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# A biomechanical and spectroscopic study of bone from rats with selenium deficiency and toxicity

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**Abstract**

Selenium, being an essential mineral in the mammalian diet, is important in providing protection against oxidative damage. Numerous *in vitro* studies of selenium compounds reveal a very high correlation between catalytic activity of selenium compounds and toxicity. The present study was designed to investigate the effects of dietary selenium on the biomechanical properties of bone. New born rats of both sexes were fed with either a control, or a selenium- and vitamin E-deficient, or a selenium-excess and vitamin E-adequate diet. We obtained the stiffness (modulus of elasticity) of bones (femur and tibia) by tensile test for all groups considered. Both the deficient and the excess groups have decreased biomechanical strength with respect to the control group. To support our biomechanical results for both experimental groups, X-ray diffraction analysis and FTIR spectroscopic study were performed on the femurs and tibiae. The X-ray diffraction analysis showed that the intensities of the peak observed at around $2\theta^\circ = 31.820$, in the control femur and tibia are stronger than the intensities of the corresponding peak of two experimental groups. In FTIR spectroscopy, the disappearance and/or reduction of the intensities of some carbonate bands in the two experimental groups indicate that there is a decrease in crystallinity and mineral contents which, together with X-ray diffraction analysis, correlate very well with the biomechanical data.

## Introduction

Selenium, known to be essential for mammals since 1957, is an integral component of glutathione peroxidase (GSH-Px), an important enzyme of the cellular protecting system against lipid peroxide-initiated damage. Following absorption, selenium makes complexes with plasma proteins and is rapidly distributed into all tissues (Venugopal & Luckey 1974) where it is retained and believed to replace sulphur in sulphur containing compounds (Cummins & Martin 1967). In animals suffering from selenium deficiency diseases, GSH-Px activities in organs and tissues are decreased, suggesting that peroxidative damage of cells and tissues is responsible for at least some of the symptoms. Although similarly well-defined primary selenium deficiency diseases have this far not been observed in humans, many authors consider it to be a contributing factor in a variety of human pathological conditions. In certain regions of China, severe selenium deficiency is associated with an endemic disease known as Keshan disease (Chen *et al.* 1980) and Kashin-Beck disease, an osteoarticular disease. Kashin-Beck disease is a case of endemic, chronic, degenerative osteoarthrosis which is characterized by the selective necrosis of articular and growth plate chondrocytes (Sokoloff 1985).

Multiple degenerative and necrotic lesions within the articular cartilage and the growth plate represent the initial pathological changes associated with distributed mineralization and disfiguration of joints. In the study of an animal model of Kashin-Beck disease (Yang *et al.* 1993a), irregular bone formation was observed by radiography and morphometry as well as biochemical analysis showing overmodified lysine residues in collagen I from bone and in collagen II from cartilage. In the histological and biochemical studies of bone and articular cartilage specimens obtained from rats fed a low-selenium diet, it was shown that there were some noticeable changes in bone mineral density and in some biochemical parameters of the serum and the changes were related with the diet and the feeding duration (Sasaki *et al.* 1994). In addition to the other histopathological studies, Yang *et al.* (1993b) carried out histologic staining to detect mineralized and unmineralized bone and cartilage in the mice fed a selenium deficient diet and fulvic acid supplemented drinking water. The results of this study have shown that the degeneration of the articular cartilage in the knee joints of these mice resembles the early stages of osteoarthrosis.

Selenium is unusual among elements in that it is both toxic and beneficial to animals under natural conditions of living. Extremes of selenium uptake by animals are related to the content of the element in food plants and in the soil. These concentrations are varied and localized. Selenium toxicity in liverstock that consumed selenium accumulator plants can be traced back to Marco Polo (Spallholz 1994). When dietary selenium is in excess of 4 ppm in mammalian diet (Olson *et al.* 1970; Olson & Kobayoshi 1992), this effect is more pronounced. Experimental chronic selenium toxicity in animals affects the major organs; more particularly sodium selenite has been shown to cause cellular dysfunction in a number of tissues (Young *et al.* 1981; Anundi *et al.* 1982; Lin-Shiau *et al.* 1989; Turan *et al.* 1996, 1997a, b, 1998). The mechanisms of selenium toxicity as well as deficiency are not clearly established yet. It is thought to be related to its ability to form covalent linkages with intracellular proteins and reduced glutathione (Dickson & Tappel 1969).

Although various effects of selenium deficiency or excess have been reported in humans and animals, most of the studies give little information on the status of vitamin E in those cases. This point is of importance because vitamin E is known to decrease the threshold dietary selenium needed to elicit selenium deficiency symptoms in several species (Scott *et al.* 1967; Hakkarainen *et al.* 1978; Ytrehus *et al.* 1988). The present study was therefore designed to investigate the effects of combined deficiency of dietary selenium and vitamin E and of sole selenium excess on some biomechanical characteristics of rat bones. In this study, we obtained the stiffness (modulus of elasticity) of bones by using tensile test. We also used a variety of physical and chemical techniques, including conventional X-ray diffraction analysis, Fourier transform infrared spectroscopy and light microscopy to support the biomechanical observations from these bones.

