



Title	Determinants of serum 25-hydroxyvitamin D in Hong Kong
Author(s)	Xu, C; Perera, RAPM; Chan, YH; Fang, J; Ng, S; Ip, DKM; Kam, AM; Leung, GM; Peiris, JSM; Cowling, BJ
Citation	The British Journal of Nutrition, 2015, v. 114 n. 1, p. 144-151
Issued Date	2015
URL	http://hdl.handle.net/10722/211641
Rights	The British Journal of Nutrition. Copyright © Cambridge University Press.

1 **Determinants of serum 25-hydroxyvitamin D in Hong Kong**

2
3 Cuiling Xu¹, Ranawaka A. P. M. Perera¹, Yap-Hang Chan^{1,2}, Vicky J. Fang¹, Sophia Ng³, Dennis
4 K. M. Ip¹, Andrea May-Sin Kam¹, Gabriel M. Leung¹, J. S. Malik Peiris¹, Benjamin J. Cowling^{1*}

5
6 **Affiliations:**

- 7 1. School of Public Health, The University of Hong Kong, Pokfulam, Hong Kong Special
8 Administrative Region, China.
9 2. Division of Cardiology, Department of Medicine, Queen Mary Hospital, The University
10 of Hong Kong, Hong Kong, China
11 3. School of Public Health, University of Michigan, Ann Arbor, Michigan.

12
13 ***Corresponding author:**

14 Dr B. J. Cowling, School of Public Health, Li Ka Shing Faculty of Medicine, The University of
15 Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong.

16 Tel: +852 3917 6711; Fax: +852 3520 1945; email: bcowling@hku.hk

17
18 Running title: Determinants of vitamin D in Hong Kong

19 Key words: Vitamin D, 25-hydroxyvitamin D, age, solar radiation, Hong Kong

20 Abbreviations: 1,25(OH)₂D, 1,25-dihydroxy-vitamin D ; 25(OH)D , 25-hydroxyvitamin D.

21 Word count (abstract): 250

22 Word count (main text): 2,986

1 **ABSTRACT**

2 Vitamin D plays an important role in skeletal health throughout life. Some studies have
3 hypothesized that vitamin D may reduce the risk of other diseases. Our study aimed to estimate
4 age-specific and sex-specific serum 25-hydroxyvitamin D (25(OH)D) status and to identify the
5 determinants of serum 25(OH)D status in Hong Kong, a subtropical city in southern China. In
6 2009-10, households in Hong Kong were followed up to identify acute respiratory illnesses, and
7 sera from 2694 subjects were collected in 3-4 different study phases to permit measurement of
8 25(OH)D levels at different times of the year. A questionnaire survey on diet and lifestyle was
9 conducted among children with simultaneous serum collection in April and May 2010. The mean
10 of serum 25(OH)D levels in age groups ranged from 39-63 nmol/L throughout the year with the
11 mean values in all age groups in spring below 50 nmol/L. Children 6-17y, and girls and women
12 had significantly lower serum 25(OH)D levels, than adults, and boys and men respectively (all
13 $p < 0.001$). We estimated that serum 25(OH)D levels in Hong Kong followed a lagged pattern
14 relative to climatic season by 5 weeks with lowest observed levels in early spring (March). For
15 children 6-17y, reporting a suntan, having at least 1 servings of fish per week, and having at least
16 1 serving of egg per week, were independently associated with higher serum 25(OH)D levels.
17 Adequate sunlight exposure and increased intake of dietary vitamin D could improve vitamin D
18 status, especially for children and females in the winter and spring.

19

20

1 **Introduction**

2 Vitamin D plays an important role in skeletal health, and vitamin D deficiency is known to be a
3 cause of rickets and osteoporosis ⁽¹⁾. In addition, a wide range of tissues and cells have been
4 found to possess vitamin D receptors. Observational studies have suggested that low 25-
5 hydroxyvitamin D values are associated with an increased risk for several non-skeletal diseases,
6 including cancer ^(2,3), infectious diseases ⁽⁴⁻⁷⁾, and cardiovascular disease ⁽⁸⁾. Vitamin D
7 inadequacy is being increasingly recognized worldwide, and remains common in children and
8 adults ^(1,9,10).

