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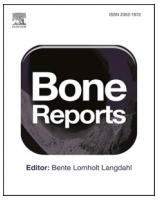

Elevated sclerostin levels contribute to reduced bone mineral density in non-ambulatory stroke patients

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ABSTRACT

Osteoporosis following stroke is a significant impediment to patient recovery. Decreased mechanical loading and locomotion following the onset of paralysis in stroke patients, especially those who are non-ambulatory, contributes greatly to bone loss. Sclerostin, a protein encoded by the SOST gene, accumulates as a result of reduced mechanical loading and inhibits bone formation. This study explores the relationship between mechanical unloading, sclerostin levels, and bone mineral density (BMD) in stroke patients, utilizing three cohorts. Analysis of Cohort 1, consisting of patients with available sclerostin level measurements, found significantly elevated sclerostin levels in non-ambulatory patients compared to ambulatory patients, indicating the influence of ambulatory status on sclerostin regulation. Cohort 2, consisting of patients with BMD measurements, demonstrated that prolonged mechanical unloading in non-ambulatory patients resulted in a greater decline in BMD over time. Analysis in Cohort 3 patients, who had bilateral BMD measurements available, revealed that hemiplegic sides subjected to reduced mechanical loading exhibited lower BMD compared to non-hemiplegic sides. These findings collectively confirm the hypothesis that reduced mechanical loading elevates sclerostin levels and accelerates bone loss. By integrating data across the three cohorts, this study underscores the critical impact of mechanical unloading on bone health, particularly in chronic stroke patients with limited mobility. Our study provides clinical insights for treatments integrating ambulatory status, sclerostin levels, and BMD in chronic stroke patients and highlights an increased need for therapeutics targeting mechanical loading pathways and sclerostin accumulation which can be administered to treat chronic osteoporosis following stroke.

1. Introduction

Reduced mobility and mechanical load are major contributors to bone loss, a serious clinical manifestation in chronic stroke patients. Due to limited or impaired mobility and use of the limbs after a stroke, patients are often exposed to reduced mechanical load following increased reliance on implements such as wheelchairs or beds. Thus, chronic stroke patients are more vulnerable to reduced bone mineral density (BMD) as they experience less physical activity (Lee and Joo, 2022; Hamdy et al., 1995; Yang et al., 2020; Pang et al., 2007), especially in the hemiplegic side of the body (Jorgensen et al., 2000a). Concurrently, there is a strong association between ambulation and bone mineral density. BMD on the paretic side fell significantly more than the non-paretic side and correlated with motor impairment in stroke patients

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(Yang et al., 2020; Jorgensen et al., 2000a; Pang et al., 2010). Ambulatory status of patients as measured during the first year following a stroke was found to directly correlate with changes in BMD, with patients who relearned to walk following stroke having a significantly reduced loss of BMD in the paretic side of the body (Jorgensen et al., 2000a; Jorgensen et al., 2000b). These conditions of diminished mechanical loading increase patient vulnerability to bone loss and osteoporosis (Lee and Joo, 2022; Tomasevic-Todorovic et al., 2016), the latter of which is defined by an imbalance of bone loss and resorption leading to low BMD and bone degradation (Suen and Qin, 2016). Bone loss, diminished bone strength, and altered bone geometry following stroke not only increase the risk of fracture, but also the risk of further medical complications (Yang et al., 2020; Pang et al., 2007; Jorgensen et al., 2000a; Pang et al., 2010; Ramnemark et al., 1998; Pang et al., 2008; Kanis et al., 2001). Despite the high risk for such serious complications, there is no standard treatment for bone loss in patients with stroke.

In healthy individuals, bone resorption in the body is a tightly regulated process. While necessary under non-pathogenic conditions for bone remodeling and maintenance, loss of physical activity and mechanical loading due to causes including injury or paralysis, in addition to factors such as aging, hormonal imbalance, autoimmune disease, cancer, and certain drugs such as glucocorticoids, can all increase bone resorption by osteoclasts, contributing to bone loss, osteopenia and osteoporosis (Boyle et al., 2003; Yokota, 2024; Moller et al., 2020; Kameda et al., 1997; Kim et al., 2020; Jilka et al., 1992; Gado et al., 2022; Cheng et al., 2022; Ponzetti and Rucci, 2021). The critical regulator of osteoclastogenesis, RANKL, is among those secreted by osteocytes (Boyle et al., 2003; Ponzetti and Rucci, 2021; Takayanagi, 2021; Choi et al., 2021; Cabahug-Zuckerman et al., 2016; Winkler et al., 2003; Delgado-Calle and Bellido, 2015) and is increased in response to mechanical unloading and bone damage. Consequent accumulation of sclerostin (Suen and Qin, 2016; Boyle et al., 2003; Choi et al., 2021; Cabahug-Zuckerman et al., 2016; Qin et al., 2020; Sasaki et al., 2020), which is both a product as well as a positive regulator of RANKL upregulation, accelerates bone resorption (Winkler et al., 2003) and is therefore associated with decreased patient BMD (Suen and Qin, 2016). Sclerostin, a protein encoded by the *SOST* gene, is a modulator of bone turnover via competitive inhibition of the Wnt pathway for osteoblast differentiation. By binding to LRP5/6 receptors on osteoblasts, binding of Wnt is prevented, which releases the GSK3/APC/axin complex bound to Frizzled-family receptors and disallows for β-catenin translocation into the nucleus, leaving it to be degraded (Suen and Qin, 2016; Choi et al., 2021; Pederson et al., 2008). Thus, at the same time that osteoblast differentiation and proliferation are suppressed, increased RANKL secretion promotes osteoclast activity. This biangular response induced by sclerostin, by simultaneously allowing for increased osteoclast activity and stalled bone formation, ultimately results in bone loss.

