Selenium Deficiency Decreases Antioxidative Capacity and Is Detrimental to Bone Microarchitecture in Mice

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Abstract

Selenium (Se), an essential mineral, plays a major role in cellular redox status and may have beneficial effects on bone health. The objective of this study was to determine whether Se deficiency affects redox status and bone microarchitecture in a mouse model. Thirty-three male C57BL/6J mice, 18 wk old, were randomly assigned to 3 groups. Mice were fed either a purified, Se-deficient diet (SeDef) containing ~0.9 µg Se/kg diet, or Se-adequate diets containing ~100 µg Se/kg diet from either selenomethionine (SeMet) or pinto beans (SeBean) for 4 mo. The Se concentration, glutathione peroxidase (GPx1) activity, and GPx1 mRNA in liver were lower in the SeDef group than in the SeMet or SeBean group. The femoral trabecular bone volume/total volume and trabecular number were less, whereas trabecular separation was greater, in the SeDef group than in either the SeMet or SeBean group (P < 0.05). Bone structural parameters between the SeMet and SeBean groups did not differ. Furthermore, Serum concentrations of C-reactive protein, tartrate-resistant acid phosphatase, and intact parathyroid hormone were higher in the SeDef group than in the other 2 groups. These findings demonstrate that Se deficiency is detrimental to bone microarchitecture by increasing bone resorption, possibly through decreasing antioxidative potential. J. Nutr. 142: 1526–1531, 2012.

Introduction

Selenium (Se), a trace mineral essential for animals and humans, has been shown to regulate cellular processes by being a component of selenoproteins (1–3). Most of these known selenoproteins are Se-dependent antioxidant enzymes such as glutathione peroxidase 1 (GPx1) and thioredoxin reductase (TRR) (1–3). These enzymes eliminate H2O2 and damaging lipid and phospholipid hydroperoxides generated in vivo by free radicals and other oxygen-derived species during normal metabolism in the body (3,4). The deficiency of Se is associated with increased oxidative stress and increased levels of reactive oxygen species (ROS) (3,5). In contrast, Se supplementation to Se-deficient animals restores the antioxidative capacity, reduces inflammatory stress response, and prevents cell damage in bone marrow stromal cells, precursors of osteoblasts (3,5–7). Oxidative stress and ROS directly stimulate osteoclast activity and bone resorption (8–10). Reduced oxidative defense, such as knockout of the antioxidant enzyme superoxide dismutase (SOD1), decreased femoral bone strength and increased the activity of tartrate-resistant acid phosphatase (TRAP), a bone resorption enzyme (11).

Although it has been reported that Se deficiency results in growth retardation and low bone mineral density (12,13), the effect of Se deficiency on bone microarchitecture has not been determined and its underlying molecular process on bone metabolism is not fully understood. In this study, we determined whether Se deficiency affects redox status and bone microarchitecture in a mouse model. We also determined whether Se from pinto beans (SeBean), a crop rich in Se and widely consumed throughout the world, is as bioavailable as Se in selenomethionine (SeMet) in supporting normal bone development.

Materials and Methods

Mice, diets, and treatments. The pinto beans used in this study were obtained from the Northarvest Bean Growers Association. Beans were cooked in deionized water in glass containers. The cooked beans were then homogenized in a stainless steel, Waring-type blender, frozen at −80°C, and lyophilized to <10% moisture. The dried material was ground in a centrifugal grinder to a powder. By analysis (in triplicate),
the cooked, dried, ground pinto beans contained 415 ± 15 µg Se/kg dry weight.

Thirty-three male C57BL/6 mice, 18 wk old, were obtained from Charles River Laboratories. All mice were housed in Plexiglas ventilated cages (2 animals/cage) in an environmentally controlled, pathogen-free facility with a 12-h-light/dark cycle. The animal protocol for the study was approved by the USDA-Agricultural Research Service Grand Forks Human Nutrition Research Center Animal Care and Use Committee. The mice were maintained and processed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Mice were allowed to acclimate to our animal facility for 3 d with Purina Rat Chow #5012 (Ralston-Purina) before being randomly assigned to 3 treatment groups. Mice were fed purified AIN-93G-based diets that were either Se deficient (SeDef; n = 10) or with 0.4 µg Se/g diet or Se adequate containing ~100 µg Se/kg diet (by analysis) supplemented as selenomethionine (SeMet; n = 11). Mice consumed the experimental diets ad libitum for 4 mo and had free access to deionized water throughout the study. The body weights of the mice were recorded weekly.