## Materials and methods

### *Animals, diets, tissue preparation, and analysis*

Both-sex weanling Wistar rats were divided randomly into three groups and housed in stainless-steel, wire-bottomed cages initially at a density of three per cage and, as they grew, were then caged individually. They were maintained at an ambient air temperature of $22 \pm 1$ °C and a 12 h light/dark cycle. All experiments were performed in accordance with the guiding principles in 'Care and use of animals' published in *Am. J. Physiol.*

The deficient diet was obtained commercially. Selenium and vitamin E were supplemented in adequate- and rich- diets with sodium selenite and -tocopherol acetate. The Se content of the diets was determined by using a graphite furnace atomic absorption spectrometer. Based on analysis of random batches of diet, the Se concentration of the deficient diet was 9.8 $\mu$g/kg diet and the adequate and rich diets contained 225 $\mu$g Se/kg diet and 4.2 mg Se/kg diet, respectively (Turan *et al.* 1999). Deionized distilled water was given to the animals which contained very little Se ($< 1$ $\mu$g/l). The animals were fed with either an adequate diet (Group I: control group), a deficient diet (Group II: Se- and Vitamin E-deficient group), or a rich diet (Group III: Se-rich and vitamin E-adequate group). The animals were permitted free access to the food and water for about 12 to 14 weeks.

Rats were heparinized and anesthetized with sodium pentobarbital (30 mg/kg). Plasma vitamin E concentrations were determined by high-performance liquid chromatographic (HPLC) technique. Selenium levels were measured using the same graphite furnace atomic absorption spectrometer mentioned above. The results of Se and vitamin-E determinations were presented in our previous study (Turan *et al.* 1999).

*Biomechanical testing*

The bones of the animals were taken to the mechanical testing laboratory immediately after removal from the body. The muscles surrounding the bones were carefully removed by lancet. The two ends of the bones were immerged into an adhesive material (Sunfix) poured into a special die so that the shape of the two ends of the bone became appropriate to be held by the grips of the tensile testing machine used (Lloyd LS-500, Southampton, UK). The sunfix material containing the ends of the bone was kept in the die for 3 h at room temperature. The 'axes' of the rat bones considered were not straight. This fact, of course, causes a natural eccentricity of the loading during the tensile tests creating bending stresses in addition to the pure normal tensile stress. This undesired eccentricity of the bone between the two dies was minimized adjusting the locations of the ends in the dies during solidification. As a result of the alignment of the tension axis with that of the ends of the bones, the axial (tensile) load passes through the center of the cross section of the bone at the midpoint of its length where bone breaking generally occurs. In other words, at the section of breakage there is no eccentricity of the load and we have pure tension. Obviously, at other sections of the bone we have combined axial load and bending case; however, we are interested in the section of failure which turned out to be generally at the midlength in our experiments. The stresses and strains reported in our work all refer to this breakage section and they are both pure tensile. The adhesion between the sunfix and the bone was properly established and the sunfix solidified totally in 3 hours. This procedure was applied to all the specimens tested. Three separate bone groups of tibiae, femora and humera were selected as the test groups. The geometrical properties of the bones, such as span length, wall thickness and cross-sectional dimensions, were recorded. The cross sections of the tibiae were considered to be in the form of a circular tube of uniform thickness whereas the cross sections of the femora and of the humera were treated as elliptical tubes having uniform thicknesses. The specimens were tested using the material testing machine mentioned above. The tensile tests were performed on all specimens with a constant speed of 2 mm/min and the loading was of displacement-controlled type. The tests were undertaken using a 500-N load cell. Data sampling rate was 3.3 Hz. The measured force and deflection data throughout the tests were collected using a Pentium 166 MHz computer connected to the testing machine via an RS232 serial communication port. The force versus deflection curves obtained from the tests were transformed into stress versus strain diagrams using the data postprocessing program, version 3.21, available in the testing machine. The modulus of elasticity of the bone under consideration was calculated from the slope of the linear region of the stress-strain curve.

*Fourier transform infrared spectroscopy and X-ray diffraction analysis*

The femura and tibiae specimens from all animals of each group under consideration were removed from freshly killed animals. The bones were carefully removed from the surrounding soft tissue, placed in a freezer, and then were placed in an Edwards vacuum freeze dryer. The samples were powdered in a nitrogen mill to an approximate mesh size of 600 and stored in a dessicator. Fourier Transform Infrared spectra (FTIR) were recorded in the range 4000–400 $cm^{-1}$ on a Bomem MB157 spectrometer purged with dry air. Interferograms were averaged for 100 scans at 2 $cm^{-1}$ resolution. KBr pellet technique (2.5 mg of sample per 300 mg KBr) was used. X-ray diffraction patterns of all powdered samples were recorded on a Rigaku model diffractometer using $CuK_a$ radiation. The selenium (Se), calcium (Ca) and phosphate ($PO_4$) concentrations of the bone samples were measured by atomic absorption spectrometry.

