The Relationship between Exercise, Bone Mineral Density and Antioxidant Enzyme Activity of Postmenopausal Women

Haeng-Shin Lee¹, Da-Hong Lee^{2†} and Mi-Hyun Kim³

¹Nutrition Research Team, Korea Health Industry Development Institute, Seoul 156-800, Korea ²Department of Food and Nutrition, Wonkwang University, Iksan 570-749, Korea ³Department of Food and Nutrition, Kangwon National University, Samcheok 245-711, Korea

Abstract

This study was carried out to elucidate the relationship among exercise, bone mineral density and antioxidant enzyme activity of postmenopausal women. 60 women residing in the Iksan, Korea area were recruited. The questionnaires were designed to find out exercise habits. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry. Parameters of antioxidative capacity, including the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and total antioxidant capacity (TA) were analyzed in fasting blood. The mean age, height, weight, and BMI of subjects were 65.0 years, 151.1 cm, 59.5 kg 26.0 m/kg², respectively. The mean BMDs of subjects were 0.85 g/cm² (lumbar spine), 0.6 g/cm² (Femoral neck), 0.49 g/cm² (trochanter), and 0.40 g/cm² (Ward's triangle). There was a significant difference in BMD among different age groups (50's, 60's and 70's) showing lower value with increasing age (p<0.05). The mean SOD, GPx, and CAT activities were 138.5 U/mL, 1,273.8 U/mL and 314.3 kU/L respectively, and TA was 1.16 mmol/L. TA of the group which exercised 3~4 times a week was significantly higher than those of the other exercise groups (p<0.05). The subjects with higher SOD activity also have a higher the T values in the lumbar spine, femoral neck, trochanter, and Ward's triangle. In conclusion, this study revealed that the levels of antioxidant enzyme activity were closely associated with the exercise status and bone mineral density in postmenopausal women.

Key words: bone mineral density, superoxide dismutase, catalase, total antioxidant capacity

INTRODUCTION

Osteoporosis, the decrease of bone density, is an increasingly important problem as the population ages. The list of leading causes of mortality causes for Korea shows that 2.1 persons per one hundred thousand

Korea shows that 2.1 persons per one hundred thousand persons in the population in 1989 had diseases of the musculoskeletal system and conexus substanita. This figure has increased to 4.4 persons in 2001. For women, the rate was tripled from 2.5 to 7.5 persons per one hundred thousand at this time (1).

It is estimated that a number of chronic diseases, including aging, have a loose relationship with oxidative stress in the body. Some studies on anti-oxidative factors (2-5) are being conducted.

These investigate the relationship between osteoporosis and oxidative damage, on the assumption that oxidative stress affects osteoporosis caused by aging. They suggest free radicals as the major cause of osteoporosis (6-9). Osteoporosis is caused by various factors, such

as genetic factors, menopause and nutritional status (10, 11). There are few studies on the activation of anti-oxidative enzymes by general nutrition conditions, except for antioxidant vitamin and minerals. According to the studies of Datta et al. (6) and Garrett et al. (9), Reactive Oxygen Species (ROS) may directly affect the process of bone resorption through destruction of the collagen in the bone, or facilitating the secretion of protease by osteoclasts. Osteoclasts reacts with parathormone, interleukin-1, tumor necrosis factor (TNF) and 1,25-(OH)₂ vitamin D₃ and creates superoxide. This superoxide facilitates bone resorption by increasing the creation and activation of osteoclasts. Also, Veille et al. (12) reported that estrogen increased oxide synthase activity and decreased the activity of SOD in the womb of lambs. Damoulis and Hauschka (13) and Ralston et al. (14) reported nitric oxide increased the activation of osteoblasts at low levels, while it hindered its activation or toxicity at high levels. Therefore, in the decrease of bone density after menopause caused by lack of estrogen, the creation of free radicals may affect the creation and activation of osteoclasts and be the major cause of decreasing of bone mineral density, caused by inharmonious remodeling.

Limited study has been conducted on the possible effect of the "free radicals" on bone mineral density, especially among postmenopausal women. Research on the relationship between bone density and antioxidant enzyme activity is greatly needed.

This study focused on the relationship between the anthropometric measurement, lifestyle, bone mineral density, and activity of the antioxidant enzyme of postmenopausal women.

