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ORIGINAL ARTICLE

Vitamin D Status and Bone Health in Postmenopausal Women Working in Greenhouses

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Objectives: Greenhouse workers synthesize less vitamin D because ultraviolet light is blocked by the vinyl that covers the greenhouses. This study aimed to investigate the vitamin D status and bone health of postmenopausal women working in greenhouses.

Methods: This observational study enrolled women living in urban (n = 70, group 1) and rural areas (n = 91, group 2) and those working in greenhouses (n = 112, group 3). Serum levels of total and bioavailable 25-hydroxy-vitamin D [25(OH)D] and vitamin D binding protein were measured. T-scores of the lumbar spine and femur neck were measured by dual-energy X-ray absorptionetry.

Results: Heights were shorter in group 2 and group 3 than in group 1. Bioavailable 25(OH)D levels were higher and T-scores of the spine and femur were lower in both group 2 and 3 than in group 1. T-scores of the femur in group 3 were lower than those in group 2. The linear regression analysis showed that levels of bioavailable 25(OH)D significantly correlated with the spine T-scores but not with the femur T-scores. The prevalence of spinal osteoporosis was higher in both groups 2 and 3 compared to group 1. Group 3 demonstrated greater femur osteoporosis compared to groups 1 and 2.

Conclusions: Vitamin D plays an important role in spinal bone health. Moreover, working conditions are likely to play an important role in femur bone health. Therefore, appropriate working conditions and nutrition are paramount to improve bone health in postmenopausal women working in greenhouses.

Key Words: Bone mineral density, Greenhouse, Menopause, Vitamin D

INTRODUCTION

Most vitamin D in humans is generated by exposure to sunlight. Considering the features of one's lifestyle, living environment, and occupation, greater exposure to sunlight is expected in people living in rural areas than in urban areas. Previous studies reported significant differences in mean serum 25-hydroxy-vitamin D [25(OH)D] levels between urban and rural dwellers, with greater vitamin D deficiencies in urban residents than in rural dwellers [1,2]. Even in the same rural area, exposure times to sunlight vary, depending on one's working environment. In Korea, many crops are grown in greenhouses, and Korean greenhouses are made of vinyl, not glass. Thus, there is a likely difference in one's exposure to ultraviolet (UV) light, which is important for the synthesis of vitamin D. Unfortunately, there are few studies on the levels of vitamin D in women working in greenhouses in Korea, and there are few studies on bone health directly related to vitamin D.

Vitamin D is closely related to bone health. In particular, vitamin D is known to play an important role in

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bone mineral density (BMD) by being involved in calcium and phosphorus metabolism. The general function of vitamin D is the stimulation of calcium absorption from the intestine, which combines to form hydroxyapatite crystals that mineralize and strengthen bones. Vitamin D is lipid-soluble and most of it is bound to vitamin D binding protein (VDBP), so it does not have biological activity. In recent studies, there have been reports that biologically active vitamin D are significantly associated with various disease [3,4]. In consideration of this, research related to VDBP was also conducted by measuring VDBP level.

In the southern region of South Korea, many vegetables are grown in greenhouses made of vinyl (polyethylene), which absorbs UV light to increase its durability. UV light is required for the synthesis of vitamin D from cholesterol, especially at wavelengths between 290 and 350 nm. Thus, the following hypotheses were established. First, women living in rural areas have higher serum levels of vitamin D than women living in urban areas. Second, women working inside greenhouses have lower serum levels of vitamin D than women in urban or rural areas. Third, serum vitamin D levels vary greatly, depending on the levels of VDBP.

We therefore measured BMD and serum levels of total, bioavailable vitamin D and VDBP and determined their relationships in women living in urban areas and rural areas and women working in greenhouses.

