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Research

Suboptimal vitamin D status in Korean adolescents: A nation-wide study on its prevalence, risk factors including cotinine verified smoking status and association with atopic dermatitis and asthma

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Short title: Suboptimal vitamin D status in Korean adolescents

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ABSTRACT

Objective: To determine the prevalence and risk factors for suboptimal vitamin D status in Korean adolescents, and to assess its relationship with atopic dermatitis and asthma.

Design: This is a cross-sectional study with data from the Korean National Health and Nutrition Examination Survey. Information regarding socioeconomic characteristics, clinical data and environmental factors was collected. Blood and urine samples were taken for vitamin D and cotinine respectively. Descriptive and multivariable logistic regression was performed on the data.

Setting: Korea (Nation-wide)

Participants: 2,515 individuals aged 10-18 years who participated in the Korean National Health and Nutrition Examination Survey from 2008-2011.

Main outcome measures: Vitamin D status was determined through measurement of serum 25(OH)D. Smoking status was classified based on the urine cotinine level. Physician diagnosed AD and asthma were assessed using a questionnaire.

Results: Overall, 73.3% of the subjects were vitamin D deficient. Older age (p<0.0001), female gender (p<0.0001), urban residence (p=0.0189), higher body mass index (p=0.0034) and sampling in winter months (November-March) (p<0.0001) were independently associated with low serum 25(OH)D levels. With cotinine verification, 18.2% of the participants were classified as active smokers, 43.4% passive smokers, and 38.4% non-smokers. After adjusting for potential cofounders, serum 25(OH)D status showed no association with AD or asthma at the national level.

Conclusion: Vitamin D deficiency is highly prevalent in Korean adolescents, which should be a matter of public health concern. The cotinine-verified prevalence of smoking was also high in this population, but its relationship with vitamin D deficiency was not confirmed in our study. Our results provide epidemiologic evidence against the association of vitamin D status with AD and asthma at the national level among Korean adolescents.

Article summary

Strengths and limitations of this study

- One of the first studies to identify the prevalence and risk factors for vitamin D deficiency in Korean adolescents.
- The relationship of vitamin D with AD and asthma were assessed at a national level.
- Cotinine-verified smoking status was adopted in this study.
- Limitations of the study include its cross-sectional design and lack of data on dietary and supplemental vitamin D intake.
- Also, the study has recall bias because diagnoses of allergic conditions (AD and asthma) were self-reported.

INTRODUCTION

Vitamin D is essential for skeletal health and bone growth and its deficiency has been associated with skeletal deformities in children and fracture risk in adults. Recent studies have also identified the previously unanticipated roles of vitamin D in the immune system, cardiovascular system and cancer prevention.[1,2] Despite growing health concerns over low vitamin D status, few nation-wide studies have been performed to evaluate vitamin D deficiency in the Korean pediatric population.[3,4] Suboptimal vitamin D level was reported in 70% of the US children (9% vitamin D deficient, 61% vitamin D insufficient),[5] and it was found in 41.7% of the children from New Zealand).[6] Korean adolescents are theoretically at increased risk for vitamin D deficiency because of the high latitude (34-38ºN), culturally vigorous sun protection, reduced outdoor activity and lack of vitamin D-fortified food.

The increasing prevalence of allergic diseases is a world-wide phenomenon and it is strikingly more evident in the younger population compared to adults.[7] Vitamin D has immunomodulatory functions, and its relationship with allergic disease has been evaluated in a number of studies.[8-15] While some authors have reported about the protective role of vitamin D in atopic dermatitis (AD), asthma, allergic rhinitis (AR) and allergic sensitization in childhood, other authors have shown a deleterious effect. Although the exact cause of such conflicting results is not known, racial difference may be a contributing factor. Unfortunately, there are limited nation-wide studies on vitamin D and allergic disease and they were mainly performed in western countries.

In this study, we aimed to identify the prevalence and risk factors for vitamin D deficiency in Korean adolescents and to assess its relationship with AD and asthma at the national level. Cotinine-verified smoking status was adopted in this study.

METHODS

Study population

This study was based on data acquired from the Korean National Health and Nutrition Examination Survey (KNHANES), a survey conducted by the Korea Centers for Disease Control and Prevention to provide nationally representative and reliable statistical data regarding the health, behavior associated with health, nutrition, and food intake status of the Korean population. Data were collected from 2008 to 2011, which corresponds to the second and third year of KNHANES IV (2007-2009) and the first and second year of KNHANES V (2010-2012). The survey included a health interview, a nutritional survey, physical examination, and blood and urine tests. The institutional review board at the Korea Centers for Disease Control and Prevention approved the protocol, and all participants signed informed consent forms.

Both KNHANES IV and V adopted the stratified multistage cluster sampling design by using the rolling-survey sampling method. The rolling sample collected each year is the probability sample representing the general Korean population, and it is homogeneous and independent of each other. In 2008, 2009, 2010 and 2011, a total of 37,753 individuals were sampled. The study population was further limited to 4,598 adolescents aged 10-18 years. Among the 4,598 participants, we subsequently excluded the following participants: those whose 25(OH)D levels were not measured; those without urine cotinine levels; those who did not completely answer the questions regarding AD or asthma; those without body mass index (BMI) measurement; and those who had a chronic disease that may affect vitamin D metabolism. Finally, a total of 2,515 participants (1,314 males and 1,201 females) were eligible for analysis.

Study variables

Blood samples were collected from the antecubital vein, refrigerated immediately, transported to the central testing facility in cold storage, and analyzed within 24 hours of sampling. Serum 25(OH)D levels were measured as described previously[16] and categorized as sufficient (≥30 ng/mL), insufficient (20-29.9 ng/mL) or deficient (<20 ng/mL).[17]

Factors were categorized to analyze the risk factors for vitamin D deficiency. Age and BMI were

continuous variables while season of sampling was categorized into winter months (November-March) and summer months (April-October).[18] The region of residence of each participant was grouped as follows: urban (Seoul, Gyeonggi, Busan, Daegu, Incheon, Gwangju, Daejeon, and Ulsan) and rural (Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju).[19] Monthly income was standardized according to the number of family members (monthly income/number of family members) and it was divided into the following 4 quartile groups: lowest, lower middle, higher middle, and highest. Participants who performed moderate physical activity for more than 30 minutes per day on more than 5 days a week and/or strenuous physical activity for more than 20 minutes per day on more than 3 days a week were assigned to the regular exercise group. Regular walking was designated as "yes" for those who walked for more than 30 minutes per day on more than 5 days a week.[19] Smoking status was divided into three groups based on the urine cotinine level, which are as follows: non-smokers (<5 ng/mL), passive smokers (secondhand smoking) (5-100 ng/mL) and active smokers (>100 ng/mL).[19-21] Urine cotinine level was measured by chromatography mass spectrometry using the Perkin Elmer Clarus 600T (PerkinElmer, Turku, Finland). All data were measured in a standardized manner and reviewed by the central quality control center.

The following question was used to assess physician-diagnosed AD in each participant: "Have you been diagnosed with AD by a doctor?" or "Have you been told by a doctor that (your child) had AD? Physician-diagnosed asthma was also determined using similar questions.

Statistical analysis

Statistical analyses were performed using an SAS survey procedure (version 9.2; SAS Institute, Inc., Cary, NC, USA), and 2-sided *p* values of less than 0.05 were considered statistically significant. To produce unbiased national estimates representing the general Korean population, we used KNHANES sample weights accounting for the complex sampling design to each participant.

To compare the mean serum 25(OH)D levels among categories of each possible predicting factor, Student's *t*-test or ANOVA (followed by Tukey-Kramer for multiple comparison) was used.

Univariate analysis was performed to evaluate the association of the possible predicting factors, AD and asthma with vitamin D deficiency. Participants' characteristics were described using means and

standard errors for continuous variables and numbers and percentages for categorical variables. Here, the Student's *t*-test or ANOVA was used for comparing continuous variables, as appropriate, and Rao-Scott χ^2 test was used for comparing categorical variables. Variables with a *p*-value <0.05 in univariate analyses were included in the multivariate regression model for exploring factors associated with serum 25(OH)D.

To estimate the mean serum 25(OH)D levels in participants with and without AD and asthma, we performed simple and multiple linear regression analyses using the generalized linear model for a complex survey design. The estimated means were calculated as follows: no adjustment for potential confounders; and confounder adjustment for age, gender, region, BMI, smoking, and season of sampling.

To estimate the odds ratios (ORs) for AD and asthma according to quartiles of serum 25(OH)D levels, we conducted simple and multivariate logistic regression analyses by using the generalized linear model for a complex survey design. The ORs and 95% CIs were calculated in the following ways: no adjustment for potential confounders; and confounder adjustment for age, gender, region, BMI, smoking, and season of sampling.

RESULTS

General characteristics

A total of 2,515 subjects (1,314 males and 1,201 females, age: 14.4 ± 0.1 years) were included in the study. Table 1 summarizes the baseline data of the participants. The mean BMI of the study population was 20.9 kg/m^2 (SE: 0.1). Blood samples were drawn more frequently in April-October (58.4%) than in November-March (41.62%). The urine cotinine-verified smoking statuses were as follows: active smoking 18.2%, passive smoking 43.4%, and non-smoking 38.4%. The prevalences of AD and asthma in the study population were 10.6% and 4.1%, respectively.