*Statistics*

The results were expressed as means $\pm$ standard deviation (SD). Data were analysed statistically by using student's $t$-test and the differences of $p < 0.05$ were considered statistically significant.

## Results

*Animal characteristics*

Most of the animals fed with the selenium- and vitamin E-deficient diet and the selenium rich and vitamin E-adequate diet showed hair loss in both cases. Some of the animals from the deficient group showed testicular hypertrophy. Body weights of the animals from these two groups were significantly lower than those of the control group (Turan *et al.* 1999). Selenium deficiency and selenium excess were verified by selenium concentration measured in plasma. Vitamin E

was also confirmed by vitamin E level measured in plasma. Both selenium and vitamin E levels in the deficient group were about 50% of the corresponding levels of the control group. In the selenium rich group, the plasma selenium level was increased significantly (280% of the control) while the vitamin E level was comparable to that of the control group.

*Effect of selenium on bone mechanics*

The results of the tensile tests on bone mechanics were presented as stress versus strain curves. During the tests, most fractures occurred near the midpoint of the span and very few occurred near one of the grips generally in the transverse direction. The data are presented in Figures 1A, B, and C for femur, tibia and humerus, respectively. For each bone type, the three test groups I, II, and III are presented with different centre symbols and the corresponding average slope of the group data. Ten points from each tensile test are selected randomly and plotted in these graphs. Ninety points in one graph represent 9 tensile tests performed. The slope specifies the stiffness of the bone under consideration. The slopes of the graphs for femur and tibia show that the stiffnesses in the experimental groups (Groups II and III) are different from the stiffnesses of the corresponding bones of the control group (Group I). On the other hand, the stiffnesses of humerus in the experimental groups are not significantly different from the corresponding control value. It should be stressed that the values presented as moduli of elasticity have a relative implication for comparison. In bone, being not an isotropic material, there is an easy and difficult direction. In other words, the bones tested are structures showing anisotropic behavior. Because of this anisotropy, the modulus of elasticity varies with direction and the bone specimens used in the present experiments must, therefore, be considered as structures (macro size) rather than materials (micro size). The stiffness that we are talking about is the structural stiffness of the bone as a whole not the stiffness of the individual materials constituting the bone. Thus, we are comparing the macro level properties of the bones from various groups. To point out the differences in stiffness more clearly, the results on the moduli of elasticity of femur, tibia and humerus are examined separately for each one of the two diet groups and then compared with those of the other diet group. The mean values of the moduli of elasticity, including the standard deviations, of all bone samples in all three groups are presented in Table 1. In