Accordingly, sclerostin levels are elevated in patients with decreased ambulation, such as stroke, spinal cord injury, and cerebral palsy, due to paralysis or decreased mobility of paretic limbs and reduced overall mechanical load (Shin et al., 2017; Morse et al., 2012; Gaudio et al., 2010). Further, patients with sclerosteosis, which is caused by a rare loss-of-function mutation in the *SOST* gene, exhibit increased bone mass and elevated BMD (Brunkow et al., 2001; Balemans et al., 2001). Studies in *SOST* knock-out mice also showed increased BMD, high bone mass, and bone formation (Li et al., 2008; Lin et al., 2009), while overexpression causes a decrease in bone mass, formation, and strength (Winkler et al., 2003; Kramer et al., 2010). Consequently, methods to reduce or prevent sclerostin accumulation have been developed, including treatment with anti-sclerostin antibodies and other pharmacological interventions. In rat models of bone loss induced by SCI, bone deterioration was completely prevented when anti-sclerostin antibody (Scl-Ab) was administered following injury (Qin et al., 2016; Beggs et al., 2015). Romosozumab, a monoclonal antibody targeting human sclerostin, has been used in Phase 3 clinical studies to prevent bone deterioration due to osteoporosis and restore BMD (Fabre et al., 2020). Further research targeting the cause of sclerostin accumulation demonstrated a reduction in SOST and sclerostin levels following mechanical stimulation in models such as osteocytic cell lines (Sasaki et al., 2020) and the rodent ulna (Galea et al., 2017). While some previous studies have investigated the correlation between sclerostin and BMD, these focused mainly on hemodialysis patients (Lu et al., 2022), patients who have calcific atherosclerosis (Catalano et al., 2020; Register et al., 2014), or post-menopausal women (Kalem et al., 2017). Gaudio, Agostino, et al. showed that long-term immobilized patients present higher sclerostin levels associated with reduced bone formation and lower BMD (Gaudio et al., 2010), but did not serially follow BMD levels. To date, few studies have considered sclerostin activity in response to reduced mechanical loading when analyzing bone loss following stroke. Unlike previous studies, this study shows that non-ambulatory stroke patients have higher sclerostin levels, leading to low BMD scores. This study aims to examine the association between mechanical unloading and sclerostin levels and BMD in stroke patients, providing new insights into bone loss and osteoporosis in stroke patients to inform improved therapeutic and rehabilitative practices.

2. Materials and methods

2.1. Participants

Every participant came to our institution to receive treatment for low bone mineral density or concerns about osteoporosis. Following screening, participants who did not meet the requirements for our study were eliminated based on the following criteria: age (1) younger than 20 years old; (2) use of drugs or medications in the last 12 months that may impact bone metabolism, such as glucocorticoids, anticonvulsants, and bisphosphonates; (3) any preexisting diseases that might affect bone metabolism, such as diabetes, hyperparathyroidism, hypercortisolism, rheumatoid arthritis, and bone tumors; and (4) systemic illness, such as kidney, liver, and thyroid diseases.

This study utilizes a hybrid approach by integrating prospective and observational study designs. In cohort 1, patients were prospectively selected, with blood sampling and data collection beginning at the start of the study, and participants were actively followed over a specific period to observe outcomes. The observational component of the study involved analysis of both bilateral BMD and BMD over a long-term period in cohorts 2 and 3. Ultimately, 91 patients with stroke were determined to be eligible for our study among 145 patients with stroke in enrollment. Out of 145 patients, 54 were excluded for not meeting the screening test (Table 1, Fig. 1) These 54 patients excluded comprised patients with no Q-CT follow-up data. The selection of patients into cohorts was based on available data. Eligible patients who were

Table 1
Baseline characteristics of total stroke patients.

Parameter	Ambulator ($n = 31$)	Non-ambulator ($n = 60$)	p-value
Age (Years)	60.81 ± 3.15	53.92 ± 2.74	0.124
Sex (male %) M/F	45.16 (14/17)	61.67 (37/23)	0.136
BMI (kg/m^2)	23.02 ± 0.59	21.82 ± 0.62	0.214
Weight (kg)	60.92 ± 2.21	60.51 ± 1.99	0.897
Height (cm)	162.23 ± 1.54	166.17 ± 1.25	0.060
Onset duration (year)	4.07 ± 0.86	3.50 ± 0.72	0.629
BMD			
Lumbar total	−0.60 ± 0.23	−0.59 ± 0.16	0.970
Hip total	−0.66 ± 0.27	−1.55 ± 0.24	0.026*
Femur neck	−0.41 ± 0.26	−0.99 ± 0.23	0.120
Femur trochanter	−0.68 ± 0.30	−1.52 ± 0.48	0.041*
Femur intertrochanter	−0.60 ± 0.28	−1.41 ± 0.25	0.048*

Note. Data are presented as mean ± SEM; $p < 0.05$*; Significant difference by independent t-test; BMI: body mass index; BMD: bone mineral density; BMC: bone mineral content.

