding the possibility of osteoporosis or were referred for osteoporosis that had been diagnosed during routine examinations. Menopause was defined as the absence of menstruation for at least 1 year and was confirmed by measuring serum follicle-stimulating hormone levels. Women with premature menopause (<40 years of age) and those who had taken drugs that could affect bone metabolism (e.g., bisphosphonate, systemic glucocorticoid, hormone-replacement therapy) for more than 6 months or within the previous 12 months were excluded from the study. Subjects with diseases that might affect bone metabolism (e.g., cancer, hyperparathyroidism, rheumatoid arthritis) were also excluded. Osteophyte formation greater than Nathan classification grade 4 and/or severe facet joint osteoarthritis in the lumbar spine, as determined using conventional spine radiography, were also excluded. Subjects were also excluded if they had a fever (oral temperature $\geq$38.0 °C) or abnormal findings on complete blood counts (e.g., <4.0 or >10.0 $\times$ 10^9/L leukocytes, <150 or >350 $\times$ 10^9/L platelets). Finally, abnormal liver, kidney, or thyroid function, and abnormal serum calcium or phosphorus concentrations were reasons also for exclusion. These criteria were used to rule out systemic illness.

We obtained patient information using a questionnaire in order to assess smoking habits (current smoker), alcohol use ($\geq$3 units/day), regular outdoor exercise ($\geq$30 min/day), history of medication use, previous medical or surgical procedures, and reproductive status (including menstruation). We collected information about parental history of fragility fractures using an interviewer-assisted questionnaire in order to exclude all non-osteoporotic fractures (i.e., fractures due to cancer or an accident, such as a motor vehicle accident, and all fractures affecting the fingers, faces, skull, and toes). After excluding these subjects, 474 women were considered eligible for participation. Among these women, we identified 73 cases defined as subjects with radiological VF. To perform this case–control study, controls were randomly selected from the remaining 401 subjects and matched 1:1 to cases according to both age (within 2.5 years) and body mass index (BMI; within 1.0 kg/m^2). This study was approved by the AMC Institutional Review Board. All enrolled subjects provided written informed consent.

Radiological assessment of osteoporotic VFs

We examined any prevalent morphological VFs in our study subjects using lateral thoracolumbar (T4–L4) radiographs, which were obtained in accordance with the recommendations of the Working Group on Vertebral Fractures [18]. Radiographs were assessed at AMC by expert radiologists who were blind to this study. VF was quantitatively defined as >20 % reduction in any measured vertebral height (anterior, middle, or posterior) [19]. Fractures clearly caused by major trauma, such as motor vehicle accidents or falls from standing height or higher, were also excluded.

BMD measurement

Areal BMD (g/cm^2) was measured at the lumbar spine (L1–L4) and proximal femur (femoral neck, total femur, trochanter, shaft, and ward) by dual energy X-ray absorptiometry (DXA) using Lunar equipment (running software version 9.30.044; Prodigy, Madison, WI, USA). The precision values of the equipment, in terms of the coefficients of variations (CVs), were 0.67 and 1.25 % for the lumbar spine and femoral neck, respectively, which were determined in 17 volunteers who were not enrolled in this study. Each volunteer underwent five scans on the same day, and were required to get on and off the table between examinations.

BMD measurements provided absolute values for each anatomic site, which were then compared with those of healthy young Korean adults (T score). The reference population consisted of 590 women between the ages of 20–39 years, and the reference BMD values at the lumbar spine, femoral neck, total femur, trochanter, and ward were 1.148 $\pm$ 0.119, 0.942 $\pm$ 0.121, 0.974 $\pm$ 0.120, 0.737 $\pm$ 0.112, and 0.841 $\pm$ 0.138 g/cm^2, respectively. Using the World Health Organization definition, osteopenia was diagnosed as $-2.5 < T$ score < -1.0 standard deviations (SD), and osteoporosis was diagnosed as T score ≤ -2.5 SD at the lumbar spine, femoral neck, or total femur.

