

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016409
Article Type:	Research
Date Submitted by the Author:	12-Feb-2017
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Dermatology, Smoking and tobacco, Health services research, Paediatrics
Keywords:	vitamin D deficiency, atopic dermatitis, cotinine, smoking, adolescent

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Manuscripts

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4 **1 Research**

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6 **2 Suboptimal vitamin D status in Korean adolescents: A nation-wide**
7 **3 study on its prevalence, risk factors including cotinine verified**
8 **4 smoking status and association with atopic dermatitis and asthma**
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25 **11 Short title: Suboptimal vitamin D status in Korean adolescents**
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4 23 **ABSTRACT**

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6 24 **Objective:** To determine the prevalence and risk factors for suboptimal vitamin D status in Korean
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8 25 adolescents, and to assess its relationship with atopic dermatitis and asthma.

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10 26 **Design:** This is a cross-sectional study with data from the Korean National Health and Nutrition
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12 27 Examination Survey. Information regarding socioeconomic characteristics, clinical data and
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14 28 environmental factors was collected. Blood and urine samples were taken for vitamin D and cotinine
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16 29 respectively. Descriptive and multivariable logistic regression was performed on the data.

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18 30 **Setting:** Korea (Nation-wide)

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20 31 **Participants:** 2,515 individuals aged 10-18 years who participated in the Korean National Health and
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22 32 Nutrition Examination Survey from 2008-2011.

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24 33 **Main outcome measures:** Vitamin D status was determined through measurement of serum 25(OH)D.
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26 34 Smoking status was classified based on the urine cotinine level. Physician diagnosed AD and asthma
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28 35 were assessed using a questionnaire.

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30 36 **Results:** Overall, 73.3% of the subjects were vitamin D deficient. Older age ($p<0.0001$), female
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32 37 gender ($p<0.0001$), urban residence ($p=0.0189$), higher body mass index ($p=0.0034$) and sampling in
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34 38 winter months (November-March) ($p<0.0001$) were independently associated with low serum
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36 39 25(OH)D levels. With cotinine verification, 18.2% of the participants were classified as active
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38 40 smokers, 43.4% passive smokers, and 38.4% non-smokers. After adjusting for potential cofounders,
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40 41 serum 25(OH)D status showed no association with AD or asthma at the national level.

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42 42 **Conclusion:** Vitamin D deficiency is highly prevalent in Korean adolescents, which should be a
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44 43 matter of public health concern. The cotinine-verified prevalence of smoking was also high in this
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46 44 population, but its relationship with vitamin D deficiency was not confirmed in our study. Our results
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48 45 provide epidemiologic evidence against the association of vitamin D status with AD and asthma at the
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50 46 national level among Korean adolescents.

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4 47 **Article summary**

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6 48 **Strengths and limitations of this study**

7
8 49 One of the first studies to identify the prevalence and risk factors for vitamin D deficiency in Korean
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10 50 adolescents.

11 51 The relationship of vitamin D with AD and asthma were assessed at a national level.

12 52 Cotinine-verified smoking status was adopted in this study.

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14 53 Limitations of the study include its cross-sectional design and lack of data on dietary and
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16 54 supplemental vitamin D intake.

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18 55 Also, the study has recall bias because diagnoses of allergic conditions (AD and asthma) were self-
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20 56 reported.

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58 INTRODUCTION

59 Vitamin D is essential for skeletal health and bone growth and its deficiency has been associated with
60 skeletal deformities in children and fracture risk in adults. Recent studies have also identified the
61 previously unanticipated roles of vitamin D in the immune system, cardiovascular system and cancer
62 prevention.^{1,2} Despite growing health concerns over low vitamin D status, few nation-wide studies
63 have been performed to evaluate vitamin D deficiency in the Korean pediatric population.^{3,4}
64 Suboptimal vitamin D level was reported in 70% of the US children (9% vitamin D deficient, 61%
65 vitamin D insufficient),⁵ and it was found in 41.7% of the children from New Zealand).⁶ Korean
66 adolescents are theoretically at increased risk for vitamin D deficiency because of the high latitude
67 (34-38°N), culturally vigorous sun protection, reduced outdoor activity and lack of vitamin D-fortified
68 food.

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

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

80 **METHODS**

81 **Study population**

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

91 Both KNHANES IV and V adopted the stratified multistage cluster sampling design by using the
92 rolling-survey sampling method. The rolling sample collected each year is the probability sample
93 representing the general Korean population, and it is homogeneous and independent of each other. In
94 2008, 2009, 2010 and 2011, a total of 37,753 individuals were sampled. The study population was
95 further limited to 4,598 adolescents aged 10-18 years. Among the 4,598 participants, we subsequently
96 excluded the following participants: those whose 25(OH)D levels were not measured; those without
97 urine cotinine levels; those who did not completely answer the questions regarding AD or asthma;
98 those without body mass index (BMI) measurement; and those who had a chronic disease that may
99 affect vitamin D metabolism. Finally, a total of 2,515 participants (1,314 males and 1,201 females)
100 were eligible for analysis.

