

# Relationship Among Nutritional Intake, Duration of Outdoor Activities, Vitamin D Status and Bone Health in High School Girls

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This study investigated the interactions of bone health with several variables such as outdoor activity hours, nutritional status including habitual intake of calcium and vitamin D status in 72 high school girls aged 16-17 years attending day classes or night classes. The subjects consisted of 39 day-class students and 33 night-class students. Dietary nutrient intakes were estimated using the 24-hour recall method. The daily activities of each subject were assessed using an activity questionnaire. Urinary calcium and creatinine excretion were assayed from subjects' 24-hour urine, while 25-OH-vitamin D [25-(OH)-D] and osteocalcin were measured from the subjects' fasting blood. Intake of energy, iron, vitamin A and vitamin C were worse in the night-class students. There was no significant difference in dietary calcium between the subjects in the different class types. Time spent on outdoor activities was significantly less in subjects attending night classes. Urinary calcium excretion of the night-class subjects was significantly higher than that of the day-class subjects ( $p < 0.05$ ). There was no significant difference in serum 25-(OH)-D level according to class type. Serum osteocalcin for night-class subjects was significantly higher than that for day-class subjects ( $p < 0.01$ ). It appeared that the night-class students had poorer dietary habits as well as fewer outdoor activities. Even though the estimated bone health of both groups of subjects appeared to be normal, the overall nutritional intake and duration of outdoor activities appeared to be important for maintaining bone health and lowering the future risk of osteoporosis.

**Key words:** Nutrient intake, Urinary calcium, Serum 25-(OH)-Vitamin D, Osteocalcin, Outdoor activity

## INTRODUCTION

Public concern for osteoporosis is growing in Korea.<sup>1)</sup> It is generally agreed that there are few effective treatments for osteoporosis and, therefore, that more prevention efforts are needed.<sup>2,3)</sup> Peak bone mass is attained at 20-30 years, and thereafter skeletal mass normally decreases with age.<sup>2,4)</sup> In this context, acquisition of peak bone mass during the growth period is regarded as the key factor in preventing osteoporosis in later life. Bone mass in adult life is dependent upon several factors including genetics, nutrition, physical activities and hormonal status.<sup>5-7)</sup> Although literature suggests that a metabolic imbalance of calcium plays an important role in the pathogenesis of osteoporosis, there are growing concerns on the decline of physical activity intensity in modern society as well.<sup>8-12)</sup> It has been also suggested that the decline of vitamin D status is associated with the age-related decline of participation in outdoor activities, which may cause inefficient calcium absorption, as vitamin D and parathyroid hormone are interrelated with the maintenance of calcium homeostasis.<sup>13-15)</sup> However, there have not been enough studies on the relationship between outdoor

activity and calcium status in humans.<sup>16)</sup>

Finding strategies with the potential to have positive implications in attaining maximum bone mass during puberty has long been a focus of research, including in western societies where the incidence of osteoporosis is greater.<sup>17,18)</sup> In Korea, the relationship of bone status to nutritional factors in adult women has been extensively reported. However, limited information is available as to the nutritional factors involved in the bone health of Korean adolescent girls.<sup>19-21)</sup> Studies conducted on adolescent girls and college women confirmed the importance of nutritional adequacy, optimal calcium intake and physical activity for bone health.

Recently it has been reported that better maintenance of bone density in teenagers was linked to more physical activity and vitamin D status.<sup>22,23)</sup> Serum levels of 25-OH-vitamin D, which is sensitive to UV light exposure, are widely accepted as the most useful indicator of vitamin D status.<sup>24)</sup> Therefore, seasonal changes, latitude of residence and work type classified by the extent of sunlight exposure are regarded as important factors contributing to vitamin D status.<sup>25,26)</sup>

In Korea, there is growing concern about the vulnerability of bone health status among adolescents associated with decreased outdoor activity for reasons such as college entrance examination preparation and transition to inactive lifestyles. Moreover, the National Health and

Nutrition Examination Survey conducted recently in Korea indicated that dietary calcium intake remains inadequate in Korea regardless of age group.<sup>27,28)</sup> In this study, we investigated the relationship among dietary intake, duration of outdoor activity, vitamin D status and bone health in order to provide strategies to improve dietary and activity patterns to attain desirable bone mass during the adolescent period.

