Effects of whole-body vibration on plasma sclerostin level in healthy women*

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1. Introduction
Sclerostin, a SOST gene protein, potently inhibits Wnt canonical signaling by binding to low-density lipoprotein receptor-5 and is a potent inhibitor of bone formation (1–3). It is produced almost exclusively by osteocytes. Sclerostin expression is also regulated by mechanical strain in adult bone. Osteocytes, the most abundant cells in bone, have mechanosensory properties, and mechanical loading triggers the cells to modulate bone homeostasis (1,2,4,5).

It has been recently shown that mechanical stimulation of long bones in mice suppresses sclerostin expression in osteocytes and simultaneously induces bone formation in rodents (2,6,7). Conversely, mechanical unloading causes upregulation of sclerostin activity in mice and disuse osteoporosis in humans (1,4).

Sclerostin acts in a local paracrine fashion in the bone microenvironment. Mirza et al. showed that sclerostin also enters the circulatory system, where it may regulate bone mass by acting as an endocrine hormone. They also demonstrated that sclerostin can be measured in peripheral serum (8).

Plasma sclerostin level may be used as an indicator to evaluate the bone response to mechanical loading. Despite a growing understanding of the basic biology of sclerostin, no data are available on circulating sclerostin levels in humans exposed to cyclic mechanical loading.

In this study, we aimed to determine whether plasma sclerostin level is affected by whole-body vibration (WBV) in healthy women.

2. Materials and methods
Ethical approval was obtained from the Institutional Review Board (Ethics Committee of Istanbul University's Istanbul Medical Faculty, 2011/228-473). All testing

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procedures and the experimental protocol were explained, and written informed consent was received.

To determine the earliest changes in plasma sclerostin levels, we designed a pilot study on 33 voluntary adult healthy women working in our hospital. In our pilot study, plasma sclerostin levels tended to increase during the vibration and at 10 and 30 min after vibration, even though the elevation was not statistically significant. However, the increase in sclerostin level was more obvious 10 min after vibration in each of the 3 groups. Based on these findings, the present study was planned as a prospective, randomized, controlled, single-blind, single-center experimental trial.

2.1. Participants
Among young-adult healthy women working in our hospital, 34 subjects who voluntarily agreed to participate were assessed for eligibility. None of subjects who participated in the pilot study were enrolled in this study. Exclusion criteria are given in Table 1.

The participants meeting the criteria were randomized into the WBV group and the control group. Only one investigator was involved in the randomization process, which employed a computerized random number generator. This study followed the guidelines of the CONSORT statement for individual randomized, controlled trials with no pharmacological treatment (Figure 1).

The mean age of the participants was 28.8 ± 6.5 years in the WBV group (n = 8) and 29.3 ± 4.2 years in the control group (n = 8) (P = 0.859). The body mass index was 22.2 ± 1.9 kg/m² in the WBV group and 21.0 ± 2.2 kg/m² in the control group (P = 0.281)

2.2. WBV application
During WBV, the subjects were asked to stand upright on a vibration platform. Their ankles and knees were in a neutral position. The hip joint was held in abduction so that there was 30 cm between the heels. Subjects were barefooted, and no sponge or foam was placed between the vibration platform and their feet (Figure 2). A synchronous WBV device was used in the present study. The whole plate oscillated with a linear movement upward and downward. The frequency of vibrations was set to 40 Hz, with a peak-to-peak amplitude of 2 mm and acceleration of 2.7 × g. WBV was applied at a rate of one session per day from Monday to Friday. WBV was applied on the 1st and 2nd days for 60 s (2 × 30 s treatments), on the 3rd day for 120 s (4 × 30 s treatments), on the 4th day for 150 s (5 × 30 s treatments), and on the 5th day for 210 s (3 × 30 s treatments + 2 × 60 s treatments). After each 30-s application of vibration, the subjects were told to relax for 10 s without changing their position (rest period).

2.3. Blood samples
In the WBV group, blood samples were obtained before WBV (pre-WBV) and at the 10th minute after WBV (post-WBV10) in all participants on the 1st, 2nd, and 5th days by inserting an intravenous cannula into the antecubital vein.