101 **Study variables**

102 Blood samples were collected from the antecubital vein, refrigerated immediately, transported to the
103 central testing facility in cold storage, and analyzed within 24 hours of sampling. Serum 25(OH)D
104 levels were measured as described previously¹⁶ and categorized as sufficient (≥ 30 ng/mL), insufficient
105 (20-29.9 ng/mL) or deficient (< 20 ng/mL).¹⁷

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

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4 107 continuous variables while season of sampling was categorized into winter months (November-
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6 108 March) and summer months (April-October).¹⁸ The region of residence of each participant was
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8 109 grouped as follows: urban (Seoul, Gyeonggi, Busan, Daegu, Incheon, Gwangju, Daejeon, and Ulsan)
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10 110 and rural (Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and
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12 111 Jeju).¹⁹ Monthly income was standardized according to the number of family members (monthly
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14 112 income/number of family members) and it was divided into the following 4 quartile groups: lowest,
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16 113 lower middle, higher middle, and highest. Participants who performed moderate physical activity for
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18 114 more than 30 minutes per day on more than 5 days a week and/or strenuous physical activity for more
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20 115 than 20 minutes per day on more than 3 days a week were assigned to the regular exercise group.
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22 116 Regular walking was designated as “yes” for those who walked for more than 30 minutes per day on
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24 117 more than 5 days a week.¹⁹ Smoking status was divided into three groups based on the urine cotinine
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26 118 level, which are as follows: non-smokers (<5 ng/mL), passive smokers (secondhand smoking) (5-100
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28 119 ng/mL) and active smokers (>100 ng/mL).¹⁹⁻²¹ Urine cotinine level was measured by chromatography
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30 120 mass spectrometry using the Perkin Elmer Clarus 600T (PerkinElmer, Turku, Finland). All data were
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32 121 measured in a standardized manner and reviewed by the central quality control center.

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34 122 The following question was used to assess physician-diagnosed AD in each participant: “Have you
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36 123 been diagnosed with AD by a doctor?” or “Have you been told by a doctor that (your child) had AD?”
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38 124 Physician-diagnosed asthma was also determined using similar questions.

39 40 41 125 **Statistical analysis**

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43 126 Statistical analyses were performed using an SAS survey procedure (version 9.2; SAS Institute, Inc.,
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45 127 Cary, NC, USA), and 2-sided *p* values of less than 0.05 were considered statistically significant. To
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47 128 produce unbiased national estimates representing the general Korean population, we used KNHANES
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49 129 sample weights accounting for the complex sampling design to each participant.

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51 130 To compare the mean serum 25(OH)D levels among categories of each possible predicting factor,
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53 131 Student’s *t*-test or ANOVA (followed by Tukey-Kramer for multiple comparison) was used.

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55 132 Univariate analysis was performed to evaluate the association of the possible predicting factors, AD
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57 133 and asthma with vitamin D deficiency. Participants’ characteristics were described using means and
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4 134 standard errors for continuous variables and numbers and percentages for categorical variables. Here,
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6 135 the Student's *t*-test or ANOVA was used for comparing continuous variables, as appropriate, and Rao-
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8 136 Scott χ^2 test was used for comparing categorical variables. Variables with a *p*-value <0.05 in
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10 137 univariate analyses were included in the multivariate regression model for exploring factors
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12 138 associated with serum 25(OH)D.

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14 139 To estimate the mean serum 25(OH)D levels in participants with and without AD and asthma, we
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16 140 performed simple and multiple linear regression analyses using the generalized linear model for a
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18 141 complex survey design. The estimated means were calculated as follows: no adjustment for potential
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20 142 confounders; and confounder adjustment for age, gender, region, BMI, smoking, and season of
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22 143 sampling.

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24 144 To estimate the odds ratios (ORs) for AD and asthma according to quartiles of serum 25(OH)D levels,
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26 145 we conducted simple and multivariate logistic regression analyses by using the generalized linear
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28 146 model for a complex survey design. The ORs and 95% CIs were calculated in the following ways: no
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30 147 adjustment for potential confounders; and confounder adjustment for age, gender, region, BMI,
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32 148 smoking, and season of sampling.
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149 RESULTS

150 General characteristics

151 A total of 2,515 subjects (1,314 males and 1,201 females, age: 14.4 ± 0.1 years) were included in the
152 study. Table 1 summarizes the baseline data of the participants. The mean BMI of the study
153 population was 20.9 kg/m^2 (SE: 0.1). Blood samples were drawn more frequently in April-October
154 (58.4%) than in November-March (41.62%). The urine cotinine-verified smoking statuses were as
155 follows: active smoking 18.2%, passive smoking 43.4%, and non-smoking 38.4%. The prevalences of
156 AD and asthma in the study population were 10.6% and 4.1%, respectively.

157 Serum 25(OH)D levels in the study population

158 The mean concentration of serum 25(OH)D in the 2,515 subjects was 16.7 ng/mL (SE: 0.2), with a
159 range of 3.0 to 46.2 ng/mL . Overall, 1,843 subjects (73.3%) were vitamin D deficient, 613 subjects
160 (24.4%) were vitamin D insufficient, and 59 subjects (2.3%) were vitamin sufficient (Figure). Girls
161 had a significantly lower mean serum 25(OH)D level than boys ($16.0 \pm 0.2 \text{ ng/mL}$ vs 17.3 ± 0.2
162 ng/mL , $p < 0.0001$), and subjects sampled in winter months (November-March) had lower serum
163 25(OH)D levels than those sampled in summer months (April-October) ($14.7 \pm 0.2 \text{ ng/mL}$ vs $18.1 \pm$
164 0.2 ng/mL , $p < 0.0001$). Passive smoking (vs non-smoking) and urban residence (vs rural) were also
165 associated with statistically lower serum 25(OH)D levels ($p = 0.0382$ and $p = 0.0024$, respectively)
166 (Table 2).

