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Research Article

Parathyroid Hormone Regulates Circulating Levels of Sclerostin and FGF23 in a Primary Hyperparathyroidism Model

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Abstract

Parathyroid hormone (PTH) increases fibroblast growth factor 23 (FGF23), mediated both by protein kinase A (PKA) and Wnt signaling, and decreases expression of sclerostin, a Wnt antagonist derived from osteocytes. Patients with primary hyperparathyroidism (PHPT) have lower serum sclerostin levels than healthy controls, consistent with the idea of *SOST* downregulation by PTH. Nevertheless, the relationship between FGF23 and sclerostin in PHPT is still unclear. We examined this issue in a mouse model of PHPT. PHPT mice had increased FGF23 and decreased sclerostin expression in calvaria and in their serum concentrations compared with wild-type (WT) mice. In UMR106 osteoblasts, PTH increased *Fgf23* expression and decreased *Sost* expression, as well as forskolin, a PKA agonist, whereas inhibition of PKA reversed the changes in *Fgf23* and *Sost* expression, stimulated by PTH. Sclerostin treatment had no effect on *Fgf23* expression, but when it was added together with PTH, it statistically significantly abrogated the increase in *Fgf23* expression. By contrast, there was no statistically significant correlation between serum FGF23 and sclerostin, whereas PTH was positively and negatively correlated with serum FGF23 and sclerostin, respectively. These results indicate that the high level of PTH in PHPT mice leads to increased FGF23 and decreased sclerostin expression in serum and calvaria. A decrease of sclerostin may further augment FGF23 in vitro; however, there was no statistically significant association between circulating FGF23 and sclerostin. It is suggested that the pathogenesis of increased FGF23 expression in PHPT mice may be modified by not only sclerostin, but also other regulatory factors modulated by PTH.

Key Words: parathyroid hormone, fibroblast growth factor 23, sclerostin, protein kinase A, Wnt signaling

Abbreviations: DMP1, dentin matrix protein 1; ELISA, enzyme-linked immunosorbent assay; FGF23, fibroblast growth factor 23; IgG, immunoglobulin G; mRNA, messenger RNA; PCR, polymerase chain reaction; PKA, protein kinase A; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; PTHR1, parathyroid hormone receptor 1; WT, wild-type.

Parathyroid hormone (PTH) has calciotropic effects of direct action on bone and kidney and indirect action on the intestine to regulate calcium and phosphate homeostasis [1]. The skeletal effect of PTH is an increase of bone remodeling, stimulating osteoblasts and osteocytes via activation of protein kinase A (PKA) and Wnt signaling. Furthermore, PTH increases fibroblast growth factor 23 (FGF23), a major phosphaturic factor secreted from mature osteoblasts and osteocytes [2, 3]. Healthy individuals injected with 1-34 PTH developed hypophosphatemia and an increase of serum FGF23, along with elevated 1,25-dihydroxyvitamin D levels [4]. Circulating levels of FGF23 are statistically significantly elevated and positively correlated with serum calcium and PTH levels in a mouse model and patients with primary hyperparathyroidism (PHPT), who are characterized by hypercalcemia and hypophosphatemia due to excessive secretion of PTH from one or more parathyroid tumors or hyperplasia. After parathyroidectomy, FGF23 levels were statistically significantly lower than preoperative levels in these mice and patients with PHPT [5-7]. Patients with chronic kidney disease develop secondary hyperparathyroidism accompanied by renal function decline, and their circulating FGF23 levels also increase substantially and positively correlate with serum calcium and PTH levels [3]. Total parathyroidectomy can reverse the increased serum FGF23 levels in patients undergoing hemodialysis who have advanced secondary hyperparathyroidism [8]. According to these observations, excessive secretion of PTH is considered to contribute to the increased FGF23 levels in hyperfunctioning parathyroid diseases [3].

Sclerostin, encoded by the *Sost* gene, is a secreted Wnt antagonist mainly produced by osteocytes, and is a negative regulator of bone formation [9-11]. It abrogates the terminal differentiation of osteoblasts and promotes their apoptosis by binding to low-density lipoprotein receptor-related protein 5/6, resulting in inhibition of the canonical

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Wnt/β-catenin signaling pathway, which plays an essential role in osteoblast differentiation, proliferation, and activity [12, 13]. PTH decreases *Sost* messenger RNA (mRNA) levels in vitro, and in mice treated with continuous and intermittent administration of PTH [14, 15]. Similarly, transgenic mice with constitutive activation of parathyroid hormone receptor 1 (PTHR1) specifically in osteocytes exhibit increased bone mass and remodeling and decreased *Sost* expression [16]. Serum sclerostin levels negatively correlate with PTH levels in postmenopausal women [17], and intermittent PTH 1-34 treatment of postmenopausal women is associated with a reduction in circulating sclerostin levels [18]. Serum sclerostin levels in patients with PHPT are lower than in healthy controls and negatively correlated with plasma PTH levels [19], whereas serum sclerostin in these patients increased immediately after parathyroidectomy, returning to normal levels by the tenth day postoperatively [7]. These findings indicate that the expression and secretion of sclerostin is decreased by PTH stimulation in bone.

Based on these findings, it has been demonstrated that PTH has substantial effects on increased FGF23 and decreased sclerostin expression and secretion in bone. In addition, these effects involve both PKA and Wnt signaling, because sclerostin has an inhibitory effect on PTH to increase FGF23 expression [2]; however, their mechanistic interrelationships in the setting of hyperfunctioning parathyroid disease remain unclear. To elucidate whether decreased sclerostin levels affect increased FGF23 levels in PHPT, we sought to determine circulating levels of sclerostin and FGF23 and their respective gene expression patterns in bone, and analyze their relationship, using a mouse model of PHPT. We also investigated the involvement of sclerostin and PKA signaling in the regulation of FGF23 expression in the mature osteoblast cell line, UMR106, following PTH stimulation.