Serum 25(OH)D levels in the study population

The mean concentration of serum 25(OH)D in the 2,515 subjects was 16.7 ng/mL (SE: 0.2), with a range of 3.0 to 46.2 ng/mL. Overall, 1,843 subjects (73.3%) were vitamin D deficient, 613 subjects (24.4%) were vitamin D insufficient, and 59 subjects (2.3%) were vitamin sufficient (Figure). Girls had a significantly lower mean serum 25(OH)D level than boys (16.0 ± 0.2 ng/mL vs 17.3 ± 0.2 ng/mL, p<0.0001), and subjects sampled in winter months (November-March) had lower serum 25(OH)D levels than those sampled in summer months (April-October) (14.7 ± 0.2 ng/mL vs 18.1 ± 0.2 ng/mL, p<0.0001). Passive smoking (vs non-smoking) and urban residence (vs rural) were also associated with statistically lower serum 25(OH)D levels (p=0.0382 and p=0.0024, respectively) (Table 2).

Univariate and multivariate analyses of factors associated with serum 25(OH)D

Univariate analysis revealed that age (p<0.0001), gender (p=0.0031), region of residence (p=0.0108), season of sampling (p<0.0001), BMI (<0.0001) and smoking status (p=0.0075) were associated with vitamin D deficiency (Table 1).

Multivariate linear regression analysis of serum 25(OH)D levels was performed using variables that had a p-value <0.05 in univariate analyses. Older age (p<0.0001), female gender (p<0.0001), urban residence (p=0.0189), higher BMI (p=0.0034) and sampling in winter months (November-March) (p<0.0001) were independently associated with low serum 25(OH)D levels (Table 3). Multivariate ordinal logistic regression analysis of serum 25(OH)D showed similar results (Table 4).

Relationship of serum 25(OH)D with AD and asthma

Univariate analysis demonstrated that vitamin D deficiency was not significantly associated with AD (p=0.4675) or asthma (p=0.5376) (Table 1).

Multivariate logistic regression showed that vitamin D deficiency had no association with AD and asthma after adjusting for age, gender, region of residence, BMI, smoking, and season of sampling. The adjusted odds ratio for AD and asthma increased across categories of serum 25(OH)D (1.00 [reference] for ≥ 30 ng/mL, 1.14 and 4.87 each for 20-29.9 ng/mL, and 1.52 and 5.31, respectively for <20 ng/mL), but it was not statistically significant (p=0.8078 (AD) and p=0.1360 (asthma) for 20-29.9 ng/mL and p=0.4271 (AD) and p=0.1042 (asthma) for <20 ng/mL) (Table 6).

Univariate or multivariate analyses with serum 25(OH)D levels as a continuous variable showed similar results, regardless of the adjustment (Table 6).

DISCUSSION

Vitamin D deficiency has been reported to be highly prevalent in a number of countries[1] and there are studies suggesting a possible link between vitamin D status and allergic diseases.[8-15] In this study, we examined the prevalence of vitamin D deficiency in Korean adolescents and set out to determine its relationship with AD and asthma at the national level. Our study results emphasize a public health issue and suggest that a substantial proportion of Korean children are vitamin D deficient. It is significant in those who are older in age, females, who live in the city, have a higher BMI and subjects sampled in winter months (November-March). Our findings are in line with those from prior studies[3,4] which also claimed that lack of vitamin D supplementation and parental vitamin D deficiency are risk factors for vitamin D deficiency in Korean adolescents. With respect to the relationship with allergic diseases, our data does not support association of vitamin D status with AD and asthma.

This nation-wide study is meaningful as it is the first to measure both serum 25(OH)D and urine cotinine in a population of 2,515 Korean adolescents. Smoking has been quoted as a significant determinant of serum 25(OH)D in a number of studies[20-23] including the one from Taiwan,[23] where passive smoking was independently associated with low serum 25(OH)D. To identify the effect of smoking on serum 25(OH)D in Koreans, we adopted urine cotinine, which is presently the biomarker of choice for assessing tobacco smoke exposure.[24-27] KNHANES normally assesses a person's smoking status via a survey. For those who are aged 19 years and above, detailed questions are asked regarding the presence and history of active and passive smoking, but for those who are aged less than 19 years, the questions are much more limited. There is currently no data with regards to passive smoking in Korean adolescents and the survey only determines the presence of active smoking in subjects aged between 12-18 years. Omissions and false responses are common in this age group[28] which impede accurate assessment of smoking status, and in order to improve accuracy, we adopted the cotinine-verified smoking status in our study. Urine cotinine level was measured for a limited time period (2008-2011) and this measurement has only been performed for those aged 10 years and above, which resulted in an inevitable reduction of our study population. We used a urine cotinine cut off value of more than 100 ng/mL to discriminate smokers from non-smokers and 5 ng/mL as a threshold

for non-smokers exposed to secondhand smoking based on published values.[24-26] With cotinine verification, 18.2% of the participants were classified as active smokers, 43.4% of the participants were classified as passive smokers, and 38.4% of the participants were classified as non-smokers, where the percentage of smokers (active and passive) was higher than what we expected.

In this study, the mean concentration of serum 25(OH)D was 16.7 ng/mL with 73.3% of the subjects being vitamin D deficient (serum 25(OH)D <20 ng/mL). Potential explanations for this remarkably low vitamin D status observed in Korean children include, but are not limited to pigmented skin (skin pigmentation is known the reduce the skin's production of vitamin D in East Asian populations),[3] vigorous use of sunscreens and possibly dietary factors. The Dietary Reference Intakes for vitamin D from the Institute of Medicine (2010) and the American Academy of Pediatrics state that the Adequate Intake (AI) of vitamin D is 15 μg/day for children.[29,30] However, in the Dietary Reference Intakes for vitamin D for Koreans published in 2010, the AI of vitamin D for children is claimed to be 5 μg/day,[31] which we feel is too low. Our study results call for careful consideration of vitamin D supplementation, particularly in at-risk children to further optimize their health.

Exposure to sunlight, specifically UVB, leads to vitamin D synthesis in the skin, which provides most of the vitamin D requirement of an individual. In Korea (latitude: 34-38ºN), vitamin D is synthesized mostly between April and October (summer months),[32] which was apparent in our study.

The finding of an inverse relationship of age, female gender, urban residence, and BMI with vitamin D status is in accordance with previous studies.[3,4,23,33,34] It has been previously shown that there is a consistent decline in physical outdoor activity over the school age years. The prevalence of physical inactivity is higher in girls than in boys and it is greater in obese children than in their normal-weight peers. The lifestyle in big cities is also sedentary, and it is therefore possible that age, gender, place of residence and BMI may act as surrogate indicators for sunlight exposure through their association with physical activity. In addition to limited sunlight exposure due to physical inactivity, the inverse association of BMI and urban residence with vitamin D levels can be explained by sequestration of vitamin D in fat tissues[35] and UVB blockade due to air pollution.

Smokers have been reported to have a significantly higher risk of vitamin D deficiency,[20-23] but this

was not confirmed in our study. Since active smoking is usually done outdoors, this can result in greater exposure to sunlight which may alter the true effect of smoking on serum 25(OH)D. Passive smoking in adolescents is more likely to occur indoors, and interestingly enough, passive smoking was reported to be independently associated with low serum 25(OH)D in Taiwanese children/adolescents.[23] Results can also be influenced by cotinine cut-off values and higher thresholds such as 10 ng/mL or 30 ng/mL may be considered in the future when testing children as this population can have higher cotinine concentrations than adults due to differences in body distribution and nicotine metabolism.[36]

There is accumulating evidence indicating that an adequate concentration of vitamin D is protective against allergic disease in children.[37,38] However, most of these findings have been reported in western countries and only a few studies have been performed in Asia. We consider our investigation important because differences among ethnicities and subjects of various age groups can possibly affect the results.

In this national level study, AD and asthma were not associated with serum 25(OH)D. The present study results, as well as previous studies showing an association between high vitamin D levels and AD, raise questions on the suggestion that vitamin D may be used to prevent or treat AD. In fact, a Cochrane review found no evidence for an effect of vitamin D supplementation on AD.[39] The beneficial effect of sunlight on AD has been well documented, but this is probably due to the anti-inflammatory and antimicrobial effects of UV and not those of vitamin D.

Consistent with our results, Hollams *et al.*[40] showed no significant cross-sectional association between serum vitamin D levels and current asthma in 6- or 14-year-old children in UK. Further studies examining the role of serum 25(OH)D levels as predictors of subsequent asthma are needed during the longitudinal follow-up of cohort subjects.

The prevalences of AD and asthma in the present study (10.6% and 4.1%, respectively) differed slightly from those in the other population-based studies in Taiwan[23] and Japan.[41] The variation in prevalence in different countries may be caused by several factors, including genetic, socioeconomic and environmental factors.

This study has several notable strengths. The sampling of adolescents across a broad age range, a large sample size with robust data collection, incorporation of objective markers of smoking status, and a thorough analysis strengthen the results of this study. Limitations of the study include its cross-sectional design and lack of data on dietary and supplemental vitamin D intake. Also, the study is prone to recall bias because diagnoses of allergic conditions (AD and asthma) were self-reported.

CONCLUSION

Vitamin D deficiency is highly prevalent in Korean adolescents. It is a significant public health concern, and optimal vitamin D intakes to maintain sufficient vitamin D status should be examined. The cotinine-verified prevalence of smoking was also high in this population, but its relationship with vitamin D deficiency was not confirmed in our study. Above all, our results provide epidemiologic evidence against the association of vitamin D status with atopic dermatitis and asthma in Korean adolescents. The finding is obtained at the national level and is meaningful, but at the same time, it merits further study.