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

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

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

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4 176 **Relationship of serum 25(OH)D with AD and asthma**

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6 177 Univariate analysis demonstrated that vitamin D deficiency was not significantly associated with AD
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8 178 ($p=0.4675$) or asthma ($p=0.5376$) (Table 1).

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10 179 Multivariate logistic regression showed that vitamin D deficiency had no association with AD and
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12 180 asthma after adjusting for age, gender, region of residence, BMI, smoking, and season of sampling.

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14 181 The adjusted odds ratio for AD and asthma increased across categories of serum 25(OH)D (1.00
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16 182 [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
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18 183 <20 ng/mL), but it was not statistically significant ($p=0.8078$ (AD) and $p=0.1360$ (asthma) for 20-29.9
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20 184 ng/mL and $p=0.4271$ (AD) and $p=0.1042$ (asthma) for <20 ng/mL) (Table 6).

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22 185 Univariate or multivariate analyses with serum 25(OH)D levels as a continuous variable showed
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24 186 similar results, regardless of the adjustment (Table 6).
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DISCUSSION

Vitamin D deficiency has been reported to be highly prevalent in a number of countries¹ 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 studies²⁰⁻²³ 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 group²⁸ 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

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4 214 for non-smokers exposed to secondhand smoking based on published values.²⁴⁻²⁶ With cotinine
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6 215 verification, 18.2% of the participants were classified as active smokers, 43.4% of the participants
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8 216 were classified as passive smokers, and 38.4% of the participants were classified as non-smokers,
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10 217 where the percentage of smokers (active and passive) was higher than what we expected.
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12 218 In this study, the mean concentration of serum 25(OH)D was 16.7 ng/mL with 73.3% of the subjects
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14 219 being vitamin D deficient (serum 25(OH)D <20 ng/mL). Potential explanations for this remarkably
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16 220 low vitamin D status observed in Korean children include, but are not limited to pigmented skin (skin
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18 221 pigmentation is known to reduce the skin's production of vitamin D in East Asian populations),³
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20 222 vigorous use of sunscreens and possibly dietary factors. The Dietary Reference Intakes for vitamin D
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22 223 from the Institute of Medicine (2010) and the American Academy of Pediatrics state that the Adequate
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24 224 Intake (AI) of vitamin D is 15 µg/day for children.^{29,30} However, in the Dietary Reference Intakes for
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26 225 vitamin D for Koreans published in 2010, the AI of vitamin D for children is claimed to be 5 µg/day,³¹
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28 226 which we feel is too low. Our study results call for careful consideration of vitamin D
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30 227 supplementation, particularly in at-risk children to further optimize their health.
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32 228 Exposure to sunlight, specifically UVB, leads to vitamin D synthesis in the skin, which provides most
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34 229 of the vitamin D requirement of an individual. In Korea (latitude: 34-38°N), vitamin D is synthesized
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36 230 mostly between April and October (summer months),³² which was apparent in our study.
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38 231 The finding of an inverse relationship of age, female gender, urban residence, and BMI with vitamin
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40 232 D status is in accordance with previous studies.^{3,4,23,33,34} It has been previously shown that there is a
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42 233 consistent decline in physical outdoor activity over the school age years. The prevalence of physical
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44 234 inactivity is higher in girls than in boys and it is greater in obese children than in their normal-weight
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46 235 peers. The lifestyle in big cities is also sedentary, and it is therefore possible that age, gender, place of
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48 236 residence and BMI may act as surrogate indicators for sunlight exposure through their association
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50 237 with physical activity. In addition to limited sunlight exposure due to physical inactivity, the inverse
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52 238 association of BMI and urban residence with vitamin D levels can be explained by sequestration of
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54 239 vitamin D in fat tissues³⁵ and UVB blockade due to air pollution.
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56 240 Smokers have been reported to have a significantly higher risk of vitamin D deficiency,²⁰⁻²³ but this
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4 241 was not confirmed in our study. Since active smoking is usually done outdoors, this can result in
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6 242 greater exposure to sunlight which may alter the true effect of smoking on serum 25(OH)D. Passive
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8 243 smoking in adolescents is more likely to occur indoors, and interestingly enough, passive smoking
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10 244 was reported to be independently associated with low serum 25(OH)D in Taiwanese
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12 245 children/adolescents.²³ Results can also be influenced by cotinine cut-off values and higher thresholds
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14 246 such as 10 ng/mL or 30 ng/mL may be considered in the future when testing children as this
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16 247 population can have higher cotinine concentrations than adults due to differences in body distribution
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18 248 and nicotine metabolism.³⁶
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20 249 There is accumulating evidence indicating that an adequate concentration of vitamin D is protective
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22 250 against allergic disease in children.^{37,38} However, most of these findings have been reported in western
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24 251 countries and only a few studies have been performed in Asia. We consider our investigation
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26 252 important because differences among ethnicities and subjects of various age groups can possibly
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28 253 affect the results.
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30 254 In this national level study, AD and asthma were not associated with serum 25(OH)D. The present
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32 255 study results, as well as previous studies showing an association between high vitamin D levels and
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34 256 AD, raise questions on the suggestion that vitamin D may be used to prevent or treat AD. In fact, a
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36 257 Cochrane review found no evidence for an effect of vitamin D supplementation on AD.³⁹ The
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38 258 beneficial effect of sunlight on AD has been well documented, but this is probably due to the anti-
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40 259 inflammatory and antimicrobial effects of UV and not those of vitamin D.
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42 260 Consistent with our results, Hollams *et al.*⁴⁰ showed no significant cross-sectional association between
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44 261 serum vitamin D levels and current asthma in 6- or 14-year-old children in UK. Further studies
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46 262 examining the role of serum 25(OH)D levels as predictors of subsequent asthma are needed during the
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48 263 longitudinal follow-up of cohort subjects.
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50 264 The prevalences of AD and asthma in the present study (10.6% and 4.1%, respectively) differed
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52 265 slightly from those in the other population-based studies in Taiwan²³ and Japan.⁴¹ The variation in
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54 266 prevalence in different countries may be caused by several factors, including genetic, socioeconomic
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56 267 and environmental factors.
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4 268 This study has several notable strengths. The sampling of adolescents across a broad age range, a
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6 269 large sample size with robust data collection, incorporation of objective markers of smoking status,
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8 270 and a thorough analysis strengthen the results of this study. Limitations of the study include its cross-
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10 271 sectional design and lack of data on dietary and supplemental vitamin D intake. Also, the study is
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12 272 prone to recall bias because diagnoses of allergic conditions (AD and asthma) were self-reported.

