Biological Trace Element Research (2024) 202:5000–5005
https://doi.org/10.1007/s12011-023-04055-7

RESEARCH

Selenomethionine, a Trace Element, Increases Osteoblastic Activity of hFOB 1.19 Cells (an In Vitro Study)

Erhan Sahin[1] · **Mahmoud Arafat**[2] · **Ayse Tansu Koparal**[2]

Received: 25 October 2023 / Accepted: 30 December 2023 / Published online: 11 January 2024

Abstract

Osteoporosis and resulting fractures affect a significant group of people in the world. It has been shown in many studies that selenium has positive effects on bone metabolism. Based on this information, the aim of this study is to investigate whether bone differentiation will start in a shorter time by applying selenomethionine (SeMet) to hFOB cells.

First, hFOB 1.19 cells were cultured. Safe doses of SeMet were determined by MTT and LDH tests. Ossification levels were determined by alizarin red staining and measurement of alkaline phosphatase enzyme levels. The results were analyzed with statistical tests.

It was observed that SeMet increased cell viability at concentrations of 10, 25, 50, 100, and 200 µM in 24 h. At these concentrations, cell viability increased above the control, the viabilities were as follows: 109.4%, 104.9%, 104.3%, 103.15%, and 100.27%. High doses of SeMet significantly reduce cell viability. According to Alizarin red staining, SeMet increases the amount of calcium deposits in hFOB cells in a dose-dependent manner. In the experimental groups, the highest ALP enzyme was determined in the 7-day SeMet application. The most effective dose was measured as 15 µM.

It was determined that SeMet, which is found as a trace element in living things in nature, increases the viability of hFOB cells, which are osteoblast cell precursors, and increases osteoblastic differentiation and osteoblastic activity in these cells. Our results are at a level that sheds light on an important problem in public health.

Keywords hFOB 1.19 cells · Selenomethionine · Osteogenic differentiation · Osteoblastic activity · ALP enzyme

Introduction

Selenium is an important trace element that takes part in many biological reactions in the body. Selenomethionine (SeMet) is one of the main forms of selenium found in living organisms. Selenium participates in the structure of 25 proteins in humans [1]. It is very important in body defense as it participates in the structure of antioxidant proteins. Deficiency in selenium intake causes a decrease in the synthesis of these proteins and therefore their biological activities [2]. On the other hand, too much selenium in the diet causes toxic effects and can lead to selenium poisoning [3]. Considering the amount of selenium that should be taken per day, it is recommended that adults take 55 µg/day in the USA and 75 µg/day in the UK. To ensure adequate selenium intake, it is important to include foods such as grains, vegetables, meat, milk, seafood, and nuts in our meals [4]. The positive effect of selenium on many organs makes researchers think about its effects on ossification.

Bones, a special type of tissue, are the main component of the protection, support, and movement system in humans. It is responsible for the production of cellular elements of the blood with the bone marrow in its medulla. Bone tissue stores almost all the calcium in the body and the daily calcium requirement is met from here. In normal physiology, bone formation and destruction are in balance. Two types of ossification occur in our embryonal and adult life, endochondral ossification and intramembranous ossification [5]. In our daily lives, disruption of bone integrity for any reason is referred to as bone fracture. The rate of bone healing can be increased with various surgical procedures [6]. Accelerating fracture healing is a very important

✉ Erhan Sahin
erhansahinn@gmail.com

[1] Histology and Embryology Department, Faculty of Medicine, Bilecik Seyh Edebali University, Bilecik, Turkey

[2] Department of Medical Services and Techniques, Yunus Emre Vocational School of Health Services, Anadolu University, Eskisehir, Turkey

point. After skeletal muscles, selenium is found mostly in bones. Many studies in the literature have shown that selenium levels in the body increase bone mineral density and have a positive effect on bone by reducing the risk of osteoporotic fractures [7]. In two very important reviews compiled from many clinical and preclinical studies, it is stated that selenium and selenoproteins are very important beneficial trace elements for bone health [8, 9]. Although there are many studies in the literature on the bone metabolism of selenium and selenoproteins, the effects of selenium on bone metabolism are controversial. For example, in the clinical trial conducted by Walsh et al. on 120 postmenopausal women over the age of 55, it was observed that the musculoskeletal health parameters of postmenopausal women who took 200 and 50 µg selenite once a day did not change [10]. In order to clarify the relationship between selenium and bone metabolism, more in vitro and in vivo studies are needed in this area. Finding new treatment approaches and auxiliary molecules that will increase the speed of bone healing will be of great benefit to healthcare providers.

