Patients with non-ambulatory cerebral palsy have higher sclerostin levels and lower bone mineral density than patients with ambulatory cerebral palsy

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A b s t r a c t

Bone loss is a serious clinical issue in patients with cerebral palsy (CP). Sclerostin has garnered interest as a key mechanosensor in osteocytes, leading to considerations of the therapeutic utilization of anti-sclerostin medications. This study was undertaken to determine associations among mechanical unloading, sclerostin levels, and bone imbalance in patients with CP. A total of 28 patients with CP participated in this cross-sectional study. The following measurements were taken: anthropometrics, clinical diagnosis of CP subtype and ambulatory status, bone mineral density (BMD) z-scores at the lumbar spine and hip, and blood biochemical markers, including sclerostin, parathyroid hormone (PTH), osteocalcin, C-terminal telopeptide, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, creatinine, calcium, and phosphorus. In analysis according to CP subtype, patients with spastic CP showed significantly lower BMD z-scores at the lumbar spine and femur neck regions than patients with dyskinetic CP. In analysis according to ambulatory status, patients with non-ambulatory CP showed significantly lower BMD z-scores at all lumbar spine and femoral sites, lower PTH and creatinine levels, and higher plasma sclerostin levels than patients with ambulatory CP. In regression analysis, ambulatory status was a significant determinant of plasma sclerostin levels. This study is the first to report on sclerostin levels and BMD in patients with CP, based on the hypothesis that patients who lack sufficient weight-bearing activities would show increased sclerostin levels and decreased BMD scores, compared with patients who sustain relatively sufficient physical activity. Therefore, this report may provide clinical insights for clinicians considering ambulatory status, sclerostin levels, and bone loss in patients with CP.

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1. Introduction

Cerebral palsy (CP) from neonatal brain events results in a profound loss of bone balance due to prolonged conditions of paralysis and limited weight bearing in adulthood. Dysregulated muscle tone and posture following brain disturbance interfere with normal movement and bone health [1,2]. Bone loss in adulthood CP increases not only the risk of fracture but also the risk of medical complications [3,4]. Despite these serious problems, there is no standard treatment for bone loss in patients with CP.

Recently, sclerostin has garnered more interest than any other biological markers of bone. Sclerostin has newly been identified as a key molecule in osteocytes: it acts as a mechanosensor regulating bone balance in response to weight loading under gravity [5–8]. Sclerostin potentially inhibits bone formation by competing with Wnt and LRP5 or LRP6 receptor signaling cascades, which are related to osteogenesis, in vivo and in vitro. In these molecular pathways, sclerostin inhibits osteoblast differentiation and proliferation, ultimately resulting in bone loss [5–7,9–11].

Several reports have shown that sclerostin levels change in response to partial or complete mechanical unloading in human subjects [8,12–14]. Gaudio et al. [8] revealed that higher sclerostin levels were negatively associated with bone formation markers and positively associated with bone resorption markers in patients who were immobilized in
beds or wheelchairs after stroke. Spatz et al. [13] showed that sclerostin levels increased in response to prolonged skeletal disuse for 90 days in healthy adults. The increase in serum sclerostin was accompanied by a decrease in serum PTH, increase in urinary bone resorption marker and calcium, and decrease in bone mineral density (BMD). Power et al. [14] showed that osteocyte sclerostin was inversely correlated with the bone formation marker alkaline phosphatase in musculoskeletal diseases, such as osteoarthritis and femur neck fracture. Morse et al. [15] found that circulating sclerostin levels were lower in patients with complete spinal cord injury who required the use of wheelchair than in patients with incomplete injury who did not require the use of wheelchair. These previous studies suggest that the association between sclerostin and bone imbalance is stronger in severe disease conditions that interfere with normal weight bearing through the lower extremities.

To date, few studies have taken into account sclerostin activity in response to mechanical loading when analyzing bone loss following brain injury. Our study aimed to elucidate the associations among mechanical unloading, sclerostin level, and bone imbalance in patients with CP. Significant bone loss has been found in patients with spastic CP and patients with non-ambulatory CP due to their limited movement [2,16,17]. Our cross-sectional study was undertaken to compare bone metabolic changes and BMD differences across two parameters (CP subtype and ambulatory status) and to investigate the role of sclerostin in patients with CP. With this rationale, we hypothesized that patients with spastic CP and patients with non-ambulatory CP would show higher sclerostin levels and lower BMD, in favor of bone resorption, than patients with dyskinetic CP and patients with ambulatory CP.