MATERIALS AND METHODS

Subjects

60 postmenopausal women an age $50\sim77$, attending a seniors' college and living at Iksan, Jeon-buk in July 2002 were selected as the main subjects. The subjects experienced natural menopause without hysterectomy, except those who had thyroid and kidney problems. We studied anthropometric measurements, collected blood and details of food intake for three days.

Questionnaire interview

Each participant completed a questionnaire conducted by investigators. The questionnaire included the participant's age, health status, past and current use of medication (including estrogen), lifestyle factors such as sleeping hours and exercise status.

Anthropometric measurements

The subjects took their heights and weight in an upright position without shoes using an automatic physical measuring machine (DS-102, JENIX, Korea). The BMI {body mass index=weight (kg)/height (m)²} was calculated based on height and weight. The percentage of body fat (body fat %) was calculated based on age and height using a body fat measuring instrument (TBF-105 TANITA, Japan). Waist and hips were measured with a measuring tape. On the basis of this, WHR (waist hip ratio) was calculated. Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) were measured using an automatic sphygmomanometer (BP-750A, NISSEI, Japan). The survey was designed to measure the age, maternal factors, the lifestyle, the frequency of the exercise, and the exercise hours.

Measurement of the bone mineral density

The age, height and weight of the participants were recorded. The bone mineral density was measured in the following areas: the lumbar spine (L2~L4), femoral neck, trochanter, intratrochanter and Ward's triangle using dual

energy X-ray absorptiometry (DEXA, Hologic, USA).

Analysis of antioxidant enzyme activity

Superoxide dismutase: The activity of SOD was measured using heparin-treated plasma 1.0 mL based on Floh method (15). Xanthin created superoxide by xanthine oxidase. This superoxide radical forms Formazan dye through reacting with I.N.T (2-[4-iodophenyl]-3-[4-introphenol]-5phenyl-tetrazolium chloride). We measured the degree of suppression of this reaction as an indicator of SOD activity.

Glutathione peroxidase: GPx activity was determined by a spectrophotometric method using heparin-treated 0.05 mL plasma based on Paglia and Valentine's method (16). We measured the degree of decrease in optical density at 340 nm when glutathione is reduced by GR and NADPH.

Serum catalase: Serum catalase activity was determined according to Aebi's method (17). We placed 50 mmol/L Na-K phosphate buffer (pH 7.0) and a substrate, 1.0 mL H₂O₂, into 0.2 mL serum and activated it at 37°C for 1 minute. Then 32.4 mmol/L ammonium molydate solution was added and held at 37°C for 1 minute. Optical density then measured at 405 nm using a spectrophotometer (Photometer 4020, Japan).

Total antioxidant capacity: We cultivated ABTS [2,2'-Azino-dl-(3-ethylbinzthiazoline sulphonate)] with peroxidase and H₂O₂, and measured the appearance of a positive ion at 600 nm, which forms a stable bluish green molecule creates by ABTS.

Statistical analysis

Statistical comparisons were performed using SAS software (Version 8.2). Significance of differences among three groups was conducted with Duncan's multiple range test at a p<0.05 level minimum after ANOVA.

RESULTS AND DISCUSSION

Anthropometric measurements

Sixty subjects (Table 1) were divided into 3 age groups of 20 (age group, $50 \sim 59$), 21 (age group, $60 \sim 69$) and 19 (age group, $70 \sim 77$). The average age, height and weight of all subjects were 65.0, 151.1 cm and 59.5 kg, respectively; which was a little shorter, but relatively heavier for their age than the 154 cm and 54 kg suggested by Korean physical standards for citizens aged $65 \sim 74$ (18). Therefore, average BMI was 26.0, which was higher than the 22.8 suggested for the age group by Korean physical standards (18). The content of body fat was also higher at 38.4%. WHR was within a normal range $(75 \sim 90\%)$ at an average of 87.4%. The average