Biochemical measurements

Serum calcium concentrations were measured using the cresolphthalein complexone method on a Toshiba 200FR

Autoanalyzer (Toshiba Medical Systems Co., Ltd, Tokyo, Japan). The intra- and inter-assay CVs were 1.24 and 2.06 %, respectively, and the reference interval was 2.07–2.50 mmol/L. Serum phosphorus concentrations were measured using the phosphomolybdate ultraviolet method (Toshiba 200FR instrument). The intra- and inter-assay CVs were 1.28 and 2.54 %, respectively, and the reference interval was 0.81–1.45 mmol/L. Serum alkaline phosphatase (ALP) concentrations were measured according to the Bowers and McComb method at 37 °C in AMP buffer (Toshiba 200 FR Autoanalyzer). The intra- and inter-assay CVs were 0.7 and 1.3 %, respectively, and the reference interval was 40–120 U/L.

Measurement of plasma PAF concentrations

Fasting venous blood samples were obtained. After centrifugation, we carefully collected the supernatants to exclude cellular components. All samples demonstrating hemolysis or clotting were discarded. Plasma samples were stored at -80 °C prior to determining PAF concentrations. PAF levels were measured using the PAF competitive ELISA kit (Echelon Biosciences Inc, Salt Lake, UT, USA) according to the manufacturer's instructions. The kit's lower limit of detection was 82.1 pg/mL, and the intra- and inter-assay coefficients of variations (CVs) were 6.3 and 6.7 %, respectively. Duplicate samples were assayed, and all results are reported as the mean values.

Statistical analysis

All data are presented as the mean ± standard deviation (SD) or numbers and percentages, unless otherwise specified. The baseline characteristics of the cases and controls were compared using Student's *t* tests for continuous variables and chi square tests for categorical variables. Multivariate-adjusted least-square mean PAF levels [95 % confidence interval (CI)] in terms of VF status were estimated using analysis of covariance (ANCOVA) after adjusting for well-known demographic and behavioral factors that might affect bone metabolism. These factors included age, BMI, current smoking habits, alcohol intake, regular outdoor exercise, and parental history of osteoporotic fracture. This is an age- and BMI-matched case–control study; however, because age and BMI are well-known and very strong determinants of osteoporotic fracture and/or bone mass, we were concerned about the possible residual effects of age and BMI on bone metabolism, even after matching, and included these factors in the multivariate-adjusted model. To test our hypothesis that higher PAF levels might be associated with the risk of osteoporotic VF, we categorized subjects into four groups according to plasma PAF concentrations. We then performed multiple logistic regression analyses to generate odds ratios (ORs) (95 % CI) and compare the odds of developing VF in subjects in the higher three PAF quartiles to subjects in the lowest quartile after adjusting for confounders. The correlations between plasma PAF concentrations and BMD values at various sites, and plasma PAF and total serum ALP concentrations, were analyzed using Pearson's and partial correlation analyses before and after adjusting for confounders, respectively. For these analyses, plasma PAF concentrations were log-transformed because the distribution was positively skewed. All statistical analyses were performed using SPSS statistical software (SPSS Inc. Chicago, IL, USA), and $P < 0.05$ is considered statistically significant.

Results

Clinical characteristics of the study subjects according to their VF status

The baseline characteristics of the study subjects are shown in Table 1. The mean ages for the 73 cases and 73 controls were 65.2 ± 6.8 years (range 47–77 years) and 65.0 ± 7.0 years (range 49–79 years), respectively. There were no significant differences between groups in terms of the measured calcium and phosphorus levels, weight, height, BMI, behavioral factors, or parental history of osteoporotic fractures. The BMD values obtained at all measured sites were significantly lower in subjects with VF ($P = 0.029$ to <0.001). Consistently, the percentage of subjects with osteoporosis was higher among subjects with VF ($P = 0.006$).