2. Materials and methods

2.1. Participants

This study was designed as a cross-sectional study. All subjects visited our institution for concerns regarding osteoporosis or treatment for low BMD. After screening, subjects who were not eligible in our study were excluded in accordance with the following criteria: (1) age under 20 years; (2) history of drug or medication use that could affect bone metabolism within the previous 12 months, such as bisphosphonate, anticonvulsants, and glucocorticoid; (3) any diseases that might affect bone metabolism, such as diabetes, hyperparathyroidism, hypercortisolism, rheumatoid arthritis, and bone tumor; and (4) systemic illness, such as kidney, liver, and thyroid diseases. Ultimately, 28 patients were determined to be eligible for our study. A healthy control group of 18 age-matched subjects were also recruited. The demographic characteristics of the healthy controls are provided as follows: median age (31.50 years), height (165.50 cm), weight (58.50 kg), and body mass index (BMI) (21.09 kg/m²).

This research was carried out with human subjects by complying with the Declaration of Helsinki. This study was approved by the Institutional Review Board (4-2013-0404) of Yonsei University Health System. Informed consent was obtained from all participants, who approved all procedures of this study.

2.2. Clinical categories of patients with cerebral palsy

All participants were placed into two clinical categories according to subtype of cerebral palsy and ambulatory status. Regarding CP subtype, a participant who showed impairment of joint mobility due to hypertonic muscle was categorized as spastic CP. A participant who showed repetitive involuntary movement due to mixed hypo- and hyper-tonicity was categorized as dyskinetic CP. Regarding ambulatory status, a participant who was able to walk independently or with crutches and walkers was categorized as ambulatory CP. A participant who was able to move around via a wheelchair was categorized as non-ambulatory CP.

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 parathyroid hormone (PTH) was measured by immunoradiometric assay (IRMA) using commercial kits (Biosource, Nivelles, Belgium); (2) serum osteocalcin (OCN) concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) (CIS Bio International, Gif-sur-Yvette, France); (3) serum C-telopeptide of type I collagen (CTX) concentrations were measured by ELISA (Osteomark, Ostex International, Seattle, WA, USA); (4) serum 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) were measured by radioimmunoassay (RIA) using commercial kits (D or D3-RIA-CT, Biosource, Nivelles, Belgium); (5) serum calcium and phosphate were measured by standard routine chemistry techniques using an automatic device (Hitachi, Tokyo, Japan); and (6) serum creatinine was also measured by a standard routine chemistry technique using an automated chemistry analyzer (Hitachi, Tokyo, Japan) to identify renal function in all participants.

2.4. Measurement of plasma sclerostin concentrations

Peripheral blood samples were obtained from each participant. After centrifuging whole blood for 10 min at 2000–2500 g, the supernatants were collected. All plasma samples were immediately frozen and stored at −80 °C before ELISA, a method to determine sclerostin concentrations. Plasma sclerostin levels were determined using an immunoperoxidase assay kit (E-80SST, Immunology Consultants Laboratory Inc., Portland, USA) following the manufacturer’s instructions (http://www.icllab.com/human-sclerostin-elisa-kit.html). Individual sclerostin concentration results were obtained by calculating the mean value from sample duplication.

2.5. Measurement of bone mineral density

The BMD was measured at the lumbar spine (L1-L4), proximal femur (femoral neck, intertrochanter), and total femur by dual-energy X-ray absorptiometry (Delphi A, Hologic, Waltham, MA, USA) with software version 12.6.

2.6. Statistics

All statistical analyses were performed with SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). Median and interquartile ranges (25th–75th percentiles) were used for non-normally distributed variables, and p < 0.05 was considered statistically significant. Chi-square test was used to identify differences in sex distribution between groups. Mann-Whitney U tests were used to compare the difference between two groups in regards to demographic parameters, biochemical markers, and BMD. Regression analysis was used to identify relationships between multiple variables and sclerostin and to investigate predictive variables of sclerostin concentration.

3. Results

3.1. Comparison according to subtype of CP

The clinical characteristics of our subjects are shown in Table 1 and Table 2. Twenty-eight patients diagnosed with CP (median age, 38.00 years) participated in this study. Baseline characteristics are shown according to two clinical criteria, namely CP subtype and ambulatory status. Sixteen patients with spastic CP and 12 patients with dyskinetic CP participated in this study (Table 1). There were no significant differences in demographic parameters, such as age, sex, and BMI, blood biochemistry markers, or plasma sclerostin levels between groups.
3.3. Association between ambulatory status and sclerostin levels

Thirteen patients with ambulatory CP and 15 patients with non-ambulatory CP participated in this study (Table 2). There were no significant differences in demographic parameters. In the analysis of biochemistry markers, there were no significant differences in the univariate model. Furthermore, patients with non-ambulatory CP showed higher sclerostin levels (390.68 vs. 237.71 pg/mL) than patients with ambulatory CP.