MATERIALS AND METHODS

Study subjects

This study included 203 healthy women working in agriculture and 70 healthy women with other occupations in Jinju city. All participants working in rural were registered with the National Farmers Health Examination Program, a free government-funded program. The agricultural workers were divided into 91 women in rural areas who work outside greenhouses, such as rice farming (rural group), and 112 women who work in a greenhouse (greenhouse group). In Korea, a greenhouse is a structure covered with transparent plastic (vinyl) on a steel frame. It is used for the purpose of increasing plant yield by concentrating the sun's heat inside the vinyl to create a high-temperature environment so that the plants can grow actively. Strawberries and paprika chilis are the main crop varieties in greenhouses located in Gyeongsangnam-do, in the southern region of the Korean peninsula. The professions of the 70 women in urban areas (urban group) were as follows: 40 homemakers, 17 clerks, and 13 self-employed professionals. All participants visited the Total Healthcare Service Center of Gyeongsang National University Hospital for a health examination between May and September of 2019. This study was approved by the Gyeongsang National University Hospital Institutional Review Board (IRB No. GIRB-A16-Y-0012) and written informed consent was obtained by all participants enrolled.

Demographics and clinical data

This study was performed retrospectively via the analysis of medical records. Menopause was defined as cessation of menstruation for more than one year with estrogen deficiency symptoms. Women diagnosed with osteoporosis or who were receiving medications, such as bisphosphonate or calcium, were excluded from the study. In addition, women with kidney disease, hyperparathyroidism, rheumatoid joint disease, cancer, hyperthyroidism, long-term use of glucocorticoids, and artificial spinal implants as a result of spinal disease were also excluded from the study.

During a health examination at the Total Healthcare Service Center, self-reported questionnaires were administered to get medical histories and lifestyle information, including history of disease, medication history, physical activity, alcohol consumption, and smoking status, from all study participants. Alcohol consumption was defined as drinking more than once per month and smoking status was defined as more than a cigarette per day. Height and body weight were measured while standing barefoot with outer garments removed using an automatic height-weight-body mass index (BMI)-measuring device (JENIX DS-102; DS Medical JENIX, Seoul, Korea). BMI was derived calculating by dividing body weight (kg) by the square of the height in meters (m^2). All body measurements were rounded up to one decimal place.

Diagnoses of osteopenia and osteoporosis

Dual-energy X-ray absorptiometry (Horizon CI; HOLOGIC, Marlborough, MA, USA) of the femur neck and lumbar spine (vertebrae L1–L4) was used for measuring BMD and analyzed according to International Society for Clinical Densitometry guidelines. The accuracy of the instrument was determined using two measurements from 30 participants. The precision error at the spine was 0.007 g/cm^2, and the least significant change was measured as 0.019 g/cm^2 at the 95% confidence interval. BMD was categorized as normal (T-score ≥ −1.0 SD), osteopenia (T-score −1.0 to −2.5 SD), and osteoporosis (T-score ≤ 2.5 SD).

Total 25(OH)D and VDBP measurements

Each of serum sample was aliquoted into two tubes and stored at −80°C until the analyses of total 25(OH)D and VDBP levels were performed. Serum total 25(OH)D levels were analyzed using the Elecsys® Vitamin D total II assay and Cobas e602 analyzer (Roche Diagnostics, Mannheim, Germany). Bioavailable 25(OH)D concentrations were calculated using equations reported in previous studies and based on albumin level, total 25(OH)D and VDBP derived from the medical records [2,3]. VDBP concentrations were measured using the Human Vitamin D BP Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), according to protocol of manufacturer.

Statistical analyses

Analyses were conducted using R software, version 4.0.3 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria), with a significance level of *P* less than 0.05. The following analyses were performed to compare differences between groups. For quantitative data analysis, ANOVA and Student's *t* tests were performed when the distribution assumption was satisfied, and Kruskal–Wallis and Mann–Whitney *U* tests (Wilcoxon rank-sum test) were performed when the distribution assumption was not satisfied. For qualitative data analysis, a chi-square test was performed when the distribution assumption was satisfied, and Fisher's exact test was performed when the distribution assumption was not satisfied. Analyses were performed using a multiple linear regression model to find factors affecting the T-scores.