The aim of this study is to investigate the effects of selenomethionine on the osteoblastic properties of hFOB cells, which are osteoblast cells.

Material and Method

Cell Culture

hFOB 1.19 cells were cultured in 75 cm^2 flasks in phenol red-free DMEM:Ham's F12 (1:1) medium containing 10% fetal bovine serum, 0.3 mg/ml geneticin (Gibco, G418), 2.5 mM L-glutamine (5% CO_2 and 34 °C). When the cells in the flasks reached 70% density, they were taken into the experiment.

Preparation of SeMet Doses

Stock solution was prepared by dissolving SeMet (Sigma) in phosphate buffer (PBS). Concentrations of 10–25-50–100-200–400-800–1000 and 2000 µM were prepared from the main stock by diluting with PBS. Since SeMet is dissolved in PBS, PBS was used as a negative control. SeMet's stock solution was stored at − 20 °C.

Effects of SeMet on hFOB Cells (Viability Assays)

The effects of SeMet on cell viability were determined by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) test. SeMet concentrations applied in MTT experiments were as follows: 10, 25, 50, 100, 200, 400, 800, 1000, and 2000 µM. Cells were allowed to proliferate in incubators with 95% air, 5% CO_2, and 34 °C. After the cells covered 70% of the flask surface, they were removed from the bottom of the flask with trypsin–EDTA to obtain a cell solution. To determine the number of cells, cells were counted 3 times with the help of a Thoma slide. Five thousand cells were planted in each well of the 96-well cell culture plates. It was waited 24 h for the cells to stick to the ground. After 24 h of incubation, the media on the adherent cells was removed by inverting the plates. Cells were kept in different concentrations of SeMet for 24 and 48 h. At the end of the period, SeMet media were removed. A 0.5 mg/ml MTT solution was added to the wells and left in the incubator for 2 h. At the end of the period, MTT was removed and 0.1 ml DMSO was added to the wells. The optical density of the wells was read on an ELISA reader at 570 nm. The cell viability rate of the control group, which was not treated with the test substance, was accepted as 100%, and the viability rates of the experimental cells were expressed as percentages. To minimize variability within the experiment, each dose of selenomethionine was administered to 8 separate wells in a 96-well plate. Then, the results from these 8 wells were evaluated for each dose. The MTT assays were repeated three times to increase the reliability of the results.

Cytotoxicity Experiments (Lactic Dehydrogenase (LDH) Released Assay)

LDH Cytotoxicity Detection Kit (Roche, Germany, Cat. No. 04744926001) was used to perform the lactate-dehydrogenase (LDH) assay. Five thousand cells were planted in each well of the 96-well cell culture plates. It was waited 24 h for the cells to stick to the ground. After 24 h of incubation, the media on the adherent cells was removed by inverting the plates. Cells were kept in different concentrations of SeMet for 24 and 48 h. The supernatant was collected after treatment with concentrations of SeMet, and the assay was completed in accordance with the precise instructions included in the kit. To determine the LDH enzyme released, the OD values of the plates were measured at a wavelength of 492 nm using a microplate reader.

Stimulation of Osteogenic Differentiation in hFOBs

To differentiate cultured cells, cells were cultured in the presence of osteogenic differentiation cocktail (OD(+)) containing phenol red-free DMEM:Ham's F12 (1:1) medium containing 10% fetal bovine serum, 0.3 mg/ml geneticin (Gibco, G418), 2.5 mM L-glutamine, 10^{-8} M dexamethasone, 0.05 mM ascorbic acid 2-phosphate, and 10 mM β-glycerophosphate. The OD(+) medium was renewed every 3–4 days. At the end of the 7th, 14th, and 28th days, the samples were frozen for subsequent biochemical analyses or fixed for staining. The content of the medium that did not contain the osteogenic differentiation cocktail (OD(−)) was as follows: phenol red-free DMEM:Ham's F12 (1:1) medium containing 10% fetal bovine serum, 0.3 mg/ml geneticin (Gibco, G418), and 2.5 mM L-glutamine.