3.3. Association between ambulatory status and sclerostin levels

Linear regression analysis was conducted to identify significant variables affecting sclerostin levels in patients with CP. The final model revealed a significant association between sclerostin and ambulatory status ($p = 0.023$), which was significant in the univariate model ($p = 0.014$), after adjustment for demographical factors, including age, sex, and BMI (Table 3).

3.4. Comparison of BMD in ambulatory status and CP subtype

In comparison of bone densities between different CP types, the spastic CP group had significantly lower BMD z-scores for the lumbar spine (−1.60 vs. −0.80) and femur neck (−1.75 vs. −0.55) than the dyskinetic CP group (Fig. 1-A).

Comparing bone densities between ambulatory/non-ambulatory groups, results showed that patients with non-ambulatory CP had significantly lower BMD z-scores than patients with ambulatory CP within

### Table 1
Baseline characteristics of participants according to subtype of cerebral palsy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Spastic CP</th>
<th>Dyskinetic CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>–</td>
<td>26.00 (21.25–44.50)</td>
<td>42.00 (36.25–46.00)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>8/8</td>
<td>7/5</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.5–25</td>
<td>22.57 (18.47–24.38)</td>
<td>22.14 (19.95–24.52)</td>
</tr>
<tr>
<td>Sclerostin concentration (pg/mL)</td>
<td>15–65</td>
<td>347.83 (250.54–418.70)</td>
<td>256.39 (185.17–369.38)</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>14–42</td>
<td>28.29 (20.90–31.88)</td>
<td>19.56 (14.55–25.59)</td>
</tr>
<tr>
<td>CTx (ng/mL)</td>
<td>0.584</td>
<td>0.52 (0.33–0.77)</td>
<td>0.30 (0.22–0.51)</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (ng/mL)</td>
<td>Deficiency &lt;9</td>
<td>12.54 (10.59–23.10)</td>
<td>12.44 (8.89–17.60)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.49–0.91</td>
<td>0.62 (0.51–0.71)</td>
<td>0.69 (0.61–0.73)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.5–10.5</td>
<td>9.30 (9.00–9.58)</td>
<td>9.10 (8.70–9.50)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>2.5–4.5</td>
<td>3.90 (3.58–4.10)</td>
<td>3.50 (3.40–3.90)</td>
</tr>
</tbody>
</table>

Note. Median (interquartile range); * $p < 0.05$; BMI: body mass index; PTH: parathyroid hormone; OCN: osteocalcin; CTx: C-telopeptide of type I collagen.

### Table 2
Baseline characteristics of participants according to ambulatory status.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Ambulatory CP</th>
<th>Non-ambulatory CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>–</td>
<td>36.00 (23.50–43.00)</td>
<td>39.00 (24.00–48.00)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>8/5</td>
<td>7/8</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.5–25</td>
<td>23.53 (20.14–24.81)</td>
<td>20.00 (18.02–24.22)</td>
</tr>
<tr>
<td>Sclerostin concentration (pg/mL)</td>
<td>–</td>
<td>237.71 (195.50–295.59)</td>
<td>390.68 (328.46–457.61)*</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>15–65</td>
<td>50.60 (36.15–62.80)</td>
<td>37.70 (30.70–45.40)*</td>
</tr>
<tr>
<td>CTx (ng/mL)</td>
<td>14–42</td>
<td>23.51 (15.49–32.22)</td>
<td>25.59 (18.69–30.57)</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (ng/mL)</td>
<td>Deficiency &lt;9</td>
<td>10.65 (8.13–14.87)</td>
<td>17.95 (10.70–23.12)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.49–0.91</td>
<td>0.70 (0.66–0.75)</td>
<td>0.57 (0.46–0.69)*</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.5–10.5</td>
<td>9.15 (8.73–9.50)</td>
<td>9.30 (9.00–9.60)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>2.5–4.5</td>
<td>3.70 (3.33–3.98)</td>
<td>3.90 (3.50–4.10)</td>
</tr>
</tbody>
</table>

Note. Median (interquartile range); * $p < 0.05$; BMI: body mass index; PTH: parathyroid hormone; OCN: osteocalcin; CTx: C-telopeptide of type I collagen.