Materials and Methods

Experimental Animals

Transgenic mice overexpressing the human cyclin D1 oncogene specifically in the parathyroids of FVB/N mice were used in this study as a model of PHPT, as previously described [20]. PHPT and WT littermates were previously demonstrated to have no significant differences in morphology, weight, or growth, and alterations of biochemical parameters in PHPT mice are similar between males and females. PHPT mice older than 60 to 75 weeks, and even 87 to 118 weeks, have obviously higher serum PTH and FGF23 levels than younger mice aged 27 to 33 weeks, whereas serum PTH levels in these mice at earlier ages are not significantly increased compared to WT mice of the same age [5, 21]. Thus, we used mice aged 75 to 96 weeks for in vivo experiments. All mice were kept under constant conditions at room temperature (23 ± 2 °C) and 50% to 60% relative humidity. They were fed a commercially available rodent diet (CE-2, Crea Japan) containing 1.03% calcium and 0.97% phosphorus, and were provided food and water ad libitum under specific pathogen-free conditions. Because there were no significant sex differences in terms of phenotype, both male and female samples were collected and stored for statistical analysis. All in vivo experiments were approved by the appropriate institutional animal care committees at Osaka City University Graduate School of Medicine (protocol No. 08093).

Measurement of Biochemical Parameters

Blood samples were collected using cardiac puncture of anesthetized mice and were stored at – 20 °C until chemical analysis. Serum calcium, phosphate, and creatinine levels were determined using the Calcium E-TEST, the Phospha-C TEST, and the LaboAssay Creatinine test (Wako Pure Chemical Industries Ltd), respectively. Serum PTH, FGF23, and sclerostin were measured using the Mouse PTH 1-84 enzyme-linked immunosorbent assay (ELISA) kit (Quidel, previously Immutopics), Human FGF23 ELISA Kit (Kinos) [21], and Mouse/Rat SOST Quantikine ELISA Kit (R&D Systems), respectively. All kits were used according to the manufacturers' instructions.

Cell Culture

UMR106 rat osteogenic sarcoma cells (CRL-1661; American Type Culture Collection) [22] were cultured in α-Minimum Essential Medium (α-MEM; Gibco) containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco) at 37 °C in a humidified atmosphere of 5% CO_2. Cells were seeded into the wells of 12-well plates at 1×10^5 cells/well.

For the analysis of *Fgf23* and *Sost* expression in response to PTH treatment, UMR106 cells were cultured with 10^{-8} M, 10^{-7} M, and 10^{-6} M 1-34 PTH for 24 hours. In the time-course experiment, UMR106 cells were cultured with 10^{-7} M 1-34 PTH for 4, 24, 48, 96, and 168 hours. For PKA signaling analysis, UMR106 cells were cultured with 10^{-6} M, 10^{-5} M, and 10^{-4} M forskolin (Wako Pure Chemical Industries Ltd) or with 10^{-5} M H89 dihydrochloride (Tocris Bioscience) and/or 10^{-7} M 1-34 PTH for 24 hours. Further, these cells were also cultured with 4.4×10^{-8} M (1 μg/mL) recombinant human sclerostin (R&D Systems) and/or 10^{-7} M 1-34 PTH for 24 hours. Cells were collected to extract total RNA and/or protein for analyses.

RNA Extraction and Quantitative Real-Time Reverse Transcription–Polymerase Chain Reaction

Mouse bone tissues were frozen in liquid nitrogen immediately after surgical resection and stored at – 80 °C until analysis. Total RNA from bone tissue specimens and cultured cells was extracted using Trizol (Life Technologies Inc) and the RNeasy Mini RNA isolation kit (Qiagen), according to the manufacturers' instructions. Total RNA was reverse-transcribed to complementary DNA using the Moloney murine leukemia virus (M-MLV) reverse transcriptase kit (Invitrogen), as described previously [23]. Complementary DNA was analyzed by quantitative real-time reverse transcription–polymerase chain reaction (PCR), using the StepOnePlus real-time PCR System (Applied Biosystems) and specific primers for mouse and rat *Fgf23*, *Sost*, and *18S* rRNA genes (Applied Biosystems).

Immunoblotting Analysis

Calvariae were dissected from WT and PHPT mice, and chopped into smaller pieces in radioimmunoprecipitation assay buffer (Santa Cruz Biotechnology Inc) to extract protein. Total protein was also extracted from UMR106 cells with radioimmunoprecipitation assay buffer. A total of 20 μg of protein was loaded into the lanes of 12% sodium dodecyl sulfate gels, and the resolved proteins were transferred onto poly (vinylidene fluoride) membranes (Bio-Rad

Laboratories Inc) using the Trans-Blot Turbo System (Bio-Rad Laboratories Inc). Membranes were incubated with blocking solution (5% nonfat dry milk in Tris-buffered saline/Tween-20 [TBS-T]) for 1 hour. The antibodies used in the present study are listed in Table 1. Primary antibodies against FGF23 (Abcam) [24], sclerostin (Abcam) [25], and β-actin (Abcam) [26] were diluted in 5% bovine serum albumin in TBS-T, and membranes were incubated overnight at 4 °C. Membranes were then washed and incubated with appropriate horseradish peroxidase–conjugated immunoglobulin G (IgG) antibodies for 1 hour. The blots were washed and visualized with the Immobilon Western Chemiluminescent detection system (Thermo Fisher Scientific), with signal intensity determined using ImageQuant LAS 4000 (GE Healthcare UK Ltd).

