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Increasing vitamin D deficiency in children from 1995 to 2011

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Serum concentrations of 25-hydroxy vitamin D_3 [25(OH)D_3] and vitamin D deficiency have changed over time in Korean children. This study assessed serum 25(OH)D_3 concentrations and the prevalence of vitamin D deficiency in children. Serum samples were obtained during 1995 to 2011, and 25(OH)D_3 concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Tests of 948 serum samples showed that median 25(OH)D_3 concentrations decreased significantly ($P<0.001$), and the rates of vitamin D deficiency/insufficiency increased significantly ($P<0.001$), over 15 years. Median serum 25(OH)D_3 was significantly higher in males than in females in 2005–2006 and 2010–2011 ($P<0.001$), whereas the rates of vitamin D deficiency/insufficiency were higher in subjects aged 11–15 years than in the other two age groups after the year 2000. These increases over time in vitamin D deficiency/insufficiency may be due to the changing lifestyles of children. Outdoor physical activity should be strongly encouraged.

***Key words:** vitamin D, vitamin D deficiency, children, adolescent.*

Vitamin D is a fat-soluble vitamin that is important for maintaining calcium homeostasis through actions in the intestine, bone, kidney, and parathyroid gland[1]. Vitamin D is also essential for normal growth and skeletal development[2]. More recently, vitamin D status has been linked to cancer, cardiovascular disease, autoimmune disease, and infection[3]. This vitamin is acquired through nutritional supplements (10–20%) and by cutaneous synthesis from exposure to sunlight (80–90%)[4].

In children, causes of rickets have changed from low exposure to sunlight and intake of vitamin D to inappropriate supplementation or metabolic disorders, including hepatic disease, renal disease, and metabolic problems with calcium or phosphorus. However, vitamin D deficiency and nutritional rickets are re-emerging as a major public health problem throughout the world[5-9]. The prevalence of vitamin D deficiency in normal healthy individuals is high in many countries[6,7,9].

Risk factors for low vitamin D status in Korean children from 2006 to 2013 have been reported to include obesity 10[10-12], female gender [11,13], low physical activity[11,12], winter season[11], and older age [11,14]. The effects of older age on vitamin D deficiency regardless of weight may be due to the reduced opportunity of older Korean children to participate in outdoor activities[14].

To date, no study has assessed changes over time of vitamin D levels in Korean children. Vitamin D levels are routinely tested by measuring the serum concentration of the major circulating form of vitamin D, 25-hydroxy vitamin D_3 [25(OH)D_3]. This study was designed to investigate the changing pattern of serum 25(OH)D_3 levels in children from 1990 to 2011 and to evaluate the prevalence of vitamin D deficiency in this population.

Material and Methods

Clinical samples

The study population consisted of patients aged 1–15 years who visited the Department of Pediatrics of Gyeongsang National University Hospital (GNUH) in Jinju, Korea, from June to October of each year. As a member of the National Biobank of Korea, the GNUH collects serum samples from patients randomly and stores them at −80°C. Serum samples collected in 1990–1991, 1995–1996, 2000–2001, 2005–2006, and 2010–2011 were stratified by age groups, subjects aged 1–5 years (preschoolers), 6–10 years (elementary school children), and 11–15 years (adolescents) (Table 1). Stored data included age, sex, diagnosis, and month of sample collection (from June to August). Patients with specific conditions, such as malignant disorders, chronic kidney disease, parathyroid disease, inflammatory bowel disease, or severe immunodeficiency, were excluded. Finally, 1008 samples were selected, 525 from boys and 483 from girls. The number of samples ranged from 60 collected in 1990–1991 to 298 samples collected in 2005–2006 and in 2010–2011 (Table 1).

Vitamin D deficiency has been defined as a 25(OH)D_3 concentration <20 ng/ml, whereas vitamin D insufficiency has been defined as a 25(OH)D_3 concentration of 21–29 ng/ml[12]. The 20 ng/ml cut-off was based on studies showing that lower concentrations may be associated with rickets and impaired bone development, whereas the 30 ng/ml cut-off value was the level below which parathyroid hormone levels begin to increase in adults, thereby affecting calcium absorption.

There was no detectable degradation of 25(OH)D_3 in human samples stored at −20°C for more than 10 years[15] or in serum samples stored for 6–24 years at −25°C[16]. The extreme stability of 25(OH)D_3 in serum is likely due to its tight binding to vitamin D binding protein, and the opacity of serum in tubes to ultraviolet (UV) irradiation[15]. Moreover, repeated freezing and thawing did not significantly affect serum concentrations of 25(OH)D_3[17].