### Table 3
Linear regression analysis for predicting sclerostin levels from clinical variables.

<table>
<thead>
<tr>
<th>Variables (reference)</th>
<th>Coefficient (SE)</th>
<th>$p$-value</th>
<th>Coefficient (SE)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.51 (2.37)</td>
<td>0.832</td>
<td>−0.48 (2.40)</td>
<td>0.842</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>35.91 (40.84)</td>
<td>0.478</td>
<td>21.21 (50.56)</td>
<td>0.679</td>
</tr>
<tr>
<td>BMI</td>
<td>−1.81 (7.15)</td>
<td>0.802</td>
<td>3.20 (7.09)</td>
<td>0.656</td>
</tr>
<tr>
<td>Ambulatory status (ambulatory)</td>
<td>117.54 (44.75)</td>
<td>0.693</td>
<td>121.57 (49.94)</td>
<td>0.634</td>
</tr>
<tr>
<td>PTH</td>
<td>−1.78 (1.39)</td>
<td>0.211</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>OCN</td>
<td>1.47 (3.26)</td>
<td>0.057</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CTx</td>
<td>−47.90 (99.46)</td>
<td>0.634</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>25-hydroxyvitamin D</td>
<td>0.83 (3.60)</td>
<td>0.820</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Creatinine</td>
<td>−81.88 (205.42)</td>
<td>0.693</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Calcium</td>
<td>63.65 (60.49)</td>
<td>0.303</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>−8.45 (48.10)</td>
<td>0.862</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Note. * $p < 0.05$; SE (standard error); BMI: body mass index; PTH: parathyroid hormone; OCN: osteocalcin; CTx: C-telopeptide of type I collagen.
Bone mineral density (BMD) z-scores in the lumbar spine and femur neck were significantly lower in patients with spastic CP, compared to patients with dyskinetic CP (A. a & c). All BMD levels were significantly lower in patients with non-ambulatory CP than in patients with ambulatory CP (B. a-d). Statistical analysis was performed using Mann-Whitney U test. Median (interquartile range); *p < 0.05.

Fig. 1. Group comparison of lumbar and femur bone mineral density scores according to subtype of CP (A) and ambulatory status (B). Bone mineral density (BMD) z-scores in the lumbar spine and femur neck were significantly lower in patients with spastic CP, compared to patients with dyskinetic CP (A. a & c). All BMD levels were significantly lower in patients with non-ambulatory CP than in patients with ambulatory CP (B. a-d). Statistical analysis was performed using Mann-Whitney U test. Median (interquartile range); *p < 0.05.
regions of the lumbar spine (−1.80 vs. −0.80), femur neck (−1.80 vs. −0.50), femur inter-trochanter (−2.60 vs. −1.20), and total hip (−2.00 vs. −0.90) (Fig. 1-B).

4. Discussion

In the present study, we discovered that patients with non-ambulatory CP show significantly higher circulating levels of sclerostin than patients with ambulatory CP. Further, plasma sclerostin concentrations were found to be correlated with ambulatory status in regression analysis. These findings suggest that the main contributing factor to circulating sclerostin in the murine bone is the mechanical stimulation of habitual weight bearing in adults with CP. Furthermore, BMD levels of the lumbar spine and hip were lower in patients with non-ambulatory CP than patients with ambulatory CP. These results lend support to our hypothesis that patients who have limited weight bearing due to a wheelchair-depend-ent life would show higher sclerostin levels and lower BMD than patients who sustain relatively sufficient physical activity.

We also found that patients with CP show significantly lower circulating sclerostin levels than healthy controls (p < 0.01; 309.83 vs. 532.42), in line with the previous report [15]. Morse et al., however, suggested that lower sclerostin levels in chronic patients with complete paralysis were due to a greater loss of osteocytes in an acute stage, compared to patients with incomplete paralysis. In other words, the greater loss of osteocyte numbers following severe bone loss could lead to lower sclerostin levels under the extreme osteoporosis condition in spinal cord injured patients with complete paralysis. We speculate that these discrepancies might stem from etiological heterogeneity between the different cohorts. In our study, patients with non-ambulatory CP were not in completely paralyzed conditions unlike complete spinal cord injury. Therefore, we suspect the difference in the numbers of osteocyte between subgroups based on injury severity was less than in spinal cord injured patients. Taken together, osteocytes might be similar in patients with CP regardless of ambulatory status, and the number thereof might differ only between normal controls and patients with CP. Therefore, we suggest that differences in mechanical loading between subgroups in CP might significantly affect sclerostin levels, in line with the previous report in chronic patients with stroke [8].