Immunohistochemical Analysis

Calvariae from WT and PHPT mice were fixed with 4% paraformaldehyde in 1 × phosphate-buffered saline, decalcified in 10% EDTA at 4 °C for 2 weeks, and then embedded in paraffin. Longitudinal sections (3 μm) were cut and mounted onto glass slides. Deparaffinized sections were treated with 3% hydrogen peroxide solution for 5 minutes to inhibit endogenous peroxidase activity. Antigen retrieval was performed by L.A.B. Solution (Polysciences) for 5 minutes, followed by blocking with rabbit or goat normal serum, as appropriate for the primary antibody. Sections were incubated with antibodies against FGF23, sclerostin, goat, or rabbit control IgG [27, 28] overnight. After washing with phosphate-buffered saline, sections were incubated with antigoat or antirabbit horseradish peroxidase–conjugated antibodies (Histofine, Nichirei Bioscience), as appropriate, for 60 minutes at room temperature. Sections were stained using DAB (Vector Laboratories) and counterstained with hematoxylin.

Statistical Analysis

Data are the mean ± SD. Differences were evaluated using a *t* test or analysis of variance followed by Dunnett test or Tukey-Kramer test. *P* values less than .05 were considered statistically significant.

Results

Serum Fibroblast Growth Factor 23, Sclerostin, and Biochemical Parameters in Wild-type and Primary Hyperparathyroidism Mice

PHPT mice exhibited typical biochemical features of hyperparathyroidism, such as hypercalcemia (PHPT vs WT: 11.78 ± 1.20 vs 9.09 ± 0.34 mg/dL), hypophosphatemia (PHPT vs WT: 6.74 ± 1.22 vs 9.02 ± 1.79 mg/dL), accompanying elevated serum PTH levels (PHPT vs WT: 554 ± 173 vs 196 ± 84 pg/mL), compared to WT mice. PHPT mice also had statistically significantly higher levels of serum FGF23 than WT mice (PHPT vs WT: 584 ± 310 vs 167 ± 54 pg/mL), as we reported previously [5, 21], whereas serum sclerostin level was statistically significantly lower in PHPT mice than WT mice (PHPT vs WT: 85.5 ± 12.3 vs 99.5 ± 7.9 pg/mL). Serum creatinine levels were not statistically significantly different between the 2 groups (Table 2).

Expression of Fibroblast Growth Factor 23 and Sclerostin/*Sost* in Bone Tissues in Wild-type and Primary Hyperparathyroidism Mice

We next investigated *Fgf23* and *Sost* gene expression in the calvariae, lumbar vertebrae, and femurs of WT and PHPT mice using quantitative real-time PCR. In the calvariae, *Fgf23* expression was statistically significantly higher whereas *Sost* expression was statistically significantly lower in PHPT mice as compared with WT mice, but these expression levels in the lumbar vertebrae and femurs were unchanged (Fig. 1A and 1B). Similarly, immunohistochemical analyses of calvarial tissues in PHPT mice showed high levels of FGF23 expression in osteoblasts/osteocytes as previously described [21], whereas low levels of sclerostin expression were observed in osteocytes of PHPT mice, compared to WT mice (Fig. 1C, 1D, 1F, and 1G). There was no positive staining in samples incubated with goat or rabbit control IgG instead of the primary antibody (Fig. 1E and 1H). Western blot analysis of calvarial extracts also showed similar changes in protein expression between the 2 groups (Fig. 1I).

Relationship Between Circulating Levels of Sclerostin, Fibroblast Growth Factor 23, and Parathyroid Hormone in Wild-type and Primary Hyperparathyroidism Mice

Serum FGF23 levels were significantly positively correlated with serum PTH ($r = 0.678$, $P = .0010$; Fig. 2A) and calcium ($r = 0.756$, $P = .0001$) and negatively correlated with serum phosphate ($r = –0.492$, $P = .0274$), consistent with previous report [5]. In contrast, serum sclerostin levels were significantly negatively correlated with serum PTH ($r = –0.476$, $P = .0337$; Fig. 2B) and had borderline significance with serum calcium ($r = –0.416$, $P = .0678$) and positively correlated with serum phosphate ($r = 0.480$, $P = .0321$). There was no significant correlation between serum FGF23 and sclerostin levels ($r = –0.238$, $P = .3115$).

Effects of Parathyroid Hormone on *Fgf23* and *Sost* Expression

To study the direct effect of PTH on *Fgf23* and *Sost* expressions, we incubated UMR106 cells with 1-34 PTH. We found that PTH (10^{-7} M) treatment led to sustained alterations,

Table 1. Antibodies

Name of antibody	Source of antibody	Host; monoclonal/polyclonal	Antibody ID	Dilution for WB and/or IHC
FGF23	Abcam	Goat; polyclonal	RRID: AB_880086 [24]	WB (1:1000); IHC (1:1000)
Sclerostin	Abcam	Rabbit; polyclonal	RRID: AB_956321 [25]	WB (1:1000); IHC (1:1000)
β-Actin	Abcam	Mouse; monoclonal	RRID: AB_867494 [26]	WB (1:20000)
IgG from goat serum	Sigma-Aldrich	Goat; polyclonal	RRID: AB_1163599[27]	Same concentration of FGF23 for IHC
IgG from rabbit serum	Sigma-Aldrich	Rabbit; polyclonal	RRID: AB_1163659 [28]	Same concentration of sclerostin for IHC

Abbreviations: FGF23, fibroblast growth factor 23; IgG, immunoglobulin G; IHC, immunohistochemistry; RRID, Research Resource Identifier; WB, Western blotting.