The purified SeDef and SeMet diets (Supplemental Table 1) were formulated based on the AIN-93G diet (14). The formulation of the SeMet diet, which provided the same amount of Se as the SeMet diet, was based on the analyzed Se content of the pinto beans. The cooked pinto bean powder was incorporated into the diet at ~24% (Supplemental Table 1). Different amino acid (Supplemental Table 2) and mineral mixes (Supplemental Table 3) were used for the SeDef and SeMet diets to obtain a similar nutrient composition in the diets based on pinto bean composition tables (15). A proximate analysis of pinto beans performed at this center (16) indicated that the use of the food composition tables was appropriate.

Sample preparation. At the end of the study, mice were killed with a ketamine cocktail [1.37:1 mixture of ketamine (Animal Health)/sylazine (Phoenix Scientific)]. Blood samples were collected and centrifuged at 1500 × g for 20 min at 4°C to obtain the serum that was stored at −80°C until analysis. The left femur of each mouse was removed and cleaned of adherent tissue and stored at −20°C before being scanned by micro computerized tomography (µCT) as described below. Liver samples were immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

Measurement of mRNA levels in liver. Total RNA was purified from liver samples by using Trizol reagent according to the manufacturer's instructions. Denatured total RNA from cells (2 µg) was reverse transcribed with the following components from Applied Biosystems: 1× RT buffer, 5.5 mmol/L MgCl₂, 500 µmol/L of each dNTP, 2.5 µmol/L random hexamers, 0.4 U in 1 µL RNase inhibitor, and 1.25 U in 1 µL MultiScribe RT enzyme in a 100-µL reaction volume with the following protocol: hexamer incubation at 25°C for 10 min, RT at 37°C for 1 h, and heat inactivation of reverse transcriptase at 95°C for 5 min.

Following RT, the cDNA (2 µL) was amplified and quantified by using a Sequence Detection System (SDS 7300) and a PCR universal protocol as follows: AmpliTaq Gold activation at 95°C for 15 s and annealing/extension at 60°C for 1 min. The fluorescence of the double-stranded products accumulated was monitored in real time. The relative mRNA levels were normalized to levels of GAPDH mRNA in the same sample. The sense and antisense primer sequences were as follows:

**GPx1**: 5'-GGG ACT ACA CCA TGA ACG A-3', and 5'-ACC ATT CAT TCC GCA CTT A-3'; for **Gpx4**, 5'-TCT GGC ACG CAC CAT GTG T-3', and 5'-GGG CCA TGC ACG A-3' for catalase (CAT), 5'-GCG TCC TGT GGC CTT A-3'; and 5'-TCA GGG TGG ACG TCA GGA AA-3' for TRR, 5'-AGG GGT GTC TCA CAC TAC CCT TCT-3'; and 5'-ACA GTC AAC TCC CTT GTC CCT-3' for glutathione reductase (GR), 5'-GCT TCA GTG GGA GGA GTT GC-3', and 5'-CCA CCG ATG ATG AGT TCT TT-3'; for **SOD1**, 5'-TGG GGT CCA CCG CCA TGA-3', and 5'-ACC GTC TCT TCC AGC AGT CA-3'; for **SOD2**, 5'-ATT ACG CCG CAG ATC ATG CA-3', and 5'-TGG CCA CCA CCA AAC TAC TT-3'; for **GAPDH**, 5'-TGG ACC ACC ACC TGC TTA G-3', and 5'-GGA TGC AGG GAT GAT GTC C-3'. All oligonucleotides primers for PCR amplification were synthesized by Integrated DNA Technologies with HPLC purification.

**Biochemical measurements.** Serum concentrations of C-reactive protein (CRP), intact parathyroid hormone (PTH), and osteoprotegerin (OPG) were measured by mouse ELISA kits from ALPCO Diagnostics. Serum TRAP5b was determined with kits from Immunodiagnostic System according to the manufacturer's instructions.

Liver samples were homogenized in 0.1 mol/L Tris-HCl buffer, pH 7.4, at 4°C and centrifuged at 10,000 × g for 15 min. The supernatant was collected for GPx1 enzymatic activity assays. Blood for GPx1 activity assay was drawn into an EDTA-treated tube. Liver and blood GPx1 activity were determined by the modified method (17) developed by Paglia and Valentine (18). The activity in liver was expressed as units/mg protein or units/mg hemoglobin for blood. Protein concentrations were determined by the Bradford method (BioRad). One unit of activity is defined as the amount of enzyme required to oxidize 1.0 µmol NADPH/min.