RESULTS

Demographics and clinical factors

Between May 2019 and September 2019, 274 women agreed to complete the survey, and one participant excluded due to overlapping. Of these 273 women, 70 (25.6%) lived and worked in urban areas, 91 (33.3%) worked outdoors in rural areas, and 112 (41.0%) worked inside greenhouses. The demographics and clinical factors for each group of participants are shown in Table 1. There were no significant differences in age, body weight, or BMI between the groups. Shorter height was found in the rural and greenhouse groups than in the urban group (156.6 ± 5.8 cm and 155.3 ± 5.9 cm vs. 158.2 ± 4.9 cm, respectively; P = 0.003). Parity and menopausal periods were slightly higher and longer, respectively, in women in the rural and greenhouse groups than in the urban group, although these trends were not statistically significant.

Laboratory and bone health results

Laboratory findings for each group are listed in Table 2. Plasma albumin and calcium concentrations were higher in the urban group than in the rural and greenhouse groups (P < 0.05). Total 25(OH)D concentrations were not significantly different between the groups. However, bioavailable 25(OH)D concentrations were statistically higher in the rural and greenhouse groups than in the urban group (12.3 ± 5.3 ng/mL and 12.4 ± 4.2 ng/mL vs. 6.0 ± 4.6 ng/mL, respectively; P < 0.001). The BMD values were reanalyzed using T-scores derived from Korean data and are shown in Table 2. Lumbar spine T-scores from the rural and greenhouse groups were significantly lower than T-scores from the urban group (–0.8 ± 1.3 and –0.9 ± 1.3 vs. –0.1 ± 1.1, respectively; P < 0.001), but there were no differences between the rural and greenhouse groups. The femur neck T-scores were lower in the rural and greenhouse groups than in the urban group (–0.3 ± 0.9 and –0.5 ± 1.0 vs. 0.1 ± 0.9, respectively; P < 0.001). Unlike the results in the lumbar spine, the femur neck T-score was statistically significantly lower in greenhouse group than in rural group.

Multiple linear regression for T-scores of the lumbar spine and femur neck

The T-scores of the lumbar spine in the rural and greenhouse groups were lower than the T-score of the urban group (decreases of 0.558 and 0.708, respectively) after adjusting for menopausal period, receipt of hormone therapy, and serum levels of total 25(OH)D, calcium, phosphate, and albumin in the multiple linear regression analysis (P < 0.01). Menopausal period and BMI were statistically associated with the lumbar spine T-score in the multiple linear regression analysis (P < 0.05). When menopausal period and BMI each increased by one-point, lumbar spine T-score decreased by 0.084 and increased by 0.06, respectively (P < 0.05). There was a significant correlation between serum levels of bioavailable vitamin D and lumbar spine T-score

Table 1. Demographic and clinical factors of participants by group

Variable	Urban (n = 70)	Rural (n = 91)	Greenhouse (n = 112)	*P* value
Age (y)	58.6 ± 4.2	58.4 ± 6.6	58.4 ± 6.3	0.777
Height (cm)	158.2 ± 4.9[a]	156.6 ± 5.8[b]	155.3 ± 5.9[b]	0.003*
Body weight (kg)	60.4 ± 8.6	58.9 ± 9.5	58.7 ± 8.6	0.298
BMI (kg/m^2)	24.1 ± 3.2	23.9 ± 3.5	24.5 ± 3.5	0.381
Parity	2.7 ± 1.1	2.8 ± 1.5	2.8 ± 1.3	0.597
Menopausal period (y)	7.5 ± 4.1	8.8 ± 6.7	9.0 ± 7.1	0.801
Agricultural period (y)	-	27.6 ± 14.1	26.7 ± 12.0	0.787
MHT				0.068
No	68 (97.1)	88 (96.7)	106 (94.6)	
Yes	2 (2.9)	3 (3.3)	6 (5.4)	
Smoking (≥ 1 cigarette/day)				0.312
No	67 (95.7)	83 (91.2)	100 (89.3)	
Yes	3 (4.3)	8 (8.8)	12 (10.7)	
Alcohol (> 1 time/mo)				0.527
No	27 (38.6)	43 (47.3)	47 (42.0)	
Yes	43 (61.4)	48 (52.7)	65 (58.0)	

Values are presented as the mean ± SD or number (%).
BMI: body mass index, MHT: menopausal hormone therapy.
**P* < 0.05.
[a,b]Different characteristics indicate a statistical difference (*P* < 0.05).

