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ORIGINAL RESEARCH

Teriparatide Therapy Reduces Serum Phosphate and Intima-Media Thickness at the Carotid Wall Artery in Patients with Osteoporosis

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Abstract Although cross-sectional and longitudinal studies report a relationship between osteoporosis and cardiovascular disorders (known as the bone-cardiovascular axis), the benefits of osteoporosis treatment on atherosclerosis are largely unclear. Teriparatide is a bone-forming agent that increases urinary phosphate excretion. Because elevated serum phosphate is associated with the development of atherosclerosis, the purpose of our study was to examine the relationship among lumbar spine bone mineral density (LS-BMD), intima-media thickness at the carotid artery (CA-IMT), and phosphate metabolism in response to daily teriparatide therapy. Osteoporotic patients (n = 28) with low LS-BMD (T-score < -2.5) and/or at least one vertebral fracture were treated with teriparatide (20 μg/day) for 12 months. Metabolic bone markers, LS-BMD, and CA-IMT were measured over the course of treatment. The LS-BMD significantly increased by 0.046 ± 0.038 g/cm^2 over the 12-month period ($P < 0.001$). CA-IMT decreased from 0.701 mm (interquartile range: 0.655–0.774 mm) at baseline to 0.525 mm (0.477–0.670 mm) at 12 months ($P < 0.05$); however, CA-IMT change was not significantly associated with LS-BMD change. Serum phosphate decreased after 1 month of teriparatide administration, and the change in serum phosphate at 1 months was associated with the change in CA-IMT at 12 months (ρ = 0.431, P = 0.025). Teriparatide improved LS-BMD and CA-IMT, suggesting the existence of the bone–cardiovascular axis. The association between serum phosphate and CA-IMT suggests that the teriparatide decreased CA-IMT in part by reducing serum phosphate, a well-known vascular toxin, in addition to the improvement of bone–cardiovascular axis.

Keywords Atherosclerosis · Intima-media thickness · Osteoporosis · Phosphate · Teriparatide

Introduction

Daily administration of teriparatide, also known as PTH 1–34, is an established therapy for severe osteoporosis because it decreases the risk of fractures and increases vertebral and femoral BMD. Teriparatide increases P1NP, a marker of bone formation, followed by urinary N-telopeptide corrected for creatinine (NTX), a marker of bone resorption [1]. Increase in P1NP after 1 month of teriparatide administration was strongly correlated with increased LS-BMD at 12 months [2], indicating a strong relationship between early change in P1NP and subsequent change in LS-BMD.

Cross-sectional and longitudinal studies show that low BMD is significantly associated with atherosclerosis or cardiovascular calcifications in the general population [3–6] as well as in patients with CKD [7–9]. The connection between osteoporosis and cardiovascular disorders may be due to the similar biological mechanisms behind the pathogenesis between bone and arterial abnormalities, often termed the bone–cardiovascular axis.

Treatment of osteoporosis with bisphosphonates confers beneficial effects on the cardiovascular system. Oral daily risedronate prevents the progression of brachial–ankle pulse wave velocity, and intima-media thickness of the carotid

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artery (CA-IMT), an early quantitative marker of generalized atherosclerosis [10], whereas these parameters increased significantly in non-treated controls [11]. Additionally, oral risedronate taken weekly for 6 months inhibits the progression of atherosclerosis by improving large and small artery elasticity index and systemic vascular resistance [12]. Furthermore, a population-based matched cohort analysis shows that patients who receive bisphosphonate therapy have a lower risk of acute myocardial infarction during the 2-year follow-up period (hazard ratio = 0.35) [13]. These reports indicate that bisphosphonate has beneficial effects on both bone and cardiovascular systems. However, whether bisphosphonate or simply the general treatment of osteoporosis improves the cardiovascular system is unclear.

Hyperphosphatemia in CKD is associated with increased all-cause mortality as well as cardiovascular morbidity and mortality [14]. Higher serum phosphate (Pi), even within the normal range, is associated with increased risk of cardiovascular disease (CVD), even after adjusting for established CVD risk factors such as age, sex, smoking, high blood pressure, and dyslipidemia [15]. We previously reported that greater serum Pi is a significant and an independent risk factor for increased CA-IMT [16]. Dietary Pi overload accelerates aortic sinus atheroma [17] and attenuates smooth muscle functions [18] in vivo without calcification, suggesting that excessive Pi may accelerate atherogenesis without vascular calcification.

The purpose of this study was to investigate whether teriparatide improves indicators of atherosclerosis, as well as BMD.