Measurement of serum 25(OH)D_3

Serum levels of 25(OH)D_3 were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), a method simpler, and with greater rapidity, accuracy, sensitivity, and cost-effectiveness, than commercial radioimmunoassays[18]. Another important advantage of the LC-MS/MS method is its ability to separate 25(OH)D_3 and 25(OH)D_2 [18]. The LC-MS/MS system consisted of an Agilent 1260 HPLC system (Agilent, Germany) with an Agilent 6460 triple quadrupole mass spectrometer (Agilent, Singapore) equipped with an electrospray ionization source. Calibration curves of 25(OH)D_3 were linear over the ranges studied, with r^2>0.999. The coefficients of variations of the 25(OH)D_3 (intra-batch and inter-batch) were below 6.12%, and the accuracy ranged from 90.8% to 113%. Cut-off values for serum 25(OH)D_3 were set at 20 ng/ml (deficiency) and 30 ng/mll(insufficiency).

Statistical analysis

All statistical analyses were performed by using IBM SPSS 21 Statistics1 software (IBM, USA). Serum 25(OH)D_3 concentrations are reported as mean and standard deviation (SD). The normality and equality of variances of 25(OH)D_3 were tested using the Shapiro-Wilk test and Levene's test, respectively. Linear changes in mean 25(OH)D_3 concentrations were analyzed by the General Linear Model (GLM) method when the rates of vitamin D deficiency and insufficiency differed over time among all subjects or by age or sex. Differences among age groups in mean serum 25(OH)D_3 over the total time period were assessed by analysis of variance (ANOVA). Changes in rates of vitamin D deficiency and insufficiency in the total population, age group, and sex were analyzed using the linear trend test. Differences in serum 25(OH)D_3 concentrations among age groups and by sex were determined using the X^2-test.

Ethics Statement

The study protocol was approved by the Institutional Review Board of the GNUH (GNUHIRB-2013-02-002). An agreement exemption was applied because the serum samples included no genetic information and were derived from the National Biobank of Korea. Thus, under institutional review board-approved protocols, these samples were obtained with informed consent.

Results

A total of 948 serum samples from children aged 1 to 15 years were tested (Table 1). The mean concentrations of serum 25(OH)D_3 decreased significantly over time (P<0.001), being 37.5 ng/ml in 1995–1996, 34.9 ng/ml in 2000–2001, 31.4 ng/ml in 2005–2006, and 22.5 ng/ml in 2010–2011. Mean serum 25(OH) D_3 concentrations decreased in all age groups from 1995–1996 to 2010–2011 (P <0.001). Excluding 1995–1996, the mean level of serum 25(OH)D_3 decreased as subject age increased (P<0.001, Fig. 1).

Mean serum 25(OH)D_3 concentrations were higher in males than in females among total subjects (P<0.001, Fig. 2). The mean level and distribution of serum 25(OH)D_3 were similar in males and females during 1995–1996 (P=0.420) and 2000–2001 (P=0.478), but were lower in females after 2005–2006 (P<0.001, Table 1). After 2000–2001, the mean level of serum 25(OH)D_3 was lowest in the 11–15 years' age group (P<0.001, Fig. 1), being particularly lower in this age group in 2010–2011 than in the other three periods.

The proportions of subjects with vitamin D deficiency increased from 1995–1996 to 2010–2011 (P<0.001, Fig.3), being 3.6% in 1995–1996, 10.7% in 2000–2001, 15.1% in 2005–2006, and 38.6% in 2010–2011. Similarly, the proportions of vitamin D insufficiency increased, being 26.4%, 28.5%, 30.5%, and 45.0%, respectively, during these four time periods. The proportion of subjects with vitamin D deficiency and insufficiency was highest in the 11–15 yrs. age group after 2000. In 2010–2011, 93.0% of children aged 11–15 yrs. and 90.9% of children aged 6–10 yrs. had vitamin D deficiency or insufficiency. The proportion of subjects with vitamin D deficiency and insufficiency increased with age (P<0.001) and was significantly higher in females than in males regardless of study period (P<0.001, Table 2).

Discussion

These results show that, from 1995 to 2011, serum concentrations of 25(OH)D_3 have declined significantly, while the rates of vitamin D deficiency and insufficiency have increased significantly in Korean children aged 1 to 15 yr. After 2000, the prevalence of vitamin D deficiency and insufficiency was significantly higher in subjects aged 11–15 yrs. than in the other two age groups.