Table 2. Serum biochemistries of experimental mice

Genotype	No.	Calcium, mg/dL	Phosphate, mg/dL	PTH, pg/mL	FGF23, pg/mL	Creatinine, mg/dL	Sclerostin, pg/mL
WT	10	9.09 ± 0.34	9.02 ± 1.79	196 ± 84	167 ± 54	0.29 ± 0.20	99.5 ± 7.9
PHPT	10	11.78 ± 1.20[a]	6.74±1.22[a]	554 ± 173[a]	584 ± 310[a]	0.19 ± 0.14	85.5 ± 12.3[a]

WT mice aged 75, 87, and 96 weeks (n = 3, 5, 2); PHPT mice aged 75, 87, and 96 weeks (n = 6, 3, 1).
Data are mean ± SD.
Abbreviations: FGF23, fibroblast growth factor 23; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; WT, wild-type.
[a]*P* less than .05 using *t* test.

with elevated *Fgf23* expression and reduced *Sost* expression (Fig. 3A and 3B); *Fgf23* expression increased and *Sost* expression decreased significantly in a dose-dependent manner with 1-34 PTH (10^{-8} M to 10^{-6} M; Fig. 3C and 3D). PTH treatment also increased and decreased protein levels of FGF23 and sclerostin in UMR106 cells, respectively (Fig. 3E).

Regulation of *Fgf23* and *Sost* Expression via Protein Kinase A Pathways and Effects of Sclerostin Treatment

Treatment with forskolin, an activator of PKA signaling [29], led to an increase in *Fgf23* expression and a decrease in *Sost* expression in UMR106 cells in a dose-dependent manner (Figs. 4A and 4B). Furthermore, treatment with H89, an inhibitor of PKA [29], led to inhibition of Fgf23 or partial restoration of the alterations in *Sost* expression, stimulated with 10^{-7} M 1-34 PTH (Figs. 4C and 4D). Treatment of UMR106 cells with 4.4×10^{-8} M sclerostin had no effect on *Fgf23* expression; however, sclerostin added together with PTH (10^{-7} M) blunted the increased gene expression and protein levels of FGF23 induced by PTH (Figs. 4E and 4F).

Discussion

We examined the relationship between FGF23 and sclerostin in PHPT using a mouse model of PHPT and UMR106 mature osteoblast cell line, and found that in PHPT the excessive secretion of PTH leads to increased FGF23 and decreased sclerostin expression in serum and calvaria.

Our previous studies showed that serum PTH and calcium levels correlated highly with serum FGF23 levels in PHPT mice and that serum FGF23 levels decreased in the PHPT mice after parathyroidectomy [5]. There was a positive correlation between *Fgf23* expression and the expression of osteoblastic markers, such as *Alp* and *osteocalcin* [5], whereas the expression of dentin matrix protein 1 (DMP1), one of the osteocytic markers, was significantly decreased in calvaria in PHPT mice [21]. These studies indicated that excessive secretion of PTH increases calvarial expression of *Fgf23* and increases serum FGF23 levels in PHPT, and that activities of osteoblasts and/or osteocytes might be involved in the stimulation of FGF-23 by PTH. In the present study, we demonstrate that in PHPT mice the persistently high level of PTH leads to increased FGF23 and decreased sclerostin expression in serum and calvaria compared with WT mice (see Fig. 1 and Table 1). WT mice had higher expression levels of *Sost* in calvaria than lumbar spine and femur in accordance with previous findings in vivo [14] and in vitro [30]. In contrast, PHPT mice exhibit decreases in *Sost*/sclerostin expression in calvaria, and have lower circulating sclerostin levels than WT mice (see Figs. 1B, 1F, and 1G and Table 2), similar to rodent studies in which continuous and intermittent PTH treatment decreased both *Sost* expression and protein levels of sclerostin in bone tissue [15, 31]. No studies in PHPT have investigated the expression of *Sost* in various parts of bone tissue, despite the higher expression of *Sost* in cortical bone than trabecular bone. Keller and Kneissel [14] reported that *Sost* expression in WT mouse calvaria was higher than femur diaphysis, and our results also showed that *Sost* expression in calvaria was higher than in other bone tissues in WT mice, although there was no significant difference in the expression of *Fgf23* or *Sost* in the femoral or vertebral tissues unlike calvaria, suggesting that mRNA samples extracted from vertebrae and whole femur may not be accurately evaluated because of contamination of trabecular bone and bone marrow. In human studies of PHPT, several studies documented lower sclerostin levels compared to control individuals, and parathyroidectomy normalized circulating sclerostin levels as well as markers of bone turnover [7, 19, 32]. These findings suggested that persistently high levels of PTH decrease *Sost*/sclerostin expression in calvaria and lower circulating levels of sclerostin in PHPT mice.