Table 2. Laboratory parameters and bone health of participants by group

Variable	Urban (n = 70)	Rural (n = 91)	Greenhouse (n = 112)	*P* value
Albumin (g/dL)	4.6 ± 0.2[a]	4.4 ± 0.3[b]	4.4 ± 0.2[b]	<0.001*
Calcium (mg/dL)	9.5 ± 0.4[a]	9.4 ± 0.3[b]	9.4 ± 0.3[b]	0.011*
Phosphate (mg/dL)	3.7 ± 0.5	3.7 ± 0.5	3.7 ± 0.4	0.754
Total 25(OH)D (ng/mL)	24.8 ± 10.4	25.1 ± 12.1	23.7 ± 9.5	0.858
Bioavailable 25(OH)D (ng/mL)	6.0 ± 4.6[a]	12.3 ± 5.3[b]	12.4 ± 4.2[b]	<0.001*
VDBP (μg/mL)	201.2 ± 74.2	213.8 ± 59.0	210.4 ± 54.0	0.366
T-score of spine (L1–L4)	–0.1 ± 1.1[a]	–0.8 ± 1.3[b]	–0.9 ± 1.3[b]	<0.001*
T-score of femur neck (left)	0.1 ± 0.9[a]	–0.3 ± 0.9[b]	–0.5 ± 1.0[c]	<0.001*

Values are presented as the mean ± SD.
25(OH)D: 25-hydroxy-vitamin D, VDBP: vitamin D binding protein, L1–L4: lumbar vertebrae 1–4.
**P* < 0.05.
[a,b,c]Different characteristics indicate a statistical difference (*P* < 0.05).

(*P* < 0.05) (Table 3).

The T-score of the femur neck was decreased 0.366 in the rural group compared with the urban group after the same adjustments (*P* < 0.05). The femur T-score of the greenhouse group was significantly lower than that of the rural group (decrease of 0.246; *P* < 0.05). Menopausal period and BMI were statistically associated with femur neck T-score (*P* < 0.05). When menopausal period and BMI each increased by one point, femur neck T-score decreased by 0.041 and increased by 0.035, respectively (*P* < 0.05).

Table 3. Linear regression model for T-scores of the spine and femur neck

Site	Variable	Adjusted estimate (with CI)	Adjusted *P* value
Spine	Rural vs. Urban	–0.558 (–0.203 to –0.912)	0.002*
	Greenhouse vs. Urban	–0.708 (–1.045 to –0.036)	< 0.001*
	Greenhouse vs. Rural	–0.143 (–0.460 to 0.175)	0.377
	Menopausal period	–0.084 (–0.106 to –0.063)	< 0.001*
	MHT	0.348 (–0.516 to 1.212)	0.428
	BMI (kg/m^2)	0.062 (0.021 to 0.102)	0.003*
	Bioavailable 25(OH)D	0.015 (0.002 to 0.027)	0.026*
Femur neck	Rural vs. Urban	–0.366 (–0.087 to –0.645)	0.011*
	Greenhouse vs. Urban	–0.812 (–1.033 to –0.045)	< 0.001*
	Greenhouse vs. Rural	–0.246 (–0.436 to 0.063)	0.048*
	Menopausal period	–0.041 (–0.058 to –0.023)	< 0.001*
	MHT	0.093 (–0.587 to 0.772)	0.788
	BMI (kg/m^2)	0.035 (0.004 to 0.066)	0.029*
	Smoking	–0.523 (–0.923 to –0.123)	0.011*
	Bioavailable 25(OH)D	0.007 (–0.003 to 0.017)	0.179

Adjusted by menopausal period, MHT, and serum levels of total 25(OH)D, calcium, phosphate, and albumin in the multiple linear regression analysis.
MHT: menopausal hormonal therapy, BMI: body mass index, 25(OH)D: 25-hydroxyl-vitamin D, CI: confidence interval.
*$P < 0.05$.