In the control group, 2 blood samples were obtained on the same day. The second samples were obtained 10 min after the first sample.

Table 1. Exclusion criteria.

<table>
<thead>
<tr>
<th>1. Lower extremity problems</th>
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<tbody>
<tr>
<td>a. Orthopedic problems: shortness of legs, congenital anomalies, etc.</td>
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<tr>
<td>b. Joint disease (arthritis, joint prosthesis, etc.), other painful pathologies in the lower extremities (fractures, tendinitis, bursitis, etc.)</td>
</tr>
<tr>
<td>c. Circulation problems in the lower extremities</td>
</tr>
<tr>
<td>2. Dorsolumbar diseases: Vertebral fractures, disk hernias, spondylodiscitis, etc.</td>
</tr>
<tr>
<td>3. Systemic disease cases</td>
</tr>
<tr>
<td>a. Systemic bone disease: osteoporosis, osteomalacia, Paget’s disease</td>
</tr>
<tr>
<td>b. Hypertension (&gt;135 mmHg systolic, &gt;85 mmHg diastolic), heart diseases</td>
</tr>
<tr>
<td>c. Infectious diseases</td>
</tr>
<tr>
<td>d. Endocrine diseases (diabetes mellitus, etc.)</td>
</tr>
<tr>
<td>4. Neurological diseases</td>
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<tr>
<td>5. Menstrual cycle disorders, amenorrhea, lactation, oral contraceptive use</td>
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<td>6. During the ovulatory period (11th–16th day of menses)</td>
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<tr>
<td>7. Obesity (BMI &gt; 30 kg/m²) or low BMI (BMI &lt; 20 kg/m²)</td>
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<tr>
<td>8. Vertigo</td>
</tr>
<tr>
<td>9. Cognitive function disorders</td>
</tr>
<tr>
<td>10. Nonpalpable antecubital vein</td>
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</tbody>
</table>

BMI: Body mass index.
2.4. Plasma sclerostin, estradiol, and parathyroid hormone levels

Plasma was collected using EDTA and was centrifuged for 15 min at 400 × g within 30 min of collection. Aliquots of plasma were added to Eppendorf tubes and stored at –20°C.

Plasma sclerostin levels were measured blindly by only one investigator. No information was given to the lab workers regarding blood sample identification or time when the blood was taken. Plasma sclerostin levels were measured using a Human Sclerostin ELISA kit (Cusabio, Catalog No. CSB-E13146h, Newark, DE, USA). All assays were performed according to the manufacturer’s instructions. The minimum detectable concentration of human sclerostin is typically <0.012 ng/mL. None of the measured sclerostin values in our subjects were below the limit of detection for this assay. Intraassay precision is less than 8%.

Figure 1. Flow chart of participants considered for inclusion.

Figure 2. Posture of the participants during WBV.
Plasma parathyroid hormone (PTH) and estradiol (E2) levels were measured to test whether hemoconcentration or hemodilution occur during or after WBV. PTH and E2 were analyzed in EDTA-plasma samples using an electrochemiluminescence immunoassay (Elecsys PTH, Elecsys-Estradiol II; Roche Diagnostics, Mannheim, Germany) and the Cobas 601 analyzing system (Roche Diagnostics).

2.5. Statistical analysis
Continuous variables were summarized as the arithmetic mean and standard deviation (SD). The Kolmogorov–Smirnov test was used to confirm that data were normally distributed. Within-group comparisons were done using paired t-tests and general linear model repeated measures. Pearson’s correlation coefficient was used to test for associations between variables. A P-value of <0.05 was considered statistically significant. The software package used for data management was PASW Statistic 18.

3. Results
In the control group, plasma sclerostin levels measured in the 1st and 2nd blood samples were 0.835 ± 0.218 ng/mL and 0.878 ± 0.279 ng/mL, respectively (P = 0.602).