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Contributors EJB, SHC, JDL, and HSK made substantial contributions to the conception or design of the work. JYH and HSK had role in the acquisition, analysis and interpretation of data tor the work. EJB, SHC, JDL, and HSK drafted and revised the work and all authors gave final approval and agreed to be accountable for all aspects of the work.

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Competing interests None declared.

Ethics approval The institutional review board at the Korea Centers for Disease Control and Prevention approved the protocol

Data sharing statement All data from the study, published and unpublished, are available to the principle investigators. The data are managed under the Department of Dermatology, Incheon St. Mary's Hospital, The Catholic University of Korea, Incheon, Korea.

REFERENCES

1. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 257: 266-81.
2. Cheng HM, Kim S, Park GH, et al. Low vitamin D levels are associated with atopic dermatitis, but not allergic rhinitis, asthma, or IgE sensitization, in the adult Korean population. *J Allergy Clin Immunol* 2014; 133: 1048-55.
3. Kim SH, Oh MK, Namgung R, *et al.* Prevalence of 25-hydroxyvitamin D deficiency in Korean adolescents: association with age, season, and parental vitamin D status. *Public Health Nutr* 2014; 17: 122-30.
4. Lee YA, Kim HY, Hong H, *et al.* Risk factors for low vitamin D status in Korean adolescents: the Korea National Health and Nutrition Examination Survey (KNHANES) 2008-2009. *Public Health Nutr* 2014; 17: 764-71.
5. Kumar J, Muntner P, Kaskel FJ, *et al.* Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. Pediatrics 2009; 124: e362-70.
6. Rockell JE, Green TJ, Skeaff CM, *et al.* Season and ethnicity are determinants of serum 25-hydroxyvitamin D concentrations in New Zealand children aged 5-14 y. *J Nutr* 2005; 135: 2602-8.
7. Asher MI, Montefort S, Bjorksten B, *et al.* Worldwide time trends in the prevalence of symptoms of asthma, allergic rhino-conjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multi-country cross-sectional surveys. *Lancet* 2006; 26: 733-43.
8. Bener A, Ehlayel MS, Tulic MK, *et al.* Vitamin D deficiency as a strong predictor of asthma in children. *Int Arch Allergy Immunol* 2012; 157: 168-75.
9. Wjst M, Hypponen E. Vitamin D serum levels and allergic rhinitis. *Allergy* 2007; 62: 1085-6.
10. El Taieb MA, Fayed HM, Aly SS, *et al.* Assessment of serum 25-hydroxyvitamin D levels in children with atopic dermatitis: correlation with SCORAD index. *Dermatitis* 2013; 24: 296-301.
11. Sharief S, Jariwala S, Kumar J, *et al.* Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. *J Allergy Clin Immunol* 2011; 127: 1195-202.
12. Berents TL, Lodrup Carlsen KC, Mowinckel P, *et al.* Vitamin D levels and atopic eczema in infancy and early childhood in Norway: a cohort study. *Br J Dermatol* 2016; 175: 95-101.
13. Barman M, Jonsson K, Hesselmar B, *et al.* No association between allergy and current 25-hydroxyvitamin D in serum or vitamin D intake. *Acta Paediatr* 2015; 104: 405-13.

14. Chiu YE, Havens PL, Siegel DH, *et al.* Serum 25-hydroxyvitamin D concentration does not correlate with atopic dermatitis severity. *J Am Acad Dermatol* 2013; 69: 40-6.
15. Yang HK, Choi J, Kim WK, *et al.* The association between hypovitaminosis D and pediatric allergic disease: A Korean nationwide population-based study. *Allergy Asthma Proc* 2016; 37: 64-9.
16. Nam GE, Kim DH, Cho KH, *et al.* 25-Hydroxyvitamin D insufficiency is associated with cardiometabolic risk in Korean adolescents: the 2008-2009 Korea National Health and Nutrition Examination Survey (KNHANES). *Public Health Nutr* 2014; 17: 186-94.
17. Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al.* Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J Clin Endrocrinol Metab* 2011; 96: 1911-30.
18. McKinney K, Breitkopf CR, Berenson AB. Association of race, body fat and season with vitamin D status among young women: a cross-sectional study. *Clin Endocrinol (Oxf)* 2008; 69: 535-41.
19. Choi HS, Oh HJ, Choi H, *et al.* Vitamin D insufficiency in Korea- a greater threat to younger generation: The Korea National Health and Nutrition Examination Survey (KNHANES) 2008. *J Clin Endocrinol Metab* 2011; 96: 643-51.
20. Kassi EN, Stavropoulos S, Kokkoris P, *et al.* Smoking is a significant determinant of low serum vitamin D in young and middle-aged healthy males. *Hormones (Athens)* 2015; 14: 245-50.
21. Manavi KR, Alston-Mills BP, Thompson MP, *et al.* Effect of serum cotinine on vitamin D serum concentrations among American females with different ethnic backgrounds. *Anticancer Res* 2015; 35: 1211-8.
22. Cutillas-Marco E, Fuertes-Prosper A, Grant WB, *et al.* Vitamin D deficiency in South Europe: effect of smoking and aging. *Photodermatol Photoimmunol Photomed* 2012; 28: 159-61.
23. Yao TC, Tu YL, Chang SW, *et al.* Suboptimal vitamin D status in a population-based study of Asian children: prevalence and relation to allergic disease and atopy. *PLoS One* 2014; 9: e99105
24. Jeong BY, Lim MK, Yun EH, *et al.* Tolerance for and potential indicators of second-hand smoke exposure among nonsmokers: a comparison of self-reported and cotinine verified second-hand smoke exposure based on nationally representative data. *Prev Med* 2014; 67: 280-7.
25. Jung S, Lee IS, Kim SB, *et al.* Urine cotinine for assessing tobacco smoke exposure in Korea: Analysis of the Korea National Health and Nutrition Examination Survey (KNHANES). *Tuberc Respir Dis (Seoul)* 2012; 73: 210-8.

26. Florescu A, Ferrence R, Einarson T, *et al.* Methods for quantification of exposure to cigarette smoking and environmental tobacco smoke: focus on developmental toxicology. *Ther Drug Monit* 2009; 31: 14-30.
27. Kim Y, Choi YJ, Oh SW, *et al.* Discrepancy between self-reported and urine-cotinine verified smoking status among Korean male adults: analysis of health check-up data from a single private hospital. *Korean J Fam Med* 2016; 37: 171-6.
28. Park MB, Nam EW, Lee SK, *et al.* The correlation of different cotinine levels with questionnaire results: A comparative study for different measurement methods of the adolescent smoking rate in Korea. *Asia Pac J Public Health* 2015; 27: 542-50.
29. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008; 122: 1142-52.
30. Ross AC, Manson JE, Abrams SA, *et al.* The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: what clinicians needs to know. *J Clin Endocrinol Metab* 2011; 96: 53-8.
31. Ryoo E. Adolescent nutrition: what do pediatricians do? *Korean J Pediatr* 2011; 54: 287-91.
32. Chung IH, Kim HJ, Chung S, *et al.* Vitamin D deficiency in Korean children: prevalence, risk factors, and the relationship with parathyroid hormone levels. *Ann Pediatr Endocrinol Metab* 2014; 19: 86-90.
33. Carpenter TO, Herreros F, Zhang JH, *et al.* Demographic, dietary, and biochemical determinants of vitamin D status in inner-city children. *Am J Clin Nutr* 2012; 95: 137-46.
34. Tolppanen AM, Fraser A, Fraser WD, *et al.* Risk factors variation in 25-hydroxyvitamin D3 and D2 concentrations and vitamin D deficiency in children. *J Clin Endocrinol Metab* 2012; 97: 1202-10.
35. Wortsman J, Matsuoka LY, Chen Tc, *et al.* Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; 72: 690-3.
36. Haufroid V, Lison D. Urinary cotinine as a tobacco-smoke exposure index: a minireview. *Int Arch Occup Environ Health* 1998; 71: 162-8.
37. Paul G, Brehm JM, Alcorn JF, *et al.* Vitamin D and asthma. *Am J Respir Crit Care Med* 2012; 185: 124-32.
38. Bozzetto S, Carraro S, Giordano G, *et al.* Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy* 2012; 67: 10-7.

39. Bath-hextall FJ, Jenkinson C, Humphreys R, *et al.* Dietary supplements for established atopic eczema. *Cochrane Database Syst Rev* 2012; 15: CD005205

40. Hollams EM, Hart PH, Holt BJ, *et al.* Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. *Eur respire J* 2011; 38: 1320-7.

41. Ito Y, Adachi Y, Yoshida K, *et al.* No association between serum vitamin D status and the prevalence of allergic diseases in Japanese children. *Int Arch Allergy Immunol* 2013; 160: 218-20.