14 273 **CONCLUSION**

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16 274 Vitamin D deficiency is highly prevalent in Korean adolescents. It is a significant public health
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18 275 concern, and optimal vitamin D intakes to maintain sufficient vitamin D status should be examined.
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20 276 The cotinine-verified prevalence of smoking was also high in this population, but its relationship with
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22 277 vitamin D deficiency was not confirmed in our study. Above all, our results provide epidemiologic
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24 278 evidence against the association of vitamin D status with atopic dermatitis and asthma in Korean
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26 279 adolescents. The finding is obtained at the national level and is meaningful, but at the same time, it
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28 280 merits further study.

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4 281 **Acknowledgements** This study was supported by a grant of the Korean Healthcare technology R&D
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6 282 project, Ministry of Health & Welfare, Republic of Korea (Grant No.: HN15C0105). The funders had
7
8 283 no role in study design, collection, analysis and interpretation of data, writing of the manuscript, or
9
10 284 decision to submit for publication.
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14 286 **Contributors** EJB, SHC, JDL, and HSK made substantial contributions to the conception or design of
15
16 287 the work. JYH and HSK had role in the acquisition, analysis and interpretation of data for the work.
17
18 288 EJB, SHC, JDL, and HSK drafted and revised the work and all authors gave final approval and agreed
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20 289 to be accountable for all aspects of the work.
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24 291 **Funding** This study was supported by a grant of the Korean Healthcare technology R&D project,
25
26 292 Ministry of Health & Welfare, Republic of Korea (Grant No.: HN15C0105)
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30 294 **Competing interests** None declared.
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34 296 **Ethics approval** The institutional review board at the Korea Centers for Disease Control and
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36 297 Prevention approved the protocol
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40 299 **Data sharing statement** All data from the study, published and unpublished, are available to the
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42 300 principle investigators. The data are managed under the Department of Dermatology, Incheon St.
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44 301 Mary's Hospital, The Catholic University of Korea, Incheon, Korea.
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399 **FIGURE LEGENDS**

400 **Figure** Serum 25(OH)D levels: histogram

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

	Total (n=2515)	Vit D Level			P value
		Deficiency (<20ng/ml)	Insufficiency (≥20 - <30ng/ml)	Sufficiency (≥30ng/ml)	
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.					

403 **Table 2. Vitamin D levels in the study population**

	Vit D	<i>P</i> value ⁽¹⁾	<i>P</i> 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 highest : 0.0218
Lower middle	16.63 ± 0.29		lower middle vs highest : 0.1576
Higher middle	16.47 ± 0.24		higher middle vs highest : 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			

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406 **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.			

408 **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.			

413 **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)	<i>P</i> value	Estimated mean	Difference (Estimated mean)	<i>P</i> 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.						

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

		Unadjusted		Adjusted	
		OR (95%CI)	<i>P</i> value	OR (95%CI)	<i>P</i> 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.					