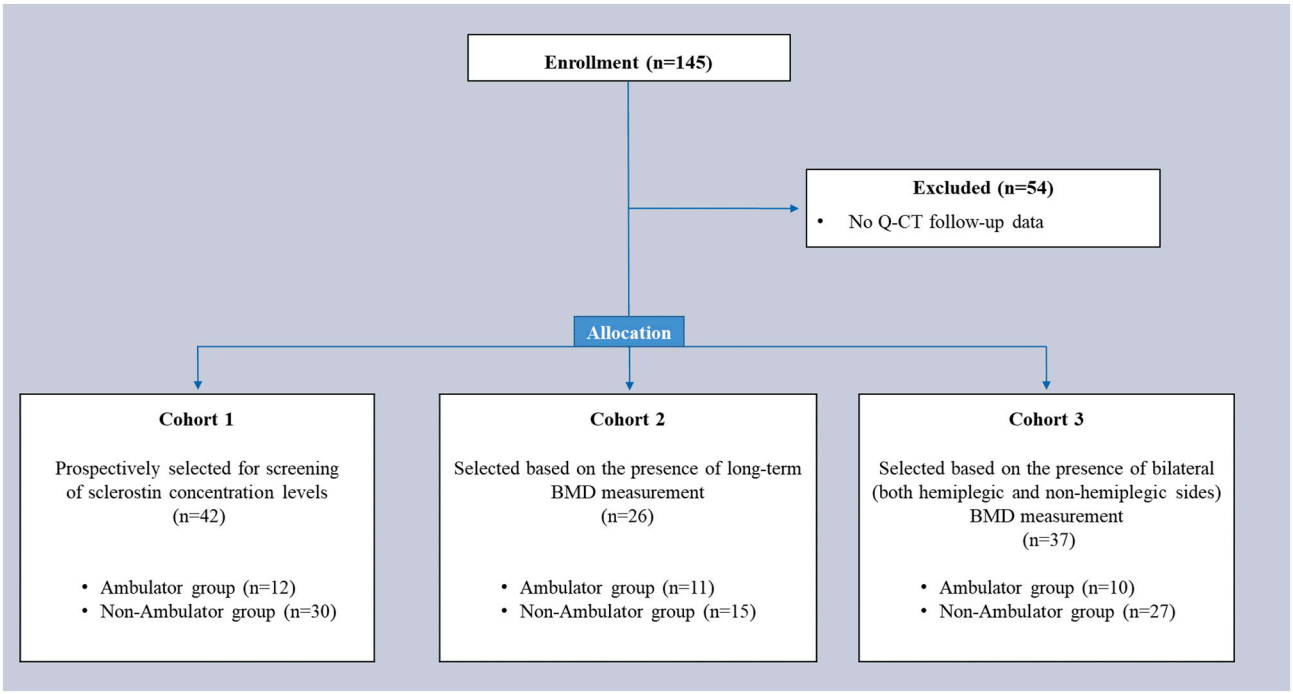

Fig. 1. Patient disposition graph.
The selection of patients into cohorts was based on available data.

individuals of at least 20 years of age, diagnosed with stroke, and had quantitative computed tomography (Q-CT) data were divided into three cohorts: cohort 1 which consisted of patients with measured sclerostin levels ($n = 42$), cohort 2 consisting of patients having undergone repeated observation of BMD over time ($n = 26$), where the period from the first measurement to the second measurement was 2 to 5 years, with an average of 3.53 ± 1.28 years, and cohort 3, in which bilateral BMD of both hemiplegic and non-hemiplegic sides was measured to characterize BMD differences according to the intensity of mechanical load ($n = 37$). Notably, there were overlaps among the cohorts: 3 patients were common to both Cohorts 1 and 2, 8 patients to Cohorts 1 and 3, 5 patients to Cohorts 2 and 3, and 2 patients were included in all three cohorts. Although there is limited overlap among the three cohorts, the presence of shared patients highlights meaningful connections between them. Specifically, a small number of patients appear in multiple cohorts, suggesting that certain clinical or biological characteristics span across groups. These shared patients provide valuable connectivity in a study where there was a limited availability of patient data. Together, the three cohorts offer an integrated and multidimensional understanding of the research objectives. This research was carried out with human subjects following the stipulations in the Declaration of Helsinki. This study was approved by the Institutional Review Board (4-2013-0404) of the Yonsei University Health System. Informed consent was obtained from all participants, who approved all procedures in this study.

2.2. Clinical categories of patients with stroke

Ambulatory stroke patients are classified as those who are able to walk independently or with the assistance of crutches or walkers. Patients who are only able to move around in a wheelchair or are otherwise unable to walk are classified as non-ambulatory stroke patients.

2.3. Measurement of biochemical markers

Participants were asked to fast 8 h prior to blood sampling. Blood samples were obtained via venous puncture and analyzed according to the institutional standard guidelines as follows: (1) serum sclerostin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA); (2) serum parathyroid hormone (PTH) was measured by immunoradiometric assay (IRMA) using commercial kits (Biosource, Nivelles, Belgium); (3) serum 25-hydroxyvitamin D (25(OH)D) were measured by radioimmunoassay (RIA) using commercial kits (D or D3-RIA-CT, Biosource, Nivelles, Belgium); (4) serum calcium and phosphate were measured by standard routine chemistry techniques using an automatic device (Hitachi, Tokyo, Japan); (5) serum C-telopeptide of type I collagen (CTx) concentrations were measured by ELISA (Osteomark, Ostex International, Seattle, WA, USA); and (6) serum procollagen type 1 N-terminal propeptide (P1NP) concentrations were measured by ELISA (Osteomark, Ostex International, Seattle, WA, USA) to identify renal function in all participants.

2.4. Measurement of plasma sclerostin concentrations

Peripheral blood samples were obtained from each participant under standardized conditions to ensure consistency. Blood was collected into EDTA-coated tubes and immediately stored on ice to maintain sample integrity. Whole blood was centrifuged for 10 min at 2000–2500 g, and the supernatant, consisting of blood plasma, was collected. All plasma samples were frozen immediately and kept at −80 °C until analysis via ELISA to measure sclerostin concentrations. Plasma sclerostin levels were determined using a Human SOST/Sclerostin Quantikine ELISA Kit (DSST00, R&D Systems, Inc., Minneapolis, MN, USA) following the manufacturer's instructions. Standards and samples were run in duplicate. Results for each individual's sclerostin concentration were determined by averaging the values from duplicate samples.

2.5. Measurement of bone mineral density

BMD was measured at the proximal femur (femoral neck, trochanter, intertrochanter), and total femur and lumbar by quantitative computed tomography (Q-CT) (Delphi A, Hologic, Waltham, MA, USA) with software Rervion 12.6. Standard BMD data was collected on the same side in cohorts 1 and 2. Cohort 3 was selected based on the availability of data

for both hemiplegic and non-hemiplegic sides. The location of the brain lesion as well as muscle strength was checked to determine the hemiplegic and non-hemiplegic side.