FIGURE LEGENDS

Figure Serum 25(OH)D levels: histogram

Table 1. Characteristics of the study population. Univariate and multivariate analyses of factors associated with serum 25(OH)D

		Vit D Level			
	Total (n=2515)	Deficiency (<20ng/ml)	Insufficiency (≥20 - <30ng/ml)	Sufficiency (≥30ng/ml)	*P* value
Age	14.39 ± 0.07	14.66 ± 0.08	13.46 ± 0.15	14.07 ± 0.52	<.0001
Sex					
Male	1314 (53.39)	914 (51.20)	359 (59.74)	41 (71.46)	0.0031
Female	1201 (46.61)	929 (37.45)	254 (40.26)	18 (28.54)	
Region of residence					
Urban	1709 (71.98)	1297 (73.59)	379 (67.94)	33 (50.84)	0.0108
Rural	806 (28.02)	546 (26.41)	234 (32.06)	26 (49.16)	
Regular exercise					
No	1861 (73.83)	1375 (74.94)	449 (70.20)	37 (69.98)	0.1912
Yes	654 (26.17)	468 (25.06)	164 (29.80)	22 (30.02)	
Regular walking					
No	1354 (53.23)	981 (52.98)	339 (54.37)	34 (50.44)	0.8708
Yes	1161 (46.77)	862 (47.02)	274 (45.63)	25 (49.56)	
Income					
Lowest	306 (13.87)	229 (14.33)	71 (12.78)	6 (6.83)	0.0996
Lower middle	604 (27.04)	452 (27.66)	133 (24.41)	19 (31.94)	
Higher middle	803 (30.39)	580 (30.92)	210 (29.54)	13 (17.44)	
Highest	802 (28.71)	582 (27.09)	199 (33.27)	21 (43.79)	
Season					
April - October	1465 (58.38)	930 (51.36)	485 (81.49)	50 (82.62)	<.0001
November - March	1050 (41.62)	913 (48.64)	128 (18.51)	9 (17.38)	
BMI	20.88 ± 0.10	21.12 ± 0.13	20.08 ± 0.19	20.12 ± 0.47	<.0001
Smoking					
Active	382 (18.22)	293 (19.08)	75 (13.99)	14 (32.22)	0.0075
Passive	1200 (43.39)	913 (44.36)	261 (39.98)	26 (42.56)	
No	933 (38.40)	637 (36.56)	277 (46.03)	19 (25.22)	
Atopic dermatitis					
No	2247 (89.44)	1648 (88.99)	546 (90.83)	53 (92.28)	0.4675
Yes	268 (10.56)	195 (11.01)	67 (9.17)	6 (7.72)	
Asthma					
No	2412 (95.87)	1771 (95.85)	583 (95.68)	58 (99.26)	0.5376
Yes	103 (4.13)	72 (4.15)	30 (4.32)	1 (0.74)	

Data are presented as mean ± SE, n (weighted %) .

Statistics were carried out using Rao-Scott Chi-square test.

Table 2. Vitamin D levels in the study population

	Vit D	*P* value(1)	*P* value(2)
Total	16.68 ± 0.17		
Sex			
Male	17.26 ± 0.22	<.0001	
Female	16.01 ± 0.21		
Region of residence			
Urban	16.34 ± 0.20	0.0024	
Rural	17.53 ± 0.34		
Regular exercise			
No	16.52 ± 0.18	0.0565	
Yes	17.13 ± 0.31		
Regular walking			
No	16.62 ± 0.21	0.687	
Yes	16.74 ± 0.24		
Income			
Lowest	16.09 ± 0.41	0.0043	lowest vs hightest : 0.0218
Lower middle	16.63 ± 0.29		lower middle vs hightest : 0.1576
Higher middle	16.47 ± 0.24		higher middle vs hightest : 0.0449
Highest	17.22 ± 0.31		
Season			
April - October	18.11 ± 0.21	<.0001	
November - March	14.67 ± 0.21		
Smoking			
Active	16.52 ± 0.42	0.0062	active vs passive : 0.9439
Passive	16.37 ± 0.23		active vs no : 0.4260
No	17.10 ± 0.23		passive vs no : 0.0382
Atopic dermatitis			
No	16.68 ± 0.18	0.8887	
Yes	16.63 ± 0.32		
Asthma			
No	16.70 ± 0.18	0.4507	
Yes	16.22 ± 0.62		
Data are presented as mean ± SE, n (weighted %) .			
P value (1) : t-test or anova (variable : Income, Smoking) result			
P value (2) : multiple comparison : Tukey-Kramer			

Table 3. Multivariate linear regression analyses of serum 25(OH)D levels

	Coefficient	95% Confidence Interval	*P* value
Age	-0.52	-0.65 , -0.40	<.0001
Sex			
Male	reference		<.0001
Female	-1.40	-1.88 , -0.91	
Region of residence			
Urban	-0.85	-1.55 , -0.14	0.0189
Rural	reference		
BMI	-0.10	-0.17 , -0.03	0.0034
Smoking			
Active	0.47	-0.42 , 1.36	0.3017
Passive	-0.04	-0.62 , 0.53	0.8842
No	reference		
Season			
April - October	reference		<.0001
November - March	-3.38	-3.97 , -2.79	
Statistics were carried out using Multivariable linear regression.			
Model : adjusted for age, sex, region, BMI, smoking, season.			

Table 4. Multivariate ordinal logistic regression analyses of categories of serum 25(OH)D levels

	Odds Ratio	95% Confidence Interval	*P* value
Age	1.22	1.13 , 1.31	<.0001
Sex			
Male	reference		0.0002
Female	1.68	1.28 , 2.21	
Region of residence			
Urban	1.32	0.96 , 1.81	0.0890
Rural	reference		
BMI	1.07	1.03 , 1.12	0.0011
Smoking			
Active	0.89	0.54 , 1.47	0.6539
Passive	1.01	0.74 , 1.37	0.9535
No	reference		
Season			
April - October	reference		
November - March	4.49	3.25 , 6.22	<.0001
Data are presented OR (95%CI).			
Statistics were carried out using Logistic regression.			
Serum 25(OH)D levels were categorized into 3 ordinal categories of vitamin D status			
(<20 : Deficiency , ≥20 - <30 : Insufficiency, 30≥ : Sufficiency)			
Model : adjusted for age, sex, region, BMI, smoking, season.			

Table 5. The estimated mean serum 25(OH)D levels and their differences according to AD and asthma

	Unadjusted			Adjusted		
	Estimated mean	Difference (Estimated mean)	*P* value	Estimated mean	Difference (Estimated mean)	*P* value
Atopic dermatitis						
Yes	16.68 ± 0.18	0.05 ± 0.34	0.8887	16.87 ± 0.16	0.19 ± 0.33	0.5776
No	16.63 ± 0.32			16.68 ± 0.32		
Asthma						
Yes	16.70 ± 0.18	0.48 ± 0.63	0.4507	16.88 ± 0.15	0.72 ± 0.48	0.1313
No	16.22 ± 0.62			16.15 ± 0.47		
Data are presented as numeric, mean ± SD.						
Statistics were carried out using Simple linear regression and Multivariable linear regression.						
Adjusted for age, sex, region, BMI, smoking, season.						

Table 6. ORs and 95% CIs of AD and asthma according to serum 25(OH)D levels

		Unadjusted		Adjusted	
		OR (95%CI)	*P* value	OR (95%CI)	*P* value
Atopic dermatitis	Vit D Level				
	Sufficiency	reference		reference	
	Insufficiency	1.21 (0.43 , 3.39)	0.7228	1.14 (0.41 , 3.19)	0.8078
	Deficiency	1.48 (0.54 , 4.02)	0.4441	1.52 (0.54 , 4.22)	0.4271
Asthma	Vit D Level				
	Sufficiency	reference		reference	
	Insufficiency	6.02 (0.75 , 48.25)	0.0908	4.87 (0.61 , 39.05)	0.1360
	Deficiency	5.79 (0.76 , 43.96)	0.0898	5.31 (0.71 , 39.86)	0.1042
Data are presented OR (95%CI).					
Statistics were carried out using Logistic regression.					
Adjusted for age, sex, region, BMI, smoking, season.					

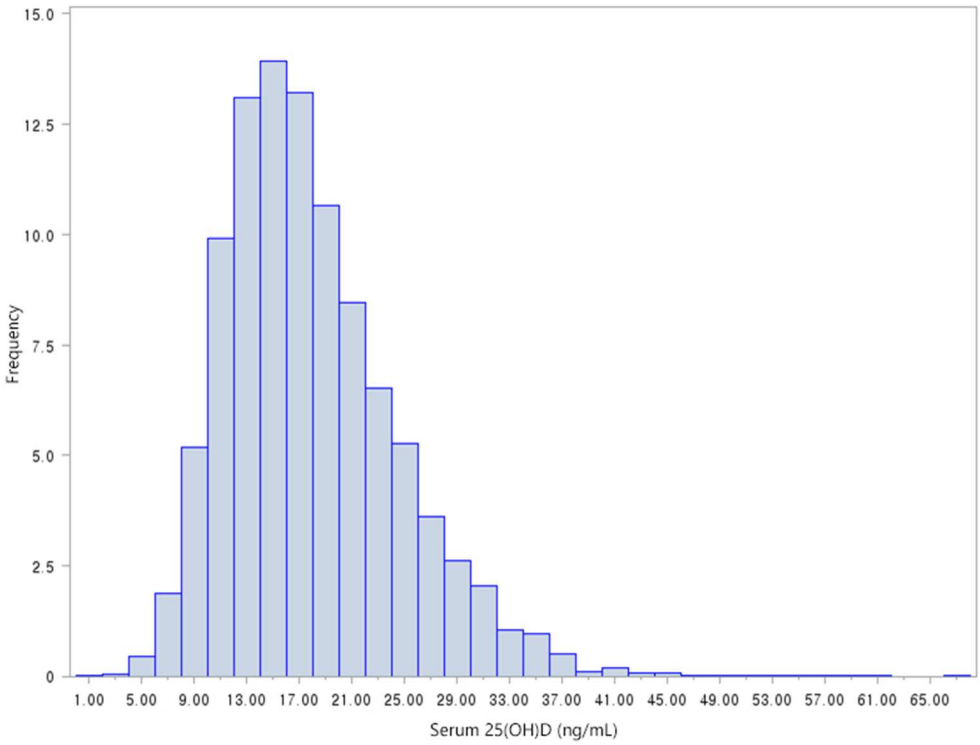

Figure Serum 25(OH)D levels: histogram

282x211mm (72 x 72 DPI)

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Suboptimal vitamin D status in Korean adolescents: A nation-wide study on its prevalence, risk factors including cotinine verified smoking status, and association with atopic dermatitis and asthma

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Research

Suboptimal vitamin D status in Korean adolescents: A nation-wide study on its prevalence, risk factors including cotinine verified smoking status, and association with atopic dermatitis and asthma

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Short title: Suboptimal vitamin D status in Korean adolescents

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ABSTRACT

Objective: To determine the prevalence and risk factors for suboptimal vitamin D status in Korean adolescents, and to assess its relationship with atopic dermatitis (AD) and asthma at a national level.