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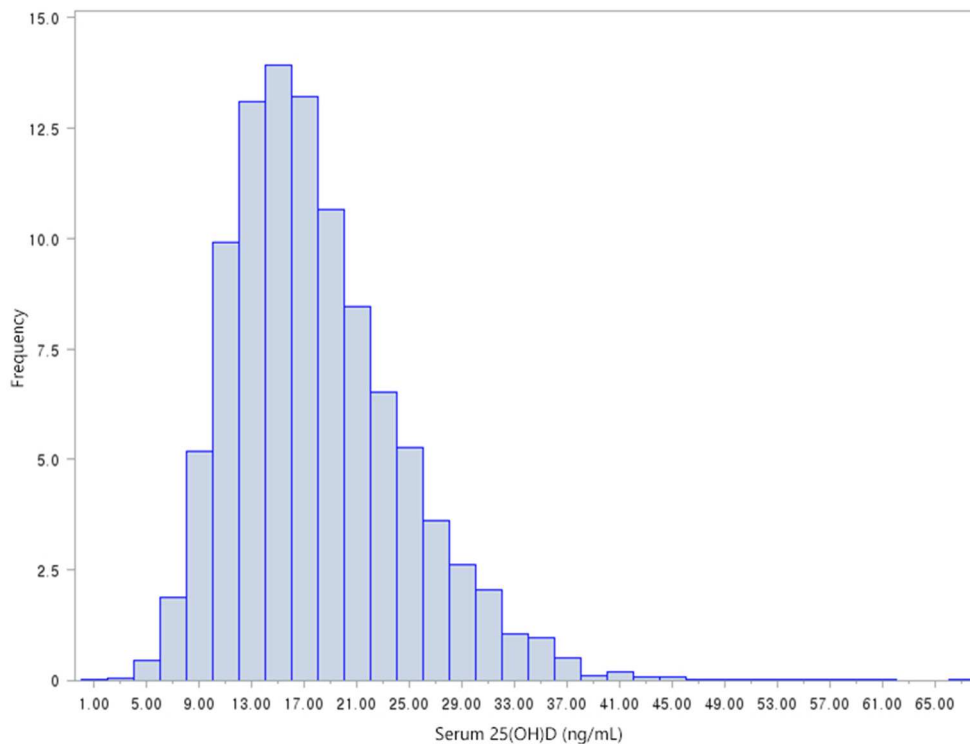


Figure Serum 25(OH)D levels: histogram

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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016409.R1
Article Type:	Research
Date Submitted by the Author:	23-May-2017
Complete List of Authors:	Byun, Eun Jung Heo, Jin Young Cho, Sang Hyun Lee, Jeong Deuk Kim, Hei Sung; Dermatology
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Dermatology, Smoking and tobacco, Health services research, Paediatrics
Keywords:	vitamin D deficiency, atopic dermatitis, cotinine, smoking, adolescent

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6 **2 Suboptimal vitamin D status in Korean adolescents: A nation-wide**
7 **3 study on its prevalence, risk factors including cotinine verified**
8 **4 smoking status, and association with atopic dermatitis and asthma**
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25 **11 Short title: Suboptimal vitamin D status in Korean adolescents**
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23 ABSTRACT

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

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

30 **Setting:** South Korea (Nation-wide)

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

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

36 **Results:** Overall, 73.3% of the subjects were vitamin D deficient (25OHD < 20 ng/mL) and 24.4% of
37 the subjects were vitamin D insufficient (25OHD, 20-29.9 ng/mL). Older age ($p<0.001$), female
38 gender ($p<0.001$), urban residence ($p=0.019$), higher body mass index ($p=0.003$) and sampling in
39 winter months (November-March) ($p<0.001$) were independently associated with low serum 25OHD
40 levels. With cotinine verification, 18.2% of the participants were classified as active smokers, and
41 43.4% were classified as passive smokers. After adjusting for potential cofounders, serum 25OHD
42 status showed no association with AD or asthma.

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

47 **Strengths and limitations of this study**

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

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

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4 50 nicotine) is a specific marker of smoking with high sensitivity.

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6 51 The limitation of the study is its cross-sectional design and the lack of data on the population's dietary
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8 52 and supplemental vitamin D intake. Also, the study has recall bias because the diagnoses of allergic
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10 53 conditions (AD and asthma) were self-reported.

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12 54 The definition of asthma and AD were physician-diagnosed asthma and AD which may have missed
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14 55 some children with symptomatic asthma and AD who have not been diagnosed.

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58 INTRODUCTION

59 Vitamin D is essential for skeletal health and bone growth and its deficiency has been associated with
60 skeletal deformities in children and fracture risk in adults. Recent studies have also identified the
61 previously unanticipated roles of vitamin D in the immune system, cardiovascular system and cancer
62 prevention.^{1,2} Despite growing health concerns over low vitamin D status, few nation-wide studies
63 have been performed to evaluate vitamin D deficiency in the Korean pediatric population.^{3,4}
64 Suboptimal vitamin D level was reported in 70% of the US children (9% vitamin D deficient, 61%
65 vitamin D insufficient),⁵ and it was found in 41.7% of the children from New Zealand).⁶ Korean
66 adolescents are theoretically at increased risk for vitamin D deficiency because of the high latitude
67 (34-38°N), culturally vigorous sun protection, reduced outdoor activity and lack of vitamin D-fortified
68 food.

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

77 In this study, we aimed to identify the prevalence and risk factors for vitamin D deficiency in Korean
78 adolescents and to assess its relationship with AD and asthma at the national level. Cotinine-verified
79 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 previously¹⁶ and categorized as sufficient (≥ 30 ng/mL), insufficient (20-29.9 ng/mL) or deficient (< 20 ng/mL).¹⁷