**Se analysis.** Se in diets and liver samples was determined as previously described (19). Briefly, samples were digested on a hot plate with 10 mL 16 mol/L nitric acid, 10 mL magnesium nitrate solution (40% in deionized water), and 2.0 mL of 12 mol/L HCl. The samples then were dried and ashed. The ashed samples were dissolved in 25 mL of 12 mol/L HCl and analyzed by hydride generation atomic absorption spectrometry.

**Bone structure determined by µCT.** The left femur from each mouse was cleaned of adherent tissue and placed in a holder with a 10.2-mm diameter and scanned by a Scanco µCT scanner as previously described (20–22). The gray-scale images were processed by using a low-pass Gaussian filter (σ = 0.8, support = 1) to remove noise and a fixed threshold of 220 was used to extract the mineralized bone from soft tissue and marrow phase. The reconstruction and 3D quantitative analyses were performed by using software provided by Scanco. The same settings for scan and analysis were used for all samples. The evaluation of 3D trabecular and cortical structure was done as previously described (20–22). We followed the recommended guidelines for µCT scanning (23) and bone histomorphometry nomenclature (24).

**Data analysis.** Data are expressed as mean ± SD. For body weight, a repeated-measures ANOVA was used to test for effects of treatment, week, and a treatment × week interaction. All other outcomes were analyzed using 1-way ANOVA, with treatment as the fixed effect (JMP, version 9.0.0, SAS Institute). Homogeneity of variances was tested using the Brown-Forsythe test. If variances were not homogeneous, then Welch’s test was used to compare means. Tukey-Kramer’s multiple comparison procedure was used for post hoc comparison of group means. In all of the analyses, P < 0.05 was considered significant.

**Results.**

**Body weight.** The initial body weight did not differ among the 3 treatment groups (Supplemental Fig. 1). All animals gained weight regardless of diet. Body weight between the SeDef and SeMet groups did not differ throughout the experimental period. Mice in the SeBean group had a higher body weight than that in the SeDef group at wk 14 and 15 and mice in the SeMet group from wk 11 to the end of the study (P < 0.05).

**Liver Se concentration and GPx1 activities in liver and blood.** The Se concentration in liver (Fig. 1A) and GPx1 activities in liver (Fig. 1B) and blood (Fig. 1C) were lower in the SeDef group than in the other 2 groups. Compared with the SeMet group, liver GPx1 activity was 19-fold greater in both the SeMet and SeBean groups and the liver Se concentration was 13- and 11-fold greater in the SeMet and SeBean groups, respectively (P < 0.0001). Blood GPx1 activity was ~6- and 5-fold higher in the SeMet and SeBean groups, respectively, than in the SeDef group. Although the SeMet group had a higher liver Se concentration than that in the SeBean group (P < 0.05), GPx1 activity was 13- and 11-fold greater in the SeMet and SeBean groups, respectively (P < 0.0001).
activity in liver and blood did not differ between the SeMet and SeBean groups.

**Liver mRNA levels of Se-containing or antioxidant enzymes.** Compared with the SeDef group, the expression of *Gpx1* in either the SeMet or SeBean group was ~2-fold higher (*P* < 0.0001), whereas the expression of *Gpx4* and *Cat* did not change with diet (Fig. 2). The mRNA levels of *Trx*, *Sod1*, and *Sod2* in both the SeMet and SeBean groups were higher than those in the SeDef group (*P* < 0.05). The expression of *Gr* in liver was lower in the SeDef group compared with the SeBean group (*P* < 0.05). However, the difference in *Gr* mRNA levels between the SeMet and SeDef groups was not significant (*P* > 0.08). The mRNA levels of *Gpx1* and *Gr* did not differ between the SeMet and SeBean groups.

**Serum markers of bone metabolism.** The Se concentrations of CRP (Fig. 3A), TRAP (Fig. 3B), and intact PTH (Fig. 3C) were greater in the SeDef group than in the other 2 groups (*P* < 0.05). Compared with the SeMet group, the SeBean group had lower serum TRAP (*P* < 0.05) but similar serum intact PTH concentrations. The serum OPG concentration was higher (*P* < 0.05) in the SeBean group than in the SeDef and SeMet groups, which did not differ (Fig. 3D).

**Bone microstructure.** Bone volume (BV)/tissue volume (TV) and trabecular number (Tb.N) were less, whereas trabecular separation (Tb.Sp) was greater in the SeDef group than in the other 2 groups (*P* < 0.05) (Table 1). Femoral trabecular BV, TV, trabecular thickness, and structure model index were not significantly affected by Se deficiency. There were no statistical differences in any trabecular variables between the SeMet and SeBean groups. Compared with the SeDef group, trabecular connectivity density was higher in the SeBean group (*P* < 0.05) but not in the SeMet group. Se status did not significantly affect any cortical bone structural variables of the mid-shaft femurs.