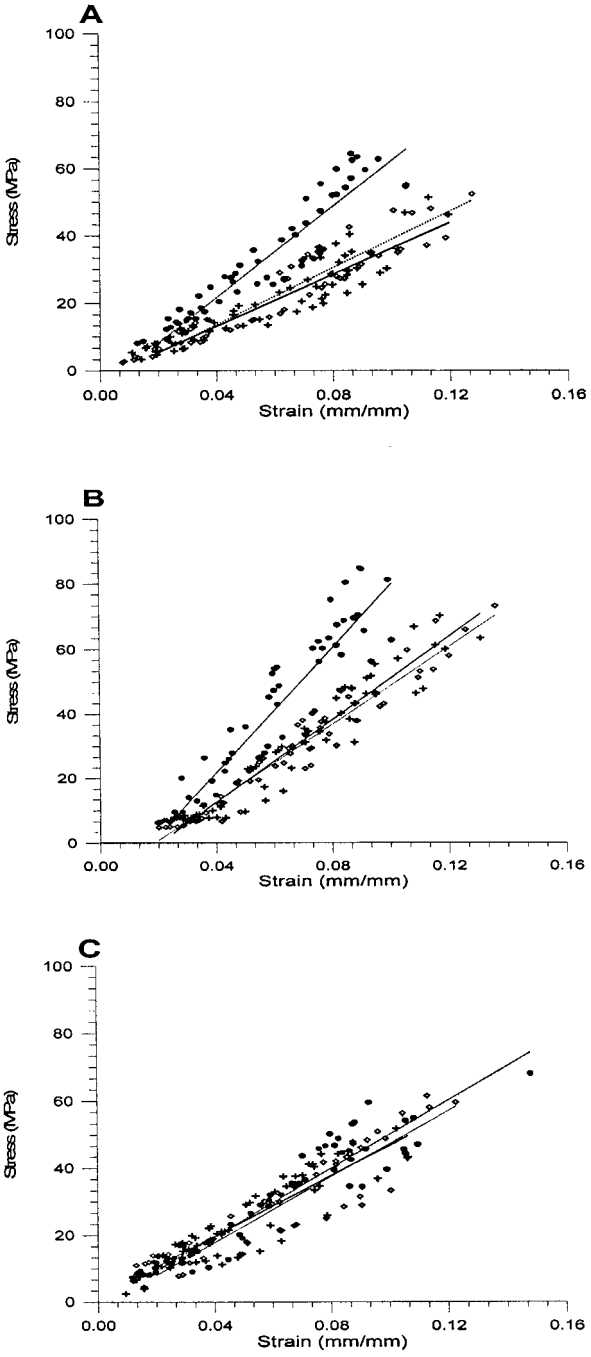


*Fig. 1.* Effect of dietary selenium on the stress versus strain diagrams of femora (A), tibiae (B), and humera (C). 50 samples data from each group are selected. Group I: Control group. Group II: Selenium and vitamin E deficient group. Group III: Selenium- and vitamin E-excess group.

*Table 1.* Effect of dietary selenium on modulus of elasticity of rat bones.

| Rats | Femur ($n = 14$) | Tibia ($n = 14$) | Humerus ($n = 14$) |
|---|---|---|---|
| Group I ($n = 10$) | $684.4 \pm 130.6$ | $1036.4 \pm 154.2$ | $639.9 \pm 143.2$ |
| Group II ($n = 8$) | $527.1 \pm 85.4^{**}$ | $653.2 \pm 142.8^{**}$ | $570.6 \pm 110.0$ |
| Group III ($n = 7$) | $494.4 \pm 106.8^{**}$ | $726.8 \pm 115.7^{**}$ | $566.1 \pm 93.4$ |

Values are given as mean $\pm$ SD with units of MPa. Modulus of elasticity is calculated from the slopes of the stress-strain curves.
Group I : animals fed with selenium and vitamin E adequate diet, control group.
Group II: animals fed with selenium and vitamin E deficient diet.
Group III: animals fed with selenium excess and vitamin E adequate diet.
** $p < 0.01$ compared with control rats.
*** $p < 0.001$ compared with control rats.
$n$: number of the animals and also of the bones.

*Table 2.* FTIR assignments.

| Peak number (see graphs) | Wavenumber ($cm^{-1}$) | Assignment |
|---|---|---|
| 1 | 3450–3300 | O-H stretch |
| 2 | 2900–3000 | Carbonate and lipid |
| 3 | 1800 | Carbonate |
| 4 | 1700–1600 | Amide I |
| 5 | 1550 | Amide II |
| 6 | 1250 | P-OH deformation |
| 7 | 1082 | Carbonate |
| 8 | 1050 | P-O stretch in $PO_4$ |
| 9 | 550–600 | $PO_4$ bend |

all the cases considered, the control group always has the highest modulus of elasticity. The two diet groups have lower averages but the difference between them is not statistically significant. The statistical analysis of the data is also given in Table 1 to potentiate the effect of selenium more clearly. A study of Figures 1A, B, and C together with Table 1 reveals that both selenium- and vitamin E-deficient diet and selenium rich diet decreased the biomechanical strengths of femur and tibia significantly while the corresponding bones belonging to the control group always had the highest modulus of elasticity.

### *Results of Fourier transform infrared spectroscopy and X-ray diffraction analysis*

Fourier transform infrared spectra (FTIR) were recorded from all the bone specimens considered and they were analyzed by comparing the vibrational bands of each sample. The common changes observed