Design: This is a cross-sectional study with data from the Korean National Health and Nutrition Examination Survey. Information regarding socioeconomic characteristics, clinical data and environmental factors was collected. Blood and urine samples were taken for vitamin D and cotinine respectively. Descriptive and multivariable logistic regression was performed on the data.

Setting: South Korea (Nation-wide)

Participants: 2,515 individuals aged 10-18 years who participated in the Korean National Health and Nutrition Examination Survey from 2008-2011.

Main outcome measures: Vitamin D status was determined through measurement of serum 25-hydroxyvitamin D (25OHD). Smoking status was classified based on the urine cotinine level. Physician diagnosed AD and asthma were assessed using a questionnaire.

Results: Overall, 73.3% of the subjects were vitamin D deficient (25OHD < 20 ng/mL) and 24.4% of the subjects were vitamin D insufficient (25OHD, 20-29.9 ng/mL). Older age (p<0.001), female gender (p<0.001), urban residence (p=0.019), higher body mass index (p=0.003) and sampling in winter months (November-March) (p<0.001) were independently associated with low serum 25OHD levels. With cotinine verification, 18.2% of the participants were classified as active smokers, and 43.4% were classified as passive smokers. After adjusting for potential cofounders, serum 25OHD status showed no association with AD or asthma.

Conclusion: Vitamin D deficiency is highly prevalent in Korean adolescents. Cotinine-verified prevalence of smoking was also high, but its relationship with vitamin D deficiency was not confirmed in our study. Above all, our results provide epidemiologic evidence against the association of vitamin D status with AD and asthma at the national level among Korean adolescents.

Strengths and limitations of this study

The Strength of this study is that a nationally representative sample was used as the study population.

This study is unique in that a cotinine-verified smoking status was adopted. Cotinine (a metabolite of

nicotine) is a specific marker of smoking with high sensitivity.

The limitation of the study is its cross-sectional design and the lack of data on the population's dietary and supplemental vitamin D intake. Also, the study has recall bias because the diagnoses of allergic conditions (AD and asthma) were self-reported.

The definition of asthma and AD were physician-diagnosed asthma and AD which may have missed some children with symptomatic asthma and AD who have not been diagnosed.

INTRODUCTION

Vitamin D is essential for skeletal health and bone growth and its deficiency has been associated with skeletal deformities in children and fracture risk in adults. Recent studies have also identified the previously unanticipated roles of vitamin D in the immune system, cardiovascular system and cancer prevention.[1,2] Despite growing health concerns over low vitamin D status, few nation-wide studies have been performed to evaluate vitamin D deficiency in the Korean pediatric population.[3,4] Suboptimal vitamin D level was reported in 70% of the US children (9% vitamin D deficient, 61% vitamin D insufficient),[5] and it was found in 41.7% of the children from New Zealand).[6] Korean adolescents are theoretically at increased risk for vitamin D deficiency because of the high latitude (34-38ºN), culturally vigorous sun protection, reduced outdoor activity and lack of vitamin D-fortified food.

The increasing prevalence of allergic diseases is a world-wide phenomenon and it is strikingly more evident in the younger population compared to adults.[7] Vitamin D has immunomodulatory functions, and its relationship with allergic disease has been evaluated in a number of studies.[8-15] While some authors have reported about the protective role of vitamin D in atopic dermatitis (AD), asthma, allergic rhinitis (AR) and allergic sensitization in childhood, other authors have shown a deleterious effect. Although the exact cause of such conflicting results is not known, racial difference may be a contributing factor. Unfortunately, nation-wide studies on vitamin D and allergic disease are limited and are mostly from western countries.

In this study, we aimed to identify the prevalence and risk factors for vitamin D deficiency in Korean adolescents and to assess its relationship with AD and asthma at the national level. Cotinine-verified smoking status was adopted in this study.

METHODS

Study population

This study was based on data acquired from the Korean National Health and Nutrition Examination Survey (KNHANES), a survey conducted by the Korea Centers for Disease Control and Prevention to provide nationally representative and reliable statistical data regarding the health, behavior associated with health, nutrition, and food intake status of the Korean population. Data were collected from 2008 to 2011, which corresponds to the second and third year of KNHANES IV (2007-2009) and the first and second year of KNHANES V (2010-2012). The survey included a health interview, a nutritional survey, physical examination, and blood and urine tests. The institutional review board at the Korea Centers for Disease Control and Prevention/ Incheon St. Mary's Hospital, The Catholic University of Korea (IRB number: OC17ZESI0055) approved the protocol, and all participants and their parents signed informed consent forms.

Both KNHANES IV and V adopted the stratified multistage cluster sampling design by using the rolling-survey sampling method. The rolling sample collected each year is the probability sample representing the general Korean population, and it is homogeneous and independent of each other. In 2008, 2009, 2010 and 2011, a total of 37,753 individuals were sampled. The study population was further limited to 4,598 adolescents aged 10-18 years. Among the 4,598 participants, we subsequently excluded the following participants: those whose serum 25-hydroxyvitamin D (25OHD) levels were not measured; those without urine cotinine levels; those who did not completely answer the questions regarding AD or asthma; those without body mass index (BMI) measurement; and those who had a chronic disease that may affect vitamin D metabolism. Finally, a total of 2,515 participants (1,314 males and 1,201 females) were eligible for analysis.

Study variables

Blood samples were collected from the antecubital vein, refrigerated immediately, transported to the central testing facility in cold storage, and analyzed within 24 hours of sampling. Serum 25OHD levels were measured as described previously[16] and categorized as sufficient (≥30 ng/mL), insufficient (20-29.9 ng/mL) or deficient (<20 ng/mL).[17]

Factors were categorized to analyze the risk factors for vitamin D deficiency. Age and BMI were continuous variables while season of sampling was categorized into winter months (November-March) and summer months (April-October).[18] The region of residence of each participant was grouped as follows: urban (Seoul, Gyeonggi, Busan, Daegu, Incheon, Gwangju, Daejeon, and Ulsan) and rural (Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju).[19] Monthly income was standardized according to the number of family members (monthly income/number of family members) and it was divided into the following 4 quartile groups: lowest, lower middle, higher middle, and highest. Participants who performed moderate physical activity for more than 30 minutes per day on more than 5 days a week and/or strenuous physical activity for more than 20 minutes per day on more than 3 days a week were assigned to the regular exercise group. Regular walking was designated as "yes" for those who walked for more than 30 minutes per day on more than 5 days a week.[19] Smoking status was divided into three groups based on the urine cotinine level, which are as follows: non-smokers (<5 ng/mL), passive smokers (secondhand smoking) (5-100 ng/mL) and active smokers (>100 ng/mL).[19-21] Urine cotinine level was measured by chromatography mass spectrometry using the Perkin Elmer Clarus 600T (PerkinElmer, Turku, Finland). All data were measured in a standardized manner and reviewed by the central quality control center.

The following question was used to assess physician-diagnosed AD in each participant: "Have you been diagnosed with AD by a doctor?" or "Have you been told by a doctor that (your child) had AD? Physician-diagnosed asthma was also determined using similar questions.

Statistical analysis

Statistical analyses were performed using an SAS survey procedure (version 9.2; SAS Institute, Inc., Cary, NC, USA), and 2-sided *p* values of less than 0.05 were considered statistically significant. To produce unbiased national estimates representing the general Korean population, we used KNHANES sample weights accounting for the complex sampling design to each participant.

To compare the mean serum 25OHD levels among categories of each possible predicting factor, Student's *t*-test or ANOVA (followed by Tukey-Kramer for multiple comparison) was used.

Univariate analysis was performed to evaluate the association of the possible predicting factors, AD

and asthma with vitamin D deficiency. Participants' characteristics were described using means and standard errors for continuous variables and numbers and percentages for categorical variables. Here, the Student's *t*-test or ANOVA was used for comparing continuous variables, as appropriate, and Rao-Scott χ^2 test was used for comparing categorical variables. Variables with a *p*-value <0.05 in univariate analyses were included in the multivariate regression model for exploring factors associated with serum 25OHD.

To estimate the mean serum 25OHD levels in participants with and without AD and asthma, we performed simple and multiple linear regression analyses using the generalized linear model for a complex survey design. The estimated means were calculated as follows: no adjustment for potential confounders; and confounder adjustment for age, gender, region, BMI, smoking, and season of sampling.

To estimate the odds ratios (ORs) for AD and asthma according to quartiles of serum 25OHD levels, we conducted simple and multivariate logistic regression analyses by using the generalized linear model for a complex survey design. The ORs and 95% CIs were calculated in the following ways: no adjustment for potential confounders; and confounder adjustment for age, gender, region, BMI, smoking, and season of sampling.