**Discussion**

Although Se deficiency has been shown to impair bone metabolism and decrease bone mineral density in animal models and human epidemiological studies (12,13,23). How Se affects bone microarchitecture has not been determined. With μCT methodology, we were able to separately evaluate trabecular and cortical bone and quantitatively assess 3D bone structural characteristics. We found that Se deficiency affected trabecular bone but had no effect on the cortical bone characteristics of mouse femurs. Specifically, Se deficiency decreased femoral trabecular BV:TV and Tb.N and increased Tb.Sp.

Bone mass reflects the balance between bone formation and resorption. Bone metabolism is controlled by the receptor activator of NF-κB ligand (RANKL) and OPG signaling pathway (26,27). RANKL, expressed on the surface of osteoblasts, binds to its receptor, RANK (expressed on the surface of osteoclasts), and stimulates osteoclast activity and bone resorption. OPG is a decoy receptor that binds RANKL and thus prevents the activation of RANK (26,27). Excess bone resorption relative to bone formation is the leading cause of bone loss that occurs in many skeletal diseases, including osteoporosis (28). It has been shown that the RANKL/RANK/OPG pathway is modulated by ROS and several proinflammatory cytokines (8,29–31).

The mechanisms through which Se deficiency impairs or Se supplementation enhances bone metabolism are not fully known. The available evidence suggests that Se preserves bone through its ability to modulate oxidant defenses. Se-containing antioxidant enzymes exhibit high peroxidase activity toward certain...
ROS, including H$_2$O$_2$ and fatty acid hydroperoxides, and thus play a pivotal role in the maintenance of cellular redox balance (1,2,4). Oxygen-derived free radicals directly stimulate osteoclast activity and bone resorption (9,10). Bone loss occurs when osteoclastogenesis is enhanced while the production of H$_2$O$_2$ in bone marrow is elevated and activities of GPx1 and TRR fall sharply (8). Restoring antioxidant defenses by administering antioxidants such as catalase, N-acetyl cysteine, or ascorbate inhibits RANKL-induced osteoclastogenesis (31) and prevents estrogen deficiency-induced bone loss (8). Recently, it was shown that Se directly modulated inflammatory stress in osteoblasts (7) and the antioxidative capacity in osteoblast precursors, bone marrow stromal cells (6). Furthermore, GPx1 is expressed by osteoclasts and overexpression of GPx1 inhibits osteoclast formation (8). Thus, it is conceivable that Se exerts its influence on bone metabolism primarily by inhibiting osteoclastogenesis through downregulating the OPG/RANK/RANK signaling pathway via the reduction of ROS. As expected, in this study, we found that the Se-adequate diets (SeMet and SeBean) decreased inflammatory stress such as CRP concentration in serum and restored the antioxidative capacity such as expression of GPx1, TRR, SOD1, and SOD2 in liver as well as GPx1 activity in liver, a finding similar to previous reports (1,2,6,7).

As a hormone, PTH functions to maintain normal blood calcium concentrations by stimulating osteoclast activity, increasing bone resorption, and enhancing intestinal calcium absorption (32). The finding that mice fed adequate-Se diets had a lower concentration of serum intact PTH accompanied by a lower concentration of serum TRAP, a bone resorption marker, compared with those fed an Se-deficient diet is consistent with a previous report (13), suggesting that Se deficiency enhanced osteoclast activities and bone resorption.

In this study, we also tested whether Se from pinto beans is as bioavailable as Se in SeMet in supporting normal bone development. Pinto beans are of great public and research interests because of their nutritional values and health benefits (33–37). In addition, beans can be high in Se (15). The beans used in this study, grown in the state of North Dakota, where some areas have very high concentrations of Se in the soil, were especially high in Se (0.42 mg/kg by analysis). Our findings indicate that Se from pinto beans is equally bioavailable to support normal bone development in mice as Se from SeMet, because mice fed either the SeMet or SeBean diet had higher femoral trabecular BV:total volume and Tb.N and lower Tb.Sp than mice fed the SeDef diet, and there were no significant differences in bone structural indices between the SeMet and SeBean groups.