9 Humans acquire vitamin D from exposure to sunlight, from their diet, and from dietary
10 supplements ⁽¹⁾. The main natural source of vitamin D is the sun, as vitamin D is synthesized in
11 the skin after exposure to solar ultraviolet B radiation (wavelength 290–315 nm) ⁽¹⁾. A diet high
12 in oily fish prevents vitamin D deficiency ⁽¹¹⁾. Vitamin D from the skin and diet converts to 25-
13 hydroxy-vitamin D (25(OH)D) in the liver and subsequently to 1,25-dihydroxy-vitamin D
14 (1,25(OH)₂D) in the kidney, which is the active form of vitamin D ⁽¹⁾. 25(OH)D is the major
15 form of vitamin D that circulates in the blood stream and can be used as a marker to determine
16 vitamin D status ⁽¹²⁾.

17 Hong Kong is a subtropical coastal city in southern China, with sufficient sunshine during the
18 whole year and fish is commonly consumed in the local diet. However, there are few data on
19 vitamin D status by age and sex in residents, and on the effect of dietary and sun exposures on
20 vitamin D status in Hong Kong. Seasonal variation in Vitamin D status is thought to play a role
21 in the seasonality of bone mass ^(13,14). However, there is a paucity of data on the seasonality of
22 vitamin D levels in subtropical Hong Kong, where there is relatively little variation in the hours
23 of sunlight throughout the year.

24 We conducted a household-based prospective study from September 2009 through December
25 2010 in Hong Kong ⁽¹⁵⁾. The study was primarily designed to study the direct and indirect
26 effectiveness of influenza vaccination among school-age children in preventing influenza virus
27 infections in their households. For the present study, we determined vitamin D status in stored
28 sera to describe the seasonal variation in vitamin D status in children and adults over time, and to
29 investigate the determinants of vitamin D status. Our present study also included an additional
30 questionnaire survey conducted among participating children 6-17y of age in April and May

1 2010 to collect information on sun-seeking behaviors, and dietary and supplementary habits that
2 might affect vitamin D status.

3

4 **Methods**

5 *Study participants*

6 Participants included in this study of vitamin D were part of a household-based prospective study
7 of influenza, as describe elsewhere⁽¹⁵⁾. In 2009-2010, we recruited all members of 796
8 households, and each household included a child 6-17y of age who was randomly allocated to
9 receive either a single dose of seasonal trivalent inactivated influenza vaccine (TIV) or placebo
10 in a double blind manner. Enrollment, collection of serum specimens and vaccinations were
11 performed by trained research staff at a study clinic. Serum specimens were collected at baseline
12 (September 2009 through February 2010) and after 12 months at the end of the follow-up period
13 (“post-study”, October through December 2010). Serum specimens were also collected 1 month
14 after vaccination from the children who received vaccine or placebo (“post-vaccination”,
15 October 2009 through February 2010). A subset of participants also provided blood samples
16 half-way through the study (“mid-study”, April and May 2010).

17 Using a vitamin D questionnaire designed according to previous studies in the United
18 States^(16,17), we collected data about sun-seeking behaviors, and dietary and vitamin D
19 supplementary habits from these children 6-17y who also provided mid-study serum specimens
20 in April and May 2010. The questionnaires were completed by the children together with their
21 parents.

22

23 *Ethics*

24 Written consent was obtained from all adult subjects. Proxy written consent from parents or legal
25 guardians was obtained for participants 17 years of age and younger, with additional written
26 assent from those aged 8-17 years. The study protocol was approved by the Institutional Review
27 Board of The University of Hong Kong.

28

29 *Laboratory analysis*

30 Blood from all household members were collected in tubes containing clot activator and held at
31 4-8°C from collection until receipt at the laboratory. At the laboratory, each specimen was

1 centrifuged to extract the sera, which was then frozen at -80°C . The serum specimens were
2 subsequently tested for 25(OH)D using the OCTEIA ELISA 25-hydroxyvitamin D immunoassay
3 kit manufactured by Immunodiagnostic Systems (IDS) Ltd (Boldon, United Kingdom) (18).