Table 1. Anthropometric measurements in postmenopausal women

Variables	Total (n=60)	50~59 yrs (n=20)	60~69 yrs (n=21)	70~77 yrs (n=19)
Height (cm)	$151.1 \pm 5.5^{1)}$	$152.2 \pm 5.7^{\mathrm{a2}}$	152.3 ± 5.1^{a}	148.7 ± 5.1 ^b
Weight (kg)	59.5 ± 8.6	60.3 ± 89.0	61.2 ± 7.7	56.8 ± 9.0
Weight (kg) BMI (kg/m ²) ³⁾	26.0 ± 3.2	26.1 ± 3.6	26.3 ± 2.7	25.6 ± 3.1
Waist (cm)	85.8 ± 8.9	82.9 ± 9.1	87.3 ± 6.6	87.1 ± 10.5
Hip (cm)	98.0 ± 6.6	96.5 ± 6.4	98.8 ± 5.9	98.8 ± 7.6
WHR ⁴⁾	87.4 ± 5.8	85.7 ± 5.8	88.3 ± 3.3	88.1 ± 7.7
Body fat (%)	38.4 ± 7.3	38.6 ± 8.0	38.4 ± 5.2	38.1 ± 8.8
SBP ⁵⁾ (mmHg)	145.5 ± 21.4	$133.3 \pm 17.6^{\mathrm{b}}$	$151.1\pm 20.9^{\rm a}$	$152.2\pm21.1^{\mathrm{a}}$
DBP ⁶⁾ (mmHg)	77.7 ± 10.0	76.7 ± 9.7	79.5 ± 9.0	76.7 ± 11.6

 $^{^{1)}}$ Mean \pm standard deviation.

SBP and DBP were 145.5 mmHg and 77.7 mmHg, respectively, lower than the 160 mmHg hypertension standard of WHO for SBP, higher than the Korean standard for normal SBP of under 140 mmHg.

There were no differences in height between the fifties, 152.2 cm and sixties, 152.3 cm. But the seventies age group was shorter at 148.7 cm (p<0.05). The SBP of the fifties group was significantly lower at 133.3 mmHg, compared with the sixties and seventies groups at 151.1 mmHg and 152.2 mmHg, respectively (p<0.05).

Lifestyle

The exercise survey reveled that (Table 2) 51.7 percent (31 people) said they exercised once a month. There was a significant effect of age on exercise frequency. The

reported time spent exercising per session was divided into three groups, 1) less than 30 minutes, 2) $30 \sim 60$ minutes, and 3) 60 minutes and over. Less than 30 minutes exercise was preferred by 28 subjects (46.7%). Lee et al. (19) pointed out that the intensity of physical activity was the main factor affecting the density of the bone in the lumbar spine after menopause. Lee (20) also concluded that the group who engaged in more physical activity exhibited better skeletal condition. The average sleep time of the participants was 7.8 hours, and there was no difference among ages. Twenty-seven participants (45.0%) reported sleeping less than 7 hours, 20 people (33.3%) $8 \sim 9$ hours, and 13 people (21.7%) 10 hours or more.

Table 2. Daily activity, sleeping hours and frequency of exercise in postmenopausal women

N (%)

	Variables	Total (n=60)	50~59 yrs (n=20)	60~69 yrs (n=21)	70~77 yrs (n=19)	x ^{2*}
	Mean of exercising hours (min/one time)	52 ± 51 ¹⁾	48±51	55 ± 33	52 ± 67	
Exercise	Frequency	16 (26.7)	7 (25.0)	5 (22.9)	4 (01.1)	
Excicise	once/day 3~4/week	16 (26.7) 2 (3.3)	7 (35.0) 1 (5.0)	5 (23.8) 1 (4.8)	4 (21.1) 0 (0.0)	$x^2 = 17.5722$
	$1 \sim 2$ /week	5 (8.3)	5 (25.0)	0 (0.0)	0 (0.0)	df=8, p<0.0247
	$2 \sim 3/\text{month}$	6 (10.0)	0 (0.0)	4 (19.1)	2 (10.5)	p <0.02+7
	once/month	31 (51.7)	7 (35.0)	11 (52.4)	13 (68.4)	
	Exercising hours groups					
	\leq 30 mins	28 (46.7)	11 (55.0)	8 (38.1)	9 (47.4)	$x^2 = 3.5334$
	$30\sim60$ mins	20 (33.3)	4 (40.0)	8 (38.1)	8 (42.1)	df=4,
	60 mins \geq	12 (20.0)	5 (15.0)	5 (23.8)	2 (10.5)	p<0.4728
	Mean of sleeping hours (hour/day)	7.8 ± 2.1	7.8 ± 2.2	8.0 ± 1.7	7.4 ± 2.4	
Sleeping	Sleeping hours groups					
	\leq 7 hours	27 (45.0)	9 (45.0)	8 (38.1)	10 (52.6)	$x^2 = 2.3819$
	$8 \sim 9$ hours	20 (33.3)	8 (40.0)	8 (38.1)	4 (21.1)	df=4,
	10 hours \geq	13 (21.7)	3 (15.0)	5 (23.8)	5 (26.3)	p<0.6659

¹⁾Mean ± standard deviation.