2.6. Statistical analysis

All statistical analyses were conducted using SPSS statistics 27, 28 (IBM Corporation, ArMONK, NY, USA). Independent *t*-tests were used to compare differences in the two group's demographic parameters, biochemical markers, and BMD. Single and multiple regression analysis was utilized to detect correlations among numerous variables and sclerostin, and to examine predictors of sclerostin concentration. The single and multiple analyses included independent variables, including age, sex, BMI, ambulatory status, PTH, CTx, 25-hydroxyvitamin D, calcium, phosphorus, ALP, and levels of P1NP. Multi linear regression analysis was performed using the enter method, which gives all independent variables equal importance when establishing the regression. All variables listed previously were used and the variable with the highest correlation among the others was determined.

Two-way repeated measures ANOVA and paired *t*-tests were used to analyze changes between the first and second Q-CT measurements of each group. The same analysis was applied to compare the hemiplegic side and non-hemiplegic sides ($p < 0.05$ was set as a criterion for statistical significance).

3. Results

3.1. Participants

After evaluating their eligibility for the study, the total participants of the three cohorts were divided into ambulatory patients ($n = 31$) and non-ambulatory patients ($n = 60$) groups (Table 1). The mean age of the total participants in the ambulatory patients was 60.81 ± 3.15 with a mean onset duration of 4.07 ± 0.86 years. The mean age of the participants in the non-ambulatory patient group was 53.92 ± 2.74 with a mean onset duration of 3.50 ± 0.72 years. There were no significant differences between ambulatory and non-ambulatory patients in terms of age ($p = 0.124$), sex ($p = 0.136$), BMI ($p = 0.214$), or duration of motor symptom onset ($p = 0.629$) (Table 1). After comparing bone densities between the two groups, results showed that patients with non-ambulatory stroke had significantly lower BMD z-scores than patients with ambulatory stroke as measured in regions of total hip ($p = 0.026$), femur trochanter ($p = 0.041$) and femur intertrochanter ($p = 0.048$).

3.2. Comparison according to ambulatory status in cohort 1

Twelve patients with ambulatory stroke and thirty patients with non-ambulatory stroke were sampled for this study (Table 2). There were no significant differences in demographic parameters. Out of 12 patients allocated to the ambulator group, 4 (33.3 %) were men and 8 (66.7 %) were women. Their mean age was 55.75 ± 6.28 years, average weight 58.25 ± 3.29 kg, average height 160.97 ± 2.75 cm, and body mass index (BMI) 22.39 ± 1.01 kg/m^2. Out of 30 patients allocated to the non-ambulator group, 20 (66.6 %) were men and 10 (33.4 %) were women. Their mean age was 57.37 ± 4.09 years, average weight 54.52 ± 1.93 kg, average height 165.18 ± 1.83 cm, and BMI 20.07 ± 0.62 kg/m^2. The mean age, mean weight, BMI, and sex ratio were similar in both groups. Analysis of blood biochemistry markers did not show significant differences in PTH, 25-hydroxy-vitamin D, calcium, phosphorus, ALP, CTX, or P1NP between the two groups. Only sclerostin levels differed. Patients with non-ambulatory stroke exhibited higher sclerostin levels (379.65 vs 274.07 pg/mL; $p = 0.009$) than patients with ambulatory stroke, as verified by an independent *t*-test (*$p < 0.05$).

Table 2
Cohort 1; baseline characteristics and hematological parameters in hemiplegic patients with stroke.

Parameter	Ambulator ($n = 12$)	Non-ambulator ($n = 30$)	*p*-value
Age (Years)	55.75 ± 6.28	57.37 ± 4.09	0.731
Sex (male %) M/F	33.3 (4/8)	66.7 (20/10)	0.189
BMI (kg/m^2)	22.39 ± 1.01	20.07 ± 0.62	0.055
Weight (kg)	58.25 ± 3.29	54.52 ± 1.93	0.319
Height (cm)	160.97 ± 2.75	165.18 ± 1.83	0.219
Sclerostin concentration (pg/mL)	274.07 ± 19.88	379.65 ± 22.48	0.009*
PTH (pg/mL)	31.70 ± 3.83	27.80 ± 1.43	0.470
25-hydroxyvitamin D (ng/mL)	25.44 ± 3.39	32.10 ± 3.44	0.314
Calcium (mg/dL)	8.96 ± 0.23	9.05 ± 0.64	0.401
Phosphorus (mg/dL)	4.02 ± 0.13	3.49 ± 0.13	0.072
ALP (IU/L)	107.60 ± 10.75	107.56 ± 9.99	0.179
CTx (ng/mL)	1.09 ± 0.38	0.96 ± 0.45	0.373
P1NP (ng/mL)	97.51 ± 16.27	129.73 ± 20.85	0.591

Note. Data are presented as mean ± SEM; $p < 0.05$*, significant difference by independent t-test;
BMI: body mass index; PTH: parathyroid hormone; ALP: alkaline phosphatase; CTx: C-telopeptide of type I collagen; P1NP: procollagen type 1 N-terminal propeptide.

3.3. Association between ambulatory status and sclerostin levels

Linear regression analysis was performed to find relevant variables influencing sclerostin levels in patients with stroke (Table 3). Sclerostin and ambulatory status had a significant negative correlation and association, as demonstrated by univariate analysis ($T = -2.787$, $p = 0.008$). The final model of multivariate analysis revealed a significant association between sclerostin and ambulatory status after adjustment for demographical factors, including age, sex, and BMI ($T = -2.730$, $p = 0.010$).