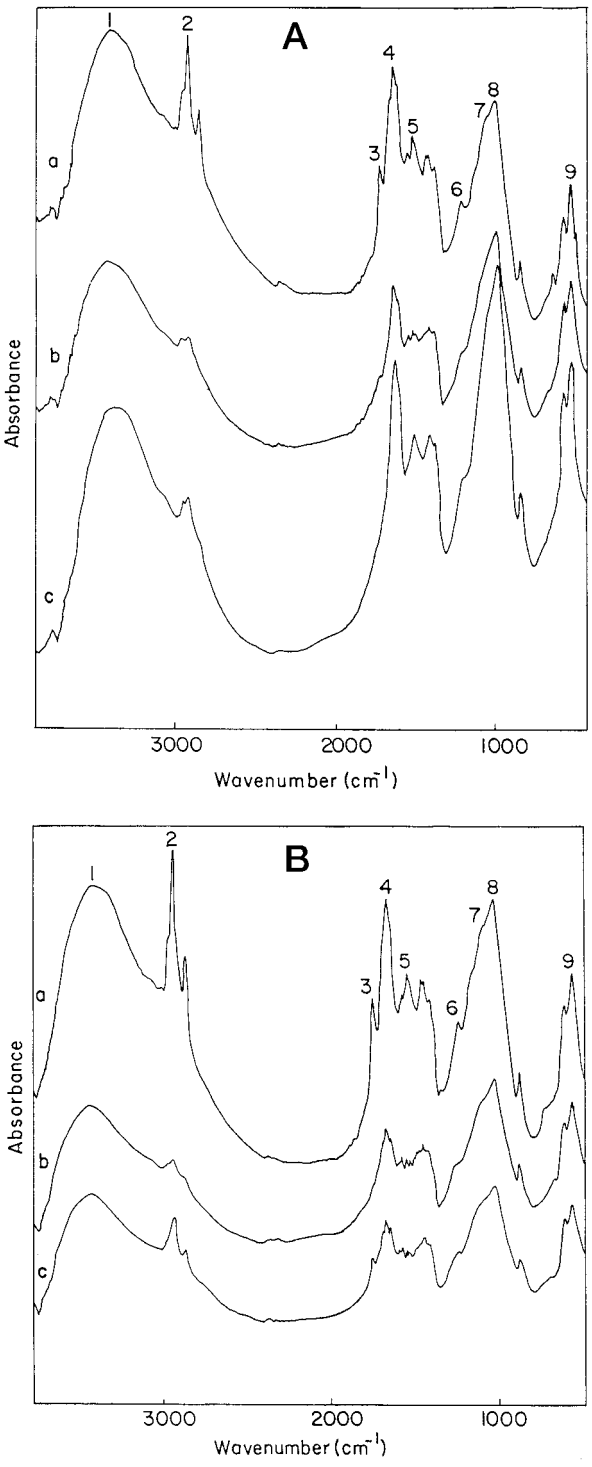


*Fig. 2.* A typical recording of FTIR spectra of the femora (A) and the tibiae (B) of all considered groups. The spectra of control, selenium-excess and vitamin E-adequate, and selenium and vitamin E deficient groups are presented in the figures as (a), (b), and (c), respectively. The peak numbers indicated on the spectra and their assignments are reported in Table 2.

in the spectra of the two experimental groups were noted and compared with the control data. Typical spectra of one femur and of one tibia from the two experimental groups and from the control group are shown in Figures 2A and B, respectively. As seen from the figures, the complex spectra consist of several vibrations of protein component and the inorganic mineral phase of the bone. The band assignments have been reported in Table 2. We have mainly assigned OH, $PO_4$ and carbonate group frequencies and amide bands which are in good agreement with previous FTIR study where more detailed band assignment was reported (Cassella *et al.* 1994). As seen from the spectra of two types of bones from control group (Figures 2A and B), the strong broad band appeared in the spectral region of 3450–3300 $cm^{-1}$ is assigned as OH stretching mode. The bands appear at 2900–3000 $cm^{-1}$, around 1800 $cm^{-1}$, 1450 $cm^{-1}$ and 871 $cm^{-1}$ are carbonate bands. The bands observed in the region 900–1250 $cm^{-1}$ and 500–650 $cm^{-1}$ are assigned as $PO_4$ group modes. The amide I and amide II absorptions occurs in the region 1600–1700 $cm^{-1}$ and at around 1550 $cm^{-1}$, respectively. As seen from the figure, similar group frequencies have also been observed for the two experimental groups under diet. In the figure, the major intensity changes according to the control group have been observed in the carbonate bands in the spectral region of 2900–3000 $cm^{-1}$, and at around 1750 $cm^{-1}$, and in the 1250 $cm^{-1}$ band which is assigned as P-OH deformation. For qualitative analysis, the intensity ratio of the each band of the died groups to the coresponding bands of the control groups were calculated after the normalization of the spectra according to the strong OH band located at 3300–3450 $cm^{-1}$ region. In the 2900–3000 $cm^{-1}$ region, the carbonate bands of the excess group have intensities smaller than the deficient group. Also intensities of the deficient group is significantly smaller than the intensities of the control group. The calculated mean ($\pm$SD) values for carbonate band (labelled 2) intensities (as percentage changes) of the excess group were $58 \pm 1\%$ and $47 \pm 2\%$ of the control values for femur and for tibia, respectively. Whilst in the deficient group, values for the carbonate band were observed to be $(42 \pm 1)\%$ and $(34 \pm 3)\%$ of the control group, for femur and tibia, respectively. For both groups, the intensity of another carbonate band observed at 1750 $cm^{-1}$ (labeled 3) was also smaller than the control. This decrease was much more profound for femur in both experimental groups. For both experimental groups, we have also observed a decrease