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4 107 Factors were categorized to analyze the risk factors for vitamin D deficiency. Age and BMI were
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6 108 continuous variables while season of sampling was categorized into winter months (November-
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8 109 March) and summer months (April-October).¹⁸ The region of residence of each participant was
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10 110 grouped as follows: urban (Seoul, Gyeonggi, Busan, Daegu, Incheon, Gwangju, Daejeon, and Ulsan)
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12 111 and rural (Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and
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14 112 Jeju).¹⁹ Monthly income was standardized according to the number of family members (monthly
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16 113 income/number of family members) and it was divided into the following 4 quartile groups: lowest,
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18 114 lower middle, higher middle, and highest. Participants who performed moderate physical activity for
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20 115 more than 30 minutes per day on more than 5 days a week and/or strenuous physical activity for more
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22 116 than 20 minutes per day on more than 3 days a week were assigned to the regular exercise group.
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24 117 Regular walking was designated as “yes” for those who walked for more than 30 minutes per day on
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26 118 more than 5 days a week.¹⁹ Smoking status was divided into three groups based on the urine cotinine
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28 119 level, which are as follows: non-smokers (<5 ng/mL), passive smokers (secondhand smoking) (5-100
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30 120 ng/mL) and active smokers (>100 ng/mL).¹⁹⁻²¹ Urine cotinine level was measured by chromatography
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32 121 mass spectrometry using the Perkin Elmer Clarus 600T (PerkinElmer, Turku, Finland). All data were
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34 122 measured in a standardized manner and reviewed by the central quality control center.
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36 123 The following question was used to assess physician-diagnosed AD in each participant: “Have you
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38 124 been diagnosed with AD by a doctor?” or “Have you been told by a doctor that (your child) had AD?”
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40 125 Physician-diagnosed asthma was also determined using similar questions.
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43 **Statistical analysis**

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45 127 Statistical analyses were performed using an SAS survey procedure (version 9.2; SAS Institute, Inc.,
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47 128 Cary, NC, USA), and 2-sided *p* values of less than 0.05 were considered statistically significant. To
48
49 129 produce unbiased national estimates representing the general Korean population, we used KNHANES
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51 130 sample weights accounting for the complex sampling design to each participant.
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53 131 To compare the mean serum 25OHD levels among categories of each possible predicting factor,
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55 132 Student’s *t*-test or ANOVA (followed by Tukey-Kramer for multiple comparison) was used.
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57 133 Univariate analysis was performed to evaluate the association of the possible predicting factors, AD
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4 134 and asthma with vitamin D deficiency. Participants' characteristics were described using means and
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6 135 standard errors for continuous variables and numbers and percentages for categorical variables. Here,
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8 136 the Student's *t*-test or ANOVA was used for comparing continuous variables, as appropriate, and Rao-
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10 137 Scott χ^2 test was used for comparing categorical variables. Variables with a *p*-value <0.05 in
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12 138 univariate analyses were included in the multivariate regression model for exploring factors
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14 139 associated with serum 25OHD.

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16 140 To estimate the mean serum 25OHD levels in participants with and without AD and asthma, we
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18 141 performed simple and multiple linear regression analyses using the generalized linear model for a
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20 142 complex survey design. The estimated means were calculated as follows: no adjustment for potential
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22 143 confounders; and confounder adjustment for age, gender, region, BMI, smoking, and season of
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24 144 sampling.

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26 145 To estimate the odds ratios (ORs) for AD and asthma according to quartiles of serum 25OHD levels,
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28 146 we conducted simple and multivariate logistic regression analyses by using the generalized linear
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30 147 model for a complex survey design. The ORs and 95% CIs were calculated in the following ways: no
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32 148 adjustment for potential confounders; and confounder adjustment for age, gender, region, BMI,
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34 149 smoking, and season of sampling.
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150 RESULTS

151 General characteristics

152 A total of 2,515 subjects (1,314 males and 1,201 females, age: 14.4 ± 0.1 years) were included in the
153 study. Table 1 summarizes the baseline data of the participants. The mean BMI of the study
154 population was 20.9 kg/m^2 (SE: 0.1). Blood samples were drawn more frequently in April-October
155 (58.4%) than in November-March (41.6%). The urine cotinine-verified smoking statuses were as
156 follows: active smoking 18.2%, passive smoking 43.4%, and non-smoking 38.4%. The prevalence of
157 AD and asthma in the study population were 10.6% and 4.1%, respectively.

158 Serum 25OHD levels in the study population

159 The mean concentration of serum 25OHD in the 2,515 subjects was 16.7 ng/mL (SE: 0.2), with a
160 range of 3.0 to 46.2 ng/mL . Overall, 1,843 subjects (73.3%) were vitamin D deficient, 613 subjects
161 (24.4%) were vitamin D insufficient, and 59 subjects (2.3%) were vitamin sufficient (Figure). Girls
162 had a significantly lower mean serum 25OHD level than boys ($16.0 \pm 0.2 \text{ ng/mL}$ vs $17.3 \pm 0.2 \text{ ng/mL}$,
163 $p < 0.001$), and subjects sampled in winter months (November-March) had lower serum 25OHD levels
164 than those sampled in summer months (April-October) ($14.7 \pm 0.2 \text{ ng/mL}$ vs $18.1 \pm 0.2 \text{ ng/mL}$,
165 $p < 0.001$). Passive smoking (vs non-smoking) and urban residence (vs rural) were also associated with
166 statistically lower serum 25OHD levels ($p = 0.038$ and $p = 0.002$, respectively) (Table 2).

167 Univariate and multivariate analyses of factors associated with serum 25OHD

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

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

176 Relationship of serum 25OHD with AD and asthma

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4 177 Univariate analysis demonstrated that vitamin D deficiency was not significantly associated with AD
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6 178 ($p=0.468$) or asthma ($p=0.538$) (Table 1).
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8 179 Multivariate logistic regression showed that vitamin D deficiency had no association with AD and
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10 180 asthma after adjusting for age, gender, region of residence, BMI, smoking, and season of sampling.