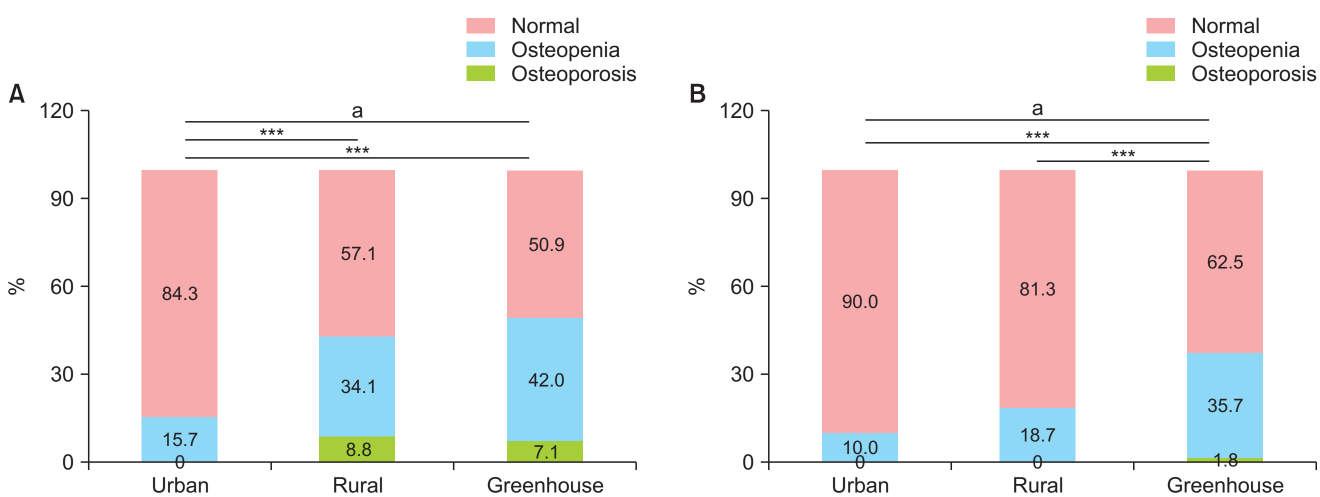

Fig. 1. Distribution of participants with osteoporosis and osteopenia of the lumbar spine (A) and femur neck (B) in each group. Normal: T-score ≥ –1; Osteopenia: –2.5 < T-score < –1; Osteoporosis: T-score ≤ –2.5. [a]Bonferroni post-hoc comparison after chi-square test between three groups. ***$P < 0.001$ between two groups.

Distribution of participants with osteopenia and osteoporosis

The distribution of participants by category of bone health (BMD) was significantly different between the three groups in terms of T-scores of the lumbar spine ($P < 0.001$) and femur neck ($P < 0.001$) (Fig. 1). For osteopenia of the lumbar spine, the greenhouse group had the highest percentage of affected individuals (42.0%), followed by 34.1% of the rural group and 15.7% of the urban group. The rural and greenhouse groups accounted for a higher proportion of lower lumbar spine BMD than the urban group. The distribution of osteoporosis and osteopenia showed a significant increase in both the rural and greenhouse groups compared to the urban group ($P < 0.001$) (Fig. 1A).

The incidence of osteopenia and osteoporosis in the greenhouse group was higher than in the rural and urban groups ($P < 0.001$), but we found no difference between the urban and rural groups (Fig. 1B).

Effects of bioavailable Vitamin D concentration on T-scores of the lumbar spine and femur neck in each group

To investigate the effects of bioavailable vitamin D on the T-scores of the lumbar spine and femur neck in each group (urban, rural, and greenhouse), we compared them using a linear model adjusted for menopausal period, hormone intake, smoking, BMI, and levels of calcium, phosphate, and albumin.

Figure 2A shows changes in each lumbar spine T-score according to the level of bioavailable 25(OH)D for each group. When the model was adjusted for other factors, the lumbar spine T-score was larger in the urban group than in the rural group (increase of 0.558) and smaller in the greenhouse group than in the rural group (decrease of 0.143). When bioavailable 25(OH) D increased by one point, the lumbar spine T-score increased by 0.015 ($P < 0.05$, Table 3, Fig. 2A).

When the model was adjusted for other factors, the T-score of the femur neck was higher in the urban group than in the rural and greenhouse groups (increases of 0.366 and 0.812, respectively). When comparing the T-score of femur neck with the greenhouse group and the rural group, it was lower in the greenhouse group (decrease of 0.246). While the T-score of the femur neck increased by 0.007 when the bioavailable 25(OH)D increased by one point, this finding was not statistically significant ($P > 0.05$) (Fig. 2B).