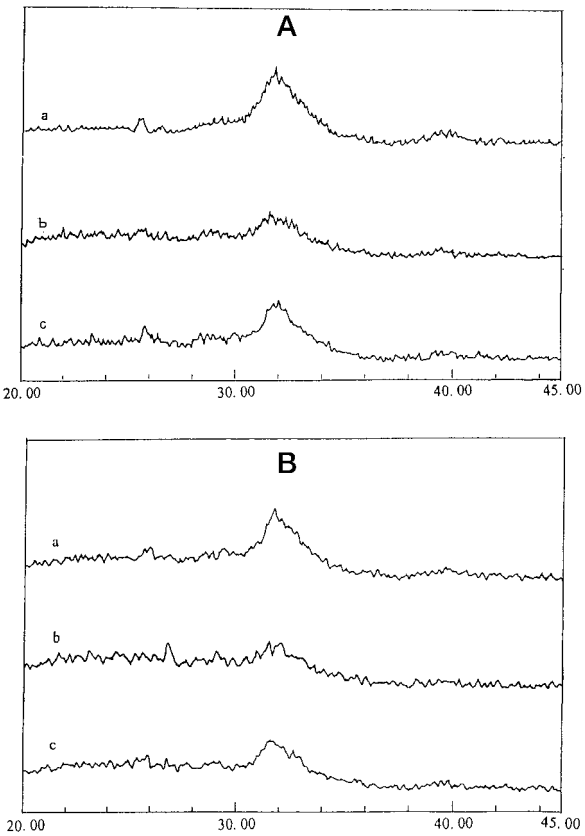


*Fig. 3.* Typical X-ray diffaction patterns (intensity versus $2\theta$) from the femora (A) and the tibiae (B) of a) control (Group I), b) selenium-excess and vitamin E-adequate (Group III), and c) selenium- and vitamin E-deficient groups (Group II). The mineral contents of the femora from all groups considered are the followings: Calcium 25.5%, 24.1%, 26.5%; phosphate 6.2%, 9.2%, 10.8%; selenium 1.1%, 0.4%, 0.2% for Group I, Group III, and Group III, respectively. The mineral contents of the tibiae are the following: Calcium 29.6%, 23.0%, 30.8%; phosphate 18.5%, 10.4%, 17.4%; selenium 2.0%, 0.9%, 0.6% for Group I, Group III, and Group II, respectively.

in the intensity of the band labeled 6. For femur, intensity of the this band is found to be $59 \pm 2\%$ and $42 \pm 1\%$ of the control group for the deficient and the excess groups, respectively. Whilst, for tibia this value was $67 \pm 4\%$ of the control group in both experimental groups.

X-ray diffraction analyses were performed on all the bone samples acquired. Representative X-ray diffraction partterns obtained from one femur and from one tibia of all the groups considered (Group I, II and III) are shown in Figures 3A and B, respectively. As seen in the figures, the intensity of the peak observed at around $2\theta^\circ = 31.820$ is the strongest in control femur with respect to the other two experimental femora, while the intensity of the same peak for the excess

group is less than those of the deficient group. The mineral contents, as percentage amount, of these specimens are also given in the figure legends of X-ray diffraction patterns.

## Discussion

Bone, which is formed by the infusion of an organic matrix, principally collagen, with calcium phosphate, performs two major functions in the body. Firstly, the combination of about 25 wt% collagen and 75 wt% calcium phosphate provides the biomechanical properties needed for body support and movement. Secondly, bone mineral is in metabolic interrelation with body fluids, serving principally as a reservoir for body minerals, storing or releasing them as the need arises. Mature bone mineral, which is an analogue of the compound hydroxyapatite (HA), is characterized by a specific surface, carbonate substitution, non-stoichiometry and internal crystal disorder. However, studies indicate that its structure is more like that of a poorly crystalline apatite and amorphous calcium phosphate (ACP) may be a component of bone. Structure of the mineral particles change with species, age and disease state, and it has been already discussed that calcium deficiency distorts the apatite structure (Betts *et al.* 1981). The present work reports the results of light microscopy, X-ray diffraction analysis, infrared spectroscopy and biomechanical studies on the structural details of bone in the case of selenium and vitamin deficiency and selenium excess.

It is known that one of the methods to quantify osteoporosis is to measure bone mass. This method has been used by many researchers (Boskey 1990; Ginsberg *et al.* 1990; Matzsch *et al.* 1990). Obviously, bone mass is related to the mechanical strength of bone (Turner & Burr 1993). However, bone mass is not the only property which affects bone strength. The architecture and the composition are also strongly related to bone strength. Thus, it is important to measure the bone mechanical properties. The results of the present study and our previous related published data indicate that both the deficiency and excess of selenium have some alternative and degenerative effects on the structure and function of tissues (Dalay *et al.* 1993; Turan *et al.* 1997a, b, 1999) besides the decreased biomechanical strength of the bones (Turan *et al.* 1997c). The results obtained from the biomechanical tests indicate that combined deficiency of selenium and vitamin E or excess of selenium in diet both have some adverse effects on the mechanical properties of femora and tibiae of the rats. The moduli of elasticity in these two experimental groups (Group II and Group III) are about 25–30% less than those of the control group. Although it is clearly known that the values that we have presented as stiffness or modulus of elasticity have only a relative implication for comparison rather than representing values for a material parameter, the results of bone biomechanical testing and the histopathological findings (data not given) in both Group II and Group III support the sendromes of the diseases such as Kashin-Beck. In the study of Sasaki *et al.* (1994), in addition to the changes in bone mineral density of the femur obtained from Kashin-Beck diseased rats which were fed a low selenium diet, biomechanical and histological changes in bone and articular specimens were also demonstrated. Similarly, Yang *et al.* (1993a, b), in their study of an animal model study of Kashin-Beck disease, investigated the altered parameters of skin, bone, and cartilage, and an irregular bone formation and a lesser force in tibia were noted.