Stimulation of PTH/PTH-related peptide type 1 receptor (PTHR1) by PTH, and subsequent activation of PKA signaling, is an important pathway to increase *Fgf23* expression in the osteoblast/osteocyte [2, 16]. Further, PTH mediates the downregulation of *Sost* expression by PKA signaling in osteoblasts/osteocytes in vitro and in vivo [14]. *Sost* expression was lower in osteocytes derived from mice expressing DMP1–constitutively active PTHR1 transgene [33] and PTH failed to suppress *Sost*/sclerostin expression levels in mice and defected the PTHR1 in osteocytes [34]. We also previously observed an increase in phosphorylation of 3′,5′-cyclic adenosine 5′-monophosphate–response element-binding protein in protein extracts obtained from calvariae of mice treated with a continuous infusion of PTH [21], suggesting the activation of PKA signaling in the calvaria of these mice. In this study, we found that 1-34 PTH significantly increased *Fgf23* expression and decreased *Sost* expression as well as activation of PKA signaling (see Figs. 3 and 4). These results indicate that not only the increase of *Fgf23* but also the decrease of *Sost* expression by PTH in osteoblasts/osteocytes is regulated via PKA signaling.

Regarding the relationship between FGF23 and sclerostin in secondary hyperparathyroidism in experimental kidney failure, Lavi-Moshayoff et al [2] demonstrated that FGF23 expression is increased by PTH via both PKA and Wnt signaling, because sclerostin has an inhibitory effect on PTH to increase FGF23 expression in vitro. In the presence of sclerostin, Wnt ligands are blocked from binding the LRP-5/6-Frizzled receptor complex, which in turn facilitates the accumulation of β-catenin degradation complex [11]. An increase of FGF23 expression by osteocyte-specific constitutively active PTH1R is abolished in mice overexpressing *Sost* in osteocytes, suggesting that PTH regulates FGF23 through a mechanism that

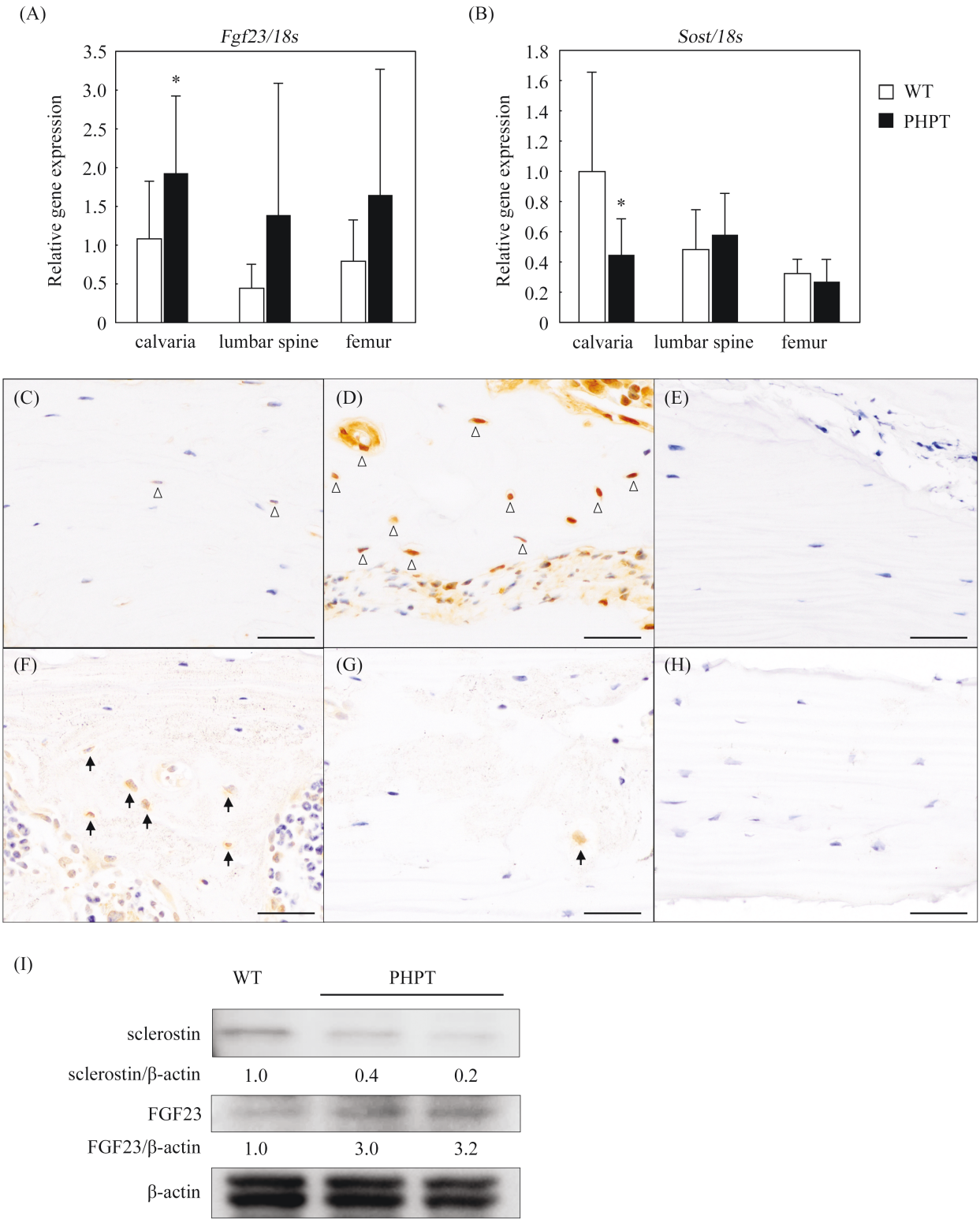

Figure 1. *Fgf23* and *Sost*/sclerostin expression in bone tissues of WT and PHPT mice. A, *Fgf23*, and B, *Sost* expression of calvaria, lumbar spine, and femur in WT and PHPT mice. Immunohistochemical analysis of C and D, FGF23, and F and G, sclerostin expression in calvaria of C and F, WT, and D and G, PHPT mice. Negative controls for FGF23 and sclerostin were performed with E, goat, and H, rabbit control immunoglobulin G. I, Western blot analysis of FGF23 and sclerostin expression in protein extracts from the calvarial tissue of WT and PHPT mice. Results are expressed relative to 18S control, and show fold-change differences in expression as compared with WT mice. Data are shown as the mean ± SD (WT, n = 10 (male/female, 6/4); PHPT, n = 10 (male/female, 9/1)); **P* less than .05 by *t* test. Arrowheads and arrows indicate FGF23 and sclerostin-positive osteocytes, respectively. C to H, Scale bars, 30 μm. FGF23, fibroblast growth factor 23; PHPT, primary hyperparathyroidism; WT, wild-type.