²⁾Means with different superscripts within a row are significantly different at @=0.05 as determined by Duncan's multiple range test.

³⁾Body mass index. ⁴⁾Waist hip ratio. ⁵⁾Systolic blood pressure. ⁶⁾Diastolic blood pressure.

^{*}Significance by x^2 -test.

Table 3. Bone mineral density in postmenopausal women

 (g/cm^2)

Skeletal sites	Total (n=60)	50~59 yrs (n=20)	$60 \sim 69 \text{ yrs}$ (n=21)	70~77 yrs (n=19)
Lumbar spine 2	$0.81 \pm 0.17^{1)}$	$0.89 \pm 0.17^{a3)}$	0.82 ± 0.09^a	0.71 ± 0.18^{b}
Lumbar spine 3	0.86 ± 0.18	$0.95\pm0.20^{\mathrm{a}}$	$0.87\pm0.10^{\rm a}$	$0.75 \pm 0.19^{\mathrm{b}}$
Lumbar spine 4	0.88 ± 0.18	$0.94 \pm 0.21^{\mathrm{a}}$	$0.90 \pm 0.09^{ m ab}$	$0.80 \pm 0.18^{\rm b}$
$LS^{2)}$ (L2~L4)	0.85 ± 0.17	0.93 ± 0.19^{a}	$0.86 \pm 0.08^{\mathrm{a}}$	$0.75 \pm 0.18^{ m b}$
Femoral neck	0.60 ± 0.10	$0.68 \pm 0.09^{\mathrm{a}}$	$0.60 \pm 0.08^{\mathrm{b}}$	$0.52\pm0.07^{\rm c}$
Trochanter	0.49 ± 0.10	$0.56 \pm 0.11^{\mathrm{a}}$	$0.49 \pm 0.07^{ m b}$	$0.42 \pm 0.08^{\circ}$
Intratrochanter	0.87 ± 0.15	$0.96 \pm 0.16^{\mathrm{a}}$	$0.89 \pm 0.12^{\mathrm{a}}$	$0.77 \pm 0.13^{\mathrm{b}}$
Ward's triangle	0.40 ± 0.14	0.50 ± 0.12^{a}	$0.40 \pm 0.13^{\mathrm{b}}$	$0.30 \pm 0.06^{\rm c}$

¹⁰ Mean \pm standard deviation. 20 Lumbar spine.

Fig. 1. Mean of bone mineral density by age in postmenopausal women.

Bone mineral density

Bone mineral density (BMD) was measured in the following: the 3 areas of the lumbar spine ($L2 \sim L4$), the femoral neck, intratrochanter, trochanter and Ward's triangle.

The mean BMD of the lumbar spine was 0.85 g/cm². Ward's triangle showed the lowest density of 0.40 g/cm² (Table 3, Fig. 1). Age was significantly related to lower BMD. Lee and Lee (21) demonstrated that bone mineral density tended to worsen in the order of lumber spine >femoral neck>trochanter>Ward's triangle for women 60 years and over. Mazess et al. (22) also mentioned that women 70 years and over showed the lowest bone mineral density in the Ward's triangle. All these studies support that aging is the main risk factor for decreased bone mineral density (23).

Participants were classified into 1) normal group (T value \geq -1), 2) osteopenia group (-2.5 \leq T value \leq -1), and 3) osteoporosis group (T value \leq -2.5) according to the standard of the osteoporosis prescribed by WHO (Table

4). For Ward's triangle, 81.7 percent of the participants turned out to have osteoporosis. For the lumbar spine, 46.7 percent of the subjects had the osteoporosis. Lee and Lee (21) studied women 60 years and over for bone mineral density. According to the report, nearly 46 percent of the participants showed the osteoporosis in the lumbar spine and thigh bone, and only nine percent of the participants maintained normal density of bones. These findings all clearly indicate the prevalence of the osteoporosis among the aged.