Demonstration of Osteogenic Differentiation With Alizarin Red Dye

The differentiation of hFOB cells and calcium accumulation metabolism were detected microscopically by the alizarin red (AR) staining method. SeMet concentrations applied in AR experiments were as follows: 5, 10, 15, and 25 µM. The medium on the cells planted in a 24-well plate and stimulated to differentiate was carefully removed at the end of the 28th day, and the plates were incubated with 70% cold ethanol at room temperature for 1 h. At the end of the period, the alcohol was carefully removed and washed twice with distilled water for 5 min. Then, water was removed and 0.1% AR was added and incubated for 30 min. Then, the AR was removed, washed 4 times with distilled water, examined under a microscope, and pictures were taken. The experiments were repeated 3 times.

Image Processing and Analysis

Images taken through the microscope were saved as JPEG. The ImageJ program was used to analyze the images. Firstly, the image to be analyzed was opened in the ImageJ program. Images were converted to 8 bits by selecting "image > type > 8-bit" from the menu in the software. "Area" and "Area fraction" options, which are the parameters we used in the calculations, were selected by going to "analyze > Set Measurements" in the software menu. In the other stage of the analysis, threshold values in the images were determined via "image > Adjust > Threshold." Results were obtained for each image from the "Analyze > Measure" tab and transferred to Excel for further statistical analysis.

Demonstration of Osteogenic Differentiation With Alkaline Phosphatase Enzyme

Alkaline phosphatase (ALP) enzyme quantification was performed for biochemical measurement of osteogenic differentiation in cultured cells. SeMet concentrations applied in ALP experiments were as follows: 5, 10, 15, 25, and 50 µM. Cells were seeded in 24-well plates at 4×10^4 cells/well with OD(+) and OD(−) media. On the 7th and 14th days, the media was removed from the incubated cells and washed once with PBS solution, and then the cells were placed in the − 80 °C freezer. Cells were removed from the freezer and left to thaw on ice for 20 min. After the lysis solution (PBS solution containing 1% Triton-X-100) was added to each well, the plates were placed in a sonicator containing ice and left in the sonication bath for approximately 50 min. The lysates were then collected into the tube by pipetting. Then, 20 µl of cell lysate was added to 96-well plates for each sample and 100 µl of ALP substrate solution (20 ml of ALP substrate buffer [(0.1 M diethanolamine, 1% triton-X-100, 1 mM $MgCl_2$, pH 9.8). After adding 1 p-nitrophenylphosphate tablet dissolved in it, it was shaken quickly and waited for 30 min at 37 °C for the ALP enzyme to convert pNpp into paranitrophenol. Experiments were repeated 3 times using at least two wells for the measurement of each sample. After 30 min, the reaction was stopped by adding 80 µl 1N NaOH, and after shaking briefly, the measurement was carried out on the ELISA device at a wavelength of 405 nm.

Statistical Analysis

All experiments were repeated three times. To calculate the data, a statistical package software was used. $p<0.05$ is considered significant. The Shapiro–Wilk test was used to test whether the data were normally distributed. One-way ANOVA followed by Tukey's multiple comparison test was used for normally distributed data.

Results

MTT Results

The 24- and 48-h effects of SeMet on hFOB cells were investigated. It was observed that SeMet increased cell viability at 10, 25, 50, and 100 µM concentrations ($p<0.05$) and viability was similar to the control group at 200 µM in 24 h. At these concentrations, cell viability increased above the control, the viabilities were as follows: 109.4%, 104.9%, 104.3%, 103.15%, and 100.27%. When exposed to high doses of SeMet concentrations such as 400 µM, 800 µM, 1000 µM, and 2000 µM for 24 h, the average viability of cells was observed to decrease compared to the control cells (86.2%, 79.8%, 87.14%, and 79.5%, respectively). It was determined that cell viability decreased gradually within 48 h of application. After 400 µM (400, 800, 1000, and 2000 µM) viability decreased significantly ($p<0.05$; viabilities, 69.2%, 68.7%, 65.9%, and 56.3%) (Fig. 1).

LDH Release Results

In parallel with the MTT tests, it was determined that SeMet doses increased cell damage in 48-h applications and that short-term (24 h) application was safer. It was observed that there was no cell damage at doses below 400 µM (Fig. 2).