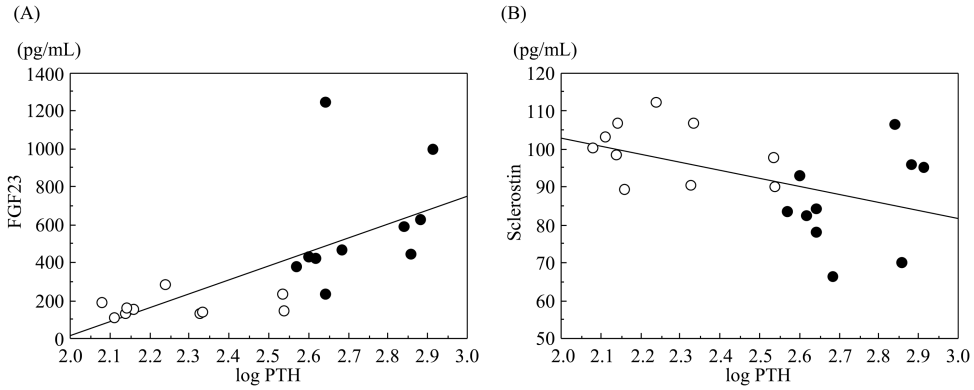

Figure 2. Relationships between circulating levels of sclerostin, fibroblast growth factor 23 (Fgf23) and parathyroid hormone (PTH) in wild-type (WT) and primary hyperparathyroidism (PHPT) mice. Graph shows the relationship between A, FGF23 and PTH ($r = 0.678$, $P = .001$) and B, sclerostin and PTH ($r = –0.476$, $P = .03$), analyzed by Pearson correlation. PTH values were log-transformed for skewness. Open circles denote WT mice, closed circles denote PHPT mice.

requires elevation of Wnt signaling [16]. Moreover, activation of Wnt signaling can increase FGF23 promoter activity in a dose-dependent manner and protein and mRNA expression of FGF23 in osteoblastic cell lines [35, 36]. Consistent with this inhibitory effect of sclerostin on FGF23 expression, we observed that the treatment of sclerostin added together with PTH prevented an increase in *Fgf23* expression (see Figs. 4E and 4F). These findings suggest that sclerostin may suppress FGF23 expression via the inhibition of Wnt signaling, and this suppressive effect of sclerostin would disappear when sclerostin expression is attenuated by PTH in osteoblasts/osteocytes, resulting in an increase of FGF23 expression, presumably resulting in aggravation of hypophosphatemia in patients with PHPT.

However, the possibility remains that PTH may increase FGF23 levels through other pathways, aside from decreasing the inhibitory effect of sclerostin. As shown in Figs. 3A and 3B, there are some differences between the kinetics of *Fgf23* and *Sost* expression. PTH treatment increased *Fgf23* expression at day 4 and decreased at day 7, whereas *Sost* expression decreased at day 2 and was almost unchanged thereafter. Sclerostin treatment without PTH did not change *Fgf23* expression, consistent with a previous report that mice overexpressing *Sost* in osteocytes did not decrease *Fgf23* expression in bone [16]. Notably, there was no correlation between serum FGF23 and sclerostin levels in WT and PHPT mice, although serum PTH had a positive correlation with serum FGF23 and a negative correlation with serum sclerostin. Mice with continuous administration of PTH for 96 hours decreased expression of sclerostin in bone tissue [15], and human studies also showed that there was a significant negative correlation between serum PTH and sclerostin in control individuals and patients with PHPT [7, 19, 37], but no study has shown the relationship between circulating FGF23 and sclerostin. FGF23 is also regulated by molecules derived from osteoblasts/osteocytes in the process of bone mineralization, and it has been reported that excessive PTH can decrease calvarial DMP1 and phosphate-regulating endopeptidase homolog X-linked (PHEX) expression [38], which may be associated with an increase in serum FGF23 levels [21, 39]. Moreover, Dickkopf 1 (DKK1), another potent inhibitor of the Wnt/β-catenin pathway secreted from osteoblasts/osteocytes, was significantly higher in patients with PHPT compared to healthy individuals, whereas decreased levels of serum sclerostin were found in these patients [32]. These observations support the notion that the increase in FGF23 caused by PHPT may be mediated, in part, by not only a decrease in sclerostin but also PTH-mediated changes of other factors regulating FGF23 expression. Furthermore, high FGF23 levels in bone are considered to contribute to bone loss via direct inhibition of osteoblastic Wnt/β-catenin signaling pathway through induction of Dkk1 [40]. PTH has the direct effect of increasing β-catenin, one of the common mediators of osteoblastic bone formation, as well as suppressing the expressions of Wnt inhibitors in osteoblasts [40, 41]. In contrast, mice receiving continuous administration of PTH exhibited severe bone loss with no increase of β-catenin in osteoblasts [42]. Attenuated sclerostin expression induced by high levels of PTH would presumably enhance FGF23 expression in osteoblasts or osteocytes and resultantly exacerbate bone loss in patients with PHPT. Further studies will be required to determine whether regulatory factors derived from osteoblasts/osteocytes affect FGF23 expression in bone tissue with PHPT.