3.4. Reduction of BMD over a long period according to ambulatory status in cohort 2

BMD z-scores within regions of the total hip, femur trochanter, and femur intertrochanter showed a time effect (F = 8.453, $p = 0.008$; F = 12.499, $p = 0.002$; F = 7.020, $p = 0.014$), group effect (F = 5.389, $p = 0.029$; F = 6.666, $p = 0.016$; F = 5.848, $p = 0.024$), and interaction effect (F = 7.950, $p = 0.009$; F = 7.432, $p = 0.012$; F = 7.133, $p = 0.013$) following two-way repeated ANOVA. There was no significant difference between the first and second BMD measurements in patients with ambulatory stroke by paired *t*-test. However, patients with non-ambulatory stroke had a lower BMD by the second measurement when compared to the first measurement by paired t-test (total hip: $p = 0.003$; femur trochanter: $p = 0.001$; femur intertrochanter: $p = 0.004$). Comparing the two groups at the point of the second BMD measurement, patients with non-ambulatory stroke had lower scores than ambulatory stroke patients by independent t-test (total hip: $p < 0.001$; femur trochanter: $p < 0.001$; femur intertrochanter: $p = 0.001$) (Table 4, Fig. 2).

3.5. Comparison of hemiplegic and non-hemiplegic sides with respect to mechanical unloading in cohort 3

To determine whether BMD decreased according to onset duration and mechanical unloading following brain damage, hemiplegic and non-hemiplegic side BMD measurements of patients with 3 years of stroke onset duration were analyzed (Fig. 3). After 3 years of onset, the BMD of the hemiplegic side was lower than the BMD of the non-hemiplegic side in the total hip, femur neck, femur trochanter, and femur intertrochanter regions in combined patients of ambulatory and non-ambulatory stroke, as determined by paired *t*-test ($p = 0.007$; $p = 0.047$; $p = 0.005$; $p =$

Table 3
Cohort 1; linear regression analysis for predicting levels from clinical variables (Dependent variable: Sclerostin concentration).

Parameter (Reference)	Univariate analysis				Multivariate analysis				
	Unstandardized coefficient		t	*p*-value	Unstandardized coefficient		Standardized coefficient	t	*p*-value
	B	SE			B	SE			
Age	1.051	0.845	1.243	0.221					
Sex (male)	2.158	37.443	0.058	0.954					
BMI	2.553	4.538	0.563	0.577					
Ambulatory status (non-ambulatory)	−105.577	37.881	−2.787	0.008**	−109.434	40.088	−0.409	−2.730	0.010*
PTH	−3.146	1.910	−1.647	0.108					
25-hydroxyvitamin D	0.760	1.104	0.689	0.495					
Calcium	−40.171	33.240	−1.209	0.234					
Phosphorus	−13.693	28.966	−0.473	0.639					
ALP	0.448	0.372	1.204	0.236					
CTx	20.612	45.721	0.451	0.655					
P1NP	0.322	0.188	1.714	0.095					

Note. $p < 0.05$*, $p < 0.01$**; SE: standard error; Sex, age, and BMI were forced into the model as confounders, while other variables were included based on a enter selection method.

Table 4
Cohort 2; comparison of BMD and BMC between ambulator and non-ambulator groups.

Variables	Ambulator ($n = 11$)		Non-ambulator ($n = 15$)		Time		Group		Time*Group	
	First measurement	Second measurement	First measurement	Second measurement	F	*p*	F	*p*	F	*p*
BMD										
Lumbar total	−0.67 ± 0.34	−0.59 ± 0.30	−0.71 ± 0.35	−1.11 ± 0.32	0.609	0.443	0.408	0.529	1.414	0.246
Hip total	0.01 ± 0.17	−0.01 ± 0.21**	−0.34 ± 0.37	−1.59 ± 0.39$^{\#\#}$	8.453	0.008^{++}	5.389	0.029^{+}	7.950	0.009^{++}
Femur neck	0.08 ± 0.15	0.01 ± 0.17	0.22 ± 0.39	−0.52 ± 0.41$^{\#}$	3.713	0.066	0.194	0.664	2.537	0.124
Femur trochanter	0.34 ± 0.20	0.14 ± 0.22***	−0.13 ± 0.42	−1.73 ± 0.41$^{\#\#}$	12.499	0.002^{++}	6.666	0.016^{+}	7.432	0.012^{+}
Femur intertrochanter	0.12 ± 0.20	0.12 ± 0.21**	−0.25 ± 0.37	−1.47 ± 0.37$^{\#\#}$	7.020	0.014^{+}	5.848	0.024^{+}	7.133	0.013^{+}

Note. Data are presented as mean ± SEM.
Abbreviations: BMD (bone mineral density), BMC (bone mineral content).
$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$ significant effect by two-way repeated measures ANOVA.
$^{\#}p < 0.05$; $^{\#\#}p < 0.01$ significant difference by paired t-test.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ significant difference by independent t-test.

0.005) (Fig. 3A). There was a significant difference in BMD comparing the hemiplegic and non-hemiplegic side in non-ambulatory stroke patients. The hemiplegic side BMD was lower than the non-hemiplegic side in patients of non-ambulatory stroke after 3 years of onset in the total hip, femur trochanter, and femur intertrochanter regions ($p = 0.028$; $p = 0.028$; $p = 0.028$) (Fig. 3B).

4. Discussion

This study provides crucial insights into the relationship between ambulatory status, sclerostin levels, and bone mineral density (BMD) in chronic stroke patients. The findings underscore the significant impact of reduced mechanical loading on bone health, particularly highlighting the role of mechanical unloading in accelerating bone loss through the elevation of sclerostin levels. Results of this study demonstrate that non-ambulatory stroke patients have significantly higher levels of sclerostin compared to their ambulatory counterparts, which is consistent with previous research in both clinical (Gaudio et al., 2010) and molecular studies (Sasaki et al., 2020; Robling et al., 2008) indicating that mechanical unloading leads to increased sclerostin production. From our findings, it is clear that accumulation of sclerostin following stroke, paralysis, or neurodegenerative disease onset is associated with loss of bone mass and can make it more difficult for patients, especially those who are elderly, to recover their mobility following stroke or disease onset.