While sclerostin levels were not sensitive to CP subtype, we found that patients with spastic CP, who suffer from hypertonicity and limited joint movement, showed lower BMD in the lumbar spine and femoral neck than patients with dyskinetic CP, who suffer from mixed muscle tones and hyperkinetic patterns. Although it was not statistically significant, the patterns of bone resorption markers in the participants in our study were consistent with previous data. Specifically, a previous study showed that adults with non-ambulatory spastic CP had lower BMD and higher concentrations of the bone resorption marker CTx than patients with ambulatory spastic CP and patients with dyskinetic CP. This result suggests that reduced weight loading and joint mobility in patients with CP can contribute to the imbalance in bone metabolism [2].

Adults with CP commonly have musculoskeletal abnormalities, including osteopenia or osteoporosis at an early age [16,18]. Although there is insufficient information about BMD and other contributing factors in adults with CP, several studies have shown an association between BMD and certain risk factors [4,19,20]. Previous studies have commonly revealed that functional decline has a strong association with BMD in adults with CP. Altogether, these results imply that ambulatory function contributes to the microenvironment for bone health in patients with CP.

In the bone microenvironment, sclerostin is nearly exclusively secreted by osteocytes and could exert a negative osteogenic effect by inhibiting Wnt-signaling under the condition of reduced mechanical loading. Moustafa et al. [21] showed that axially applied mechanical loading is associated with decreased sclerostin levels and increased new bone formation in the proximal tibia and secondary spongiosa in mice. However, these osteogenic effects were reversed by sciatric neuroectomy-induced disuse. Robling et al. [6] showed that artificially enhanced mechanical stimulation reduced SOST gene transcripts and sclerostin protein expression, especially in the high compression regions of the murine bone. These evidences suggest that sclerostin has a key role in the regulation of mechanotransduction and support the therapeutic concept of anti-sclerostin intervention in patients with CP.

An interesting finding of this study was that low serum creatinine levels were found in patients with reduced BMD, consistent with the recent study [22]. Huh et al. demonstrated that subjects who had low serum creatinine showed low bone density, presumably because of cross-talk between bone and muscle. In a parallel fashion, patients with non-ambulatory CP showed lower serum creatinine levels than patients with ambulatory CP, and this result might be related to muscle atrophy caused by limitation of physical activity [23]. We propose that the deleterious effects of CP on BMD primarily reflect a lack of physical activity and associated sclerostin response to limited mechanical loading caused by a lifelong motor deficit in adults with CP. Therefore, clinicians need to consider both exercise and anti-sclerostin treatments as a synergistic strategy to enhance the anabolic effects on bone in patients with CP [13,24–29].

This is a cross-sectional study. Longitudinal measures of sclerostin levels, BMD, and other clinical factors would be informative toward understanding the cumulative effects of weight bearing on sclerostin levels with aging in patients with CP. A larger sample size would be necessary to better investigate the relationships between sclerostin levels and bone metabolism-related factors. Despite these limitations, this study may provide clinical insights on sclerostin levels in response to prolonged mechanical unloading under the pre-existing pathological conditions found in patients with CP.

5. Conclusions

This study discloses a novel finding concerning bone health in adults with CP who showed differences in sclerostin and BMD levels according to ambulatory status. Patients with non-ambulatory CP showed higher sclerostin levels and lower lumbar spine and femur BMD than patients with ambulatory CP. Ambulatory status was a significant predictor of sclerostin levels in patients with CP. This report may provide a clinical insight for clinicians considering diagnosis and treatment for bone loss in patients with CP.

Authors’ role

All authors substantially contributed to this study. Co-primary authors: Yoon-Kyum Shin and Young Kwon Yoon. Yoon-Kyum Shin contributed to data collection and data analysis, drafted manuscript, and revised manuscript contents. Young Kwon Yoon contributed to data collection and data analysis, and drafted manuscript. Kyung Bae Chung contributed to discussion on results of this study and revision of manuscript for scholarly publication. Yumie Rhee contributed to data interpretation, and approved final version of manuscript. Sung-Rae Cho established study design, revised critically intellectual contents of the manuscript, and approved final version of manuscript.

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