RESULTS

General characteristics

A total of 2,515 subjects (1,314 males and 1,201 females, age: 14.4 ± 0.1 years) were included in the study. Table 1 summarizes the baseline data of the participants. The mean BMI of the study population was 20.9 kg/m^2 (SE: 0.1). Blood samples were drawn more frequently in April-October (58.4%) than in November-March (41.6%). The urine cotinine-verified smoking statuses were as follows: active smoking 18.2%, passive smoking 43.4%, and non-smoking 38.4%. The prevalence of AD and asthma in the study population were 10.6% and 4.1%, respectively.

Serum 25OHD levels in the study population

The mean concentration of serum 25OHD in the 2,515 subjects was 16.7 ng/mL (SE: 0.2), with a range of 3.0 to 46.2 ng/mL. Overall, 1,843 subjects (73.3%) were vitamin D deficient, 613 subjects (24.4%) were vitamin D insufficient, and 59 subjects (2.3%) were vitamin sufficient (Figure). Girls had a significantly lower mean serum 25OHD level than boys (16.0 ± 0.2 ng/mL vs 17.3 ± 0.2 ng/mL, p<0.001), and subjects sampled in winter months (November-March) had lower serum 25OHD levels than those sampled in summer months (April-October) (14.7 ± 0.2 ng/mL vs 18.1 ± 0.2 ng/mL, p<0.001). Passive smoking (vs non-smoking) and urban residence (vs rural) were also associated with statistically lower serum 25OHD levels (p=0.038 and p=0.002, respectively) (Table 2).

Univariate and multivariate analyses of factors associated with serum 25OHD

Univariate analysis revealed that age (p<0.001), gender (p=0.003), region of residence (p=0.011), season of sampling (p<0.001), BMI (<0.001) and smoking status (p=0.008) were associated with vitamin D deficiency (Table 1).

Multivariate linear regression analysis of serum 25OHD levels was performed using variables that had a p-value <0.05 in univariate analyses. Older age (p<0.001), female gender (p<0.001), urban residence (p=0.019), higher BMI (p=0.003) and sampling in winter months (November-March) (p<0.001) were independently associated with low serum 25OHD levels (Table 3). Multivariate ordinal logistic regression analysis of serum 25OHD showed similar results (Table 4).

Relationship of serum 25OHD with AD and asthma

Univariate analysis demonstrated that vitamin D deficiency was not significantly associated with AD (p=0.468) or asthma (p=0.538) (Table 1).

Multivariate logistic regression showed that vitamin D deficiency had no association with AD and asthma after adjusting for age, gender, region of residence, BMI, smoking, and season of sampling. The adjusted odds ratio for AD and asthma increased across categories of serum 25OHD (1.00 [reference] for ≥ 30 ng/mL, 1.14 and 4.87 each for 20-29.9 ng/mL, and 1.52 and 5.31, respectively for <20 ng/mL), but it was not statistically significant (p=0.8078 (AD) and p=0.1360 (asthma) for 20-29.9 ng/mL and p=0.4271 (AD) and p=0.1042 (asthma) for <20 ng/mL) (Table 5).

Univariate or multivariate analyses with serum 25OHD levels as a continuous variable showed similar results, regardless of the adjustment (Table 6).

DISCUSSION

In our cross-sectional study of Korean adolescents, Vitamin D deficiency was very common, where the mean concentration of serum 25OHD was 16.7 ng/mL with 73.3% of the subjects being vitamin D deficient (serum 25OHD < 20 ng/mL). Potential explanations for this remarkably low vitamin D status observed in Korean children include, but are not limited to pigmented skin (skin pigmentation is known the reduce the skin's production of vitamin D in East Asian populations),[3] vigorous use of sunscreens and possibly dietary factors. The Dietary Reference Intakes for vitamin D from the Institute of Medicine (2010) and the American Academy of Pediatrics state that the Adequate Intake (AI) of vitamin D is 15 μg/day for children.[22,23] However, in the Dietary Reference Intakes for vitamin D for Koreans published in 2010, the AI of vitamin D for children is claimed to be 5 μg/day,[24] which we feel is too low. Exposure to sunlight, specifically UVB, leads to vitamin D synthesis in the skin, which provides most of the vitamin D requirement of an individual. In Korea (latitude: 34-38ºN), vitamin D is synthesized mostly between April and October (summer months),[25] which was apparent in our study.

The finding of an inverse relationship of age, female gender, urban residence, and BMI with vitamin D status is in accordance with previous studies.[3,4,26-28] It has been previously shown that there is a consistent decline in physical outdoor activity over the school age years. The prevalence of physical inactivity is higher in girls than in boys and it is greater in obese children than in their normal-weight peers. The lifestyle in big cities is also sedentary, and it is therefore possible that age, gender, place of residence and BMI may act as surrogate indicators for sunlight exposure through their association with physical activity. In addition to limited sunlight exposure from physical inactivity, the inverse association of BMI and urban residence with vitamin D levels can be explained by sequestration of vitamin D in fat tissues[29] and UVB blockade due to air pollution.

This nation-wide study is particularly meaningful as it is the first to measure both serum 25(OH)D and urine cotinine in a population of 2,515 Korean adolescents. Smoking has been quoted as a significant determinant of serum 25OHD in a number of studies,[20,21,28,30] including one from Taiwan,[28] where passive smoking was independently associated with low serum 25OHD. To identify the effect

of smoking on serum 25OHD in Koreans, we adopted urine cotinine, which is presently the biomarker of choice for assessing tobacco smoke exposure.[31-34] KNHANES normally assesses a person's smoking status via a survey. For those who are aged 19 years and above, detailed questions are asked regarding the presence and history of active and passive smoking, but for those who are aged less than 19 years, the questions are much more limited. There is currently no data with regards to passive smoking in Korean adolescents and the survey only determines the presence of active smoking in subjects aged between 12-18 years. Omissions and false responses are common in this age group[35] which impede accurate assessment of the smoking status, and to improve accuracy, we adopted the cotinine-verified smoking status in our study. Urine cotinine level was measured for a limited time-period (2008-2011), and this measurement has only been performed for those aged 10 years and above, which resulted in an inevitable reduction of our study population. We used a urine cotinine cut off value of more than 100 ng/mL to discriminate smokers from non-smokers and 5 ng/mL as a threshold for non-smokers exposed to secondhand smoking based on published values.[31-33] With cotinine verification, 18.2% of the participants were classified as active smokers, 43.4% of the participants were classified as passive smokers, and 38.4% of the participants were classified as non-smokers, where the percentage of smokers (active and passive) was higher than what we expected.

Smokers have been reported to have a significantly higher risk of vitamin D deficiency,[20,21,28,30] but this was not confirmed in our study. Since active smoking is usually done outdoors, this can result in greater exposure to sunlight which may alter the true effect of smoking on serum 25OHD. Passive smoking in adolescents is more likely to occur indoors and interestingly-enough, passive smoking was reported to be independently associated with low serum 25OHD in Taiwanese children/adolescents.[28] However, results can also be influenced by cotinine cut-off values and higher thresholds such as 10 ng/mL or 30 ng/mL may be considered in the future when testing children as this population can have higher cotinine concentrations than adults due to differences in body distribution and nicotine metabolism.[36]

Some studies have indicated that an adequate concentration of vitamin D is protective against allergic disease in children.[8,9,15,37,38] However, most of these findings have been reported in western countries

and only a few studies have been performed in Asia. We consider our investigation meaningful because differences among ethnicities and subjects of various age groups can possibly affect the results.

In this national level study, the risk of AD and asthma were not dependent on serum 25OHD. The present study results, as well as prior studies which showed little association between vitamin D deficiency and AD,[10-12,28] raise questions on the suggestion that vitamin D may be used to prevent or treat AD. In fact, a Cochrane review found no evidence for an effect of vitamin D supplementation on AD.[39] The beneficial effect of sunlight on AD has been well documented, but this is probably due to the anti-inflammatory and antimicrobial effects of UV and not those of vitamin D.

As in AD, there has been considerable controversy over the relationship between vitamin D levels and asthma in children. While some studies showed an inverse relationship between vitamin D levels and current wheeze and asthma severity,[8] others such as Hollams et al.[40] showed no significant cross-sectional association between serum vitamin D levels and current asthma. Our study too did not find any relationship between vitamin D level and asthma, but further studies would be needed to determine a causal relationship and its mechanism.

This study has several notable strengths. The sampling of adolescents across a broad age range, a large sample size with robust data collection, incorporation of objective markers of smoking status, and a thorough analysis strengthen the results of this study. Limitations of the study include its cross-sectional design and lack of data on dietary and supplemental vitamin D intake. Also, the study is prone to recall bias because diagnoses of allergic conditions (AD and asthma) were self-reported. The definition of asthma and AD were physician-diagnosed asthma and AD, which may have missed some children with symptomatic asthma and AD in the population who have not been diagnosed. Although season adjusted, desasonalization of vitamin D levels with an algorithm[41] may have further increased the accuracy and strength of our study.

In conclusion, a high prevalence of vitamin D deficiency was noted in Korean adolescents aged 10 to 18 years. For high risk individuals of vitamin D deficiency, we suggest vitamin D supplementation and food fortification. Adequate outdoor activity should also be emphasized in Korean adolescents,

especially high school students, via media promotion and public health plans by schools and government. We found no apparent association between the cotinine-verified smoking status, AD, and asthma with vitamin D deficiency, which merits further study.

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Contributors EJB, SHC, JDL, and HSK made substantial contributions to the conception or design of the work. JYH and HSK had role in the acquisition, analysis and interpretation of data tor the work. EJB, SHC, JDL, and HSK drafted and revised the work and all authors gave final approval and agreed to be accountable for all aspects of the work.

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Competing interests None declared.