Effects of lifestyle and activity of the antioxidant enzymes

The antioxidant enzyme activities of the participants according to frequency of exercise, sleep hours, and coffee intake are shown in the Table 5. The subjects were divided into three groups: 1) less than 7 hours, 2) $8 \sim 9$ hours, and 3) 10 hours and over. For sleep hours, there was no significant difference in the activity of antioxidant enzyme. Exercise time had no significant effect among the groups of less than 30 minutes, $30 \sim 60$ minutes, and 60 minutes and over. However, respondents who exercised $3 \sim 4$ times a week showed the highest TA level of 1.35 mmol/L (p<0.05).

According to the Ji's study (24), vigorous muscle exercise helps to generate the ROS in the mitochondrial electronic conveyance gauge, xantine oxidase, and polymorphonuclear cells, as exhibited by increased oxygen uptake. These changes then lead to a disturbance of the prooxidant-antioxidant homeostasis, which contributes to the peroxidation of lipids in adipocytes. In doing so, vigorous exercise can impede the antioxidant defense system in the body. On the other hand, regular modest exercise enhances the immune and antioxidant defense systems in the body (25).

Antioxidant enzyme activity and exercise hours were not significantly related in this study. However, respondents who exercised $3\sim4$ times a week showed the

³⁾Means with different superscripts within a row are significantly different at =0.05 as determined by Duncan's multiple range test.

¹⁾Means with different superscripts are significantly different at 0.05 determined by Duncan's multiple range test.

Table 4. Status of bone health in postmenopausal women

Status of bone health		Total (n=60)	50~59 yrs (n=20)	60~61 yrs (n=21)	70~77 yrs (n=19)	x ^{2*}
Lumbar spine	Osteoporosis ¹⁾ Osteopenia Normal	28 (46.7) 20 (33.3) 12 (20.0)	7 (35.0) 6 (30.0) 7 (35.0)	7 (33.3) 12 (57.1) 2 (9.5)	14 (73.7) 2 (10.5) 3 (15.8)	x^2 =14.5291 df=4, p<0.0058
Femoral neck	Osteoporosis Osteopenia Normal	41 (68.3) 17 (28.3) 2 (3.3)	8 (40.0) 10 (50.0) 2 (10.0)	16 (76.2) 5 (23.8) 0 (0.0)	17 (89.5) 2 (10.5) 0 (0.0)	x^2 =13.3737 df=4, p<0.0096
Intratrochanter	Osteoporosis Osteopenia Normal	20 (33.3) 30 (50.0) 10 (16.7)	4 (20.0) 8 (40.0) 8 (40.0)	5 (23.8) 14 (66.7) 2 (9.5)	11 (57.9) 8 (42.1) 0 (0.0)	x^2 =17.2231 df=4, p<0.0017
Trochanter	Osteoporosis Osteopenia Normal	33 (55.0) 23 (38.3) 4 (6.7)	6 (30.0) 10 (50.0) 4 (20.0)	12 (57.1) 9 (42.9) 0 (0.0)	15 (79.0) 4 (21.0) 0 (0.0)	x^2 =14.5737 df=4, p<0.0057
Ward's triangle	Osteoporosis Osteopenia Normal	49 (81.7) 10 (16.7) 1 (1.7)	13 (65.0) 6 (30.0) 1 (5.0)	17 (81.0) 4 (19.0) 0 (0.0)	19 (100) 0 (0.0) 0 (0.0)	x^2 =8.8350 df=4, p<0.0654

¹⁾Osetoporosis: T-score ≤ -2.5, Ostoepenia: -2.5<T-score ≤ -1, Normal: -1<T-score.