Further supporting our conclusion that reduced mechanical load accelerates bone loss, our findings also reveal a significant decline in BMD over time in non-ambulatory stroke patients. The time, group, and interaction effects observed in the longitudinal analysis of BMD scores indicate that non-ambulatory stroke patients experience a greater reduction in BMD compared to ambulatory patients, in line with previous studies showing that immobilization and reduced mechanical loading leads to rapid bone loss and an increased risk of osteoporosis. Moreover, while related research (Lee and Joo, 2022; Wang et al., 2024; Kim et al., 2016; Potin et al., 2022) analyzed patient BMD and bone loss in the early periods following stroke (up to 12–14 months), our study uniquely focused on the effects of reduced mechanical loading in both ambulatory and non-ambulatory patients in the chronic stages of recovery, up to several years following initial onset. While sclerostin and related pathways have already been investigated in multiple studies (Gaudio et al., 2010; McClung, 2015; Marini et al., 2023; Xu et al., 2023; Bhattacharyya et al., 2018; Ke et al., 2012) as clinical targets for bone loss therapy, the long-term effects of chronically increased sclerostin levels on patient recovery and osteal integrity have been largely understudied.

The comparison between the hemiplegic and non-hemiplegic sides of the body in stroke patients reveals that the side experiencing greater mechanical unloading (the hemiplegic side) has a significantly lower BMD. This observation reinforces the idea that local mechanical loading is critical in maintaining bone density and suggests that targeted interventions to increase the mechanical load on the paretic limbs could be beneficial in preserving bone health in stroke patients. To date, despite many studies highlighting the negative impacts of reduced mechanical loading on bone health and preservation (Moller et al., 2020; Choi et al., 2021; Cabahug-Zuckerman et al., 2016; Qin et al., 2020; Sasaki et al., 2020; Benedetti et al., 2018; Pathak et al., 2015), a major portion of these studies interrogated the mechanisms for this degeneration at the in vitro and molecular level, using cell lines and animal models simulating

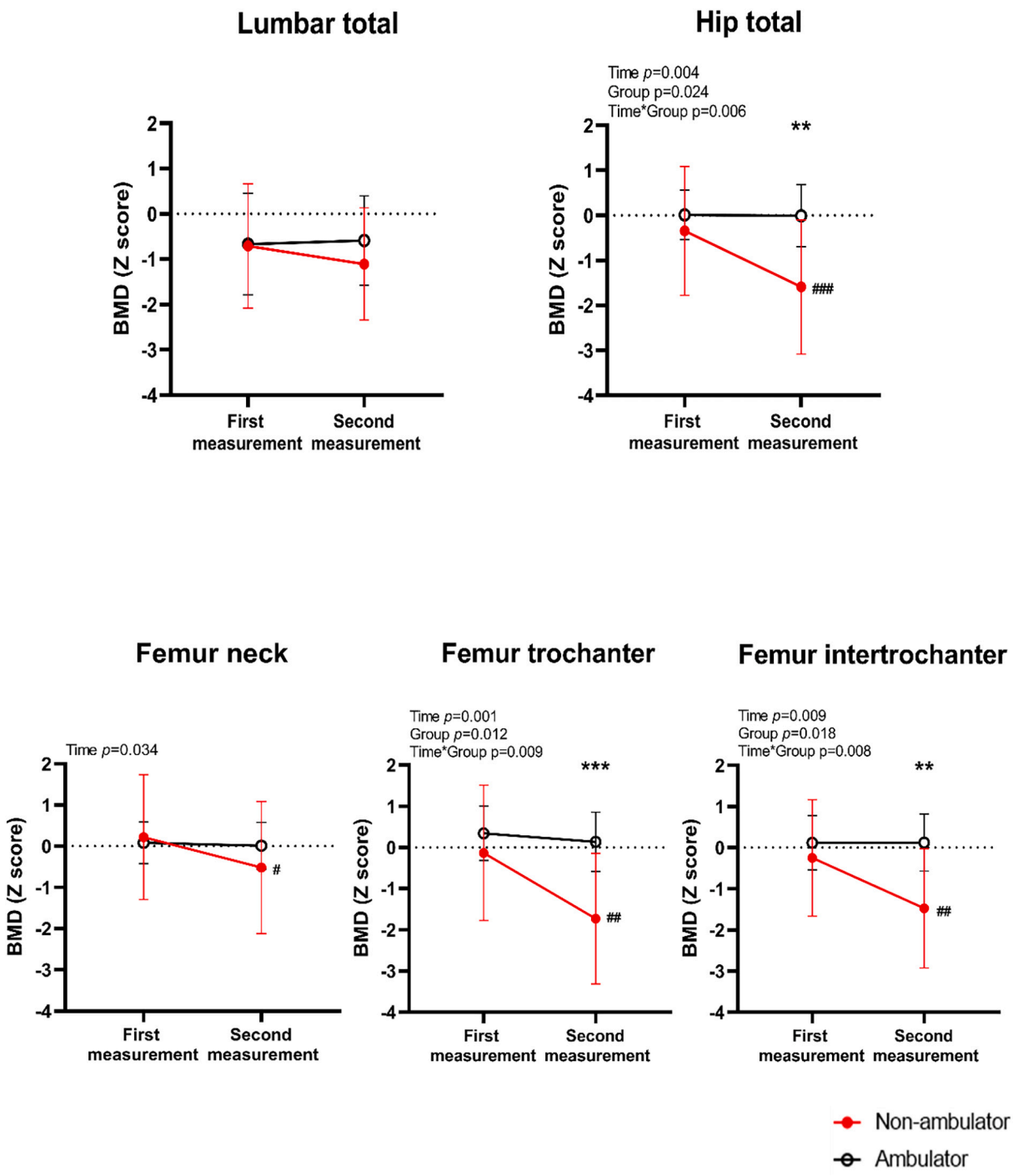

Fig. 2. Change of BMD according to ambulator status.
There was no statistical difference in the initial BMD between ambulatory and non-ambulatory groups in the first measurement. After the second measurement, the score of the total hip, femur neck, femur trochanter, and femur intertrochanter BMD decreased in the non-ambulatory group. Statistical analysis was performed using two-way repeated measures ANOVA and the group effect on each time point was clarified by independent *t*-test (**$p < 0.001$; ***$p < 0.01$). The first and second measurements were compared by paired-t-test ($^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$).

