J. Phys. Ther. Sci.
27: 2105–2107, 2015

Original Article

The effect of single extremity-vibration on the serum sclerostin level

Halil Ibrahim Cakar, PhD[1)*], Muharrem Cidem[2)], Ilhan Karacan[2)], Sadik Kara[1)]

[1)] *Institute of Biomedical Engineering, Fatih University: 34500, Büyükçekmece, Istanbul, Turkey*
[2)] *Physical Medicine and Rehabilitation Department, Bagcilar Training and Research Hospital, Turkey*

Abstract. [Purpose] Sclerostin is mechanosensitive protein that is produced exclusively by osteocytes. It was reported that the plasma sclerostin level increases in the 10th minute after the application of Whole-Body Vibration. The aim of this study was to determine whether single extremity-vibration induces any change in the serum sclerostin level. [Subjects and Methods] Eight healthy young-adult volunteers were recruited for this pilot study. The participants sat on a chair with their left hip and knee joints flexed at 90 degrees. The lower leg was exposed to vibration: 40 Hz, 4 mm, 60 s. Blood samples were collected before and after the vibration. The serum sclerostin levels were blindly measured in dual-controlled blood samples. [Results] The serum sclerostin level before vibration was 328.2±589.9 pg/ml, and it showed no significant change after vibration. [Conclusion] Unlike Whole-Body Vibration, Single-Extremity Vibration did not affect the serum sclerostin level significantly. This finding can be explained by the limited bone volume exposed to vibration. Bone volume exposed to vibration is less during Single-Extremity Vibration than during Whole-Body Vibration.
Key words: Sclerostin, Whole-Body Vibration, Bone

(This article was submitted Feb. 23, 2015, and was accepted Mar. 24, 2015)

INTRODUCTION

Sclerostin is a paracrine *SOST* gene protein that inhibits the formation of bone. Sclerostin binds to the LRP5 receptor and it inhibits Wnt canonical signaling[1–3)]. Sclerostin is produced by osteocytes. The expression of sclerostin in adult bone is regulated by mechanical strain. Osteocytes are abundant in bone tissue and have mechanosensory properties. This feature makes osteocytes responsible for bone homeostasis. Osteocytes are stimulated by mechanical loading to modulate bone homeostasis[1, 2, 4, 5)]. In a previous study, it was shown that the sclerostin expression was suppressed when the long bones of mice were stimulated mechanically. The bone formation of mice was also induced in parallel[2)]. Conversely, mechanical unloading causes up regulation of sclerostin activity in mice and disuse osteoporosis in humans[1, 4, 6, 7)].

As a source of mechanical loading for human subjects, application of the vibration leads to improvements in muscular contraction[8)] and overall muscle function[9)]. Whole body Vibration (WBV) is administered for its beneficial effects on improvement of physical strength and bone mineral density[10)]. Moreover, it has been shown that exercise with WBV elicits remarkable improvements in the balance and fear of falling of elderly people[11)]. WBV also has a positive effect on postural control[12)].

Sclerostin acts in a local paracrine fashion in the bone micro-environment. Mirza et al. showed that sclerostin enters the circulation, where it may regulate bone mass by acting as an endocrine hormone. They also demonstrated that sclerostin can be measured in the peripheral serum[13)].

The plasma sclerostin level can be used as an indicator of the bone response to mechanical loading. Cidem et al. showed that the plasma sclerostin level is detectable after 10 minutes of WBV exposure. They also suggested that evaluation of the sclerostin response of the bone to vibration may be important in terms of in vivo determination of the strength and quality of bone[14)]. However, at present, there is no study that has reported the plasma or blood sclerostin level change as a result of single extremity vibration exposure rather than WBV exposure. The characteristic change of the sclerostin level after single extremity vibration application would help to better our understanding of the exact response of the osteocytes to mechanical excitation and the evaluation of regional bone quality.

This study investigated the response of a single extremity to vibration exposure. The hypothesis of this study was that serum sclerostin level would increase after single extremity-vibration. The aim of this study was to test this hypothesis.

SUBJECTS AND METHODS

The study was approved by the ethical committee of Istanbul Medical Faculty Clinical Research Evaluation

*Corresponding author. Halil Ibrahim Cakar (E-mail: hicakar@fatih.edu.tr)

Table 1. Exclusion criteria

1. Lower extremity problems
a. Orthopedic problems: shortness of legs, congenital anomalies, etc.
b. Joint disease (arthritis, joint prosthesis, etc.), other painful pathologies in the lower extremities (fractures, tendinitis, bursitis, etc.)
c. Circulation problems in the lower extremities
2. Systemic disease cases
a. Systemic bone disease: osteoporosis, osteomalacia, Paget's disease
b. Hypertension (>135 mmHg systolic, >85 mmHg diastolic), heart diseases
c. Infectious diseases
d. Endocrine diseases (diabetes mellitus, etc.)
3. Vertigo
4. Cognitive function disorders
5. Nonpalpable antecubital vein

BMI: body mass index

Committee (Istanbul University, Istanbul; 2011/06). All the participants were informed about the study procedures which were in accordance with the ethical principles of the Declaration of Helsinki (Declaration of Helsinki, 2014).