Differences in plasma PAF levels between the cases and controls

Prior to adjusting for age, BMI, current smoking habits, alcohol intake, regular outdoor exercise, and parental history of osteoporotic fractures, subjects with VF demonstrated 33.9 % higher plasma PAF levels ($P = 0.017$) (Fig. 1). After adjusting for all of these factors, subjects with VF demonstrated 34.6 % higher plasma PAF levels in comparison with those without VF, and statistical significance persisted ($P = 0.021$).

Risk for osteoporotic VF according to plasma PAF quartiles

When subjects were categorized into four quartiles, the prevalence of VF in the lowest PAF quartile (Q1) to the highest PAF quartile (Q4) was 43.2, 44.4, 48.6, and 63.9 %, respectively. Multiple logistic regression analyses revealed that the ORs for VF linearly increased with increasing PAF quartiles

Table 1 Baseline characteristics of the study subjects according to vertebral fracture (VF) status

Characteristic	Subjects with VF ($n = 73$)	Subjects without VF ($n = 73$)	P
Age (years)	65.2 ± 6.8	65.0 ± 7.0	0.811
Weight (kg)	56.4 ± 7.5	56.5 ± 6.8	0.986
Height (cm)	153.9 ± 5.7	153.9 ± 5.3	0.938
Body mass index (kg/m^2)	23.8 ± 2.5	23.8 ± 2.6	0.879
Current smoker, no. (%)	1 (1.4)	4 (5.5)	0.172
Alcohol intake ≥3 U/day, no. (%)	7 (9.6)	5 (6.8)	0.547
Exercise ≥30 min/day, no. (%)	29 (39.7)	28 (38.4)	0.865
Parental history of osteoporotic fracture (%)	11 (15.1)	8 (11.0)	0.461
Bone mineral density (g/cm^2)			
Lumbar spine	0.804 ± 0.136	0.884 ± 0.123	<0.001
Femoral neck	0.698 ± 0.106	0.737 ± 0.108	0.029
Total femur	0.743 ± 0.123	0.812 ± 0.106	<0.001
Trochanter	0.582 ± 0.119	0.632 ± 0.106	0.008
Shaft	0.893 ± 0.154	0.989 ± 0.128	<0.001
Ward	0.476 ± 0.108	0.523 ± 0.122	0.015
WHO definition, no. (%)[a]			0.006
Normal	1 (1.4)	8 (11.0)	
Osteopenia	26 (35.6)	35 (47.9)	
Osteoporosis	46 (63.0)	30 (41.1)	
Corrected calcium level (mg/dL)[b]	9.0 ± 0.4	9.0 ± 0.3	0.599
Phosphorus (mg/dL)	3.9 ± 0.4	3.8 ± 0.5	0.795

Data are expressed as mean ± standard deviation

SI conversion factors: to convert mg/dL to mmol/L of calcium, multiply by 0.2495; to convert mg/dL to mmol/L for phosphorus, multiply by 0.3229

Fx fracture, *WHO* World Health Organization

[a] Osteopenia was diagnosed as $-2.5 < T$ score < -1.0 SD, and osteoporosis was diagnosed as T score ≤ -2.5 SD at any of the sites on the lumbar spine, femoral neck, or total femur

[b] Corrected calcium concentration (mg/dL) = total calcium concentration (mg/dL) + 0.8 × [4.0 g/dL–serum albumin concentration (g/dL)] (mg/dL)

(P for trend = 0.040), and the odds for VF were 2.88-fold higher in subjects in the highest PAF quartile in comparison with those in the lowest PAF quartile after adjusting for age, BMI, current smoking, alcohol intake, regular outdoor exercise, and parental history of osteoporotic fractures (Fig. 2).

Correlation between plasma PAF concentrations with BMD values at various sites and total serum ALP concentrations in the study subjects

Plasma PAF levels were inversely correlated with BMD values at the total femur, trochanter, and shaft ($\gamma = -0.233$ to -0.191, $P = 0.005$–0.026). However, the association between plasma PAF levels and BMD value at the lumbar spine, femoral neck, and ward were marginally significant ($\gamma = -0.163$ to -0.156, $P = 0.054$–0.062) (Table 2). After adjusting for potential confounders, plasma PAF levels were inversely correlated with BMD values at all sites ($\gamma = -0.253$ to -0.176, $P = 0.003$–0.041) except the femur neck showing marginal significance ($\gamma = -0.163$, $P = 0.055$).