4 According to the package insert of the assay, the inter-assay Coefficient of Variation for the
5 25(OH)D assay was 4.6%-8.7%, and the intra-assay Coefficient of Variation was 5.3%-6.7%. In
6 our own laboratory we found that the intra-assay Coefficient of Variation was 7.4%.

7 8 *Statistical analysis*

9 We anticipated that we would have at least 80% power to detect at least a 9 nmol/L difference in
10 serum 25(OH)D between any two groups (four age groups and male/female) in each season,
11 assuming a standard deviation of 15-18 nmol/L based on data available for mean and standard
12 deviation of serum 25(OH)D by sex in a normal population from the literature ⁽¹⁹⁾. The sample
13 size of 63 in each age or sex group would be adequate to test the difference in mean of serum
14 25(OH)D by age or sex in a single season. We anticipated that our overall study sample size of
15 2694 individuals with repeated measurements would permit reliable comparisons between
16 seasons, by age and sex, and would allow us to identify moderate effects of determinants after
17 accounting for serial correlation in the measurements.

18 The participants were categorized into four age groups, i.e. 6-17y, 18-44y, 45-64y and $\geq 65\text{y}$.
19 The four seasons were defined as spring (March-May), summer (June-August), autumn
20 (September-November), and winter (December-February) respectively. The 25(OH)D levels
21 were categorized into different seasons based on the data of specimen collection. If two
22 specimens from the same subject were categorized to the same season, we used the average
23 25(OH)D level of the two specimens. Since no blood specimens were collected in the study
24 during June through August of 2010, no data on 25(OH)D levels in the summer of 2010 were
25 available.

26 We used a generalized linear model to compare the mean of serum 25(OH) by age and sex in
27 each season to estimate age-specific and sex-specific patterns in serum 25(OH)D levels. Since
28 solar radiation can reflect climatic season, we fitted a random-effects linear regression model to
29 obtain quantitative seasonality estimates of serum 25(OH)D based on the repeated measures of
30 serum 25(OH)D, which included daily level of solar radiation as a predictive factor. Daily means
31 of solar radiation were obtained from Hong Kong observatory, and were smoothed using Kernel

1 density smoothing as a proxy measure for seasonal variation in the climate in Hong Kong⁽²⁰⁾. In
2 a separate secondary analysis, a random-effects sinusoidal linear regression model with annual
3 periodicity was fitted to characterize the seasonal variation of serum 25(OH)D. In the two
4 random-effects linear regression models used to estimate the seasonal variation of serum
5 25(OH)D, the associations of 25(OH)D with age, sex, educational attainment of the household
6 head, vaccination and chronic conditions were adjusted for. The ratio of serum 25(OH)D levels
7 between the peak season and the trough season in each age group was calculated to estimate the
8 degree of seasonal variation in serum 25(OH)D levels.

9 Since both vitamin D questionnaires and mid-study sera were collected simultaneously from a
10 subset of participating children 6-17y of age in April-May 2010, we performed univariable and
11 multivariable analyses to explore the determinants of serum vitamin D levels among children
12 using generalized linear models. A multiple linear model with backward selection was used to
13 exclude variables one by one from an initially complete model. Only the factors with p-values
14 <0.2 were included in the final model. Statistical analyses were conducted in R version 2.15.1 (R
15 Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.2 (SAS Institute, Cary,
16 NC).

17

18 **Results**

19 *Characteristics of participants*

20 In total 3030 people participated in the previous influenza household study, and 53 people from
21 14 households withdrew or were lost to follow-up. 2694/3030 participants (89%) had at least 1
22 serum specimen available for 25(OH)D testing (Table 1). Of the 2694 participants, 2459 (91%)
23 and 1341 (50%) had ≥ 2 and ≥ 3 serum specimens available for 25(OH)D testing respectively
24 (Figure 1). There was no difference in age, sex, educational attainment of household head,
25 vaccination history and chronic conditions between 3030 participants in the influenza household
26 study and 2694 participants included in the vitamin D analysis (Table 1). The median age of
27 these 2694 participants was 33 years (interquartile range, 11-43 years), and 46% were male. Of
28 these 2694 participants, 21% reported receipt of 2009-2010 seasonal influenza vaccine, and 16%
29 had a self-reported chronic condition.