Materials and Methods

Subjects

This study included 30 osteoporotic patients with low LS-BMD (T-score < -2.5) and/or at least one vertebral fracture who started daily teriparatide therapy (20 μg/day) from November 2010 to November 2012 at Osaka City University Hospital.

Previous osteoporosis therapies, including oral bisphosphonates (16 cases), selective estrogen receptor modulators (three cases), and alfacalcidol (three cases), were discontinued before the initiation of the teriparatide therapy. DXA confirmed that all participants had at least two measurable lumbar spines in the L2-4 regions.

All participants were capable of visiting the hospital independently. None had a history of unresolved skeletal diseases that affect bone metabolism, current or previous malignant neoplasms, Paget's disease of bone, skeletal exposure to therapeutic irradiation, symptomatic nephrocalcinosis or urolithiasis, abnormal thyroid and parathyroid functions not corrected by treatments, significantly impaired renal function (eGFR < 45 mL/min/1.73 m^2), treatments with heparin or warfarin at any time prior to the initiation, excessive consumption of alcohol, or abuse of drugs.

Subjects were not included if they had a disease or habit that may affect atherosclerosis, such as smoking, or a previous history of coronary or cerebral vascular events. Two were receiving an angiotensin II receptor blocker, and five received both a calcium channel blocker and an angiotensin II receptor blocker. Six participants were receiving statins. The blood pressure (BP) and serum low-density lipoprotein cholesterol (LDL-C) levels were well controlled in these patients (BP $< 140/90$ mmHg and LDL-C < 140 mg/dL, respectively) and remained stable throughout the study, and their antihypertensive and/or lipid-lowering therapies were not changed throughout the study. One participant dropped out because of a rapid allergic reaction to the teriparatide, and another relocated before completing the study. A total of 28 patients completed at least 12 months of teriparatide therapy.

Bone Mineral Density (BMD) Measurement and Spinal Radiographs

LS-BMD was assessed by DXA using QDR-2000 (Hologic Inc., MA) at baseline, 6, and 12 months. Regions of severe scoliosis and vertebral fracture sites were excluded from LS-BMD calculations. BMD of the total hip was measured in the anterior–posterior projection using DXA. Spinal radiographs were obtained at baseline, 6, and 12 months, or at unscheduled times if patients reported new or worsening symptoms suggestive of clinical vertebral fracture (e.g., back pain). Vertebral fractures were assessed using a semiquantitative technique [19].

Ultrasonographic Measurement of Intima-Media Thickness (IMT)

The CA-IMT was measured at baseline, 6, and 12 months after using an ultrasonic phase-locked echo-tracking system equipped with a high-resolution real-time 13-MHz linear scanner (ProSound SSD 6500; Aloka Corporation, Japan) as previously reported [20]. In brief, approximately 4 cm of the common carotid artery was examined bilaterally in the longitudinal and transverse projections with the images focused on the far wall of the arteries. The CA-IMT was measured in both carotid arteries at the site of the most advanced atherosclerotic lesion that exhibited the greatest distance between the lumen-intimal and the media-adventitia interfaces of the far wall, herein defined as maximum CA-IMT. The intra-observer CV for CA-IMT was 2.8 %. All scans were evaluated by a physician who was unaware of the clinical characteristics of the patients.

Physical and Biochemical Parameters

Blood pressure was determined using a conventional cuff with a mercury sphygmomanometer after the patients had rested for at least 15 min. Serum and second-void urine samples were collected in the morning after an overnight fast before teriparatide administrations. Serum samples were stored at −80 °C until assayed. Serum Ca and urinary Ca levels were determined by the colorimetric method. Serum Pi, creatinine (Cr), alkaline phosphatase (ALP), LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), and urinary Pi, and Cr levels were determined by enzymatic methods using an autoanalyzer (Hitachi 7450; Hitachi Co., Japan). Tubular maximum reabsorption of Pi per unit of glomerular filtration rate (TmP/GFR) [21] and estimated GFR (eGFR) by Modification of Diet in Renal Disease equation modified for Japanese patients [22] were calculated as previously described. Serum whole PTH was measured by an immunoradiometric assay (Scantibodies Laboratory, Inc., CA) [23]. Radioimmunoassay was used to measure serum 1,25-$(OH)_2$D (Immunodiagnostic Systems, England) and P1NP (Orion Diagnostica, Finland) [24]. Serum FGF-23 was measured using sandwich enzyme-linked immunosorbent assay kits (Kainos Laboratories, Japan) [25]. Serum whole PTH, 1,25-$(OH)_2$D, P1NP, and FGF-23 were measured concurrently to avoid inter-assay variance.