In summary, a persistently high level of PTH increases *Fgf23*/FGF23 expression in calvaria and circulating FGF23 levels, whereas it decreases *Sost*/sclerostin expression in calvaria and circulating sclerostin levels in a mouse model of PHPT. The continuous activation of PKA signaling in osteoblasts/osteocytes by PTH can alter expression of these genes, and a decrease of sclerostin may further augment FGF23 expression in vitro, whereas there was no significant association between circulating FGF23 and sclerostin. Our study proposes that the pathogenesis of increased FGF23 expression in a mouse model of PHPT may be modified not only by sclerostin, but also by other regulatory factors modulated by PTH.

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Author Contributions

Y.N., Y.I., and M.E. designed the research; Y.N., T.T., and D.M. performed the research; Y.N., Y.I., M.K., and

Figure 3.

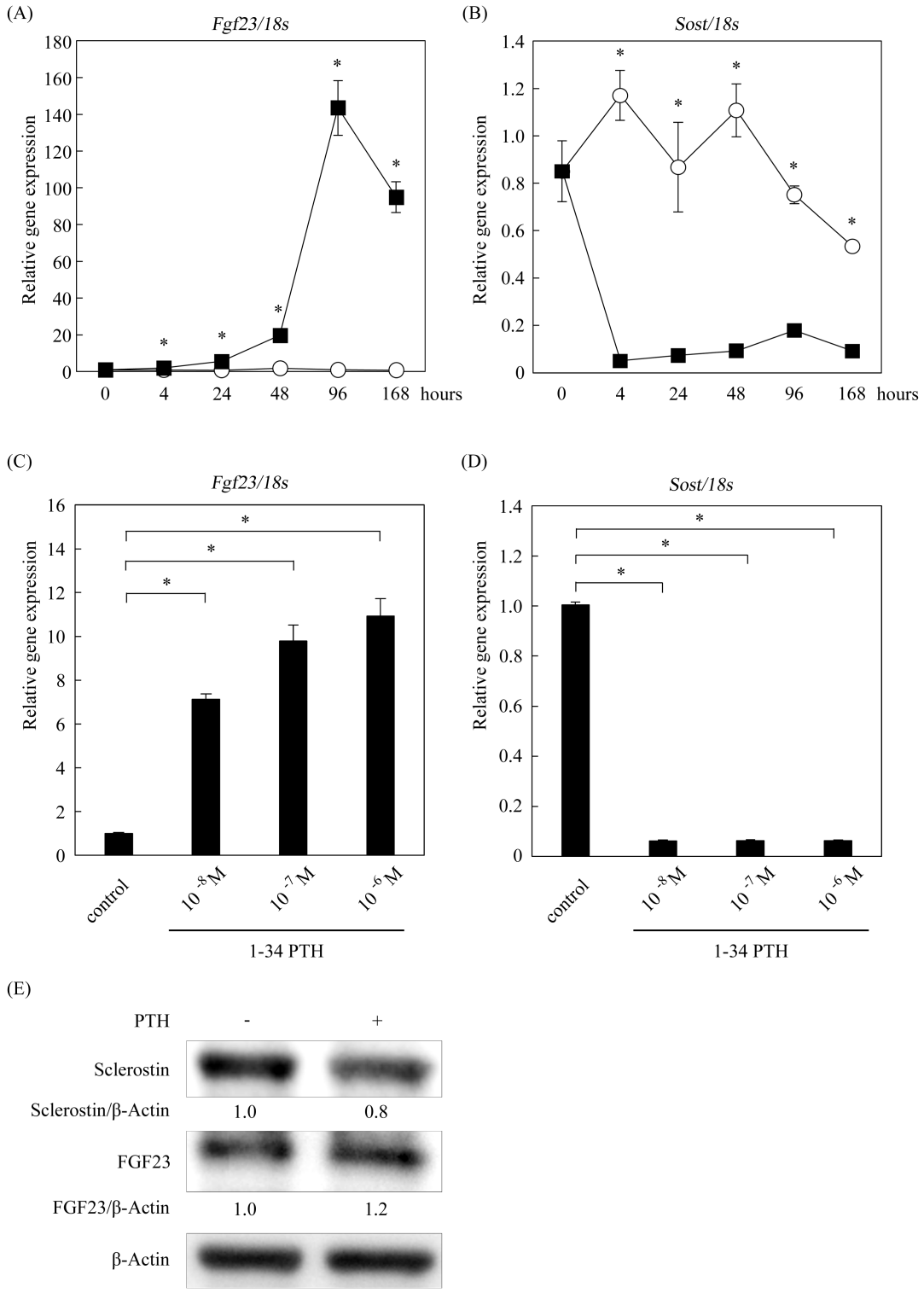

Figure 3. Parathyroid hormone (PTH) regulates fibroblast growth factor 23 (*Fgf23*) and *Sost* expression in UMR106 cells. A and B, Time-dependent changes in A, *Fgf23*, and B, *Sost* expression levels in UMR106 cells after treatment with 10^{-7} M 1-34 PTH at 4, 24, 48, 96, and 168 hours. C and D, Concentration-dependent changes in C, *Fgf23*, and D, *Sost* expression levels in UMR106 cells treated with 1-34 PTH for 24 hours. Results are relative to 18S messenger RNA and are presented as the fold-change in expression compared with control (vehicle-treated) cells. Data are shown as the mean ± SD (n = 3); **P* less than .05, by *t* test or analysis of variance with Dunnett test. E, Western blot analysis of FGF23 and sclerostin in UMR106 cells after treatment with 10^{-7} M 1-34 PTH for 24 hours.