Determination of the Effects of SeMet on Osteogenic Differentiation Using Alizarin Red Dye and Image Analysis

Two types of experimental setups were created, SeMet (5, 10, 15, and 25 µM) + with osteogenic differentiation

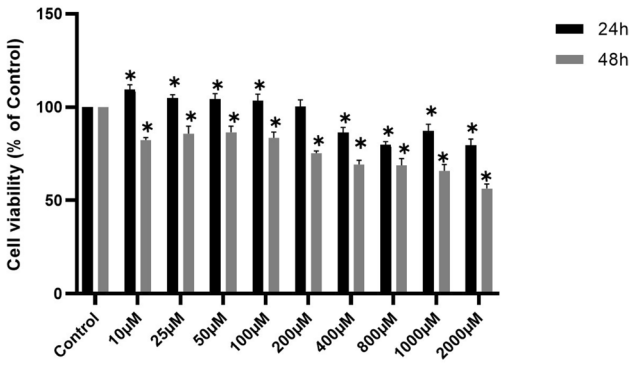

Fig. 1 MTT results. SeMet has been shown to positively affect cell viability in short-term applications at low doses (at 10, 25, 50, and 100 μM concentrations, $p < 0.05$). *Significantly different from untreated cells (control group) ($p < 0.05$)

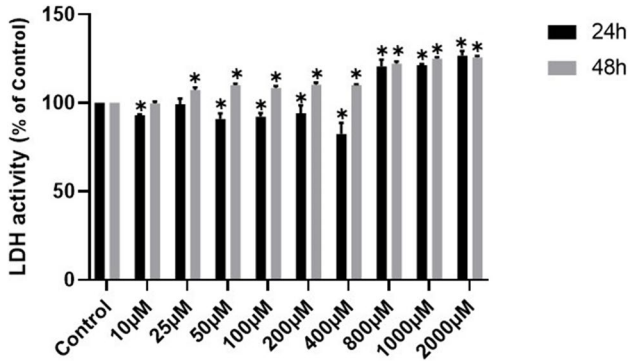

Fig. 2 LDH release results. It was determined that SeMet doses increased cell damage in 48-h applications and short-term (24 h) application was safer. No cell damage was observed at doses below 400 μM. *Significantly different from untreated cells (control group) ($p < 0.05$)

cocktail (OD +) and SeMet (5, 10, 15, and 25 μM) + without osteogenic differentiation cocktail (OD −). At the end of the 28th day, both models were stained with alizarin red, and calcium deposits were examined. It was observed that calcium deposits increased in a dose-dependent manner in both the OD(+) and OD(−) groups in which SeMet was applied (Fig. 3).

When alizarin red stainings were analyzed with the ImageJ program, it was determined that SeMet increased the amount of calcium deposits in a concentration-dependent manner in both experimental setups (SeMet concentrations + OD(+) or OD(−)), and in 5–10-15 and 25 μM SeMet + OD(+) administrations, each concentration was significant compared to the control ($p < 0.05$). In 5–10-15 and 25 μM SeMet + OD(−) administrations, the last two SeMet concentrations (15–25 μM) were determined to be significant compared to the control ($p < 0.05$) (Fig. 3).

Determination of the Effects of SeMet on Osteogenic Differentiation With ALP Enzyme

7- and 14-day experimental groups were set up for OD(+) and OD(−) groups. The highest ALP enzyme amounts in both OD(+) and OD(−) groups were determined in the 7-day SeMet application. The amount of ALP was measured higher in the OD(−) group, where low doses of SeMet (5–25 μM) were applied, than in the OD(+) group ($p < 0.05$) (Fig. 4). It was observed that the most effective dose to increase the amount of ALP in this group was 15 μM SeMet.

Discussion

In this study, the effects of selenomethionine (SeMet), a natural compound, on hFOB cells, which are osteoblastic cells, were investigated. As a result, it was shown that a low dose of SeMet increased the osteoblastic activities of hFOB cells.