Statistical Analysis

Data were analyzed using StatView5.0J (Abacus Concepts, Inc., CA). Continuous variables were expressed as the mean ± SD. Median (interquartile range) was used for ALP, whole PTH, 1,25-$(OH)_2$D, P1NP, TG, and CA-IMT because of their skewed distribution. Changes in the time course of serum Pi, TmP/GFR, and LS-BMD were analyzed by repeated measures one-way analysis of variance (ANOVA), whereas changes in the time course of CA-IMT were analyzed using Friedman's test. Fisher's protected least significant difference tests were used post hoc to confirm the statistically significant results. Differences between two points were evaluated using Wilcoxon signed-rank tests. Univariate regression analyses were performed using Spearman's rank correlation test. *P* values less than 0.05 were considered statistically significant.

Results

Baseline Characteristics of the Participants

The clinical and biochemical profiles of the participants (n = 28) are shown in Tables 1 and 2. All participants had low LS-BMD (T-score < −2.5) and/or at least one vertebral fracture, which often indicates the presence of osteoporosis. The median CA-IMT at baseline was 0.701 mm (interquartile range: 0.655–0.774 mm). Ultrasonography did not find vascular calcifications in the common carotid arteries, and chest or spinal radiographs did not reveal aortic calcifications in any participants, indicating that none of the participants had advanced atherosclerosis. Baseline serum Ca, Pi, ALP, whole PTH, 1,25-$(OH)_2$D, P1NP, and FGF-23 were within normal range.

Table 1 Clinical and biochemical profiles of the participants

Variables	
Gender (male/female)	5/23
Age (years)	67.0 ± 12.2
BMI (kg/m^2)	21.4 ± 2.8
eGFR (mL/min/1.73 m^2)	73.4 ± 20.5
LS-BMD (g/cm^2)	0.690 ± 0.163
LS-BMD (T-score)	−2.8 ± 1.4
Vertebral fractures (yes/no)	23/5

Data are expressed as mean ± SD

BMI body mass index, *eGFR* estimated glomerular filtration rate, *LS-BMD* lumbar spine bone mineral density

Changes in Biochemical and Physical Parameters in Response to Teriparatide Therapy

Serum Ca, ALP, 1,25-$(OH)_2$D, and P1NP increased significantly after 12 months of teriparatide therapy. Serum whole PTH, FGF-23, LDL-C, HDL-C, TG, systolic BP, and diastolic BP did not change significantly (Table 2).

Serum Pi and TmP/GFR decreased significantly 1 month after administration of the therapy and remained lower after 12 months (P = 0.008 and 0.029, respectively, Table 3). The change in TmP/GFR between 0 and 12 months was associated with the change in serum Pi (ρ = 0.928, $P < 0.001$, Fig. 1), but P1NP change was not significantly associated with the change in serum Pi (ρ = 0.187, P = 0.332).

Changes in LS-BMD and CA-IMT in Response to Teriparatide Therapy

The LS-BMD significantly increased by 0.046 ± 0.038 g/cm^2 after 12 months of therapy ($P < 0.001$, Fig. 2), however, no significant difference was observed in the total hip BMD (P= 0.471). The CA-IMT decreased significantly over the 12-month study period ($P < 0.001$), and decreased from 0.701 mm (0.655–0.774 mm) at baseline to 0.566 mm (0.509–0.717 mm) after 6 months ($P < 0.05$ vs. baseline) and 0.525 mm (0.477–0.670 mm) after 12 months ($P < 0.05$ vs. baseline, Fig. 2). Ultrasonography

Table 2 Changes in parameters between baseline and 12 months after the initiation of the therapy

	Reference range	Variable		*P*-value
		Baseline	12 months	
Ca (mg/dL)	8.5–10.5	9.3 ± 0.4	9.5 ± 0.4	0.026*
ALP (U/L)	115–359	181 (155–216)	238 (207–300)	0.006*
Whole PTH (pg/mL)	9.0–39.0	17.6 (12.3–22.6)	21.7 (15.8–25.6)	0.059
1,25-$(OH)_2$D (pg/mL)	28–69	43 (35–55)	71 (59–103)	<0.001*
P1NP (ng/mL)	15.0–75.0**	31.7 (23.7–55.5)	110 (73.3–149.0)	<0.001*
FGF-23 (pg/mL)	10–50	36.5 ± 10.5	37.2 ± 12.9	0.759
LDL-C (mg/dL)	70–139	105.0 ± 29.9	102.4 ± 25.4	0.933
HDL-C (mg/dL)	35–90	61.7 ± 14.9	65.8 ± 17.1	0.054
TG (mg/dL)	50–150	79 (60–101)	92 (72–103)	0.362
Systolic BP (mmHg)		124 ± 12	125 ± 16	0.999
Diastolic BP (mmHg)		72 ± 6	72 ± 7	0.909