Sample size was estimated using G*power 3.1.2 (Franz Faul, Universitat Kiel, Germany), considering a power of 80% and $\alpha = 5\%$. According to the data of Cidem et al., 8 subjects were required to detect a 0.4275 pg/ml difference and a 0.4201 standard deviation in the sclerostin level.

Eight healthy young male adults were recruited for the experiment. The exclusion criteria are given in Table 1. The mean age of the subjects was 27.3±7.63 years, their mean body height was 175.6±9.0 cm, their mean body weight was 78.7±16.1 kg, and their mean body mass index was 25.3±3.4 kg/m^2.

The subjects sat upright with their left hip and knee joints flexed at 90 degrees on an armchair that was placed in front of a vibration platform. The left leg of each subject was placed on the vibration platform and the right leg was placed on a vibration-free and mechanically isolated platform that had the same height as the vibration platform. The subjects were barefoot during the experiment. A mechanical load that was about the half weight of each subject was placed on the left knee of the each subject to load the tibia, fibula and foot bones.

Before starting the experiments a cannula was inserted in the right forearm vein of the subjects for the collection of blood samples. The first blood sample was taken before the vibration. Immediately after sampling, the left lower leg was exposed to vibration for 60 seconds of 4 mm amplitude and 40 Hz frequency. Blood samples were subsequently collected at 3, 6, 8, 10, 12, 14, 17, 20, 23 minutes after the vibration.

The blood samples were centrifuged for 10 minutes at 3,000 rpm within 30 minutes of sample collection. Aliquots of plasma were transferred to Eppendorf tubes which were stored at −80 °C till the day of the analysis. Random codes were generated for each blood sample and these codes were written on the Eppendorf tubes.

The sclerostin levels of the samples were measured blind by one expert investigator in dual-controlled blood samples. The laboratory worker was not informed about the experimental setup, the randomization of the codes or any further detail about the experiment or the subjects. The sclerostin levels were measured using a human sclerostin ELISA kit (Cusabio™, Catalog No: CSB-E13146h, Newark, DE, USA). The instructions given in the user manual of the kit were followed for the detection of the serum sclerostin levels.

Continuous variables were summarized as the arithmetic mean and standard deviation (SD). The Kolmogorov-Smirnov test was used to confirm the data were normally distributed. The serum sclerostin levels were compared using a general linear model repeated measures test. A p-value of <0.05 was considered statistically significant. The software package used for data analysis was PASW Statistic 18.

Table 2. Serum sclerostin levels before and after vibration

Blood sample collection time	Serum sclerostin level (pg/ml)
Before vibration	328.2±589.9
3 min after vibration (AV1)	166.4±249.1
6 min after vibration (AV2)	149.5±96.8
8 min after vibration (AV3)	140.9±101.9
10 min after vibration (AV4)	353.1±493.5
12 min after vibration (AV5)	101.6±74.7
14 min after vibration (AV6)	315.9±382.3
17 min after vibration (AV7)	146.7±119.5
20 min after vibration (AV8)	180.8±181.7
23 min after vibration (AV9)	81.4±91.9

$p > 0.05$. Data are expressed as the arithmetic mean ±SD

RESULTS

The serum sclerostin levels before and after the vibration are given in Table 2. The change in the serum sclerostin level after vibration was not significant.

DISCUSSION

Plasma sclerostin level increases in the 10th minute after the WBV application[14]. It was our hypothesis that the serum sclerostin level might increase after single extremity vibration, but the findings of this study do not support our hypothesis.

Cidem et al. showed there was an increment in the plasma sclerostin level 10 minutes after WBV application. They explained that the increase in plasma sclerostin level 10 minutes after WBV was due to sclerostin secretion from osteocytes entering the systemic blood circulation via lacuna–canalicular fluid[14]. Sclerostin is deposited inside osteocyte cells. Vibration stimulus can induce the secretion of sclerostin from osteocytes. Vibration causes repetitive compression and decompression of bone tissue, resulting in lacuna–canalicular fluid moving into the blood vessels. WBV also significantly increases blood flow to the tissues exposed to vibration. Relocation of lacuna–canalicular fluid and its mixture with increased blood circulation may cause more leakage of sclerostin into the blood circulation[14–17].

In the present study, blood samples were collected at specific times for 23 minutes after the cessation of vibration. It was found that there were no significant changes in the sclerostin level after vibration. Why are there non-significant changes in sclerostin after single extremity vibration when there are significant increases in the serum sclerostin level after whole body vibration? Perhaps, it is because the sclerostin response is related to the bone mass that is exposed to vibration. Osteocytes constitute over 90% of all bone cells and they are interconnected by numerous dendritic processes forming a complex cellular network[18, 19]. The amount of the bone mass (i.e., the number of osteocytes) exposed to vibration is much more during whole body vibration than during single-extremity vibration. That being the case, it would be expected that the sclerostin secreted during single-extremity vibration would be less than in WBV.