clinical conditions. There are few studies using clinical data to consider the impact of stroke on osteoporosis and bone loss with an emphasis on sclerostin accumulation and the relationship with declining BMD.

Despite clear evidence indicating the relationships between stroke and neurological disease or injury and bone loss, data from stroke patients reveals that there is still a lack of comprehensive care for osteoporosis following stroke, while a majority of patients are not screened for osteoporosis in routine practice after stroke onset (Hsieh et al., 2020; Li et al., 2023). This indicates a still-pressing need for improved therapies and care regimens to prevent accelerated bone loss in these patients. Physical therapy aimed at improving mechanical loading and purposefully subjecting the affected limbs to increased movement is an essential part of rehabilitative strategies to treat osteoporosis. However, there are cases in which patients are too old or have extensive neurodegeneration or neural injury to the point where this type of physical therapy alone may not be feasible (Benedetti et al., 2018; Multanen et al., 2014). Supplementary therapies are generally used alongside physical therapy, able to reverse or slow bone loss and resorption.

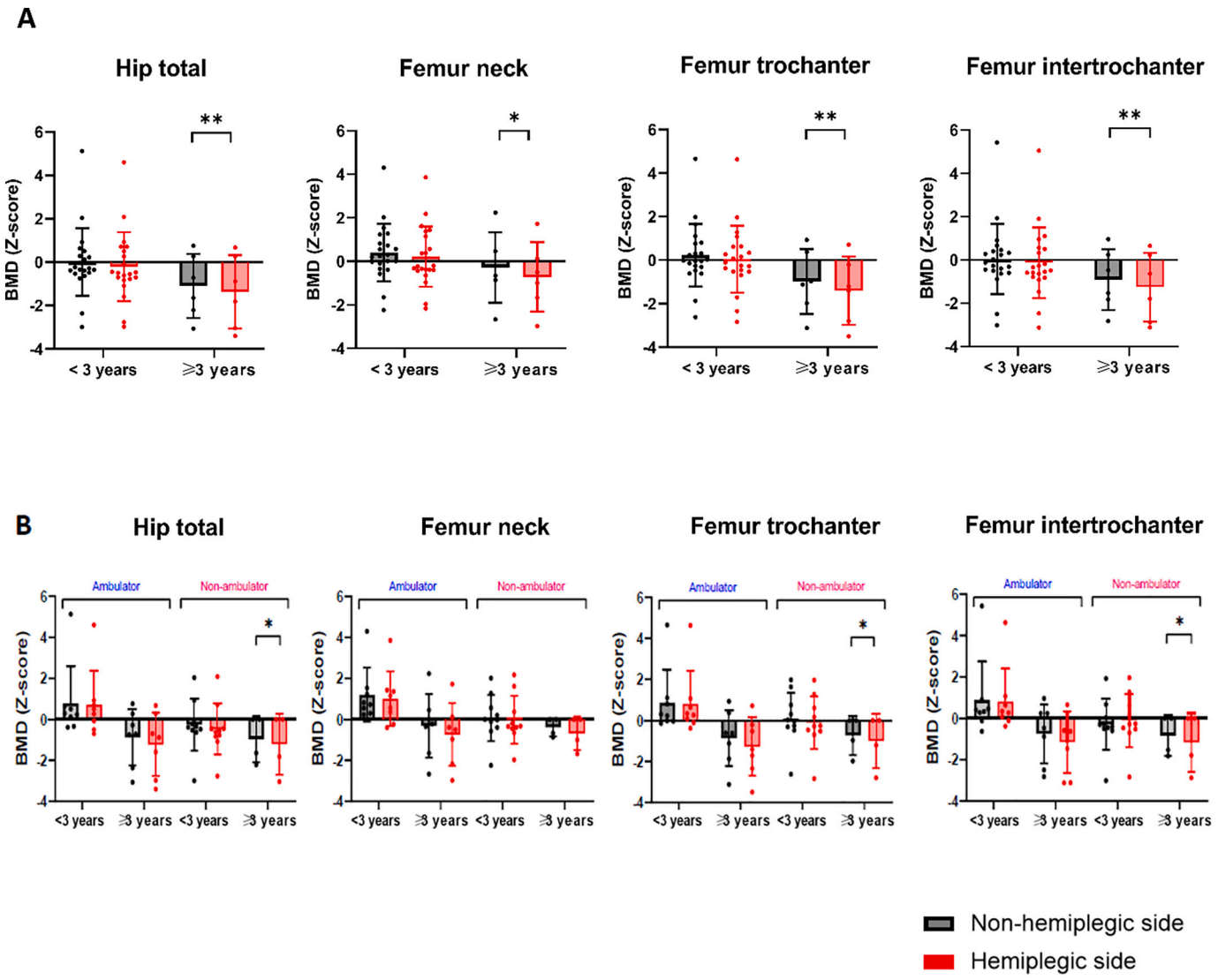

Fig. 3. Decrease of BMD on the hemiplegic side.
(A) In chronic patients with onset ≥3 years, BMD decreases more on the hemiplegic side than on the non-hemiplegic side by paired t-test (*$p < 0.05$; **$p < 0.01$). **(B)** Especially, the hemiplegic side of patients in the non-ambulatory group had a significantly decreased BMD compared to the non-hemiplegic side after 3 years of onset by paired t-test (* $p < 0.05$).