In the WBV group, the plasma sclerostin levels increased significantly 10 min after WBV on the 1st day (P = 0.024). The plasma sclerostin levels decreased significantly 10 min after WBV on the 5th day (P = 0.030). No significant change in sclerostin level with WBV was identified on the 2nd day (P = 0.159) (Table 2).

The levels of plasma sclerostin measured 10 min after WBV declined progressively during the 5 days of vibration sessions (P = 0.026). Post hoc analysis revealed that the levels of plasma sclerostin measured 10 min after WBV on the 5th day were significantly lower than those on the 1st day (P = 0.037) (Figure 3).

Only the 5-day cyclical mechanical stimulus was found to have caused a significant increase in the baseline plasma sclerostin levels (P = 0.008). Post hoc analysis revealed that the baseline levels of plasma sclerostin on the 5th day were significantly higher than those on the 1st day (P = 0.013)

No significant change in plasma E2 or PTH levels was observed after WBV (Table 3).

The effects of WBV on plasma sclerostin levels at the 10th minute were evaluated. The mean plasma sclerostin was 0.785 ± 0.302 ng/mL before WBV and 1.212 ± 0.287 ng/mL at 10 min after WBV on the 1st day. For the given effect size (plasma sclerostin level means of 0.785 vs. 1.212), SD (0.302 vs. 0.287), sample sizes (8 and 8), and alpha (0.050, 2-tailed), the power was 0.695. The mean plasma sclerostin was 1.408 ± 0.478 ng/mL before WBV and 0.832 ± 0.302 ng/mL at 10 min after WBV on the 5th day. For the given effect size (plasma sclerostin level means of 1.408 vs. 0.832), SD (0.478 vs. 0.287), sample sizes (8 and 8), and alpha (0.050, 2-tailed), the power was 0.649.

4. Discussion
In this study, the impact of cyclical mechanical loading on plasma sclerostin level was investigated for the first time. Our results revealed a significant change in plasma sclerostin levels 10 min after the cyclic mechanical stimulus. Baseline plasma sclerostin levels were also found to have increased progressively over the course of 5 days of mechanical loading sessions.

Bone tissue sclerostin levels are tightly regulated by mechanical strain (2). Sclerostin is expressed almost exclusively in osteocytes, and these osteocytes are able to detect mechanical strain (9). Osteocytes are stimulated by mechanical deformation of bone (10). The main mechanical factors determining the impact of mechanical loading on bones are strain (strain magnitude, strain rate), stress, and the frequency or the number of loading cycles (11–15). The extent of bone deformation, or strain magnitude, is directly proportional to the force magnitude to which the bone is exposed (11). According to Newton’s second law, the force magnitude is directly proportional to the acceleration (16). Subjects were asked to stand upright to create maximum mechanical loading on the lower extremity bones. The frequency of vibrations was set to 40 Hz, with acceleration of 2.7 × g in the present study.

We do not know the mechanism of the changes that occurred in the plasma sclerostin levels at post-WBV10. However, some mechanisms can be suggested. The increase in plasma sclerostin level 10 min after WBV may be explained by increased sclerostin secretion from the osteocytes and passage to the systemic blood circulation via lacuna–canalicular fluid. Sclerostin is a protein hormone (8). Protein hormones differ from steroid

<table>
<thead>
<tr>
<th>Time point</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 5</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-WBV</td>
<td>0.785 ± 0.302</td>
<td>0.998 ± 0.264</td>
<td>1.408 ± 0.478b</td>
<td>0.013b</td>
</tr>
<tr>
<td>Post-WBV10</td>
<td>1.212 ± 0.287a</td>
<td>0.903 ± 0.358a</td>
<td>0.832 ± 0.302a</td>
<td>0.037b</td>
</tr>
</tbody>
</table>

*: Comparison with pre-WBV; b: comparison with 1st day.
hormones in that they can be deposited inside the cell (17). A cyclic mechanical stimulus may trigger the secretion of sclerostin, which is mechanosensitive, from osteocytes. Cyclic mechanical loading can cause compression and decompression in bone tissue. As a result, lacuna–canalicular fluid moves into the blood vessels (18). WBV also significantly increases blood flow to tissue exposed to vibration (19,20). Relocation of lacuna–canalicular fluid and its mixture with increased blood circulation may cause more leakage of sclerostin into the blood circulation.