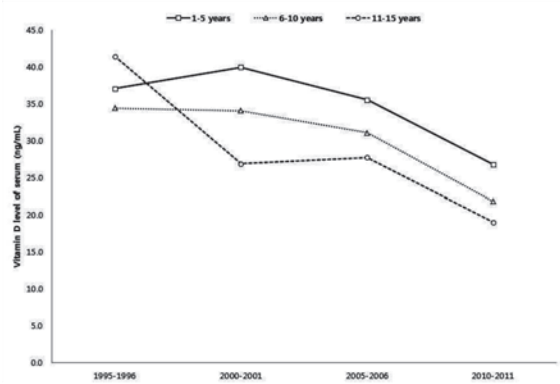

Fig. 1. Mean serum 25(OH)D_3 concentrations according to age groups during four sampling periods between 1995–1996 and 2010–2011. Mean serum 25(OH)D_3 began to decrease from 1995–1996 to 2010–2011 and was significantly lower in 2010–2011 than in the other time periods (P<0.001).

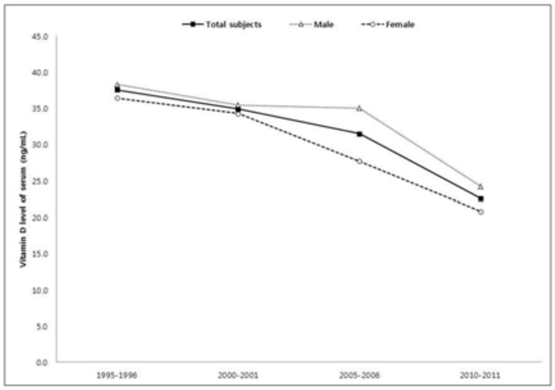

Fig. 2. Mean serum 25(OH)D_3 concentrations according to gender during four sampling periods. Mean serum 25(OH) D_3 was significantly higher in males than in females in 2005–2006 and in 2010–2011 (P<0.001).

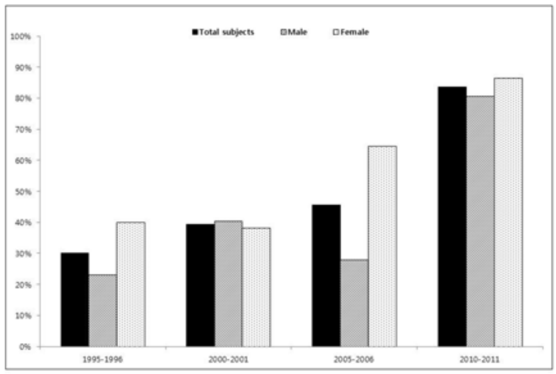

Fig. 3. Mean serum 25(OH)D_3 concentrations according to gender during four sampling periods. Mean serum 25(OH) D_3 was significantly higher in males than in females in 2005–2006, and in 2010–2011 (P<0.001).

Table I. Serum 25(OH)D_3 Concentrations During Each Time Period in Total Subjects and in Subjects Assorted by Age and Gender.

		1995-1996		2000-2001		2005-2006		2010-201		P value*	Total		P value**
		N	mean (±SD)	N	mean (±SD)	N	mean (±SD)	N	mean (±SD)		N	mean (±SD)	
All subjects		110	37.5 (±11.8)	242	34.9 (±12.5)	298	31.4 (±10.6)	298	22.5 (±8.2)	<0.001	948	30.2 (±12.0)	
Age groups	1-5 yrs.	65	37.0 (±11.3)	96	40.0 (±12.3)	100	35.5 (±10.9)	99	26.8 (±9.3)	<0.001	360	34.6 (±12.1)	<0.001
	6-10 yrs.	21	34.4 (±12.1)	94	34.0 (±11.9)	99	31.1 (±8.8)	99	21.8 (±6.6)	<0.001	313	29.3 (±10.8)	
	11-15 yrs.	24	41.4 (±12.2)	52	26.9 (±9.0)	99	27.7 (±10.7)	100	19.0 (±6.6)	<0.001	275	25.6 (±11.1)	
Gender	Male	65	38.3 (±11.4)	124	35.4 (±13.0)	154	35.0 (±9.6)	150	24.2 (±8.2)	<0.001	493	32.3 (±11.7)	<0.001
	Female	45	36.4 (±12.4)	118	34.3 (±11.9)	144	27.7 (±10.4)	148	20.8 (±7.9)	<0.001	455	28.0 (±11.8)	

*by general linear model for linear trends.
** by ANOVA.