Among these, many of the most commonly used alternative therapies, such as anti-sclerostin antibodies, bisphosphonates, or hormonal treatments are limited in their use to specific demographics. While the anti-sclerostin antibody Romosozumab has been used in Phase 3 studies in postmenopausal women, this treatment is unable to be used in patients with a history of myocardial infarction or stroke (Fabre et al., 2020). Bone loss caused by a lack of estrogen following menopause can be treated by supplemental hormonal replacement therapy (Gosset et al., 2021; Stevenson and S., 2023), but this excludes a significant portion of patients who suffer from bone loss unrelated to menopause or who should not receive hormonal replacement therapy. Bisphosphonates are most commonly administered to patients with osteoporosis due to their low cost, high effectiveness, and reasonable safety (Hsieh et al., 2020; Li et al., 2023; Johnston and Dagar, 2020; Ramchand and Leder, 2024). Bisphosphonates can be administered orally or intravenously, but the former requires the patient to remain upright for 40 min following dosage. IV bisphosphonate administration is available for patients unable to do so but may have additional adverse side effects. While bisphosphonates are generally considered safe, the risk of atypical subtrochanteric fracture increases progressively as patients continue the regimen (Johnston and Dagar, 2020). Current regimens for stroke patients include a combination of bisphosphonates, vitamin D and calcium supplementation, and rehabilitative exercise, with supplemental hormonal therapy when applicable (Hsieh et al., 2020; Li et al., 2023). Especially in non-ambulatory patients, the range of possible physical therapies and targeted exercises may be slim or nonexistent depending on their level of recovery.

Thus, as stroke patients experience bone degeneration as a consequence of their immobility due to paralysis and consequent decrease in mechanical loading, interventions targeting the mechanosensory pathways that drive osteoclast and osteoblast equilibrium should be developed for use alongside current therapies. To prevent excessive bone resorption, osteoclasts, which can directly reabsorb the bone matrix (Delaisse et al., 2021), should be in constant balance with the bone-producing osteoblasts, the cells responsible for the maintenance and formation of bone structures and bone mass (Ponzetti and Rucci, 2021). Terminally differentiated osteoblasts, which are known as osteocytes, are the main mechanosensory cells that directly respond to changes in mechanical load and shear stress (Choi et al., 2021; Qin et al., 2020) and are the master regulators of osteoclast and osteoblast homeostasis via

secretion of various factors (Takayanagi, 2021; Cabahug-Zuckerman et al., 2016; Winkler et al., 2003; Delgado-Calle and Bellido, 2015; Qin et al., 2020), including RANKL, the secretion of which is exacerbated by high levels of sclerostin (Suen and Qin, 2016; Cabahug-Zuckerman et al., 2016; Winkler et al., 2003; Delgado-Calle and Bellido, 2015; Galea et al., 2017). While the pathways governing osteoclast and osteoblast homeostasis have been heavily interrogated both in vitro and in animal models (Ponzetti and Rucci, 2021; Takayanagi, 2021; Qin et al., 2020; Pathak et al., 2015), developing therapeutics focusing on activating pathways involved in mechanical loading appears even more critical in light of our and other group's clinical assessments. Recent clinical research has shown promising results for stimulating osteoporotic bone repair via specific nanocarrier-mediated targeting of the main mechanosensitive ion channel responsible for osteocytic mechanosensation, Piezo1 (Qin et al., 2020; Sasaki et al., 2020; Guan et al., 2024). As mechanosensation by the Piezo1 channel directly suppresses sclerostin accumulation leading to osteoporosis (Sasaki et al., 2020), stimulating the channel via targeted pharmaceutical interventions looks especially relevant to developing improved treatment regimens for patients whose osteoporosis may be primarily a result of hemiplegia and quadriplegia following stroke.

5. Conclusion

Our study highlights the critical role of mechanical loading in maintaining bone health in chronic stroke patients. The elevated sclerostin levels and accelerated bone loss observed in non-ambulatory patients underscores the need for early and targeted interventions to preserve BMD and prevent osteoporosis in this vulnerable population. Our results show not only that mechanical loading plays a significant part in contributing to both elevated sclerostin levels and decreased BMD, but also that BMD tends to decrease more on the hemiplegic side of the body, further confirming our hypothesis that reduced mechanical loading, at both a local and systemic level, is a key contributor to bone loss and elevated sclerostin in stroke patients. Additionally, our study is among the first to investigate the long-term repercussions of prolonged mechanical unloading on sclerostin concentrations and BMD in chronic stroke patients. Further research is needed to explore the potential benefits of anti-sclerostin therapies and rehabilitation strategies aimed at enhancing mechanical load and improving bone health in stroke patients.

Abbreviations

BMD	bone mineral density
Scl-Ab	anti-sclerostin antibody
PTH	serum parathyroid hormone
ELISA	enzyme-linked immunosorbent assay
CTx	C-telopeptide of type I collagen
Q-CT	quantitative computed tomography
P1NP	procollagen type 1 N-terminal propeptide
BMI	body mass index

CRediT authorship contribution statement

Hye Kyoung Lee: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Investigation, Conceptualization. **Geneva Rose Notario:** Writing – review & editing, Writing – original draft, Validation. **Sun Young Won:** Visualization, Investigation, Data curation. **Jung Hwan Kim:** Visualization, Validation, Investigation, Data curation. **Su Min Lee:** Investigation, Data curation. **Ha Seong Kim:** Conceptualization. **Sung-Rae Cho:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

No conflict of interest.

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Data availability

Data will be made available on request.

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