DISCUSSION

In our study, there was no difference between women in the rural, greenhouse, and urban groups in age, weight, or BMI, but those in the rural and greenhouse groups were shorter in height than those in the urban group. Although there are several explanations for this finding, compression fractures due to osteoporosis of the lumbar spine should be considered. As the lumbar spine T-scores were lower in the rural and greenhouse groups than in the urban group, there could be a history of spinal compression fractures in the rural and greenhouse groups. This supposition is consistent with results from other studies in northern Iran and China, which reported that the incidence of osteoporosis was significantly greater in rural areas than in urban areas [5,6]. A sedentary lifestyle and certain work postures

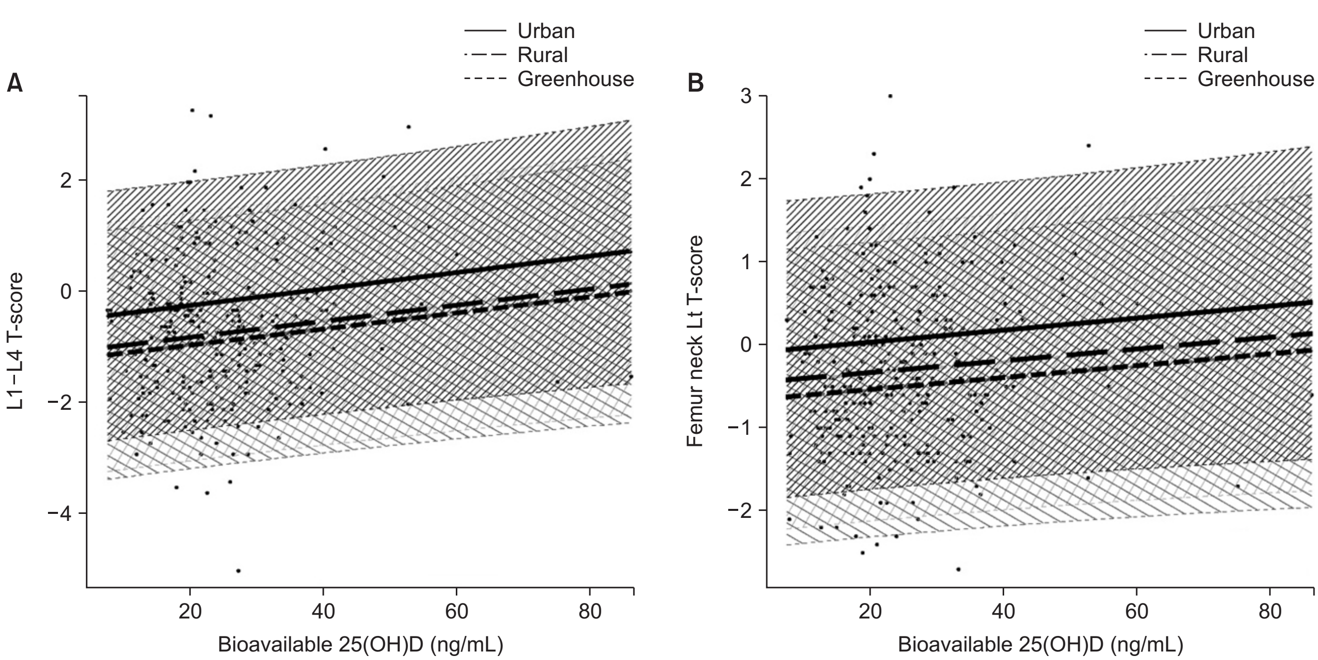

Fig. 2. The graphs showing the relationship between each T-score and level of serum bioavailable 25(OH)D concentration by group using multiple linear regression model. (A) In the lumbar spine, there was a statistically significant difference between the three groups; $P = 0.026$. (B) In the left femur neck, the difference between the three groups was not statistically significant; $P = 0.179$. 25(OH)D: 25-hydroxy-vitamin D.

such as squatting and bending are common in those living in rural areas, as these postures can lead to lumbar degenerative kyphosis, resulting in a short stature [7,8]. Compression fracture risk factors include age, premature menopause, low BMD, smoking, length of reproductive period, systemic corticosteroid use, and immobilization [9]. Particularly in women, BMD significantly decreases after menopause [10].