The major source of vitamin D for children and adults is exposure to natural sunlight[19-21]. Very few foods naturally contain or are fortified with vitamin D. Thus, the major cause of vitamin D deficiency is inadequate exposure to sunlight[22]. Among these children, the highest median serum 25(OH)D_3 concentrations and the lowest prevalence of vitamin D deficiency were found in 1995–1996 compared with the other three periods. The southern part of Korea experienced a severe drought from February 1994 to March 1996 due to extreme summer weather events[23]. In Jinju, the cumulative hours of sunshine were longest and the amount of precipitation were smallest during the summer of 1995 than in the other years studied (Meterological Database, Korea Meterological Administration).

A study of 1,212 Korean children aged 4 to 15 years from 2012 to 2013 found that rates of vitamin D deficiency increased with age, being 44.3%, 56.8%, 63.0%, and 77.0% in children aged 4–6, 7–9, 10–12, and 13–15 years, respectively[14]. This study also found that vitamin D deficiency increased with age since 2000, and that the median serum 25(OH)D_3 concentration was lower in subjects aged 11–15 yrs. than in those aged 1–5 and 6–10 yrs. A study using data from The Korean National Health and Nutrition Examination Survey (KNHANES) 2008–2009 reported that the risk factors for low vitamin D concentration in 1,510 healthy adolescents aged 12–18 years were winter season, older age, higher educational level, female gender, obesity, lack of vitamin D supplementation, lower milk consumption, and lack of physical activity[11]. The serum samples in this study were obtained from the National Biobank; thus clinical data, such as body weight, vitamin D supplementation, milk consumption, and physical activity, were unavailable for the study population. However, our finding that the prevalence of vitamin D deficiency was higher in adolescents than in younger children might be related to the tendency of Korean adolescents to limit outdoor activities as they prepare for entrance into high-ranking schools[24].

The time spent by Korean adolescents in screen-based sedentary activities has increased dramatically in the past decade[24]. At present, Korean adolescents spend most of their free time in after-school programs/tutoring (57.9%), staying home (15.9%), and online gaming

Table II. Proportions of Total Subjects, and Subjects Assorted by Age and Gender, with Vitamin D Deficiency During Each Time Period

	1995-1996			2000-2001			2005-2006			2010-2011			P value*	Total			P value**
	deficiency	insufficiency	sufficiency	deficiency	insufficiency	sufficiency	deficiency	insufficiency	sufficiency	deficiency	insufficiency	sufficiency		deficiency	insufficiency	sufficiency	
Total subject, N (%)	4 (3.6)	29 (26.4)	77 (70.0)	26 (10.7)	69 (28.5)	147 (60.7)	45 (15.1)	91 (30.5)	162 (54.4)	115 (38.6)	134 (45)	49 (16.4)	<0.001	190 (20.0)	323 (34.1)	435 (45.9)	
Age group 1 - 5 years	1 (1.5)	19 (29.2)	45 (69.2)	3 (3.1)	19 (19.8)	74 (77.1)	6 (6.0)	26 (26.0)	68 (68.0)	23 (23.2)	43 (43.4)	33 (33.3)	<0.001	33 (9.2)	107 (29.7)	220 (61.1)	<0.001
6 - 10 years	2 (9.5)	6 (28.6)	13 (61.9)	9 (9.6)	31 (33.0)	54 (57.4)	9 (9.1)	40 (40.4)	50 (50.5)	38 (38.4)	52 (52.5)	9 (9.1)	<0.001	58 (18.5)	129 (41.2)	126 (40.3)	
11-15 years	1 (4.2)	4 (16.7)	19 (79.2)	14 (26.9)	19 (36.5)	19 (36.5)	30 (30.3)	25 (25.3)	44 (44.4)	54 (54.0)	39 (39.0)	7 (7.0)	<0.001	99 (36.0)	87 (31.6)	89 (32.4)	
Gender Male	2 (3.1)	13 (20.0)	50 (76.9)	12 (9.7)	38 (30.6)	74 (59.7)	9 (5.8)	34 (22.1)	111 (72.1)	43 (28.7)	78 (52.0)	29 (19.3)	<0.001	66 (13.4)	163 (33.1)	264 (53.5)	<0.001
Female	2 (4.4)	16 (35.6)	27 (60.0)	14 (11.9)	31 (26.3)	73 (61.9)	36 (25.0)	57 (39.6)	51 (35.4)	72 (48.6)	56 (37.8)	20 (13.5)	<0.001	124 (27.3)	160 (35.2)	171 (37.6)	

*by linear trend test.
** by chi square test

(10.2%)[25]. The prevalence of internet usage among Korean adolescents aged 16 to 19 yrs. has increased dramatically, from about 50% in 1999 to 90.6% in 2002, almost reaching the market saturation point[26]. Internet usage by children aged 6–10 yrs. may be associated with the increased prevalence of vitamin D deficiency over time, from 9.1% in 2005–2006 to 38.4% in 2010–2011.

