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A Comparative Study of the Iron Nutritional Status of Female College Women according to Bone Mineral Density*

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The purpose of this study was to investigate the relationship between nutritional status of iron and bone mineral density in premenopausal women. In the study, we classified the subjects into osteopenia (-2.5<T-score<-1, n=26) and normal (T-score>-1, n=29) groups according to their lumbar spine bone mineral density. Anthropometric measurements, dietary intake analysis and blood biochemistry measurements were performed on the subjects. The average ages of those in the osteopenia and normal groups were 22.2 yrs and 23.0 yrs, respectively, with no significant difference. The average body mass index ($p<0.05$) of those in the osteopenia group (19.6) was significantly lower than that of the normal group (21.3). The mean protein intake of those in the osteopenia group was significantly lower than that ($p<0.05$) the subjects in the normal group. The osteopenia group consumed a significantly lower amount of iron ($p<0.05$) and non-heme iron ($p<0.05$) compared to the normal group. The intakes of total food, vegetables and milk of those in the osteopenia group were significantly lower than those of the subjects in the normal group. The serum ferritin ($p<0.001$) level of those in the osteopenia group was significantly lower than those of the subjects in the normal group. In conclusion, a balance of iron status may be helpful in the prevention of bone mass loss in premenopausal young women.

Key words: Iron status, Bone mineral density, Serum ferritin

INTRODUCION

In recent years, with the growing cultural emphasis on leaner bodies, the number of underweight women, especially young women, has increased.[1,2] In their efforts to lose weight and develop a leaner figure, many young woman fail to pay adequate attention to the quality and quantity of their diets. As a result, many young woman experience anemia, growth retardation,[3] hormone imbalance and mineral metabolism alterations that lead to a loss of bone mineral density and increase the risk of osteoporosis.[4,5]

Many researchers have studied the relation between bone mass density and macro minerals such as calcium, phosphorous etc[6-9] based on the fact that bone mineral density is closely linked to mineral nutrition status. Several studies[10-13] have reported that trace minerals such as iron, copper, zinc are involved in bone metabolism. Interest in trace minerals is increasing. Iron, in particular, is believed to be an essential mineral for the collagen structure, upon which bone mineralization occurs.[14] For instance, Angus *et al.'s*[15] study of the bone mineral density of premenopausal women proved the existence of a positive relation between iron intake and femur neck BMD. Although iron nutrition status can be an important factor in bone mineral density, few studies have been conducted in this area.

'There are various ways to assess the nutritional status of iron, such as hemoglobin, hematocrit, serum iron level and various RBC indices etc. Measuring the level of hemoglobin and hematocrit is the most common method of assessing iron status. However, the method is not relevant to the situation of marginal iron deficiency[16] whereas the serum ferritin level can sensitively reflect the quantity of iron storage in the body.[17,18] Therefore, serum ferritin level may be a good index of marginal iron deficiency in apparently healthy subjects.[19]

Studies[14,20] have shown that there is a relationship between iron nutritional status and bone mineral density. The previous research examined the relationship using dietary iron intake and a few studies measured the serum ferritin level to evaluate the relationship between iron status and bone mineral density. However, little research has been done on the relationship between iron nutrition and spine bone mineral density in young woman, although Ilich-Ernst *et al.'s*[21] research showed a positive relation. Therefore, studies on the relationship between iron nutritional status and the bone mineral density of Korean young woman are needed to provide basic data

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on maintaining healthy bones among women of child-bearing age.

SUBJECTS AND METHODS

1. Subjects and Length of Study

The subjects of this study were 94 college women between the ages of 19 and 30 residing in Seoul, Korea. For the selection of the study subjects, preliminary measurements of lumbar spine bone mineral density were performed. We classified the subjects into the osteopenia (-2.5<T-score≤-1) and normal (T-score>-1) groups according to WHO categories in lumbar BMD for women. Anthropometric measurements, dietary intake analysis and blood biochemistry measurements were performed on the subjects.

2. Study Content and Methods

Anthropometric measurements

The subjects were lightly clothed and measured for body weight, body mass index (BMI=weight (kg)/height (m^2)), fat mass and percent body fat (Bio-electrical impedence analyzer, TBF-300 TANITA, Japan) while in a standing position.

Survey of food and nutrient intake

Food intake for three previous days was surveyed under the guidance of investigators using a 24-hr recall method. The results were analyzed for overall nutrient intake using Can-Pro 2.0, the Computer-Aided Nutritional Analysis Program for Professionals (The Korean Nutrition Society, 2002). Intake of meat, fish, and poultry (MFP), which influences the bioavailability of iron, was calculated. Heme iron intake from MFP was calculated as 40% of total iron intake. Non-heme iron from other foods accounted for 60% of total iron intake. Iron supplement use was also surveyed.