While many other studies of bone health have included measurements of total 25(OH)D, levels of bioavailable vitamin D and VDBP were considered in this study as well. Additionally, this study further subdivided the rural group of the study population into those who live in a rural area and those who work in a greenhouse. High levels of bioavailable 25(OH)D might be the result of bone resorption, causing low BMD to maintain calcium homeostasis. Some studies have shown a correlation between levels of bioavailable 25(OH)D and BMD, but more research is needed [11-13]. Other studies in Malaysia and northern Iran reported higher levels of vitamin D in rural residents than in urban residents, as their exposure to the sun was greater [14,15]. In contrast, others reported lower levels of serum vitamin D in rural residents than in urban dwellers [16,17]. In our study, we were interested in women working in greenhouses as well as those who live in rural areas because greenhouses in Korea, which are covered with vinyl, not glass, restrict the exposure of these women to UV light, which is essential for vitamin D synthesis. In our study, the serum concentrations of total vitamin D and VDBP did not differ between the three groups, but the levels of bioavailable vitamin D were significantly higher in women in the rural and greenhouse groups than in the urban group. In addition, there were no differences between the rural and greenhouse groups, possibly because of increases in the amount of time these women were exposed to sunlight, but it may also be compensation for maintaining homeostasis due to poor bone health, such as that seen in those with osteoporosis and osteopenia.

In our study, serum vitamin D levels in postmenopausal women was 24.4 ng/mL, which is higher than average in Korea (18.3 ng/mL) [2]. Potential causes of this difference may be time of year and weather during which the samples were collected. Samples were collected from May to September, when the amount of UV light is relatively high in Korea. Further, during this period, the climate is sunny, and many people spend time outdoors, including farming in rural areas.

A comparison of T-scores of the lumbar spine and femur neck was conducted using ANOVA for the three groups. The rural and greenhouse groups had significantly lower T-scores than the urban group, but there was no difference between the rural and greenhouse groups. In this study, several factors such as menopausal period, menopausal hormone therapy, and levels of serum total vitamin D, calcium, phosphate, and albumin were adjusted, and a multiple linear regression model was used for comparisons of the groups. There were differences in femur neck T-scores between the greenhouse and rural groups, which does not seem to be related to vitamin D levels and are thought to be caused by differences in work positions, such as squatting. Because the femur is the main weight-bearing bone, it is thought that the BMD of the femur is affected to a greater extent by physical factors related to personal lifestyle, such as body posture, rather than vitamin D levels. It is presumed that this is due to differences between the spine, which is composed of trabecular bone, and the femur, which is composed of laminar bone, but further studies are needed.

An assessment of the frequency of osteoporosis and osteopenia revealed that spinal bone health was poor in the rural and greenhouses groups compared to the urban group. Femur bone health was found to be worse in the greenhouse group than in the rural group, which suggests that the working environment influences bone health. Our findings show that the role of vitamin D plays an important role in spine health and that the working environment is also an important factor for femur bone health.

There are several weaknesses in this study. The first is that this study was performed at a single tertiary hospital for a limited period of time. The second limitation is that sun exposure was not quantified according to the weather or time of year. Additionally, the materials in the greenhouses that block UV light could not be controlled. The strengths of this study are that we derived the levels of bioavailable vitamin D, the form of biologically active vitamin D, by measuring VDBP levels and comparing them between groups. We also used a linear regression model to adjust for several factors that are able to affect bone health and then conducted a comparative study between the three groups of women to derive the results.

In conclusion, no differences in vitamin D synthesis were observed in women working in greenhouses compared with women who lived in rural areas. Vitamin D plays an important role in spinal bone health, but work posture is thought to play an important role in femur bone health. Therefore, proper working posture and nutritional improvements, including vitamin D supplements, are considered important for improving bone health in postmenopausal women who work in greenhouses.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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