Table 5. Antioxidant enzyme activities by life style factors

Variables		n	SOD (U/mL)	GPx (U/mL)	CAT (kU/L)	TA (mmol/L)
Claaning	≤ 7	27	$133.6 \pm 16.8^{1)}$	1234.3 ± 308.2	308.9 ± 198.0	1.17 ± 0.16
Sleeping	8~9	20	145.9 ± 17.7	1338.4 ± 247.1	289.1 ± 176.7	1.15 ± 0.14
hours (hr)	10≤	13	139.3 ± 12.3	1274.4 ± 252.1	365.0 ± 229.5	1.17 ± 0.11
Period of	<30	28	138.9 ± 11.0	1210.7 ± 289.8	351.6 ± 196.3	1.14 ± 0.12
exercise	$30 \sim 60$	20	136.1 ± 24.0	1323.2 ± 248.3	257.1 ± 195.1	1.21 ± 0.17
hours (min)	60<	12	141.3 ± 15.7	1351.3 ± 276.9	322.4 ± 195.3	1.15 ± 0.13
	once/day	16	138.5 ± 13.6	1246.1 ± 341.3	367.5 ± 157.2	$1.15 \pm 0.12^{b2)}$
Frequency	$3 \sim 4/\text{week}$	2	148.0 ± 26.6	1094.7 ± 179.7	305.3 ± 352.9	$1.35 \pm 0.46^{\mathrm{a}}$
of exercise	$1 \sim 2/\text{week}$	5	140.0 ± 9.0	1320.2 ± 210.8	245.7 ± 130.5	$1.09 \pm 0.09^{\mathrm{b}}$
(times)	$2 \sim 3/\text{month}$	6	131.5 ± 10.5	1294.2 ± 242.3	400.1 ± 318.5	$1.19 \pm 0.06^{\mathrm{ab}}$
•	once/month	31	139.3 ± 19.5	1292.2 ± 271.8	285.1 ± 185.2	1.16 ± 0.14^{ab}

 $^{^{1)}}$ Mean \pm standard deviation.

highest TA in terms of the antioxidant enzymatic activity. This again indicates that modest exercise is the key to enhancing the antioxidant enzymatic activity. It also confirms a study on middle aged men by Kang and Park (26). People who engaged in regular modest exercise showed notably higher GPx and total radical-trapping antioxidant potential (TRAP) than the non-exercisers. Uusi-rasi et al. (27) studied 422 female in their 20's, 40's and 60's. The group who exercised in the form of jogging or aerobics for 20 minutes twice a week showed higher bone mineral density (5%) in their femoral necks compared to the non-exerciser. This result also confirms that a moderate degree of proper exercise affects both bone density and antioxidant enzyme activity.

Bone mineral density and the antioxidant enzyme activity

The participants were divided into three groups by T scores of each bone (Table 6, Fig. 2): less than -2.5, -2.5 \sim -1 and over -1. The over -1 group for the intratrochanter showed notably higher level of SOD of 152.7 U/mL compared to other two groups who were -2.5 \sim -1, and less than -2.5 showed 138.9 and 132.5 U/mL respectively (p<0.05). The higher the SOD activity was also associated with higher T scores in the lumbar spine, femoral neck, trochanter, and Ward's triangle.

These findings support the result of a cell study by Datta et al. (6) and Garrett et al. (9). First, the reactive oxygen species might destroy the collagen of the bone

^{*}Significance by x^2 -test.

²⁾Means with different superscripts within a column are significantly different from each other at \blacksquare =0.05 as determined by Duncan's multiple range test.

Status of bone health SOD (U/mL) GPx (U/mL) CAT (kU/L) TA (mmol/L) $Osteoporosis^{1)}\\$ $139.2 \pm 18.1^{2)}$ 28 1292.0 ± 301.1 334.5 ± 198.2 1.16 ± 0.13 Lumbar 1238.9 ± 261.9 Osteopenia 20 136.7 ± 12.1 320.4 ± 188.5 1.18 ± 0.13 spine $1.16 \!\pm\! 0.19$ 140.7 ± 22.3 256.4 ± 212.4 12 1300.2 ± 262.0 Normal 41 135.7 ± 17.0 Osteoporosis 1265.5 ± 283.0 302.7 ± 199.7 1.16 ± 0.16 Femoral 1.17 ± 0.11 17 143.6 ± 13.8 1283.0 ± 278.1 336.5 ± 198.5 Osteopenia neck Normal 2 $132.5 \pm 15.7^{\text{b4}}$ 1264.1 ± 302.3 329.4 ± 182.6 1.11 ± 0.13 20 Intrat-Osteoporosis 138.9 ± 16.5^{b} 30 1285.4 ± 273.8 310.0 ± 226.3 1.19 ± 0.15 rochanter Osteopenia 1247.7 ± 263.6 152.7 ± 12.1^a Normal 10 296.0 ± 105.7 1.19 ± 0.12 1279.8 ± 272.6 33 135.2 ± 18.3 Osteoporosis 289.9 ± 175.7 1.13 ± 0.13 Trochanter 23 Osteopenia 141.5 ± 12.8 1271.4 ± 295.1 340.5 ± 233.6 1.21 ± 0.15 1213.6 ± 307.3 365.5 ± 41.9 1.14 ± 0.10 Normal 4 156.7 ± 10.1 49 136.8 ± 16.7 1279.5 ± 273.4 1.16 ± 0.14 Osteoporosis 306.3 ± 202.4 Ward's Osteopenia 10 145.1 ± 13.5 1218.3 ± 326.1 345.0 ± 182.1 1.18 ± 0.15 triangle Normal 1