Partial correlation analysis revealed that serum total ALP levels increased in a dose–response manner along with increasing plasma PAF levels after considering potential confounders ($\gamma = 0.199$, $P = 0.020$).

Discussion

In this age- and BMI-matched case–control study of postmenopausal women, we reported that plasma PAF levels were markedly higher in subjects with osteoporotic VF in comparison with those without VF and were inversely correlated with the BMD values at various sites after adjusting for potential confounders. Furthermore, the risk of osteoporotic VF increased 2.88-fold among subjects in the highest PAF quartile in comparison with those in the lowest PAF quartile. To the best of our knowledge, this is the first

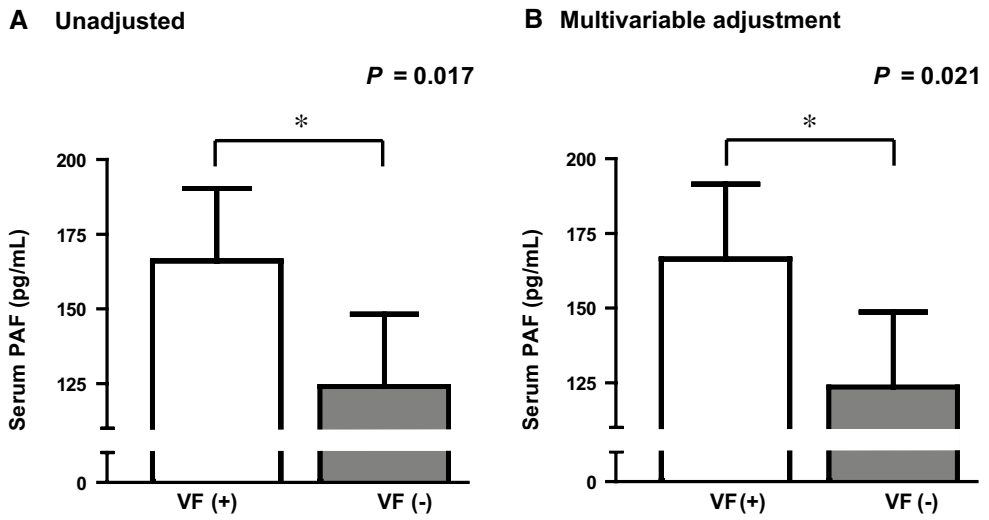

Fig. 1 Differences in plasma platelet-activating factor (PAF) levels according to vertebral fracture (VF) status **a** before and **b** after adjusting for multivariable confounding factors. Multivariable confounding factors included age, body mass index, current smoking habits, alcohol intake (≥3 units/day), regular outdoor exercise (≥30 min/day), and parental history of osteoporotic fracture. Multivariate-adjusted least-square mean (95 % CI) PAF levels were generated and compared using analysis of covariance (ANCOVA). *$P < 0.05$