## METHODS

### 1. Selection of Subjects

Seventy-two female high school students with no health problems participated in this study. We recruited 36 subjects from a vocational training high school in Daegu offering night classes, and the other 36 subjects from among those attending day classes. None was taking prescribed drugs, including vitamins, mineral supplements or oral contraceptives.

### 2. Dietary Assessment

A combination of 24-hour dietary recall method and food records was used to assess the nutritional intake of each subject. Food pictures were provided to improve the accuracy of estimation of actual intake.

Habitual intake of calcium and sodium were evaluated using the semi-quantitative food frequency method. Details of this method have been reported previously.<sup>29)</sup> The questionnaire for calcium intake consisted of 22 food items commonly consumed by Koreans. Calcium intake score was calculated from the calcium content and frequency of intake. The calcium content of each food of standard serving size was classified into three levels. Intake frequency was classified into five levels - every day, three times a week, one or twotimes a week, once a month, rarely. Foods were divided into 3 groups - foods with a calcium content of more than 100 mg per serving were scored three points, foods with a calcium content of 50-99 mg were scored two points and foods with a calcium content of less than 50 mg were scored one point. Alcohol intake was converted to the amount of daily intake of soju (360 cc bottle, alcohol content 25%).

### 3. Physical Activity Assessment

An activity questionnaire was provided for each subject to fill out all of the information on their physical activities over a 24-hour period. Average physical activity coefficient was calculated by multiplying time spent on each activity by the activity intensity. Duration of outdoor activities from 8:00 to 17:00 was also calculated.

### 4. Anthropometric Assessment

Body mass index (BMI) was calculated from measured

height and weight for each subject. Body fat content was assessed using the Bio-electrical Impedance Fatness Analyzer (GIF-891).

### 5. Biochemical Assessment

A fasting blood sample of 10 mL was collected from each subject and stored at -70°C. As bone turnover markers, we measured the 25-(OH)-vitamin D<sup>30)</sup> and osteocalcin levels in serum using an radio immuno assay kit [OSCA test Osteocalcin(BGP), BRAHMS, Germany].

Urine samples for 24 hours were collected for two days from each subject. Urinary creatinine was measured using Hawk's method.<sup>31)</sup> Urinary calcium was measured using Inductively Coupled Plasma Emission Spectroscopy (ICP)(Jobin Yvon, 38 plus, France). The analytical condition of ICP is provided in Table 1.

**Table 1.** Analytical conditions of ICP

Power	1000 W
Sheath gas flow	0.2 L/min
Plasmagene gas flow	12 L/min
MEINHARD nebulizer pressure	3 bars
Sample flow rate	1 mL/min

### 6. Statistical Analysis

The Statistical Analysis System (SAS) Package was used to analyze the relationship among bone status, dietary factors and physical activity components. Two sample t-test was used to compare the differences between the two groups of subjects and the Pearson correlation coefficient was applied to identify the factors involved in calcium status.

## RESULTS

### 1. General Characteristics of the Subjects

The physical characteristics of the subjects are described in Table 2. The mean age of the day-class attendees was 17.1 years, significantly higher than that of the night-class attendees ( $p < 0.01$ ). There were no significant differences in physical characteristics such as

**Table 2.** General characteristics of subjects

Variables	Dayclass(N=39) <sup>1)</sup>	Nightclass(N=33)	Significance
Age(years)	17.1±1.70 <sup>4)</sup>	16.2±0.71	p<0.01
Height(cm)	163.4±4.18	161.8±4.47	NS*
Weight(kg)	52.7±5.57	52.3±6.83	NS
BMI(kg/m <sup>2</sup> ) <sup>2)</sup>	19.7±1.70	19.9±2.16	NS
Body fat(%)	26.8±3.16	26.5±4.05	NS
LBM <sup>3)</sup>	38.6±4.45	38.3±3.98	NS

1) Number of subjects

2) BMI; Body mass index

3) LBM; Lean body mass

4) Mean ± S.D.