Data are expressed as mean ± SD, or medians (interquartile range) with skewed distribution

ALP alkaline phosphatase, *whole PTH* whole parathyroid hormone, *1,25-$(OH)_2$D* 1,25-dihydroxyvitamin D, *P1NP* procollagen type 1 amino-terminal propeptide, *FGF-23* fibroblast growth factor-23, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TG* triglyceride, *BP* blood pressure

* $P < 0.05$ versus baseline, ** if female

Table 3 Time course of changes in serum Pi and TmP/GFR with teriparatide therapy

	Variable			
	Baseline	1 month	6 months	12 months
Pi (mg/dL)	3.9 ± 0.4	3.6 ± 0.5*	3.5 ± 0.7*	3.6 ± 0.6*
TmP/GFR (mg/dL)	3.5 ± 0.4	3.2 ± 0.6*	3.1 ± 0.7*	3.2 ± 0.6*

Serum Pi and TmP/GFR decreased from 1 to 12 months ($P = 0.008$ and 0.029, by repeated measures one-way ANOVA, respectively)

Pi phosphate, *TmP/GFR* Tubular maximum reabsorption of Pi per unit of glomerular filtration rate

Closed circles denote mean ± SD

* $P < 0.05$ versus 0 month by Fisher's PLSD

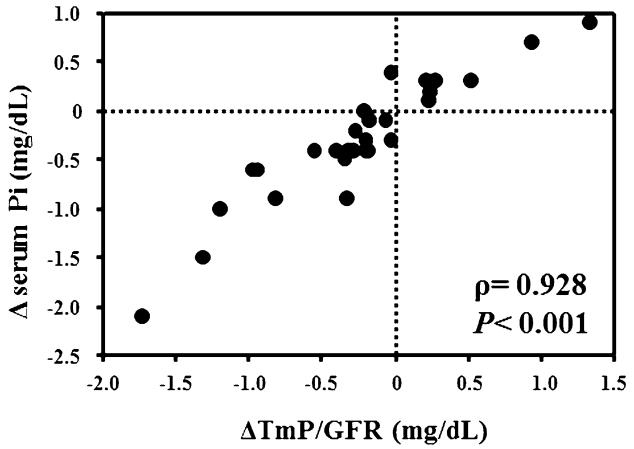

Fig. 1 Association between TmP/GFR and serum Pi changes from baseline to 12 months in response to teriparatide therapy. The change in TmP/GFR was positively correlated with change in serum Pi ($\rho = 0.928$, $P < 0.001$ by Spearman's rank correlation test)

did not find vascular calcifications in the common carotid arteries in any participants during the study periods. The changes in CA-IMT between 0 and 12 months were not significantly correlated to those in LS-BMD ($\rho = 0.033$, $P = 0.863$).

Relationship between biochemical parameters and LS-BMD and CA-IMT

The changes in P1NP between baseline and 1 month was positively associated with the change in LS-BMD between baseline and 12 months ($\rho = 0.599$, $P = 0.002$). The changes in serum Pi and the Ca × Pi product between 0 and 1 month were positively associated with the change in CA-IMT from 0 to 12 months ($\rho = 0.431$, $P = 0.025$ and $\rho = 0.405$, $P = 0.035$, respectively). None of the other parameters were associated with CA-IMT (Table 4).