Table 6. Antioxidant enzyme activities by status of bone health by T-score

Fig. 2. Comparison of superoxide dismutase activities by T-score groups.

and affect the process of the bone's absorption directly by facilitating the secretion of the protease. Second, the osteoclast generates superoxide in response to parathyroid hormone, interleukin-1, tumor necrosis factor (TNF) and 1,25-(OH)₂ vitamin D₃, and the generation and activity of the precursor osteoclast increases these production of superoxides.

This study also relates to other results of Burton and Ingold (28) who found that due to the lack of estrogen after the menopause, phenolic hydroxyl group of the estrogen itself is transferred to the lipid peroxyradical and act as a direct antioxidant by preventing the chain re-

action of the free radical. Another related study was conducted by Chamber and Hall (29). They found that the generation of ROS increases the activity of nuclear factor kappa B (NF-kB) which guides the expression of antioxidant enzymes such as glutathione synthetase, SOD and CAT in the osteoclast as a defense mechanism against ROS. The estrogen receptor then combined with NF-B blocks the activity of the NF-kB as an indirect antioxidant.

Ko (30) studied the relationship between antioxidant enzyme activity and skeletal muscle such, evaluating GR, GPx and GST using estrogen and glutathione in the skeletal muscle of ovariectomized white mice. The activities of GR and GPx were lower in the soleus muscle, among the skeletal muscles of the tested. The mice that received estrogen treatment after ovary removal also had higher activities of the GR and GPx. These findings all suggest a further need to explore the relationship between the bone mineral density and the activity of SOD through the clinical tests.

CONCLUSION

In short, the participants who exercised $3\sim4$ times a week showed the highest TA, This indicates that regular moderate exercise enhances the activities of the antioxidant enzymes. Higher bone mineral density is associated with higher SOD activities. Among the antioxidant indices, SOD and TA were the most affected by bone mineral density.

¹⁾Osteoporosis: T-score ≤ -2.5, Osteopenia: -2.5 < T-score ≤ -1, Normal: -1 < T-score

²⁾Mean ± standard deviation.

³⁾It is exempted in analysis, due to lack of numbers.

⁴⁾Means with different superscripts within a column are significantly different at $\square = 0.05$ as determined by Duncan's multiple range test.

¹⁾Means with different superscripts are significantly different at □=0.05 determined by Duncan's multiple range test.
²⁾No subject.