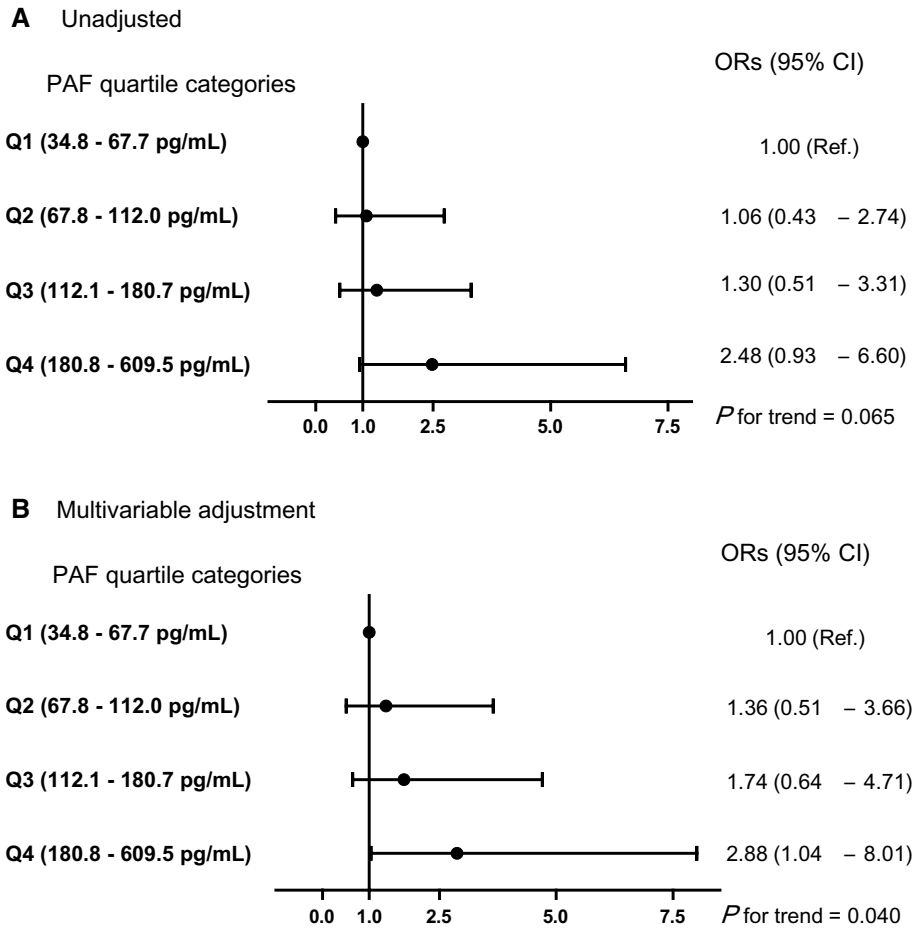

Fig. 2 Odds ratios (95 % CI) for osteoporotic vertebral facture according to plasma platelet-activating factor (PAF) quartile categories **a** before and **b** after adjusting for confounders. Multiple logistic regression analyses were performed. Multivariable confounding factors included age, body mass index, current smoking habits, alcohol intake (≥3 units/day), regular outdoor exercise (≥30 min/day), and parental history of osteoporotic fracture

Table 2 Correlation between plasma platelet-activating factor (PAF) concentration and bone mineral density (BMD) at various sites in the study subjects

Variables	γ	P^a	γ	P^b
Bone mineral density (g/cm^2)				
Lumbar spine	−0.163	0.054	−0.177	0.041
Femoral neck	−0.156	0.062	−0.163	0.055
Total femur	−0.227	0.006	−0.246	0.004
Trochanter	−0.233	0.005	−0.253	0.003
Shaft	−0.191	0.026	−0.208	0.018
Ward	−0.160	0.056	−0.176	0.039

[a] *P* values determined using Pearson's correlation analysis with respect to the log-transformed PAF concentration

[b] *P* values determined by partial correlation analysis with respect to the log-transformed PAF concentration and adjusted for age, body mass index, current smoking, alcohol intake (≥3 units/day), regular outdoor exercise (≥30 min/day), and parental history of osteoporotic fracture

clinical study to report the association between PAF and osteoporosis-related phenotypes, suggesting that plasma PAF could be a potential biomarker for predicting poor bone health in postmenopausal women.

Since establishing the critical role of estrogen deficiency in the pathogenesis of postmenopausal osteoporosis, enormous effort has focused on elucidating the mechanisms by which estrogen may modulate bone formation and resorption. While estrogen has been proven to have direct effects on bone cells [20], additional regulatory effects of estrogen that were centered at the level of the adaptive immune response have also been identified. Specifically, tumor necrosis factor-α (TNF-α), which is mainly produced by T cells in estrogen-deficiency patients, directly and indirectly stimulates osteoclastogenesis and contributes to bone loss [21]. Interleukin (IL)-1 and IL-6 are other potent stimulators of osteoclast differentiation and activation that have been linked to the accelerated bone resorption seen in postmenopausal osteoporosis [22, 23].