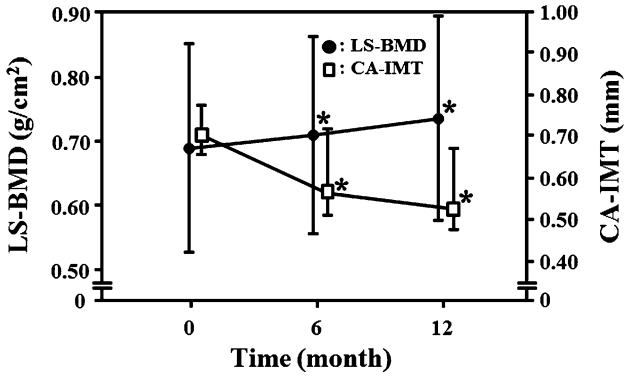

Fig. 2 Change in LS-BMD and CA-IMT after 12 months of teriparatide therapy. Teriparatide therapy increased LS-BMD (*closed circles*) ($P < 0.001$ by repeated measures one-way ANOVA), and decreased CA-IMT (*open squares*) ($P < 0.001$ by Friedman's test). *$P < 0.05$ versus baseline by Fisher's protected least significant difference test. *Closed circles* denote mean ± SD, whereas *open squares* denote median with interquartile range. LS-BMD, lumbar spine bone mineral density; CA-IMT, intima-media thickness of the common carotid artery

Table 4 Correlation between changes in biochemical parameters and LS-BMD and CA-IMT with teriparatide therapy

Measure	ΔLS-BMD		ΔCA-IMT	
	ρ	P	ρ	P
ΔCa	−0.071	0.714	−0.002	0.991
ΔPi	−0.059	0.759	0.431	0.025*
ΔCa × Pi	−0.085	0.660	0.405	0.035*
ΔTmP/GFR	0.027	0.889	0.312	0.105
ΔALP	−0.062	0.747	−0.072	0.708
Δ1,25-$(OH)_2$D	−0.032	0.868	−0.169	0.380
ΔP1NP	0.599	0.002*	−0.010	0.960

The correlation between changes in biochemical parameters from 0 to 1 month to the changes in LS-BMD and CA-IMT, respectively, from 0 to 12 months were examined by Spearman's rank correlation test

TmP/GFR Tubular maximum reabsorption of Pi per unit of glomerular filtration rate, *ALP* alkaline phosphatase, *1,25-$(OH)_2$D* 1,25-dihydroxyvitamin D, *P1NP* procollagen type 1 amino-terminal propeptide, *LS-BMD* lumbar spine bone mineral density, *CA-IMT* intima-media thickness of the common carotid artery

* $P < 0.05$

Discussion

In the present study, 12 months of teriparatide therapy improved LS-BMD and CA-IMT, suggesting this therapy for osteoporosis also improves atherosclerosis in patients who are at a high risk of sustaining osteoporotic fractures and do not have advanced atherosclerosis. Furthermore, altered Pi metabolism by teriparatide associated with the improvement of atherosclerosis.

The molecular basis for the anabolic effects of teriparatide is still being elucidated. Bone vascularization influences the osteogenic generation of new bone. An angiogenic effect of PTH has been observed *in vivo* in ovariectomized mice [26]. PTH is osteoanabolic through VEGF-related mechanisms, but did not induce bone angiogenesis, while anti VEGF antibodies blocked the anabolic effects of PTH [27], suggesting that PTH is a potential actor in vascular remodeling by impacting molecular pathways involved in post angiogenesis.

During osteogenesis, a special capillary subtype in the murine skeletal system appears in specific vessel locations and mediates growth of the bone vasculature, generates suitable microenvironments, maintains perivascular osteoprogenitors, and couples angiogenesis to osteogenesis [28]. In addition, endothelial Notch signaling promotes both angiogenesis and osteogenesis in bone [29], indicating that the synthesis of new bone requires crosstalk between bones and the vasculature. Despite the importance of PTH on bone vascularization in osteogenesis, the effect of teriparatide on general systemic vasculature function is limited. Elevated serum PTH levels were associated to higher intra-arterial and calculated central blood pressures in a community-based cohort [30], suggesting that continuous elevation of circulating PTH was one of risk factors for vascular diseases. On the other hand, teriparatide reduces the extent of both aortic and cardiac valve calcification in vivo in diabetic LDL receptor-deficient mice [31]; however, no studies have investigated the beneficial effects of teriparatide on atherosclerosis in human patients.

Transient teriparatide exposure has anabolic effects by enhancing genes associated with bone formation in osteoblasts without inducing osteoclast activity [32]. Teriparatide also has a phosphaturic effect by reducing TmP/GFR as well as PTH [33]. However, this effect is transient, and serum Pi levels are not significantly suppressed in response to osteoporosis treatment [34, 35]. However, combination therapy with teriparatide and raloxifene significantly decreased serum Pi levels in another study [34], suggesting teriparatide combined with other therapies may affect serum Pi levels. In this study, serum Pi levels were significantly lower 1 month after the initiation of teriparatide, although the range of the decrease in serum Pi was wide.