SeMet is one of the main natural forms of selenium. This compound is found in many foods, including nuts, whole grains, meat, eggs, and dairy products. In addition to its beneficial effects on health, SeMet has toxic effects when taken excessively. With the discovery of the glutathione peroxidase enzyme, the number of articles investigating the effects of SeMet on health began to increase. Its effects have been investigated in many experimental models such as metabolic diseases and cancer and positive results have been obtained [11]. It has been shown in previous studies that the microstructure of bones changes and bone fractures increase in selenium deficiency [12–14]. Although there are many studies in the literature examining the relationship between selenium and bone tissue [12, 15, 16], it seems that molecular studies in this field will produce important results and support existing studies. Based on this reality, we designed this study. When we look at our experimental results, SeMet increases the osteogenic functions of hFOB cells at low doses. Doses of 15 μM and below were effective doses. This dose study serves as the basis for many future studies. Biomaterials can be used to fix the bone in bone fractures. The materials used in such prostheses are improving day by day with the joint work of biomedical and basic sciences. Coating these biomaterials with various molecules with high osteogenic differentiation capacity will make a great contribution to bone treatments. Tran et al. showed that the osteogenic activity of human normal osteoblasts increased when they coated titanium, a biomaterial extensively used in bone fractures and treatments, with sodium selenite [15]. We think that using SeMet, another form of selenium, as a coating material in future studies will yield important results in bone treatment. Our study significantly supports this proposition. While SeMet can be considered an implant coating material in studies, it can also increase bone healing when taken systemically. At this point, drug studies that will increase bone healing, focusing on SeMet, need to be planned. Saos-2 cells are an osteoblastic cell line frequently used in in vitro bone