Ethics approval The institutional review board at the Korea Centers for Disease Control and Prevention/ Incheon St. Mary's Hospital, The Catholic University of Korea (IRB number: OC17ZESI0055) approved the protocol.

Data sharing statement All data from the study, published and unpublished, are available to the principle investigators. The data are managed under the Department of Dermatology, Incheon St. Mary's Hospital, The Catholic University of Korea, Incheon, Korea.

REFERENCES

1. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 257: 266-81.
2. Cheng HM, Kim S, Park GH, *et al.* Low vitamin D levels are associated with atopic dermatitis, but not allergic rhinitis, asthma, or IgE sensitization, in the adult Korean population. *J Allergy Clin Immunol* 2014; 133: 1048-55.
3. Kim SH, Oh MK, Namgung R, *et al.* Prevalence of 25-hydroxyvitamin D deficiency in Korean adolescents: association with age, season, and parental vitamin D status. *Public Health Nutr* 2014; 17: 122-30.
4. Lee YA, Kim HY, Hong H, *et al.* Risk factors for low vitamin D status in Korean adolescents: the Korea National Health and Nutrition Examination Survey (KNHANES) 2008-2009. *Public Health Nutr* 2014; 17: 764-71.
5. Kumar J, Muntner P, Kaskel FJ, *et al.* Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. *Pediatrics* 2009; 124: e362-70.
6. Rockell JE, Green TJ, Skeaff CM, *et al.* Season and ethnicity are determinants of serum 25-hydroxyvitamin D concentrations in New Zealand children aged 5-14 y. *J Nutr* 2005; 135: 2602-8.
7. Asher MI, Montefort S, Bjorksten B, *et al.* Worldwide time trends in the prevalence of symptoms of asthma, allergic rhino-conjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multi-country cross-sectional surveys. *Lancet* 2006; 26: 733-43.
8. Bener A, Ehlayel MS, Tulic MK, *et al.* Vitamin D deficiency as a strong predictor of asthma in children. *Int Arch Allergy Immunol* 2012; 157: 168-75.
9. El Taieb MA, Fayed HM, Aly SS, *et al.* Assessment of serum 25-hydroxyvitamin D levels in children with atopic dermatitis: correlation with SCORAD index. *Dermatitis* 2013; 24: 296-301.
10. Berents TL, Lodrup Carlsen KC, *et al.* Vitamin D levels and atopic eczema in infancy and early childhood in Norway: a cohort study. *Br J Dermatol* 2016; 175: 95-101.
11. Barman M, Jonsson K, Hesselmar B, *et al.* No association between allergy and current 25-hydroxyvitamin D in serum or vitamin D intake. *Acta Paediatr* 2015; 104: 405-13.
12. Chiu YE, Havens PL, Siegel DH, *et al.* Serum 25-hydroxyvitamin D concentration does not correlate with atopic dermatitis severity. *J Am Acad Dermatol* 2013; 69: 40-6.
13. Yang HK, Choi J, Kim WK, *et al.* The association between hypovitaminosis D and pediatric allergic disease: A Korean nationwide population-based study. *Allergy Asthma Proc* 2016; 37: 64-9.

14. Lee SJ, Kang BH, Choi BS. Vitamin D serum levels in children with allergic and vasomotor rhinitis. *Korean J Pediatr* 2015; 58: 325-9.
15. Cheon BR, Shin JE, Kim YJ, *et al.* Relationship between serum 25-hydroxyvitamin D and interleukin-31 levels, and the severity of atopic dermatitis in children. *Korean J Pediatr* 2015; 58: 96-101.
16. Nam GE, Kim DH, Cho KH, *et al.* 25-Hydroxyvitamin D insufficiency is associated with cardiometabolic risk in Korean adolescents: the 2008-2009 Korea National Health and Nutrition Examination Survey (KNHANES). *Public Health Nutr* 2014; 17: 186-94.
17. Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al.* Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J Clin Endrocrinol Metab* 2011; 96: 1911-30.
18. McKinney K, Breitkopf CR, Berenson AB. Association of race, body fat and season with vitamin D status among young women: a cross-sectional study. *Clin Endocrinol (Oxf)* 2008; 69: 535-41.
19. Choi HS, Oh HJ, Choi H, *et al.* Vitamin D insufficiency in Korea- a greater threat to younger generation: The Korea National Health and Nutrition Examination Survey (KNHANES) 2008. *J Clin Endocrinol Metab* 2011; 96: 643-51.
20. Kassi EN, Stavropoulos S, Kokkoris P, *et al.* Smoking is a significant determinant of low serum vitamin D in young and middle-aged healthy males. *Hormones (Athens)* 2015; 14: 245-50.
21. Manavi KR, Alston-Mills BP, Thompson MP, *et al.* Effect of serum cotinine on vitamin D serum concentrations among American females with different ethnic backgrounds. *Anticancer Res* 2015; 35: 1211-8.
22. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008; 122: 1142-52.
23. Ross AC, Manson JE, Abrams SA, *et al.* The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: what clinicians needs to know. *J Clin Endocrinol Metab* 2011; 96: 53-8.
24. Ryoo E. Adolescent nutrition: what do pediatricians do? *Korean J Pediatr* 2011; 54: 287-91.
25. Chung IH, Kim HJ, Chung S, *et al.* Vitamin D deficiency in Korean children: prevalence, risk factors, and the relationship with parathyroid hormone levels. *Ann Pediatr Endocrinol Metab* 2014; 19: 86-90.
26. Carpenter TO, Herreros F, Zhang JH, *et al.* Demographic, dietary, and biochemical determinants of vitamin D status in inner-city children. *Am J Clin Nutr* 2012; 95: 137-46.

27. Tolppanen AM, Fraser A, Fraser WD, Lawlor DA. Risk factors variation in 25-hydroxyvitamin D3 and D2 concentrations and vitamin D deficiency in children. *J Clin Endocrinol Metab* 2012; 97: 1202-10.
28. Yao TC, Tu YL, Chang SW, *et al.* Suboptimal vitamin D status in a population-based study of Asian children: prevalence and relation to allergic disease and atopy. *PLoS One* 2014; 9: e99105
29. Wortsman J, Matsuoka LY, Chen Tc, *et al.* Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; 72: 690-3.
30. Cutillas-Marco E, Fuertes-Prosper A, Grant WB, *et al.* Vitamin D deficiency in South Europe: effect of smoking and aging. *Photodermatol Photoimmunol Photomed* 2012; 28: 159-61.
31. Jeong BY, Lim MK, Yun EH, *et al.* Tolerance for and potential indicators of second-hand smoke exposure among nonsmokers: a comparison of self-reported and cotinine verified second-hand smoke exposure based on nationally representative data. *Prev Med* 2014; 67: 280-7.
32. Jung S, Lee IS, Kim SB, *et al.* Urine cotinine for assessing tobacco smoke exposure in Korea: Analysis of the Korea National Health and Nutrition Examination Survey (KNHANES). *Tuberc Respir Dis (Seoul)* 2012; 73: 210-8.
33. Florescu A, Ferrence R, Einarson T, *et al.* Methods for quantification of exposure to cigarette smoking and environmental tobacco smoke: focus on developmental toxicology. *Ther Drug Monit* 2009; 31: 14-30.
34. Kim Y, Choi YJ, Oh SW, *et al.* Discrepancy between self-reported and urine-cotinine verified smoking status among Korean male adults: analysis of health check-up data from a single private hospital. *Korean J Fam Med* 2016; 37: 171-6.
35. Park MB, Nam EW, Lee SK, *et al.* The correlation of different cotinine levels with questionnaire results: A comparative study for different measurement methods of the adolescent smoking rate in Korea. *Asia Pac J Public Health* 2015; 27: 542-50.
36. Haufroid V, Lison D. Urinary cotinine as a tobacco-smoke exposure index: a minireview. *Int Arch Occup Environ Health* 1998; 71: 162-8.
37. Paul G, Brehm JM, Alcorn JF, *et al.* Vitamin D and asthma. *Am J Respir Crit Care Med* 2012; 185: 124-32.
38. Bozzetto S, Carraro S, Giordano G, *et al.* Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy* 2012; 67: 10-7.

39. Bath-hextall FJ, Jenkinson C, Humphreys R, *et al.* Dietary supplements for established atopic eczema. *Cochrane Database Syst Rev* 2012; 15: CD005205

40. Hollams EM, Hart PH, Holt BJ, *et al.* Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. *Eur respire J* 2011; 38: 1320-7.

41. van der Mei IA, Ponsonby AL, Dwyer T, *et al.* Vitamin D levels in people with multiple sclerosis and community controls in Tasmania Australia. J Neurol 2007; 254: 581-90.