The significant association between the changes in TmP/GFR and the changes in serum Pi suggests that the urinary Pi excretion was the main determinant of serum Pi level. The circulating levels of FGF-23, another phosphaturic hormone, are elevated in patients with primary hyperparathyroidism [36]. PTH is a potent inducer of FGF-23 [37], and elevated circulating FGF-23 levels in response to teriparatide therapy have been reported [38]. However, serum FGF-23 levels did not increase in response to

teriparatide, suggesting that the teriparatide directly acts on the nephron to increase urinary Pi excretion. We did not measure circadian changes in FGF-23, so transient rises in FGF-23 induced by teriparatide may have also contributed to hypophosphatemia in addition to the direct effect of teriparatide on Pi excretion.

Epidemiological studies show that serum Pi levels are associated with all-cause and CV-related mortality in the general population [15] as well as in patients with CKD and that Pi binders prevent CV events [14]. The beneficial effects of phosphate binders on vascular calcification (coronary and aortic) were reported in randomized control trial (RCT) in dialysis [39, 40] and pre-dialysis CKD patients [41], indicating excess serum Pi contributes to vascular dysfunction. Although non-calcium-based binders contributed less to the development of vascular calcification than calcium based binders [42], it is still plausible whether non-calcium-containing phosphate binders are superior to calcium-containing phosphate binders, because all-cause and cardiovascular mortalities among these phosphate binders were not significantly different in RCT. [43].

In particular, serum Pi is positively associated with CA-IMT [44], an established early marker of atherosclerosis [45]. Elevated extracellular Pi concentrations give vascular smooth muscle cells an osteoblast-like phenotype, which subsequently accelerates vascular calcification [46]. Dietary Pi overload increases aortic sinus atheroma without calcification in apolipoprotein knockout mice *in vivo* [17], suggesting that excessive Pi accelerates atherogenesis without vascular calcification. Dietary Pi overload also attenuates the vascular smooth muscle response to physiological and pathological stimuli *ex vivo* in CKD C57/BL6 mice [18].

The decrement of serum Pi levels was observed after the teriparatide therapy, however, its decrement was only 8 % (from 3.9 ± 0.4 to 3.6 ± 0.5 mg/dL) and within normal ranges. Although the decrement in serum Pi concentrations was relatively small in this study, prior studies reported an elevated risk of adverse cardiovascular outcomes in association with comparably small increases in serum Pi levels [15, 47].

In this study, 12 months of teriparatide therapy increased LS-BMD and decreased CA-IMT, although LS-BMD and CA-IMT were not significantly correlated. The change in P1NP was associated with the change in LS-BMD but not with the change in CA-IMT. In addition, the serum Pi was associated with CA-IMT, but not with LS-BMD. Teriparatide activated bone tissue, resulting in increased P1NP, and also induced phosphaturia in the kidney, resulted in hypophosphatemia. Our results suggest that teriparatide ameliorated both osteoporosis and atherosclerosis. Teriparatide improved CA-IMT in part by reducing serum phosphate, a well-known vascular toxin.

Classical risk factors for atherosclerosis, such as BP, lipid profile, and smoking status, were not changed during the teriparatide therapy. Serum Pi, which was decreased with teriparatide treatment in our study, is a risk factor for vascular calcification [48]. Serum FGF-23 and soluble klotho (data not shown), two other risk factors [48], were not changed during the therapy, and other factors, such as vitamin D, vitamin K, and warfarin administration, were not performed in this study.

The limitation of this study was the study protocol. This study was not a randomized control study, and we did not have appropriate untreated control subjects. However, CA-IMT increases significantly over a 12-month period in untreated patients with postmenopausal osteoporosis [11], suggesting that the decrement in CA-IMT with teriparatide treatment in our study was clinically significant.

In conclusion, daily teriparatide administration improved CA-IMT and LS-BMD, suggesting teriparatide improves both osteoporosis and atherosclerosis. Furthermore, improvements in CA-IMT were associated with decreased serum Pi, a well-known vascular toxin.

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Conflict of interest Masaaki Inaba reports grant support and lecture fees from Eli Lilly Japan K.K. Yasuo Imanishi and Katsuhito Mori report lecture fees from Eli Lilly Japan K.K. Maki Yoda, Yuki Nagata, Masaya Ohara, Koichiro Yoda, and Shinsuke Yamada have no conflicts of interest.

Human and Animal Rights and Informed Consent All participants provided written informed consent before participating in this study, which received institutional ethics committee approval (Osaka City University Graduate School of Medicine, registration number 1775) and was conducted in accordance with the principles of the Declaration of Helsinki.

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