30

31 *Mean of serum 25(OH)D by age and sex in different seasons*

1 Table 2 presents the comparative analysis of serum 25(OH)D levels in each season by age and
2 sex. In each season children 6-17y of age had significantly lower vitamin D levels (39-53 nmol/L)
3 compared to adults 18-44y of age (42-57 nmol/L) (all $p < 0.001$). Adults 45-64y of age (47-63
4 nmol/L) had significantly higher serum 25(OH)D levels than adults 18-44y of age in the other
5 three seasons (all $p < 0.01$) except the winter of 2009-10. The mean serum 25(OH)D level in adults
6 ≥ 65 y (41-56 nmol/L) was not significantly different from adults 18-44y of age in each season.
7 Males had significantly higher serum 25(OH)D levels (3-5 nmol/L) than females in each season.
8

9 *Seasonal variation of serum 25(OH)D*

10 The pattern of daily solar radiation showed one peak (August) in Hong Kong (Figure 2). Using
11 the random-effects linear regression model, we found that the daily level of solar radiation, age
12 and sex were significantly associated with serum 25(OH)D levels after adjusting for other factors
13 (supplementary Table 1). For males and females in the age groups of 6-17y, 18-44y, 45-64y, the
14 model that included a 5-week lag in solar radiation gave the best fit to time-varying serum
15 25(OH)D levels (all $p < 0.05$) (Figure 3a-c). We identified significant seasonal fluctuation in
16 serum 25(OH)D levels for males and females in the age groups of 6-17y, 18-44y and 45-64y,
17 which peaked in September (Autumn), and dropped to lowest levels in March (Spring). 10.6% of
18 the variation in vitamin D levels was explained by the inclusion of seasonal variation in solar
19 radiation in the model. In all four age groups, the average of predicted serum 25(OH)D levels in
20 boys/men were 4-9 nmol/L higher than in girls/women (all $p < 0.05$). In a secondary analysis
21 using the random effects sinusoidal linear regression model, we found that there was similar
22 degree of seasonal fluctuation in serum 25(OH)D levels for different age and sex groups to the
23 first random-effects model while the first random-effects model incorporating solar radiation
24 better explained the seasonal variation in serum 25(OH)D levels. The ratio of serum 25(OH)D
25 levels between the spring and the autumn of 2010 in each age group varied from 1.3-1.4.
26

27 *Factors that influence serum 25(OH)D among children*

28 321 children completed vitamin D questionnaires and also provided mid-study serum specimens
29 in April and May 2010. The median age of participants in the questionnaire survey was 11y
30 (interquartile range, IQR: 9-12y). 86% of participants reported a suntan in the past year, and 20%
31 reported an average of at least 1 hour of sun exposure per day in the past week. 21%, 30% and 38%

1 of participants reported having an average of at least 1 daily serving of fish, milk and eggs
2 respectively. 9%, 6% and 60% reported the use of additional vitamin D supplements, intake of
3 multivitamins, and use of cod liver or fish oil respectively.

4 In univariable analyses, younger age, male sex, reporting a suntan, having at least 1 serving of
5 fish per week, having at least 1 serving of milk per day, and taking cod liver oil or fish oil were
6 significantly associated with higher serum 25(OH)D levels (Table 3). In multivariable analysis,
7 younger age, male sex, reporting a suntan, having at least 1 serving of fish per week and having
8 at least 1 serving of egg per week were independently associated with higher serum 25(OH)D
9 levels (Table 3).