The term PAF was originally used to describe an agent that is able to aggregate and activate platelets [24]. Since its discovery, the pleiotropic and potential biological effects of PAF have been extensively reported [10–12]. In the bone field, although several experimental studies report the direct effects of PAF on osteoclasts [15, 16, 25], the most convincing evidence of PAF's role in the pathogenesis of osteoporosis was provided by [17]. In their study, TNF-α and IL-1, which are cytokines increased after ovarian dysfunction, elevate PAF production in osteoclasts via the activation of acetyl-coenzyme A:lyso-PAF acetyltransferase. PAF then activates PAFR on osteoclasts, but not osteoblasts, in an autocrine manner and prolongs cell survival. Accordingly, PAFR-deficient mice demonstrate markedly attenuated bone resorption after ovariectomy, indicating that PAF is linked to estrogen depletion and osteoporosis in vivo [17]. Here, we show that higher PAF levels are associated with increased VF risk and lower BMD in postmenopausal women. These findings clinically validate previous experimental and animal data and demonstrate that the detrimental effects of PAF on bone occur in humans as well.

Because information about bone turnover markers was only available for a limited number of subjects, we examined the association between plasma PAF and total ALP levels as an alternative investigation. ALP activity in circulation is normally contributed by bone and liver isoforms in approximately equal amounts [26]. In general, total serum ALP is regarded as a useful marker of the degree of bone turnover in subjects without liver disease [27]. Based on this background, we performed the partial correlation analysis and found that plasma PAF concentrations were positively correlated with total serum ALP levels after adjusting for confounders. This result indirectly suggests that high VF risk and low BMD associated with high PAF may be explained by increased bone turnover rate resulting from activated bone resorption and the resultant bone formation by coupling phenomenon.

Meanwhile, in the present study, the association between plasma PAF level and femoral neck BMD value after adjusting for potential confounders was not statistically significant. This result is consistent with epidemiologic studies showing higher prevalence of VF than femur neck fracture in Asian osteoporotic patients [28, 29]. Further studies in Western countries will be highly interesting to investigate that the role of PAF on bone health could be different according to populations.

One potential concern of this study is that PAF is not entirely produced by osteoclasts. This protein is known to be synthesized by a variety of proinflammatory cells that participate in the development of inflammation, such as monocytes, macrophages, polymorphonuclear neutrophils (PMN), eosinophils, basophils, and platelets [30, 31]. Therefore, our results could be affected by conditions that increase the numbers of these cells. To minimize this possibility and appropriately investigate the pure association between PAF and bone biology in humans, we strictly excluded subjects with infectious or immune disorders according to the results of our laboratory tests and medical history review.

Some potential limitations should be considered when interpreting our data. Most importantly, because this is a case–control study, we could not determine if a causal relationship exists between plasma PAF concentrations and osteoporosis-related phenotypes. Although we assume that high PAF levels may increase the risk of VF and lower BMD based on the results of previously reported in vitro and in vivo studies, there is the possibility that our results

could have been generated due to a reverse causal relationship. However, despite this limitation, we believe that our study has important implications in that there have been no clinical studies that report the association between PAF, fracture and/or BMD, and, therefore, this study could be an important beginning and provide background information for future prospective studies. Second, the study population consisted of women who visited a referral hospital and, therefore, may not be representative of the general population and could have resulted in selection bias. Lastly, although we considered as many confounding factors as possible, we cannot exclude the possibility that the observed association could have resulted from uncontrolled factors that affect PAF and/or bone parameters, such as 25-hydroxyvitamin *D* levels or years since menopause.

In summary, we show that elevated PAF concentration is associated with an increased risk of osteoporotic VF and low BMD at various sites in postmenopausal women, supporting previous in vitro and in vivo results showing the deleterious effects of PAF on bone metabolism. Further prospective studies need to include a larger number of subjects in order to understand fully the biological role of PAF on bone metabolism in humans.

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Conflict of interest All authors have no conflicts of interest.

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