FIGURE LEGENDS

Figure Serum 25OHD levels: histogram

394 **Table 1. Characteristics of the study population. Univariate and multivariate analyses of factors associated with serum 25(OH)D**

		Vit D Level			
	Total (n=2515)	Deficiency (<20ng/ml)	Insufficiency (≥20 - <30ng/ml)	Sufficiency (≥30ng/ml)	*P* value
Age	14.39±0.07	14.66±0.08	13.46±0.15	14.07±0.52	<.0001
Sex					
Male	1314 (53.39)	914 (51.20)	359 (59.74)	41 (71.46)	0.0031
Female	1201 (46.61)	929 (37.45)	254 (40.26)	18 (28.54)	
Region of residence					
Urban	1709 (71.98)	1297 (73.59)	379 (67.94)	33 (50.84)	0.0108
Rural	806 (28.02)	546 (26.41)	234 (32.06)	26 (49.16)	
Regular exercise					
No	1861 (73.83)	1375 (74.94)	449 (70.20)	37 (69.98)	0.1912
Yes	654 (26.17)	468 (25.06)	164 (29.80)	22 (30.02)	
Regular walking					
No	1354 (53.23)	981 (52.98)	339 (54.37)	34 (50.44)	0.8708
Yes	1161 (46.77)	862 (47.02)	274 (45.63)	25 (49.56)	
Income					
lowest	306 (13.87)	229 (14.33)	71 (12.78)	6 (6.83)	0.0996
lower middle	604 (27.04)	452 (27.66)	133 (24.41)	19 (31.94)	
higher middle	803 (30.39)	580 (30.92)	210 (29.54)	13 (17.44)	
highest	802 (28.71)	582 (27.09)	199 (33.27)	21 (43.79)	
Season					
April - October	1465 (58.38)	930 (51.36)	485 (81.49)	50 (82.62)	<.0001
November - March	1050 (41.62)	913 (48.64)	128 (18.51)	9 (17.38)	

BMI	20.88 ± 0.10	21.12 ± 0.13	20.08 ± 0.19	20.12 ± 0.47	<.0001
Smoking					
Active	382 (18.22)	293 (19.08)	75 (13.99)	14 (32.22)	0.0075
Passive	1200 (43.39)	913 (44.36)	261 (39.98)	26 (42.56)	
No	933 (38.40)	637 (36.56)	277 (46.03)	19 (25.22)	
Atopic dermatitis					
No	2247 (89.44)	1648 (88.99)	546 (90.83)	53 (92.28)	0.4675
Yes	268 (10.56)	195 (11.01)	67 (9.17)	6 (7.72)	
Asthma					
No	2412 (95.87)	1771 (95.85)	583 (95.68)	58 (99.26)	0.5376
Yes	103 (4.13)	72 (4.15)	30 (4.32)	1 (0.74)	

Data are presented as mean±SE, n (weighted %) .

Statistics were carried out using Rao-Scott Chi-square test.

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Table 2. Vitamin D levels in the study population

	Vit D	*P* value(1)	*P* value(2)
Total	16.68±0.17		
Sex			
Male	17.26±0.22	<.0001	
Female	16.01±0.21		
Region of residence			
Urban	16.34±0.20	0.0024	
Rural	17.53±0.34		
Regular exercise			
No	16.52±0.18	0.0565	
Yes	17.13±0.31		
Regular walking			
No	16.62±0.21	0.687	
Yes	16.74±0.24		
Income			
lowest	16.09±0.41	0.0043	lowest vs hightest : 0.0218
lower middle	16.63±0.29		lower middle vs hightest : 0.1576
higher middle	16.47±0.24		higher middle vs hightest : 0.0449
highest	17.22±0.31		
Season			
April - October	18.11±0.21	<.0001	
November - March	14.67±0.21		
Smoking			
Active	16.52±0.42	0.0062	active vs passive : 0.9439
Passive	16.37±0.23		active vs no : 0.4260
No	17.10±0.23		passive vs no : 0.0382
Atopic dermatitis			
No	16.68±0.18	0.8887	
Yes	16.63±0.32		
Asthma			
No	16.70±0.18	0.4507	
Yes	16.22±0.62		

Data are presented as mean±SE, n (weighted %) .

P value (1): t-test or anova(variable : Income, Smoking) result

P value (2): multiple comparison : Tukey-Kramer

Table 3. Multivariate linear regression analyses of serum 25(OH)D levels

	Coefficient	95% Confidence interval	*P* value
Age	-0.52	-0.65 , -0.40	<.0001
Sex			
Male	reference		<.0001
Female	-1.40	-1.88 , -0.91	
Region of residence			
Urban	-0.85	-1.55 , -0.14	0.0189
Rural	reference		
BMI	-0.10	-0.17 , -0.03	0.0034
Smoking			
Active	0.47	-0.42 , 1.36	0.3017
Passive	-0.04	-0.62 , 0.53	0.8842
No	reference		
Season			
April - October	reference		<.0001
November - March	-3.38	-3.97 , -2.79	

Statistics were carried out using Multivariable linear regression.

Model: adjusted for age, sex, region, BMI, smoking, Season.

Table 4. Multivariate ordinal logistic regression analyses of categories of serum 25(OH)D levels

	Odds ratio	95% Confidence interval	*P* value
Age	1.22	1.13 , 1.31	<.0001
Sex			
Male	reference		0.0002
Female	1.68	1.28 , 2.21	
Region of residence			
Urban	1.32	0.96 , 1.81	0.0890
Rural	reference		
BMI	1.07	1.03 , 1.12	0.0011
Smoking			
Active	0.89	0.54 , 1.47	0.6539
Passive	1.01	0.74 , 1.37	0.9535
No	reference		
Season			
April - October	reference		
November - March	4.49	3.25 , 6.22	<.0001

Data are presented OR(95%CI).

Statistics were carried out using Logistic regression.

Serum 25(OH)D levels were categorized into 3 ordinal categories of vitamin D status

(<20: Deficiency, ≥20 - <30: Insufficiency, 30≥: Sufficiency)

Model: adjusted for age, sex, region, BMI, smoking, Season.

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Table 5. ORs and 95% CIs of AD and asthma according to serum 25(OH)D levels

		Unadjusted		Adjusted	
		OR (95%CI)	*P* value	OR (95%CI)	*P* value
Atopic dermatitis	Vit D Level				
	Sufficiency	reference		reference	
	Insufficiency	1.21 (0.43 , 3.39)	0.7228	1.14 (0.41 , 3.19)	0.8078
	Deficiency	1.48 (0.54 , 4.02)	0.4441	1.52 (0.54 , 4.22)	0.4271
Asthma	Vit D Level				
	Sufficiency	reference		reference	
	Insufficiency	6.02 (0.75 , 48.25)	0.0908	4.87 (0.61 , 39.05)	0.1360
	Deficiency	5.79 (0.76 , 43.96)	0.0898	5.31 (0.71 , 39.86)	0.1042

Data are presented OR(95%CI).

Statistics were carried out using Logistic regression.

Adjusted for age, sex, region, BMI, smoking, Season.

Table 6. The estimated mean serum 25(OH)D levels and their differences according to AD and asthma

	Unadjusted			Adjusted		
	Estimated mean	Difference (Estimated mean)	*P* value	Estimated mean	Difference (Estimated mean)	*P* value
Atopic dermatitis						
Yes	16.68 ± 0.18	0.05 ± 0.34	0.8887	16.87 ± 0.16	0.19 ± 0.33	0.5776
No	16.63 ± 0.32			16.68 ± 0.32		
Asthma						
Yes	16.70 ± 0.18	0.48 ± 0.63	0.4507	16.88 ± 0.15	0.72 ± 0.48	0.1313
No	16.22 ± 0.62			16.15 ± 0.47		

Data are presented as numeric, mean±SD.

Statistics were carried out using Simple linear regression and Multivariable linear regression.

Adjusted for age, sex, region, BMI, smoking, Season.

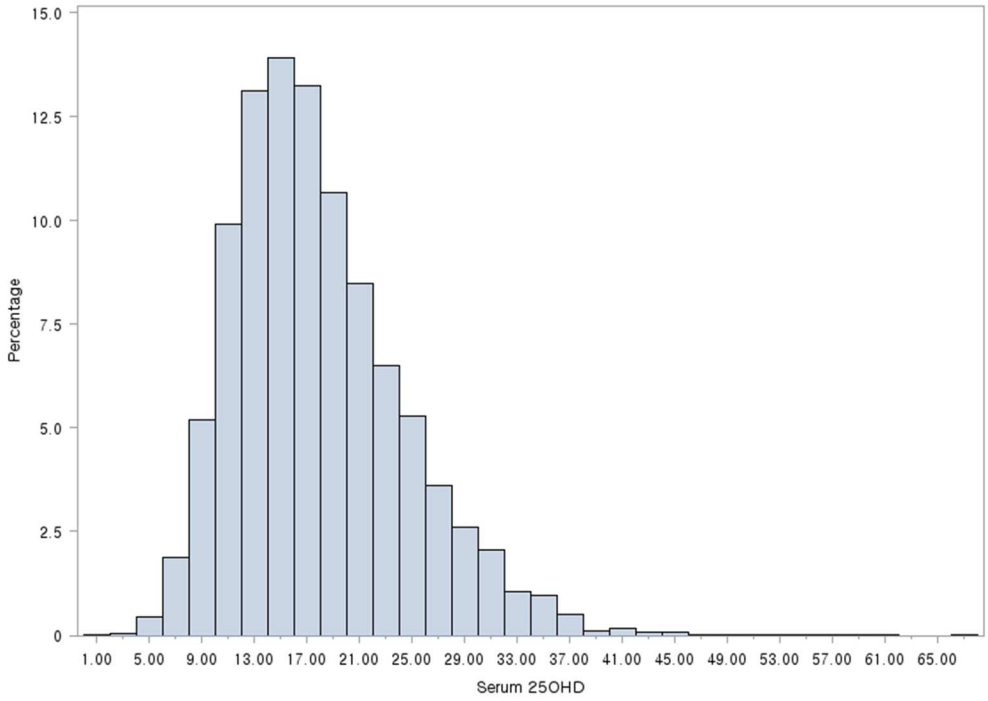

Figure Serum 25OHD levels: histogram

190x142mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6,7
		(*b*) Describe any methods used to examine subgroups and interactions	6,7
		(*c*) Explain how missing data were addressed	6,7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	6,7

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6,7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8,9
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	12,13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12,13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.