To support our biomechanical results of both femur and tibia of both experimental groups, we performed X-ray diffraction analysis and FTIR spectroscopic study. In X-ray diffraction analysis, the observed reduced intensity of the peak at around $2\theta^\circ = 31.820$ in both the femora and the tibiae for both experimental groups indicate the possible alterations in the crystallinity or a poorly crystalline substance. It is presently known that there is a close relationship between the low Ca: $PO_4$ ratio with a corresponding increase in apparent $HPO_4^{2-}$ content and the high content of tightly bound water, and the low degree of crystallinity (Glimcher 1976; Glimcher *et al.* 1981) which is usually measured in terms of diffracted X-ray intensity. Bone mineral is assumed to contain varying proportions of these two constituents, depending on its stage of development or maturity, physiological state, etc. Diseased bone, similarly to immature bone, is tought to contain more ACP, having a low Ca: $PO_4$ ratio, with a low degree of crystallinity than mature or untreated bone. Under consideration of our X-ray diffraction patterns, it is very tempting to suggest the possibly apperance of ACP in the bones of rats under our experimental conditions.

The FTIR spectra of both femora and tibiae from both experimental groups show a decrease in intensity of carbonate bands in the spectral region of 2900–3000 $cm^{-1}$, and at 1750 $cm^{-1}$ with respect to those of the controls. It is previously reported that the in-

crease in the crystallinity of the crystals is due to an increase in the carbonate ion concentration (Glimcher *et al.* 1981; Rey *et al.* 1991b). Our results, so, indicate that in the investigated bones of both excess and deficient group, there is a decrease in the crystallinity and in the mineral content of the bones. It is also observed that this decrease in the crystallinity is more profound for the excess group in both tibia and femur. Our FTIR results and X-ray diffraction analysis are in agreement and they are supporting the above conclusion. Although some studies report an inverse relation between the change in the concentration of carbonate ions and nonapatitic phosphate ions in bone mineral (Rey *et al.* 1991a), we have not observed a significant change in the intensity of the phosphate bands except that a remarkable decrease is observed in the P-OH deformation bands for Group II and III. Our X-ray diffraction analysis and the FTIR results are very encouraging when they are taken into consideration together with the biomechanical data because they correlate well with each other. A study of all of the data obtained implicates the bones as one of the major target organs in intoxication as well as deficiency syndromes, confirming the earlier reports (Sokoloff 1985; Symth *et al.* 1990; Turan *et al.* 1997a–c). When we bring our previously and presently obtained data all together along with the results of other studies on the effects of antioxidants and/or oxidants on the structure and function of tissues, we conclude that the mechanisms underlying these effects are still not clearly clarified and need to be defined.

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## References

Anundi I, Hogberg J, Stahl A. 1982 Involvement of glutathione reductase in selenite metabolism and toxicity, studied in isolated rat hepatocytes. *Arch Toxicol* **50**, 113–123

Betts F, Blumental NC, Posner AS. 1981 Bone mineralization, *J Crystal Growth* **53**, 63–73

Boskey AL. 1990 Bone mineral and matrix. Are they altered in osteoporosis? *The Orthop Clin North Am* **21** (1), 19–29

Chen X, Yang G, Chen J, Chen X, Wen Z, Ge K. 1980 Studies on the relationships of selenium and Keshan disease. *Biol Trace Element Res* **2**, 91–107

Cummins LM, Martin JL. 1967 Are selenocystine and selenomethionine synthesized *in vivo* from sodium selenite in mammals. *Biochemistry* **6**, 3163–6168

Dalay N, Turan B, Koc E, Afrasyap L, Delilbasi E. 1993 The effects of *in vivo* selenium supplementation on the amplitude of the spontaneous contractions and the responses to acetylcholine in isolated rabbit ileum. *Neurobiology* **1** (1), 83–90

Dickson RC, Tappel AL. 1969 Reduction of selenocystine by cysteine or glutathione. *Arch Biochem Biophysics* **130**, 547–550

Ginsberg JS, Kowalchuk G, Hirsch J, Edwards PB, Burrows R, Coates G, Webber C. 1990 Heparin effect on bone density. *Thrombosis Haemostasis* **64** (2), 286–289

Glimcher MJ. 1976 In Greepp RO, Astwood EBB, eds.: *Handbook of Physiology: Endocrinology*, Vol. 7, Eds. Washington, DC: Am. Physiological Soc.