Measurement of bone mineral density (BMD)

The BMDs of the lumbar spine (L2-L4) area were analyzed using Dual Energy X-ray Absorptiometry (DEXA, Hologic, USA). The average BMD value of the lumbar spine was L2-L4. The lumbar spine t-scores of those in the osteopenia and normal groups were -1.50 and -0.10, respectively ($p<0.001$).

Blood sampling and analysis

Venous blood (20 mL) was drawn from the subjects after a 12-hour fast. Samples were left at room temperature for 30 min then centrifuged for 15 min. at 2,500 rpm to separate serum and stored at -70 ℃. Total serum protein and albumin levels were determined using an autochemical analyzer (HITACHI 747, Japan). Serum ferritin concentrations were measured using double-antibody ^{125}I radio-immunoassay kits (Incstar Co, USA).

3. Statistical Aalysis

SAS program (Version 8.1) was used for statistical analysis. Significant differences between the two groups were determined using a student t-test.

RESULT AND DISCUSSION

1. General Characteristics of the Subjects

General characteristics of the subjects are shown in Table 1. The average ages of those in the osteopenia (n=26) and normal groups (n=29) were 22.2 and 23.0 years, respectively, with no significant difference between the two groups. Average weight and height of those in the osteopenia and normal groups were 161.7 cm and 51.5 kg and 161.9 cm and 53.9 kg, respectively, with no significant difference. Body mass index (BMI) of those in the osteopenia (19.6 kg/m^2) group was significantly lower than that of the subjects in the normal (21.3 kg/m^2) group ($p<0.05$). The body fat percentages of those in the osteopenia and normal groups were 25.68% and 23.38%, respectively, with no significant difference. According to Constantion,[22] the shape of a bone depends on the size and direction of pressure on the bone. In the same research, bone blood flow was found to increase and form piezoelectricity that stimulates the formation and absorption of bone and accelerates the calcification of bone substrate when pressure is exerted on the bone. Moreover, Song and Paik[23] reported that the lumbar spine BMD of female college students correlated significantly with body mass index. Lopez-Caudana *et al.'s*[24] recent research, which supports our research results, showed that the forearm BMD of premenopausal women correlated significantly with body mass index when 1,622 women between the ages of 20 and 80 were studied.

Table 1. Anthropometric measurements and spine BMD of subjects with different bone mineral density

	Osteopenia (n=26)	Normal (n=29)
Age (yrs)	22.23 ± 2.86[1)]	23.06 ±1.81
Height (cm)	161.75 ± 4.00	161.91 ±5.14
Weight (kg)	51.54 ± 4.17	53.94 ±6.45
BMI[3)] (kg/m^2)	19.67 ± 1.02	21.34 ±3.95*[,2)]
Body fat (%)	25.68 ± 5.22	23.38 ±4.85
BMD-S[4)](t-score)	-1.50 ± 0.72	-0.10 ±0.76***

1) Mean±Standard Deviation
2) Significance as determined by Student's t-test (*: $p<0.05$, ***: $p<0.001$)
3) Body Mass Index
4) Bone Mineral Density-Spine

2. Food and Nutrient Intake

The daily nutrient intake of the subjects is shown in Table 2. The mean daily intakes of energy of the subjects in the osteopenia and normal groups were 1692.5 kcal and 1748.8 kcal, respectively, with no significant difference.

Table 2. Mean daily energy and nutrient intakes of subjects with different bone mineral density

	Osteopenia (n=26)	Normal (n=29)
Energy (kcal)	1692.58 ± 428.45[1)]	1748.88 ± 322.21
Protein (g)	58.52 ± 17.76	67.67 ± 13.28*[,2)]
Fat (g)	51.25 ± 21.54	50.20 ± 17.68
Carbohydrate (g)	251.07 ± 66.33	258.42 ± 50.20
Vitamin A (R.E.)	599.62 ± 311.86	674.56 ± 332.65
Vitamin B_1 (mg)	1.11 ± 0.39	1.02 ± 0.39
Vitamin B_2 (mg)	1.17 ± 0.71	1.17 ± 0.33
Niacin (mg)	12.81 ± 5.69	14.00 ± 4.79
Vitamin B_6 (mg)	1.59 ± 0.63	1.83 ± 0.54
Vitamin C (mg)	89.83 ± 83.90	124.89 ± 72.71
Iron (mg)	10.56 ± 3.83	12.58 ± 2.97*
Animal iron (%)	26.37 ± 16.01	27.41 ± 11.76
Plant iron (%)	73.62 ± 16.01	72.58 ± 11.76