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12 181 The adjusted odds ratio for AD and asthma increased across categories of serum 25OHD (1.00
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14 182 [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
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16 183 <20 ng/mL), but it was not statistically significant ($p=0.8078$ (AD) and $p=0.1360$ (asthma) for 20-29.9
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18 184 ng/mL and $p=0.4271$ (AD) and $p=0.1042$ (asthma) for <20 ng/mL) (Table 5).
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20 185 Univariate or multivariate analyses with serum 25OHD levels as a continuous variable showed similar
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22 186 results, regardless of the adjustment (Table 6).
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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 to 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: 34-38°N), 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 tissues²⁹ 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

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4 214 of smoking on serum 25OHD in Koreans, we adopted urine cotinine, which is presently the biomarker
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6 215 of choice for assessing tobacco smoke exposure.³¹⁻³⁴ KNHANES normally assesses a person's
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8 216 smoking status via a survey. For those who are aged 19 years and above, detailed questions are asked
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10 217 regarding the presence and history of active and passive smoking, but for those who are aged less than
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12 218 19 years, the questions are much more limited. There is currently no data with regards to passive
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14 219 smoking in Korean adolescents and the survey only determines the presence of active smoking in
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16 220 subjects aged between 12-18 years. Omissions and false responses are common in this age group³⁵
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18 221 which impede accurate assessment of the smoking status, and to improve accuracy, we adopted the
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20 222 cotinine-verified smoking status in our study. Urine cotinine level was measured for a limited time-
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22 223 period (2008-2011), and this measurement has only been performed for those aged 10 years and above,
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24 224 which resulted in an inevitable reduction of our study population. We used a urine cotinine cut off
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26 225 value of more than 100 ng/mL to discriminate smokers from non-smokers and 5 ng/mL as a threshold
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28 226 for non-smokers exposed to secondhand smoking based on published values.³¹⁻³³ With cotinine
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30 227 verification, 18.2% of the participants were classified as active smokers, 43.4% of the participants
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32 228 were classified as passive smokers, and 38.4% of the participants were classified as non-smokers,
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34 229 where the percentage of smokers (active and passive) was higher than what we expected.
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36 230 Smokers have been reported to have a significantly higher risk of vitamin D deficiency,^{20,21,28,30} but
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38 231 this was not confirmed in our study. Since active smoking is usually done outdoors, this can result in
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40 232 greater exposure to sunlight which may alter the true effect of smoking on serum 25OHD. Passive
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42 233 smoking in adolescents is more likely to occur indoors and interestingly-enough, passive smoking was
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44 234 reported to be independently associated with low serum 25OHD in Taiwanese children/adolescents.²⁸
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46 235 However, results can also be influenced by cotinine cut-off values and higher thresholds such as 10
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48 236 ng/mL or 30 ng/mL may be considered in the future when testing children as this population can have
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50 237 higher cotinine concentrations than adults due to differences in body distribution and nicotine
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52 238 metabolism.³⁶
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54 239 Some studies have indicated that an adequate concentration of vitamin D is protective against allergic
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56 240 disease in children.^{8,9,15,37,38} However, most of these findings have been reported in western countries
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4 241 and only a few studies have been performed in Asia. We consider our investigation meaningful
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6 242 because differences among ethnicities and subjects of various age groups can possibly affect the
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8 243 results.

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10 244 In this national level study, the risk of AD and asthma were not dependent on serum 25OHD. The
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12 245 present study results, as well as prior studies which showed little association between vitamin D
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14 246 deficiency and AD,^{10-12,28} raise questions on the suggestion that vitamin D may be used to prevent or
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16 247 treat AD. In fact, a Cochrane review found no evidence for an effect of vitamin D supplementation on
17
18 248 AD.³⁹ The beneficial effect of sunlight on AD has been well documented, but this is probably due to
19
20 249 the anti-inflammatory and antimicrobial effects of UV and not those of vitamin D.

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22 250 As in AD, there has been considerable controversy over the relationship between vitamin D levels and
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24 251 asthma in children. While some studies showed an inverse relationship between vitamin D levels and
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26 252 current wheeze and asthma severity,⁸ others such as Hollams et al.⁴⁰ showed no significant cross-
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28 253 sectional association between serum vitamin D levels and current asthma. Our study too did not find
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30 254 any relationship between vitamin D level and asthma, but further studies would be needed to
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32 255 determine a causal relationship and its mechanism.

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34 256 This study has several notable strengths. The sampling of adolescents across a broad age range, a
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36 257 large sample size with robust data collection, incorporation of objective markers of smoking status,
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38 258 and a thorough analysis strengthen the results of this study. Limitations of the study include its cross-
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40 259 sectional design and lack of data on dietary and supplemental vitamin D intake. Also, the study is
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42 260 prone to recall bias because diagnoses of allergic conditions (AD and asthma) were self-reported. The
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44 261 definition of asthma and AD were physician-diagnosed asthma and AD, which may have missed some
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46 262 children with symptomatic asthma and AD in the population who have not been diagnosed. Although
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48 263 season adjusted, desasonalization of vitamin D levels with an algorithm⁴¹ may have further increased
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50 264 the accuracy and strength of our study.