Another potential cause of increased vitamin D deficiency and insufficiency may be increased sunscreen use by children. Sunscreens block the cutaneous absorption of UV-B radiation, thus interfering with vitamin D_3 synthesis in the skin[25,26]. In agreement with previous findings[27-29], our study found that the prevalence of vitamin D deficiency or insufficiency was higher in girls than in boys. Korean girls have been reported to engage in lower levels of physical activity and have lower calcium intake than Korean boys[29]. Girls are also more likely to use sunscreen. Serum 25(OH)D concentrations showed a negative correlation with soft drink intake in Korean schoolgirls[30]. Even in summer, 84% of 205 Korean children aged 7 to 9 yrs. had serum 25(OH)D_3 concentrations under 30 ng/ml in 2011[12], which may be related to sunscreen use.

This study found that the prevalence of vitamin D deficiency was lower in subjects aged 1–5 yrs. than in those aged 6–10 and 11–15 yrs. Few studies have assessed vitamin D deficiency in Korean children under the age of 5 years. Two studies reported that vitamin D deficiency in infants aged 1 to 6 months and in normal children under age 24 months was associated with breastfeeding[31,32]. Since clinical data, including nutritional status, could not be evaluated in our study population, we could not determine the reason for the lower rate of vitamin D deficiency in children aged 1–5 yrs. than in older children. However, children aged 1–5 yrs. may spend more time outdoors and participate in a greater degree of physical activity than children aged 6–15 yrs.

Serum concentrations of 25(OH)D_3 have been reported to be inversely associated with body mass index, waist circumference, and body fat mass[12], a finding consistent with the results of the KNHANES 2008–2009 study[11]. In Korea, height, weight, and body mass index have increased dramatically from 1965–1997 to

1997–2007[33]. Increases in height and weight may have influenced 25(OH)D_3 concentrations among our study population, although this information was not available.

Childhood vitamin D deficiency may be an important health problem because vitamin D is essential for normal growth and skeletal development[2]. Moreover, childhood vitamin D deficiency has been associated with the development of diabetes and metabolic syndrome in adults[34]. The levels of insulin, homeostasis model assessment of insulin resistance (HOMA-IR) score, and systolic blood pressure were found to be higher in Korean girls with than without vitamin D deficiency[30]. Moreover, serum 25(OH)D_3 levels were inversely associated with HOMA-IR score and concentrations of triglycerides, and low-density lipoprotein cholesterol in Korean adolescents aged 12–13 yrs.[13].

Vitamin D deficiency in children may be prevented by vitamin supplementation and participation in outdoor activities. In addition, fortification of foods with vitamin D may be effective. Appropriate guidelines for the use of topical sunscreen in children should also be established to minimize the effects of sunscreen on cutaneous vitamin D production. Schools should also change their policies and guidelines to promote outdoor activities.

This study had several limitations. First, the small sample size and the different sizes of the study subgroups may have influenced the statistical analysis. Second, the nutritional status and clinical data, including body weight, height, and body mass index, were not available. In addition, our subject sample was drawn from one geographic region in Korea, and the data were acquired from independent random samples collected for 20 years rather than from a longitudinal cohort study, precluding a determination of factors associated with vitamin D deficiency, such as insufficient dietary vitamin D intake, sunlight exposure time, or outdoor activity time. Further epidemiologic studies assessing factors associated with serum vitamin D levels in children may provide insight into this condition.

However, this study also had several strengths. For example, assessment of serum samples collected over 20 years enabled a determination of trends in serum vitamin D concentrations in children over time. Although previous studies have reported a high prevalence of vitamin D deficiency in Korean adolescents, this study, to our knowledge, is the first to evaluate secular trends in serum vitamin D levels in Korean children for two decades. Our findings clearly indicate that the prevalence of vitamin D deficiency is increasing in children in urban areas in Korea. Our data have important implications for public health recommendations.

In conclusion, this study showed that vitamin D deficiency or insufficiency in children and adolescents in Jinju, Korea, increased from 1995–1996 to 2010–2011, while mean serum vitamin D concentrations decreased, especially in adolescents. These results may be associated with changing lifestyles among Korean children and suggest that outdoor physical activity and sufficient vitamin D intake should be strongly encouraged in these children.

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