1) Mean±Standard Deviation
2) Significance as determined by Student's t-test (*: $p<0.05$)

The mean protein intake of those in the osteopenia group (58.5 g) was significantly lower than that of the subjects in the normal group (67.6 g)($p<0.05$). Tkatch *et al.*[25)] reported that protein is an important factor in maintaining healthy bones and protein deficit can be a major cause of osteoporosis. In addition, Song and Paik's research[26)] showed a positive correlation between protein intake and bone mineral density of spine BMD in female university students. The results of the present study were similar to those of the previous studies, which reported that the protein intake of those in the osteopenia group was relatively lower than that of the normal group. Fat intake in the osteopenia and normal groups was 51.2 g and 50.2 g, respectively, and carbohydrate intake was 251.0 g and 258.4 g, respectively. There were no significant differences in the intake of these nutrients.

The iron intake (10.5 mg, 65.6% of RDA) of those in the osteopenia group was significantly lower than that of the normal group (12.5 mg, 78.1% of RDA)($p<0.01$). None of the subjects took iron supplements, indicating that total iron intake came from food alone. According to Smoliar's study,[27)] deficit iron intake can cause a metabolic disorder of calcium and phosphorus and delay the maturity of collagen in the femur. Besides, iron intake through meals has a positive influence on bone mineral density. Moreover, iron is involved in transferring 25-hydroxyvitamin D to 1, 25-dihydroxyvitamin D, an active form of vitamin D which plays an important role in the absorption and control of calcium and phosphorus.[28)] Angus *et al.'s*[15)] study on the relationship between iron intake and the bone mineral density of premenopausal women showed that iron plays a positive role in femur BMD.

Plant iron accounted for 73.6% and 72.5% of the total iron intake of those in the osteopenia and normal groups, respectively, and the absorption rate of plant iron was low (5-10%) due to the form of iron and other dietary factors that reduce the utilization rate.[22,29)] However, when comparing the subjects' heme and non-heme iron intake (Table 3), the non-heme intake of those in the osteopenia group was significantly lower than that of the subjects in the normal group ($p<0.05$). This result shows that the non-heme iron in most plant iron plays a positive role in promoting bone mineral density. Consequently, Koreans who consume more vegetables than animal foods will be influenced by iron intake from vegetables rather than animals. However, further study in this area is needed.

Table 3. Iron intakes of subjects with different bone mineral density

	Osteopenia (n=26)	Normal (n=29)
Iron (mg)	10.56 ± 3.83[1)]	12.58 ± 2.97*[,2)]
Heme iron (mg)[3)]	0.59 ± 0.48	0.85 ± 0.59
Nonheme iron (mg)	9.86 ± 3.47	11.67 ± 2.73*

1) Mean±Standard Deviation
2) Significance as determined by Student's t-test (*: $p<0.05$)
3) Results calculated by Monsen's method and Cook's method (Monsen *et al.*, 1978, Cook & Monsen 1976)

3. Food Intake by Food Group

Table 4 shows the subjects' food intake by food group. The mean total food intake per day for those in the osteopenia (1076.3 g) group was lower than that of the subjects in the normal group (1319.3 g)($p<0.01$). Comparing the average food intake of females between the ages of 20 and 29 (the fig. for which is 1248.8 g/day according to the 2001 National Health and Nutrition Survey),[30)] those in the osteopenia group consumed a lower-than-average amount while those in the normal group consumed a higher-than-average amount. Milk intake of those in the osteopenia (173.4 g) group was significantly lower than that of the subjects in the normal group (190.6 g)($p<0.05$). Cadogan *et al.'s*[31)] research showed that providing 500 mL (1 pint) milk/day for 18 months to 80 young girls whose average age was 12 significantly increased bone mineral density. The authors concluded that the intake of milk increased bone mineral density and influenced the subjects' ability to maintain maximum bone mineral content. Moreover, intake of milk, which provides calcium and protein, can restrain the function of osteoclast. As a result, intake of milk and dairy products can positively influence bone mineral density, as indicated by Yamamura *et al.*.[32)] The present study

Table 4. Food intakes from each food group in subjects with different bone mineral density