51
52 265 In conclusion, a high prevalence of vitamin D deficiency was noted in Korean adolescents aged 10 to
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54 266 18 years. For high risk individuals of vitamin D deficiency, we suggest vitamin D supplementation
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56 267 and food fortification. Adequate outdoor activity should also be emphasized in Korean adolescents,
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4 268 especially high school students, via media promotion and public health plans by schools and
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6 269 government. We found no apparent association between the cotinine-verified smoking status, AD, and
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8 270 asthma with vitamin D deficiency, which merits further study.
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4 272 **Acknowledgements** This study was supported by a grant of the Korean Healthcare technology R&D
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6 273 project, Ministry of Health & Welfare, Republic of Korea (Grant No.: HN15C0105). The funders had
7
8 274 no role in study design, collection, analysis and interpretation of data, writing of the manuscript, or
9
10 275 decision to submit for publication.
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14 277 **Contributors** EJB, SHC, JDL, and HSK made substantial contributions to the conception or design of
15
16 278 the work. JYH and HSK had role in the acquisition, analysis and interpretation of data for the work.
17
18 279 EJB, SHC, JDL, and HSK drafted and revised the work and all authors gave final approval and agreed
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20 280 to be accountable for all aspects of the work.
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23
24 282 **Funding** This study was supported by a grant of the Korean Healthcare technology R&D project,
25
26 283 Ministry of Health & Welfare, Republic of Korea (Grant No.: HN15C0105)
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30 285 **Competing interests** None declared.
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34 287 **Ethics approval** The institutional review board at the Korea Centers for Disease Control and
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36 288 Prevention/ Incheon St. Mary's Hospital, The Catholic University of Korea (IRB number:
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38 289 OC17ZESI0055) approved the protocol.
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42 291 **Data sharing statement** All data from the study, published and unpublished, are available to the
43
44 292 principle investigators. The data are managed under the Department of Dermatology, Incheon St.
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46 293 Mary's Hospital, The Catholic University of Korea, Incheon, Korea.
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392 **FIGURE LEGENDS**

393 **Figure** Serum 25OHD levels: histogram

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

	Total (n=2515)	Vit D Level			P value
		Deficiency (<20ng/ml)	Insufficiency (≥20 - <30ng/ml)	Sufficiency (≥30ng/ml)	
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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397 **Table 2. Vitamin D levels in the study population**

	Vit D	<i>P</i> value ⁽¹⁾	<i>P</i> 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 highest : 0.0218
lower middle	16.63±0.29		lower middle vs highest : 0.1576
higher middle	16.47±0.24		higher middle vs highest : 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

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

	Coefficient	95% Confidence interval	<i>P</i> 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.

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

	Odds ratio	95% Confidence interval	<i>P</i> 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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410 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.

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412 **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)	<i>P</i> value	Estimated mean	Difference (Estimated mean)	<i>P</i> 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.

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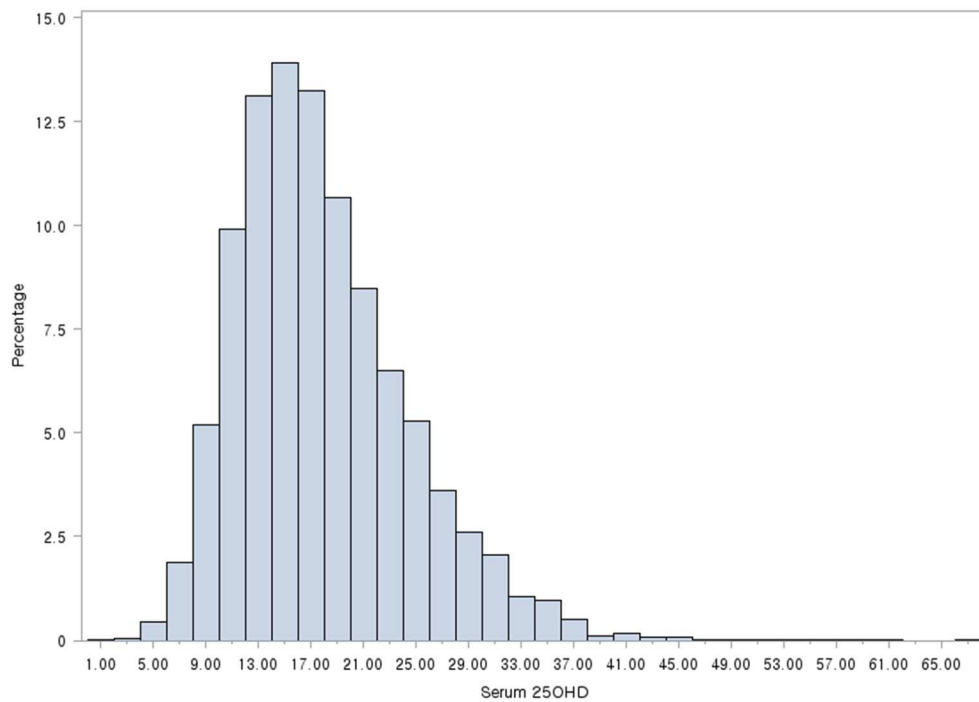


Figure Serum 25OHD levels: histogram

190x142mm (300 x 300 DPI)

ew only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	6,7

		<i>Cross-sectional study</i> —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 (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —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 (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	8,9
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.