Body weight is generally positively associated with bone mineral density (38,39), because body weight-induced mechanical loading stimulates bone formation by decreasing apoptosis and increasing proliferation and differentiation of osteoblasts and osteocytes (40). In the present study, body weight did not significantly differ between the SeDef and SeMet groups. This observation suggests that the effect of Se on bone architecture was independent of mechanical loading conferred by body weight. Interestingly, we found that mice fed the SeBean diet had significantly higher body weight than those fed the SeMet diet starting at wk 11. Similarly, compared with those fed the SeDef diet, mice fed the SeBean diet but not those fed the SeMet diet had higher trabecular connectivity density, liver GR mRNA levels, and serum OPG concentrations, changes that are positively related to bone metabolism. Upregulated OPG expression or

Selenium affects bone microstructure in mice

![FIGURE 3](https://academic.oup.com/jn/article-abstract/142/8/1526/4630951)
TABLE 1  Femoral bone structural indices in mice fed an SeDef or Se-adequate diet containing SeMet or SeBean for 4 mo1

<table>
<thead>
<tr>
<th>Indices</th>
<th>SeDef</th>
<th>SeMet</th>
<th>SeBean</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Distal femur (trabecular bone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV, mm3</td>
<td>0.63 ± 0.17</td>
<td>0.83 ± 0.23</td>
<td>0.71 ± 0.25</td>
</tr>
<tr>
<td>TV, mm3</td>
<td>3.67 ± 0.36</td>
<td>3.89 ± 0.62</td>
<td>3.51 ± 0.93</td>
</tr>
<tr>
<td>BV/TV, %</td>
<td>17.0 ± 3.4a</td>
<td>21.3 ± 3.5b</td>
<td>20.8 ± 3.6b</td>
</tr>
<tr>
<td>Tb.N, mm2</td>
<td>3.58 ± 0.45a</td>
<td>4.03 ± 0.42b</td>
<td>4.08 ± 0.20a</td>
</tr>
<tr>
<td>Tb.Th, mm</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>Tb.Sp, mm2</td>
<td>0.27 ± 0.04a</td>
<td>0.23 ± 0.03b</td>
<td>0.23 ± 0.01b</td>
</tr>
<tr>
<td>Conn.Dn, mm23</td>
<td>1.16 ± 0.30a</td>
<td>1.39 ± 0.32ab</td>
<td>1.56 ± 0.37ab</td>
</tr>
<tr>
<td>SMI</td>
<td>1.48 ± 0.17</td>
<td>1.33 ± 0.22</td>
<td>1.38 ± 0.27</td>
</tr>
<tr>
<td>Mid-shaft femur (cortical bone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV, mm3</td>
<td>0.31 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>TV, mm3</td>
<td>1.09 ± 0.04</td>
<td>1.12 ± 0.11</td>
<td>1.11 ± 0.07</td>
</tr>
<tr>
<td>BV/TV, %</td>
<td>28.3 ± 2.1</td>
<td>29.3 ± 1.8</td>
<td>29.1 ± 2.6</td>
</tr>
<tr>
<td>B.Ar, mm2</td>
<td>0.86 ± 0.06</td>
<td>0.92 ± 0.09</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>BS, mm2</td>
<td>3.38 ± 0.12</td>
<td>3.43 ± 0.16</td>
<td>3.44 ± 0.11</td>
</tr>
<tr>
<td>Ct.Th, mm2</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>

1Values are mean ± SD. Means in a row with superscripts without a common letter differ, P < 0.05. B.Ar, bone area; BS, bone surface; BV, bone volume; Conn.Dn, connectivity density; Ct.Th, cortical thickness; SeBean, selenium as pinto bean; SeDef, selenium-deficient; SeMet, selenium as selenomethionine; SMI, structure model index; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TV, total volume.

downregulated RANKL expression favors osteoblastogenesis or bone formation (30). Because dietary Se content and liver GPx1 activity were similar between the SeMet and SeBean groups, factors other than the intake of Se in the SeBean diet apparently contributed to the differences observed in the SeBean group. Although the diets were formulated with all required macro- and micro-nutrients based on the AIN-93G diet (14) and NRC recommendations (41), other factors such as the bioactive compounds in pinto beans were not taken into account. Hulls from dry edible beans contain large amounts of phenolic compounds that possess high antioxidant activity (42,43), which have beneficial effects on bone metabolism (44). Similar studies have shown that phenolic acid from blueberry polyphenols increased osteoblastic differentiation and bone formation (45,46). Therefore, the observed differences between the SeMet and SeBean groups might be caused by these phytochemicals in beans.

In conclusion, the present study demonstrated that Se deficiency is detrimental to bone microstructure by increasing bone resorption, possibly through increased oxidative stress. Se from pinto beans is highly bioavailable and provides potential benefit for promoting bone health.

Acknowledgments

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Literature Cited