	Osteopenia (n=26)	Normal (n=29)
Cereals (g)	272.88 ± 92.85[1)]	276.31 ± 67.61
Potato and Starches (g)	29.23 ± 61.69	35.65 ± 75.87
Sugars and Sweetners (g)	7.58 ± 8.08	7.88 ± 19.15
Pulses (g)	31.68 ± 35.15	44.86 ± 57.18
Nuts and Seeds (g)	4.90 ± 19.99	4.18 ± 15.68
Vegetables (g)	178.73 ± 84.82	246.25 ± 82.76*[,2)]
Fungi and Mushrooms (g)	2.61 ± 7.08	3.48 ± 9.87
Fruits (g)	93.56 ± 105.61	126.75 ± 112.27
Meats (g)	49.30 ± 59.22	55.42 ± 54.65
Eggs (g)	27.83 ± 26.07	38.22 ± 28.64
Fish and Shellfishes (g)	57.90 ± 62.82	59.68 ± 55.71
Seaweeds (g)	4.25 ± 6.42	11.48 ± 23.47
Milks (g)	173.43 ± 102.64	190.62 ± 111.54*
Oils and Fat (g)	9.98 ± 6.80	11.55 ± 7.83
Beverages (g)	47.62 ± 83.52	101.53 ± 92.31
Seasoning (g)	29.77 ± 18.71	28.69 ± 20.70
Total (g)	1076.30 ± 293.79	1319.36 ± 265.92**

1) Mean±Standard Deviation
2) Significance as determined by Student's t-test (*: p<0.05, **: p<0.01)

also suggested that increasing the intake of milk is needed to enhance the bone mineral density of young adult females, since the milk intake of those in the osteopenia group was lower than that of the subjects in the normal group.

The vegetable and fruit intake of those in the osteopenia group was (178.7 g and 93.5 g) lower than that of the subjects in the normal group (246.2 g and 126.7 g). Vegetables and fruit contain an abundance of vitamin C, which acts as coenzyme in the hydroxylation of lysine and proline to synthesize collagen. Tylavsky *et al.*[33)] reported that urinary calcium excretion decreased as vegetable and fruit intake increased, and that vegetable and fruit intake had a positive influence on bone mineral density. However, Tucker *et al.'s*[34)] four-year cohort study showed that bone mineral density was negatively affected by vegetable and fruit intake, results that are similar to those of the present study. Since vegetables contain massive quantities of dietary fiber to produce alkalic metabolite within the body, bone mineral density may be negatively affected by vegetable intake.

4. Serum Iron and Ferritin Concentrations

The results of blood analysis are shown in Table 5. There was no significant difference in total serum protein and albumin concentrations between those in the osteopenia (7.60 g/dl, 4.69 g/dl) and normal groups (7.39 g/dl, 4.62 g/dl). Serum iron concentration of those in the osteopenia (113.8 μg/dl) group was lower than that of the subjects in the normal group (114.2 μg/dl), although

Table 5. Serum parameters relating iron status in subjects with different bone mineral density

	Osteopenia (n=26)	Normal (n=29)
Total Protein (g/dl)	7.60 ± 0.36[1)]	7.39 ± 0.41
Albumin (g/dl)	4.69 ± 0.15	4.62 ± 0.23
Iron (μg/dl)	113.87 ±18.27[1)]	114.24 ± 16.32
Ferritin (ng/mL)	12.39 ± 9.01	39.32 ± 19.74***[,2)]

1) Mean±Standard Deviation
2) Significance as determined by Student's t-test (***: p<0.001)

there was no significant difference. Both groups had serum iron levels within the standard clinical range (55-180 μg/dl). Serum ferritin level of those in the osteopenia (12.39 ng/mL) group was significantly lower than that of the subjects in the normal group (39.32 ng/mL). Although the serum ferritin level of those in both groups fell within the normal range (9-446 ng/mL), the serum ferritin level of those in the osteopenia group was lower than that of the subjects in the normal group. Ferritin, a major protein for iron storage, maintains a dynamic equivalent with blood serum iron and significantly reflects the status of storage iron.[17,35)] Iron is essential for collagen synthesis leading to bone mineralization,[36)] has a positive influence on bone mineral density and prevents fractures.[37)] Furthermore, studies[14,38)] have shown that the nutritional status of iron relates positively to bone mineral density. However, little research on the relationship between nutritional status of iron and bone mineral density using serum ferritin has been conducted. Ilich-Ernst *et al.*[21)] advised that iron can play an important role in bone formation. According to the results of their research, there was a positive correlation between serum ferritin and forearm BMD in their test subjects: 354 girls between the ages of 8 and 13. Kim[39)] showed that there was a positive relationship between serum ferritin and the spine bone mineral density of postmenopausal vegetarian females.

In summary, female college students in the osteopenia group were shown to have lower BMI than the subjects in the normal group and consumed lower quantities of protein, iron, milk and dairy products, and vegetables than the subjects in the normal group. Moreover, serum ferritin level was significantly lower in the subjects in the osteopenia group than it was in the subjects in the normal group. These results suggest that the nutrition status of iron in young adult woman is important to maintain healthy bone mineral density. Further studies to broaden and deepen the knowledge of this subject are needed.

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Erratum

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