REFERENCES

- Korea National Statistical Office. 2002. Annual Report on the Cause of Death Statistics 2001. The Ministry of Health & Welfare, Korea.
- Halliwell B, Murcia MA, Chirico S, Aruoma OI. 1995.
 Free radicals and antioxidants in food and in vivo: what they do and how they work. Crit Rev Food Sci Nutr 35: 7-20.
- Thomas MJ. 1995. The role of free radicals and antioxidants: how do we know that they are working? Crit Rev Food Sci Nutr 35: 21-39.
- Schulz H. 1994. Regulation of fatty acid oxidation in heart. J Nutr 124: 165-171.
- Cho SH. 1993. Lipid peroxidation and antioxidant nutrion. Kor J Lipidology 3: 23-29.
- Datta HK, Rathod H, Manning P, Turnbull Y, McNeil CJ. 1996. Parathyroid hormone induces superoxide anion burst in the osteoclast: evidence for the direct instantaneous activation of the osteoclast by the hormone. *Endocrinology* 149: 269-275.
- Beard CJ, Key L, Newburger PE, Ezekowitz RA, Arceci RA, Miller B, Proto P, Ryan T, Anast C, Simons ER. 1986. Neutrophil defect associated with malignant infantile osteopetrosis. J Lab Clin Med 108: 498-505.
- 8. Bax BE, Alam AS, Banerji B, Bax CM, Bevis PJ, Stevens CR, Moonga BS, Blake DR, Zaida M. 1992. Stimulation of osteoclastic bone resorption by hydrogen peroxide. *Biochem Biophys Res Commun* 183: 1153-1158.
- 9. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR. 1990. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. *J Clin Invest* 85: 632-639.
- 10. Tranquilli AL, Lucino E, Garzetti GG, Romanini C. 1994. Calcium, phosphorus and magnesium intakes correlate with bone mineral with content in postmenopausal women. *Gynecol Endocrinol* 8: 55-58.
- 11. Rapuri PB, Gallagher JC, Haynatzka V. 2003. Protein intake: effects on bone mineral density and the rate of bone loss in elderly women. *Am J Clin Nutr* 77: 1517-1525.
- 12. Veille JC, Li P, Eisenach JC, Massmann AG, Figueroa JP. 1996. Effects of estrogen on nitric oxide biosynthesis and vasorelaxant activity in sheep uterine and renal arteries in vitro. *Am J Obstet Gynecol* 174: 1043-1049.
- Damoulis PD, Hauschka PV. 1994. Cytokines induce nitric oxide production in mouse osteoblasts. *Biochem Biophys Res Commun* 201: 924-931.
- Ralston SH, Todd D, Helfrich M, Benjamin N, Grabowski PS. 1994. Human osteoblast-like cells produce nitric oxide

- and express inducible nitric oxide synthase. *Endocrinology* 135: 330-336.
- Flohe L, Becker R, Brigelius R, Lengfelder E, Otting F. 1988. Convenient assasy for superoxide dismutase. In CRC Handbook of Free Radicals and Antioxidants in Biomedicine. CRC press, Boca Raton, FL. p 287-293.
- 16. Paglia DE, Valentine WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70: 158-169.
- Aebi H. 1974. Catalse. In *Methods of Enzymatic Analysis*. Bergmeyer HU, ed. Academic Press, New York. p 673-684.
- 18. The Korean Nutrition Society. 2005. The 8th Dietary Reference Intakes for Koreans. Seoul.
- Lee BK, Chang YK, Choi KS. 1992. Effect of nutrient intake on bone mineral density in postmenopausal women. *Korean J Nutr* 25: 642-655.
- Lee HJ. 1996b. The relationship of exercise to bone mineral density of Korean women in Taegu. Korean J Nutr 29: 806-820
- Lee HJ, Lee HO. 1999. A study on the bone mineral density and related factors in Korean postmenopausal women. *Korean J Nutr* 32: 197-203.
- 22. Mazess RB, Barden H, Ettinger M, Shultz E. 1988. Bone density of the radius, spine, and proximal femur in osteoporosis. *J Bone Miner Res* 3: 13-18.
- 23. Lee HJ, Choi MJ. 1996a. The effect of nutrient intake and energy expenditure on bone mineral density of Korean women in Taegu. *Korean J Nutr* 29: 622-633.
- 24. Ji LL. 1999. Antioxidants and oxidantive stress in exercise. *Proc Soc Exp Biol Med* 222: 283-292.
- 25. Lee IM. 1995. Exercise and physical health: cancer and immune function. *Res Q Exerc Sport* 66: 286-291.
- 26. Kang MH, Park EJ. 2000. Effects of regular physical exercise habits on the activities of erythrocyte antioxidant enzyme and plasma total radical-trapping antioxidant potential in health male subjects. Korean J Nutr 32: 289-295.
- 27. Uusi-Rasi K, Sievanen H, Vuori I, Pasanen M, Heinonen A, Oja P. 1998. Associations of physical activity and calcium intake with bone mass and size in healthy women at different ages. *J Bone Miner Res* 13: 133-142.
- 28. Burton G, Ingold K. 1989. Vitamin E as an in vitro and in vivo antioxidant. *Annal NY Acad Sci* 570: 7-22.
- Chambers TJ, Hall TJ. 1991. Cellular and molecular mechanisms in the regulation and function of osteoclasts. Vitamine and Hormone 46: 41-86.
- Ko YD. 1998. Differential effect estrogen on antioxidant enzymes in bone and skeletal muscle in oophorectomized rats. MS Thesis. Seoul Natiional University, Seoul.

(Received October 23, 2006; Accepted November 29, 2006)