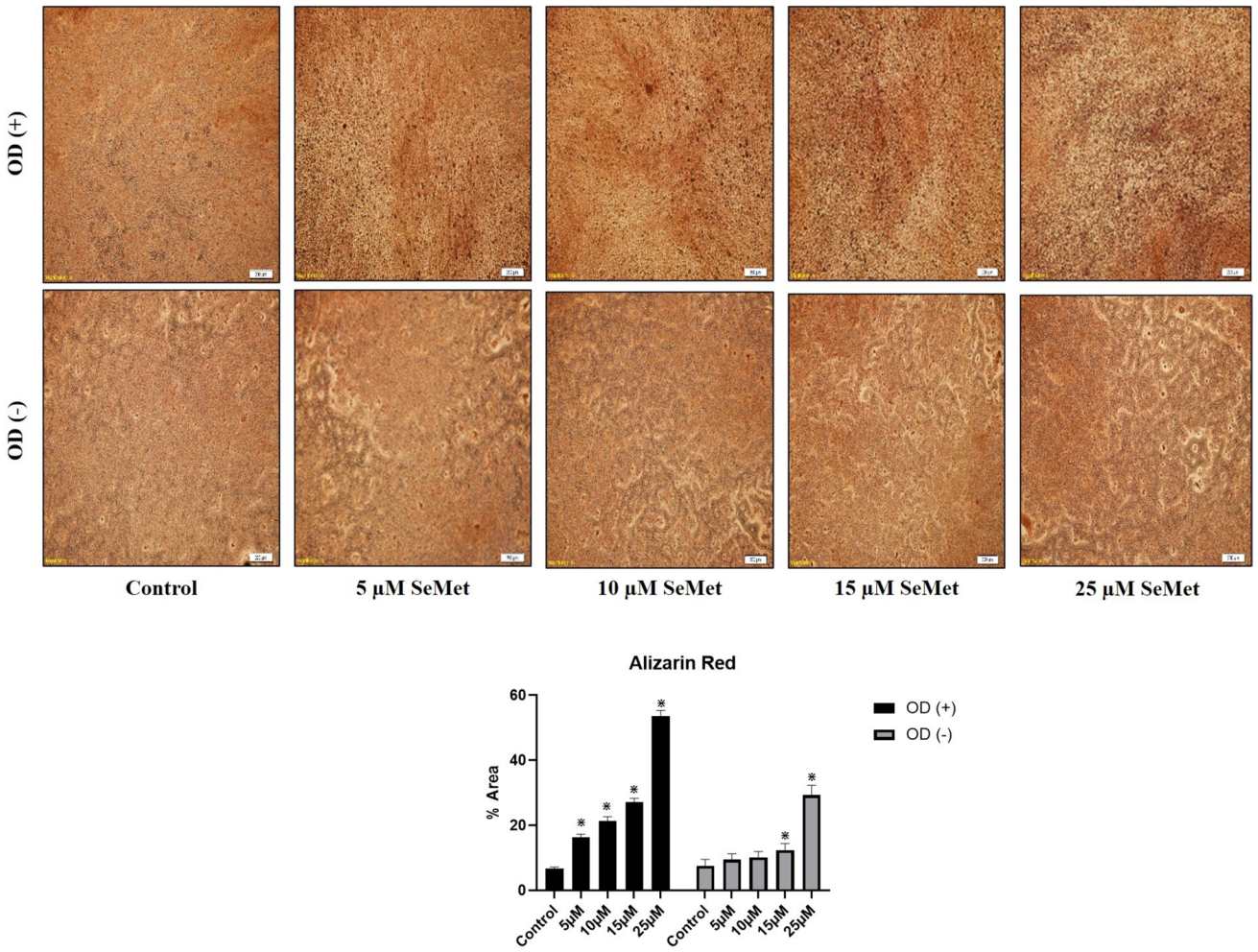

Fig. 3 Calcium deposit results (alizarin red staining). It was observed that calcium deposits increased in a concentration-dependent manner in both the OD(+) and OD(−) groups in which SeMet was applied. The significant concentrations in the OD(+) group were as follows: 5–10-15 and 25 µM. The significant concentrations in the OD(−) group were 15 and 25 µM ($p < 0.05$). All scale bars are 200 µm

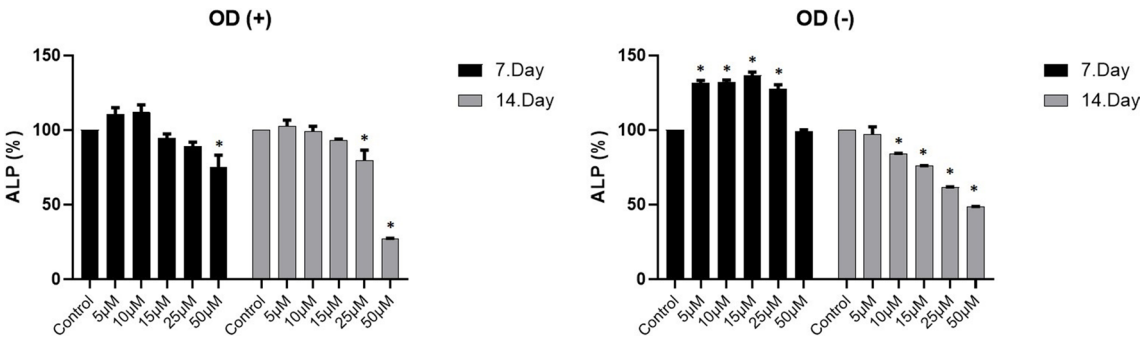

Fig. 4 ALP enzyme results. It was determined that SeMet increased the amount of ALP in both OD(−) and OD(+) groups. SeMet increased the ALP enzyme amount the most in the OD(−) experimental setup. Seven days of application was determined as the most appropriate period for the amount of ALP enzyme. *Significantly different from untreated cells (control group) ($p < 0.05$)

hypothesis studies. Yazıcı et al., in their study based on Saos-2 and selenium, found that 1 µM selenium showed therapeutic properties in 24-h experiments against the negative effects of zoledronic acid, bevacizumab, and dexamethasone, which are widely used in the treatment of bone diseases and bone cancer but cause osteonecrosis as a side effect [17]. The fact that similar studies have

yielded similar results by different experimental teams provides reassuring foundations for future and clinical studies. The daily amount of selenium required for adults is 55 mg [18]. In our study, SeMet was found to be effective at low doses, which is important in terms of the maximum values that should be taken daily. The fact that we found SeMet effective at low doses supports that SeMet or drugs derived from it can be used systemically.

Conclusion

As a result, in this study, it was determined that the metabolic activity of hFOB cells showing osteoblastic activity was increased by SeMet. We all know what a huge burden bone diseases are for the individual, society, and country's economy. Integrating molecules such as SeMet, which has a positive effect on many metabolic processes, into the treatment of diseases will provide great benefits for humanity. Based on this and similar studies in the literature, very cost-effective drugs can be developed. This study can be considered a dose reference study for future in vivo, in vitro, and clinical studies that will be designed in more detail.

Acknowledgements We commemorate our friend Mahmoud ARAFAT, who passed away at an early age and left behind great memories, with love. You are forever in our hearts.

Author Contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Mahmud Arafat, Ayse Tansu Koparal, and Erhan Sahin. The first draft of the manuscript was written by Ayse Tansu Koparal and Erhan Sahin, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by Anadolu University Scientific Research Projects Commission, Türkiye under the grant no: 1910S146.

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors declare that they have no conflict of interest.