\* NS; Not significant

height, weight, body mass index (BMI) or body fat (%) between the two groups.

## 2. Daily dietary nutrient intake

Average dietary intake of the subjects is shown in Table 3.

**Table 3.** Average daily nutrient intake of the subjects

Variables	Dayclass(N=39) <sup>1)</sup>	Nightclass(N=33)	Significance
Energy(kcal)	2028.6±605.05 <sup>2)</sup> (96.1%) <sup>3)</sup>	1663.9±383.39 (79.2%)	p<0.01
Protein(g)	71.3±27.66 (109.7%)	62.2±22.35 (95.7%)	NS*
Animal(g)	34.3±18.62	29.3±18.31	NS
Fat(g)	47.0±21.58	42.4±18.04	NS
Carbohydrate(g)	346.0±136.94	257.2±62.47	p<0.01
Calcium(mg)	706.5±106.19 (88.6%)	503.5±222.95 (62.3%)	NS
Phosphorus(mg)	961.3±387.00 (120.2%)	799.4±319.67 (99.9%)	NS
Ca/P	0.7±0.47	0.6±0.18	NS
Iron(mg)	17.5±9.65 (109.4%)	12.5±5.61 (78.1%)	p<0.05
Vitamin A(RE)	683.2±147.33 (97.6%)	495.1±150.22 (70.7%)	NS
Thiamin(mg)	1.5±1.31	1.0±0.72	p<0.05
Riboflavin(mg)	1.5±1.06	1.0±0.35	p<0.01
Vitamin C(mg)	109.8±130.25 (156.9%)	49.1±52.99 (70.1%)	p<0.05

1) Number of subjects 2) Mean ± S.D. 3) % of RDA  
\* NS; Not significant

Energy intake was 96.1% of the Korean Recommended Dietary Allowance (RDA) for day-class attendees, which was significantly higher than that for the night-class attendees, whose energy intake was only 79.2% of Korean RDA.<sup>32)</sup> Daily average protein intake, a possible dietary factor of calcium bioavailability, was not significantly different between the two groups. Calcium intake of day-class attendees (=88.5% of RDA) was slightly higher than that of night-class attendees (=62.3% of RDA), however, the difference was not significant. Ratio of Ca:P was 0.7 for day-class attendees and 0.6 for night-class attendees, which was considerably lower than the desirable level of 1:1.

Dietary intake of iron, thiamin, riboflavin and vitamin C were also significantly lower in night-class attendees.

**Table 4.** Habitual intake of Ca and Na in dayclass and nightclass subjects

Variables	Dayclass(N=39) <sup>1)</sup>	Nightclass(N=33)	Significance
Ca score	68.3±19.70 <sup>2)</sup>	64.2±32.54	NS*
Na score	513.6±177.03	485.8±205.55	NS

1) Number of subjects 2) Mean±S.D.  
\* NS; Not significant

Average intake of vitamin A and vitamin C were 70% of Korean RDA in the night-class attendees.

No significant difference between the two groups of subjects was found in terms of the habitual intake of calcium and sodium, as described in Table 4.

## 3. Daily activity patterns

The daily activity patterns of the subjects are shown in Table 5. While the average physical activity coefficient was quite similar in the two groups, time spent on outdoor activities was significantly higher in day-class attendees (=81.8 min) than in night-class attendees (=59.2 min)(p<0.01).