Glimcher MJ, Bonar LC, Grynpas MD, Landis WJ, Roufosse AH. 1981 Recent studies of bone mineral: is the amorphous calcium phosphate theory. *J Crystal Growth* **53**, 100–119

Hakkarainen J, Lindberg P, Bengtsson G, Jonsson L, Lannek N. 1978 Requirement for selenium (as selenite) and vitamin E (as a-tocopherol) in weaned pigs. III. The effect on the development of the VESD syndrome of varying selenium levels in a low-tocopherol diet. *J Anal Sci* **46**, 1001–1008

Lin-Shiau SY, Liu SH, Fu WM. 1989 Studies on the contracture of the mouse diaphragm induced by sodium selenite. *Eur J Pharmacol* **167**, 137–146

Matzsch T, Bergqvist D, Hedner U, Nilsson B, Østergaard P. 1990 Effects of low molecular weight heparin and unfragmented heparin on induction of osteoporosis in rats. *Thrombosis Haemostasis* **63** (3), 505–509

Olson JA, Kobayashi S. 1992 Antioxidants in health and disease: overview. *Poc Soc Exp Biol Med* **200**, 245–247

Olson OE, Novacek EJ, Whitehead EI, Palmer IS. 1970 Investigation on selenium im wheat. *Phytochemistry* **9**, 1181–1188

Rey C, Beshah K, Griffin R, Glimcher MJ. 1991a Structural studies of the mineral phase of calcifying cartilage. *J Bone Mineral Res* **6**, 515–525

Rey C, Renugopalakrishnan V, Collins B, Glimcher MJ 1991b Fourier Transform Infrared Spectroscopic Study of the Carbonate Ions in Bone Mineral During Aging. *Calcif Tissue Int* **49**, 251–258

Sasaki S, Iwata H, Ishigura N, Habuchi O, Miura T. 1994 Low-selenium diet, bone and articular cartilage in rats. *Nutrition* **10** (6), 538–543

Schwarz K, Foltz CM. 1957 Selenium as an integral part of factor 3 against necrotic dietary liver generation. *J Am Chem Soc* **79**, 3292–3293

Scott ML, Olson G, Krook L, Brown WR. 1967 Selenium-responsive myopathies of myocardium and of smooth muscle in the young poult. *J Nutr* **91**, 573–583

Severcan F, Kazanci N, Baykal U, Süzer S. 1995 IR and Turbidity studies of vitamin E-cholesterol-phospholipid membrane interactions. *Bioscience Reports* **15** (4), 221–229

Smyth JBA, Wang JH, Barlow RM, Humphreys DJ, Robins M, Stodulski JBJ. 1990 Experimental acute selenium intoxication in lambs. *J Comp Path* **102**, 197–209

Sokoloff L. 1985 Endemic forms of osteoarthritis. *Clin Rheum Dis* **11**, 187–212

Spallholz, JE. 1994 On the nature of selenium toxicity and carcinostatic activity. *Free Rad Biol Med* **17** (1), 45–64

Turan B, Desilets M, Açan LN, Hotomaroglu Ö, Vannier C, Vassort G. 1996 Oxidative effects of selenite on rat ventricular contractility and Ca movements. *Cardiovasc Res* **32**, 351–361

Turan B, Hotomaroglu O, Kilic M, Demirel-Yilmaz E. 1999 Cardiac dysfunction induced by low and high diet antioxidant levels comparing selenium and vitamin E in rats. *Regulatory Tox Pharmacol* **29**, 132–150

Turan B, Zaloglu N, Koc E, Saran Y, Akkas N. 1997a Dietary selenium and vitamin E induced alterations in some rabbit tissues. *Biol Trace Elem Res* **58** (3–4), 237–253

Turan B, Koc E, Zaloglu N. 1997b Deficiency and toxicity of selenium alter the acetycholine stimulated contraction of isolated rabbit ileum. *Trace Elem Electrolytes* **14** (1), 13–18

Turan B, Balcik C, Akkas N. 1997c Effect of dietary selenium and vitamin E on the biomechanical properties of rabbit bones. *Clinical Rheumatol* **16** (5), 441–449

Turner CH, Burr DB. 1993 Basic biomechanical measurements of bone. *A Tutorial Bone* **14**, 595–608

Venugopal B, Luckey TD. 1974 Toxicology of non-radioactive heavy metals and their salts. *Environ Qual Saf Suppl* **2**, 4–73

Yang C, Wolf E, Roser K, Delling G, Muller PK. 1993a Selenium deficiency and fulvic acid supplementation induces fibrosis of cartilage and disturbs subchondral ossification in knee joints of mice: an animal model study of Kashin-Beck disease. *Virchows Arch A Pathol Anat Histopathol* **423** (6), 483–491

Yang C, Niu C, Bodo M, Gabriel E, Notbohm H, Wolf E, Muller PK. 1993b Fulvic acid supplementation and selenium deficiency distrub the structural integrity of mouse skeletal tissue. *J Biochem* **289**, 829–835

Young JD, Crowley C, Tucker EM. 1981 Haemolysis of normal and glutathione-deficient erythrocytes by selenite and tellurite. *Biochem Pharmacol* **30**, 2527–2530

Ytrehus K, Ringstad J, Myklebust R, Norheim G, Mjos OD. 1988 The selenium-deficient rat heart with special reference to tolerance against enzymatically generated oxygen radicals. *Scand J Clin Lab Invest* **48**, 289–295