Figure 4.

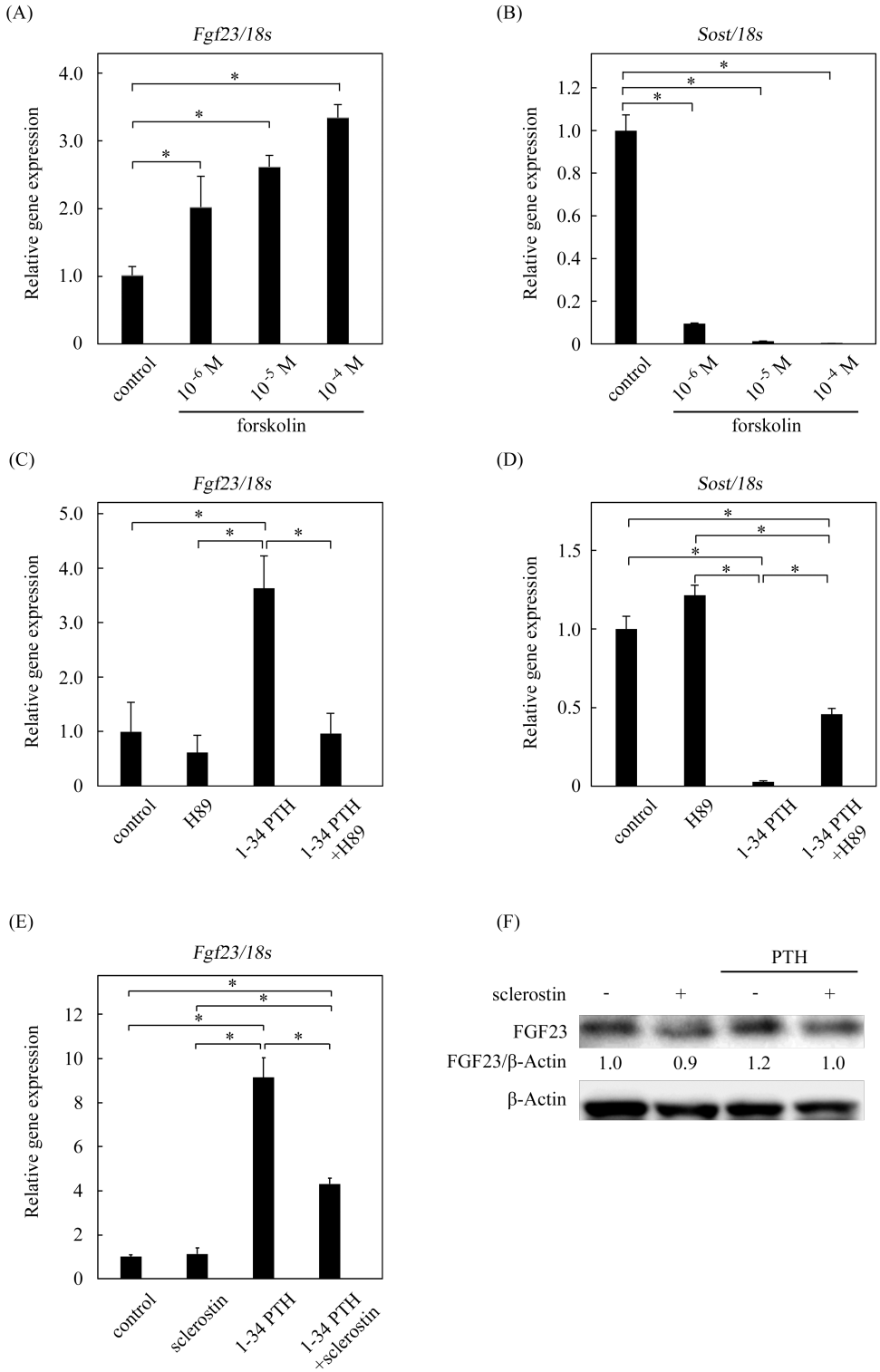

Figure 4. The regulation of fibroblast growth factor 23 (*Fgf23*) by the parathyroid hormone/protein kinase A (PTH/PKA) pathway and sclerostin in UMR106 cells. A, *Fgf23*, and B, *Sost* expression in UMR106 cells after treatment with 10^{-6} M to 10^{-4} M forskolin for 24 hours. C, *Fgf23*, and D, *Sost* expression in UMR106 cells after treatment with 10^{-7} M 1-34 PTH with or without 10 μM H89 for 24 hours. E, *FGF23* gene, and F, FGF23 protein expression in UMR106 cells after treatment with 10^{-7} M 1-34 PTH with or without 4.4×10^{-8} M sclerostin for 24 hours. Results are relative to 18S messenger RNA and are presented as the fold-change in expression compared with control (vehicle-treated) cells. Data are presented as the mean ± SD (n = 3); **P* less than .05, by analysis of variance with Tukey-Kramer test.

A.A. analyzed and interpreted the data; Y.N. and Y.I. wrote the manuscript. Y.N. takes responsibility for the integrity of the data analysis.

Disclosure Summary

All authors state that they have no conflict of interest.

Data Availability

Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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