**Table 5.** Comparison of daily physical activity

Variables	Dayclass(N=39) <sup>1)</sup>	Nightclass(N=33)	Significance
Outdoors(min)	81.8±32.07 <sup>2)</sup>	59.2±22.68	p<0.01
Physical activity coefficient	1.3±0.098	1.3±0.037	NS*

1) Number of subjects 2) Mean ± S.D.  
\* NS; Not significant

## 4. Bone Biomarker and 25-(OH)-Vit D Status

Urinary Ca excretion and creatinine levels in the urine of the two groups are compared in Table 6. Urinary Ca excretion, a clinical biomarker of bone resorption, was within the normal range (0.06-0.45) in the subjects. Average daily Ca excretion (Ca/creatinine) in the two groups - 0.13 mg/mg for day-class attendees and 0.17 mg/mg for night-class attendees respectively - was significantly different.

**Table 6.** Urinary Ca excretion, serum osteocalcin and 25-(OH) Vit D<sub>3</sub>

Variables	Dayclass(N=39) <sup>1)</sup>	Nightclass(N=33)	Significance
Urinary Ca/cr (mg/mg)	0.13±0.064 <sup>3)</sup>	0.17±0.085	p<0.05
Serum osteocalcin (ng/mL)	50.1±8.90	57.3±10.08	p<0.01
25-(OH)D <sub>3</sub> <sup>2)</sup> (ng/mL)	14.3±5.50	14.0±6.71	NS*

1) Number of subjects 2) 25-(OH)D<sub>3</sub>; 25-hydroxy vitamin D<sub>3</sub>  
3) Mean ± S.D.  
\* NS; Not significant

The osteocalcin and 25-(OH) Vit D levels in serum are also shown in Table 6. Our study indicated the osteocalcin level of night-class attendees (= 57.3 ng/mL) was significantly higher than that for day-class attendees (=50.1 ng/mL)(p<0.01). Higher excretion of Ca in the urine was also observed for night-class attendees. Average serum 25-(OH) Vit D was within the normal range<sup>24)</sup> and very similar between the two groups.

### 5. Relation Among Dietary Intake, Activity Patterns and Biomarker of Bone Status

As indicated in Table 7, protein intake positively correlated with animal protein intake ( $r=.80$ ,  $p<0.01$ ), calcium intake ( $r=.55$ ,  $p<0.01$ ) and phosphorous intake ( $r=.84$ ,  $p<0.01$ ). Meanwhile, urinary excretion of calcium was negatively related to time spent on outdoor activities ( $r=-.43$ ,  $p<0.05$ ) and serum osteocalcin ( $r=-.43$ ,  $p<0.05$ ).

**Table 7.** Correlation matrix among variables affecting bone status

Variables	Protein	Animal protein	Ca	P	Outdoor activity	25 (OH)D	Osteocalcin
Animal protein	.80**						
Calcium	.55**	.48**					
Phosphorus	.84**	.74**	.81**				
Outdoor activity	.13	-.19	-.09	-.11			
25(OH)D	.16	.23	.25	.17	-.09		
Osteocalcin	.22	-.03	.08	.05	.24	-.02	
Urinary Ca	.30	.15	.33	.24	-.43*	.02	-.43*

\*  $p<0.05$ , \*\*  $p<0.01$

## DISCUSSION

In this study, we compared the dietary intake, time spent on outdoor activities, vitamin D status and biomarkers of bone status in high school girls attending day classes or night classes.

Night-class attendees showed a poorer nutrient intake than day-class attendees. Significantly lower intakes of energy, iron, thiamin, riboflavin and vitamin C were observed in night-class attendees. Moreover, night-class attendees did not have adequate amounts of energy, calcium, vitamin A and vitamin C. A recent study also reported that bone mineral density was associated with dietary vitamin A and calcium status in Korean female college students.<sup>21)</sup> The dietary protein intake of our subjects was maintained at a slightly higher level in day-class attendees. However, we did not see any statistical significance to the difference between the two groups.