Sclerostin is reported to be higher in weight-bearing than in nonweight-bearing disciplines in athletes (3). A progressive mechanical loading was applied during the 5-day vibration sessions in the present study. Our findings showed a progressive increase in baseline plasma sclerostin levels during the 5-day vibration sessions. Relocation of lacuna–canalicular fluid and its mixture with increased blood circulation may also help explain the progressive increase in baseline plasma sclerostin levels.

We also determined that the plasma sclerostin level at the 10th minute after WBV decreased progressively during the 5-day vibration sessions (Figure 3). Despite the increased baseline level of plasma sclerostin during the 5-day vibration sessions, the plasma sclerostin level was found to decrease at 10 min after WBV on the 5th day. This finding suggests that after the 5-day, high-frequency, high-acceleration WBV treatment, cyclic mechanical stimulus may quickly inhibit sclerostin activity. A multisession, cyclic mechanical stimulus plays an anabolic and antiresorptive role in bone (21,22). Bone formation is known to increase on one side and bone resorption to decrease on the other side when the sclerostin activity decreases (23–25). Therefore, the decrease in the plasma sclerostin level at 10 min after WBV and the lower plasma sclerostin level at 10 min after WBV on the 5th day of WBV compared to the 1st day of WBV would seem to be compatible with the literature.

Some limitations should be noted. Whether WBV causes a volume change in plasma was not tested in this study. Plasma-volume alterations can change hormone concentrations. Bouts of acute exercise produce transient plasma-volume changes (26). However, Cardinale et al. reported no changes in plasma volume due to a single session of WBV (30-Hz, 4-mm amplitude for 5 sets of 1 min separated by a 1-min resting period) (27). Fricke et al. showed that WBV does not influence serum E2 levels in women (28). In this study, the absence of change in these hormone levels after WBV suggests that there was no hemoconcentration or hemodilution during or after WBV.

Bone strength and quality is important for osteoporosis (29–31). Evaluation of the response given by the bone to cyclic mechanical loading may be important in terms of in vivo determination of the strength and quality of bone. Postmenopausal osteoporosis is the most common form of osteoporosis (30). Therefore, female subjects were preferred in the study. Findings of this study may be useful for future studies investigating the bone strength and quality in patients with postmenopausal osteoporosis.

In conclusion, this study is the first to show the changes in plasma sclerostin level with WBV. Our study showed that WBV can cause changes in plasma sclerostin levels in adult women. This change is noticeable 10 min after whole-body vibration. WBV can also affect the baseline plasma sclerostin level at 10 min after WBV.

![Figure 3. Changes in baseline plasma sclerostin levels and plasma sclerostin levels at 10 min after WBV.](image-url)

### Table 3. The plasma E2 and PTH levels before and after whole-body vibration (pg/mL).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Time point</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>Pre-WBV</td>
<td>140.1 ± 77.2</td>
<td>120.8 ± 58.0</td>
<td>118.3 ± 43.0</td>
</tr>
<tr>
<td></td>
<td>Post-WBV10</td>
<td>137.5 ± 74.5</td>
<td>119.3 ± 59.3</td>
<td>115.3 ± 34.9</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.134</td>
<td>0.568</td>
<td>0.410</td>
</tr>
<tr>
<td>PTH</td>
<td>Pre-WBV</td>
<td>57.1 ± 27.0</td>
<td>60.8 ± 36.4</td>
<td>59.1 ± 30.4</td>
</tr>
<tr>
<td></td>
<td>Post-WBV10</td>
<td>54.0 ± 22.7</td>
<td>51.8 ± 24.7</td>
<td>58.6 ± 31.2</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.290</td>
<td>0.091</td>
<td>0.889</td>
</tr>
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plasma sclerostin levels when it is applied on consecutive days. However, future studies are needed to delineate the exact mechanism of changes in plasma sclerostin level with WBV.

Acknowledgment
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References