This study had some limitations. First, the parameters of vibration were selected as 40 Hz, 4 mm and 60 sec in the present study referring to the study of Cidem et al. Different frequency, amplitude and duration combinations of vibration should be used to investigate the effects of single extremity vibration on the serum sclerostin level. Second, the blood samples were collected from the antecubital vein. Sclerostin is secreted from osteocytes in the tibia, fibula and foot bones during lower leg-vibration and enters the systemic circulation via leg veins, where the total sclerostin level becomes diluted. So, it might be possible to detect a significant change in serum sclerostin level during lower leg-vibration if the blood samples were collected from the leg vein instead of the antecubital vein.

To the best of our knowledge, this is the first study to investigate the changes in the human plasma sclerostin level when a single extremity is exposed to vibration. The results show that the serum sclerostin level did not change significantly after single lower leg vibration.

ACKNOWLEDGEMENT

This study was presented at the Congress of 24th National Physical Medicine and Rehabilitation, Antalya.

REFERENCES

1) Gaudio A, Pennisi P, Bratengeier C, et al.: Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. J Clin Endocrinol Metab, 2010, 95: 2248–2253. [Medline] [CrossRef]
2) Robling AG, Niziolek PJ, Baldridge LA, et al.: Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem, 2008, 283: 5866–5875. [Medline] [CrossRef]
3) Lombardi G, Lanteri P, Colombini A, et al.: Sclerostin concentrations in athletes: role of load and gender. J Biol Regul Homeost Agents, 2012, 26: 157–163. [Medline]
4) Lin C, Jiang X, Dai Z, et al.: Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. J Bone Miner Res, 2009, 24: 1651–1661. [Medline] [CrossRef]
5) Mödder UI, Hoey KA, Amin S, et al.: Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res, 2011, 26: 373–379. [Medline] [CrossRef]
6) Moustafa A, Sugiyama T, Saxon LK, et al.: The mouse fibula as a suitable bone for the study of functional adaptation to mechanical loading. Bone, 2009, 44: 930–935. [Medline] [CrossRef]
7) Suva LJ: Sclerostin and the unloading of bone. J Bone Miner Res, 2009, 24: 1649–1650. [Medline] [CrossRef]
8) Choi Y, Kang H: The effects of sling exercise using vibration on trunk muscle activities of healthy adults. J Phys Ther Sci, 2013, 25: 1291–1294. [Medline] [CrossRef]
9) Han J, Jung J, Lee J, et al.: Effect of muscle vibration on postural balance of Parkinson's diseases patients in bipedal quiet standing. J Phys Ther Sci, 2013, 25: 1433–1435. [Medline] [CrossRef]
10) Koh HW, Cho SH, Kim CY, et al.: Effects of vibratory stimulations on maximal voluntary isometric contraction from delayed onset muscle soreness. J Phys Ther Sci, 2013, 25: 1093–1095. [Medline] [CrossRef]
11) Shim C, Lee Y, Lee D, et al.: Effect of whole body vibration exercise in the horizontal direction on balance and fear of falling in elderly people: a pilot study. J Phys Ther Sci, 2014, 26: 1083–1086. [Medline] [CrossRef]
12) Choi SJ, Shin WS, Oh BK, et al.: Effect of training with whole body vibration on the sitting balance of stroke patients. J Phys Ther Sci, 2014, 26: 1411–1414. [Medline] [CrossRef]
13) Mirza FS, Padhi ID, Raisz LG, et al.: Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. J Clin Endocrinol Metab, 2010, 95: 1991–1997. [Medline] [CrossRef]
14) Çidem M, Karakoç Y, Ekmekçi H, et al.: Effects of whole-body vibration on plasma sclerostin level in healthy women. Turk J Med Sci, 2014, 44: 404–410 [CrossRef]. [Medline]
15) Price C, Zhou X, Li W, et al.: Real-time measurement of solute transport within the lacunar-canalicular system of mechanically loaded bone: direct evidence for load-induced fluid flow. J Bone Miner Res, 2011, 26: 277–285. [Medline] [CrossRef]
16) Lohman EB 3rd, Petrofsky JS, Maloney-Hinds C, et al.: The effect of whole body vibration on lower extremity skin blood flow in normal subjects. Med Sci Monit, 2007, 13: CR71–CR76. [Medline]
17) Kerschan-Schindl K, Grampp S, Henk C, et al.: Whole-body vibration exercise leads to alterations in muscle blood volume. Clin Physiol, 2001, 21: 377–382. [Medline] [CrossRef]
18) Bonivtch AR, Bonewald LF, Nicolella DP: Tissue strain amplification at the osteocyte lacuna: a microstructural finite element analysis. J Biomech, 2007, 40: 2199–2206. [Medline] [CrossRef]
19) Nicolella DP, Feng JQ, Moravits DE, et al.: Effects of nanomechanical bone tissue properties on bone tissue strain: implications for osteocyte mechanotransduction. J Musculoskelet Neuronal Interact, 2008, 8: 330–331. [Medline]