Although day-class attendees spent more time on outdoor activities than night-class attendees, serum level of 25-(OH)-D was very similar in the two groups. In other words, serum level of 25-(OH)-D did not reflect the difference in time spent on outdoor activities. The participants in this study were healthy and homogeneous in terms of demographic characteristics. Therefore, the effect of confounding variables on bone status, associated with growth, gender, or geographical differences, was minimized. All of the subjects were selected from the same vocational training high school. In this context, it is speculated that the difference in outdoor activity duration may not have been enough to have a significant impact on vitamin D status, since their activity patterns

have been accumulated for only the two years of their attendance at high school.

It is well known that vitamin D is obtained either from dietary sources or synthesis in the skin. As we did not measure the amount of dietary vitamin D intake, it was not possible to draw a conclusion for the association among dietary inadequacy, outdoor activities and vitamin D status from this study. However, it is not likely that the influence of dietary intake on vitamin D status is more pronounced than the biosynthesized vitamin D in the Korean population, normally exposed to a sufficient amount of sunlight. A recent study of 122 adults conducted by Moon & Kim in Korea (1998) indicated that the 25-OH-D level of most subjects was within the normal range, although dietary vitamin D intake was less than 50% of Korean RDA for 23.6% of the adults.<sup>33)</sup> In their study, regression analysis demonstrated that time spent outdoors contributed to the maintenance of vitamin D status as much as vitamin D intake did.

However, serum 25-OH-vitamin D level was much lower in our adolescent subjects, regardless of whether they attended day or night classes, than the reported value for Korean adults. This is quite contrary to our expectations of declining physical activity and vitamin D status with aging. Recently, findings from other regions exposed to less sunlight have demonstrated that inadequate vitamin D is not uncommon among peripubertal girls and suggested dietary enrichment of vitamin D.<sup>22,23,34)</sup>

The finding of this study that urinary Ca excretion was lower the longer the subject spent outdoors confirmed the importance of outdoor activity for better maintenance of bone status. Protein intake is generally regarded as a possible dietary factor for calcium bioavailability. Literature has suggested that higher protein intake induces more excretion of urinary Ca.<sup>1,10,30)</sup> Positive correlation of urinary calcium excretion with protein intake was also observed in our study.

Osteocalcin, which originates from osteoblast and is vitamin K dependent, is widely accepted as a marker of bone turnover.<sup>36)</sup> Higher levels of osteocalcin are normally observed with active bone formation.<sup>17)</sup> Therefore, osteocalcin levels in children are at higher levels than those in adults.<sup>17)</sup> However, increased osteocalcin levels in the early postmenopausal stage compared to the premenopausal period have been observed and are mainly due to rapid bone turnover.<sup>37)</sup>

The average osteocalcin level of our subjects was much higher than that reported for adults,<sup>38)</sup> which indicates active bone formation during the adolescent stage.

We may conclude that active bone modeling occurred in our adolescent girls, judging from their serum osteocalcin levels. The levels were higher in this group than the reported average for college students or adults.

However, it is not possible to estimate whether they attained desirable bone mass without information on bone density.

Our study indicated significantly higher urinary calcium and serum osteocalcin levels in night-class attendees. Therefore, it is not clear whether osteocalcin is a better indicator of bone turnover than bone formation, as we did not measure the metabolic changes of bone status.

In general, the subjects of our study were more active than other Korean high school students under a more stressful environment related to their preparation for college entrance examinations. Therefore, the general activity pattern of Korean high school attendees would be less active than our subjects, who were attending a vocational high school.

Our study suggests a marginal vitamin D status among adolescent girls in Korea.<sup>22,33)</sup> Poorer dietary patterns combined with less outdoor activity were observed in night-class attendees. Therefore, more attention is needed to prevent the deterioration of bone status among Korean adolescent girls, whose activity patterns have become sedentary and indoor-based. Appropriate nutrition education is urgently needed for adolescent girls to attain dietary adequacy and to increase the amount of their outdoor activity.

Since this study was cross-sectionally conducted in a small scale, it has limitations as far as drawing a definite conclusion and further efforts, including a longitudinal study or intervention trial, are suggested.

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