References

1. Tangjaidee P, Swedlund P, Xiang J, Yin H, Quek SY (2022) Selenium-enriched plant foods: selenium accumulation, speciation, and health functionality. Front Nutr 9:962312. https://doi.org/10.3389/fnut.2022.962312
2. Zhou J, Zhang W, Cao Z, Lian S, Li J, Nie J, Huang Y et al (2023) Association of selenium levels with neurodegenerative disease: a systemic review and meta-analysis. Nutrients 15(17):3706. https://doi.org/10.3390/nu15173706
3. Hadrup N, Ravn-Haren G (2023) Toxicity of repeated oral intake of organic selenium, inorganic selenium, and selenium nanoparticles: a review. J Trace Elem Med Biol 79:127235. https://doi.org/10.1016/j.jtemb.2023.127235
4. Avery JC, Hoffmann PR (2018) Selenium, selenoproteins, and immunity. Nutrients 10(9):1203. https://doi.org/10.3390/nu10091203
5. Hadjidakis DJ, Androulakis II (2006) Bone remodeling. Ann N Y Acad Sci 1092(1):385–396. https://doi.org/10.1196/annals.1365.035
6. Clarke B (2008) Normal bone anatomy and physiology. Clin J Am Soc Nephrol 3(3):131–139. https://doi.org/10.2215/CJN.04151206
7. Yang T, Lee SY, Park KC, Park SH, Chung J, Lee S (2022) The effects of selenium on bone health: from element to therapeutics. Molecules 27(2):392. https://doi.org/10.3390/molecules27020392
8. Vescini F, Chiodini I, Palermo A, Cesareo R, De Geronimo V, Scillitani A, Gennari L et al (2021) Selenium: a trace element for a healthy skeleton - a narrative review. Endocr Metab Immune Disord Drug Targets 21(4):577–585. https://doi.org/10.2174/1871530320666200628030913
9. Zhang Z, Zhang J, Xiao J (2014) Selenoproteins and selenium status in bone physiology and pathology. Biochim Biophys Acta 1840(11):3246–3256. https://doi.org/10.1016/j.bbagen.2014.08.001
10. Walsh JS, Jacques RM, Schomburg L, Hill TR, Mathers JC, Williams GR, Eastell R (2021) Effect of selenium supplementation on musculoskeletal health in older women: a randomised, double-blind, placebo-controlled trial. Lancet Healthy Longev 2(4):212–221. https://doi.org/10.1016/S2666-7568(21)00051-9
11. Plateau P, Saveanu C, Lestini R, Dauplais M, Decourty L, Jacquier A, Blanquet S et al (2017) Exposure to selenomethionine causes selenocysteine misincorporation and protein aggregation in Saccharomyces cerevisiae. Sci Rep 7(1):44761. https://doi.org/10.1038/srep44761
12. Cao JJ, Gregoire BR, Zeng H (2012) Selenium deficiency decreases antioxidative capacity and is detrimental to bone microarchitecture in mice. J Nutr 142(8):1526–1531. https://doi.org/10.3945/jn.111.157040
13. Moreno-Reyes R, Egrise D, Boelaert M, Goldman S, Meuris S (2006) Iodine deficiency mitigates growth retardation and osteopenia in selenium-deficient rats. J Nutr 136(3):595–600. https://doi.org/10.1093/jn/136.3.595
14. Moreno-Reyes R, Egrise D, Nève J, Pasteels JL, Schoutens A (2001) Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia. J Bone Miner Res 16(8):1556–1563. https://doi.org/10.1359/jbmr.2001.16.8.1556
15. Tran PA, Sarin L, Hurt RH, Webster TJ (2010) Titanium surfaces with adherent selenium nanoclusters as a novel anticancer orthopedic material. J Biomed Mater Res A 93(4):1417–1428. https://doi.org/10.1002/jbm.a.32631
16. Wang H, Chen H, Chernick M, Li D, Ying G-G, Yang J, Zheng N et al (2020) Selenomethionine exposure affects chondrogenic differentiation and bone formation in Japanese medaka (Oryzias latipes). J Hazard Mater 387:121720. https://doi.org/10.1016/j.jhazmat.2019.121720
17. Yazıcı T, Koçer G, Nazıroğlu M, Övey İS, Öz A (2018) Zoledronic acid, bevacizumab and dexamethasone-induced apoptosis, mitochondrial oxidative stress, and calcium signaling are decreased in human osteoblast-like cell line by selenium treatment. Biol Trace Elem Res 184(2):358–368. https://doi.org/10.1007/s12011-017-1187-8
18. Meltzer H, Norheim G, Bibow K, Myhre K, Holm H (1990) The form of selenium determines the response to supplementation in a selenium replete population. Eur J Clin Nutr 44(6):435–446