11 Discussion

12 In our study, we characterized seasonal fluctuations in serum 25(OH)D levels in subtropical
13 Hong Kong at 22° latitude, identifying peaks in September and troughs in March, following a
14 lagged pattern relative to climatic seasons. We found that the mean of serum 25(OH)D levels in
15 the peak season for each age group was 1.3-1.4 times higher than that in the trough season, while
16 the peak/trough ratios tend to be slightly greater in temperate locations such as the
17 Netherlands⁽²¹⁾, Germany⁽²²⁾, Italy⁽²³⁾ and Japan⁽¹⁹⁾. In spring, the means of serum 25(OH)D in
18 each of four age groups were **below 50 nmol/L that is recommended by the Institute of Medicine**
19 **Recommended Dietary Allowance (RDA)** ⁽²⁴⁾, and in the other seasons, these values were below
20 the requirements recommended by the International Osteoporosis Foundation and the US
21 Endocrine Society (≥ 75 nmol/L)⁽²⁵⁾. In Hong Kong, the means of serum 25(OH)D in different
22 age groups were also lower than those reports at the similar age groups from Japan, Thailand and
23 Vietnam in Asia and most reports from the countries in North America^(10,26-31). Moreover, the
24 means of serum 25(OH)D our study reported were lower than that (77 nmol/L) in Taiwan where
25 the latitude (25°) is similar to Hong Kong⁽³²⁾. The reasons why living in Hong Kong with lower
26 latitudes does not appear to protect against vitamin D insufficiency is likely due to several
27 factors, potentially including less time spent outdoors, less vitamin D intake from diet or dietary
28 supplements, skin pigmentation of the local Chinese residents⁽¹¹⁾, air pollution⁽³³⁾, or other racial
29 differences in genetic polymorphism⁽³⁴⁾.

30 Similar to the findings from several temperate locations^(19,35-40), our study estimated that there
31 is substantial seasonal fluctuation in serum 25(OH)D levels in Hong Kong. Previous studies in

1 subtropical Taiwan, Florida and Hong Kong reported the differences in serum 25(OH)D level
2 between summer (or autumn) and winter^(32,41,42). However our study with 15-month study
3 duration was able to predict the year-round seasonal fluctuation by using a cyclic regression
4 model, although there was a lack of data on summer levels of serum 25(OH)D in our study. Sun
5 exposure and solar radiation are known to be a major determinant of vitamin D status⁽¹⁾ and the
6 seasonal pattern of vitamin D in Hong Kong is consistent with seasonal variation in solar
7 radiation. In Hong Kong, hours of sunlight (136 and 111 hours/month respectively) and solar
8 radiation (10 and 12 megajoule (MJ)/m² respectively) in winter and spring are lower than those
9 (182 and 182 hours/month; 16 and 14 MJ/m² respectively) in the summer and autumn⁽²⁰⁾. The
10 weather in winter and spring is suitable for outdoor activity in Hong Kong, while in the autumn
11 temperatures are still high (22–27 °C) in Hong Kong so people also reduce outdoor activity in
12 daytime. That previous study in Hong Kong in 1980's reported that the means of serum
13 25(OH)D levels in young healthy people were 26.8 µg/l and 23.4 µg/l (equal to 67.0 nmol/L and
14 58.5 nmol/L) in September and January respectively⁽⁴¹⁾, which were higher than our study for
15 the age group 18-44y at similar months.

16 Some previous studies found that aging is associated with the reduction of vitamin D synthesis,
17 however, the association of age with vitamin D status in children, young adults and middle-aged
18 adults is inconsistent^(19,43). Our study found that for adults under 65y and children 6-17y serum
19 25(OH)D levels increased with age. This could be explained by children having the capacity to
20 produce 25(OH)D and 1,25(OH)2D due to healthy renal and liver function, whereas adults may
21 produce less of these metabolites due to declining renal function and decreasing capacity of the
22 skin to produce vitamin D precursors. As in Asian and western countries, our study also provided
23 evidence that females had lower 25(OH)D levels than males^(19,42,44-46). The sex difference in
24 serum 25(OH)D status could be explained by men and boys having more sunlight exposure, and
25 more usage of sunscreen by girl or women exposure because of cosmetic concerns.

26 We identified five factors associated with higher serum 25(OH)D levels among children 6-17y
27 of age namely, younger age, male sex, reporting a suntan, having at least 1 serving of fish per
28 week, and having at least 1 serving of egg per week. Only a limited number of foods naturally
29 contain vitamin D. Oily fish and egg yolks are rich in both vitamin D3 and 25(OH)D3, which is
30 consistent with more fish and egg ingestion helping to increase serum 25(OH)D3 levels^(47,48). A
31 suntan reflect a large amount of cutaneous sun exposure so children reporting a suntan had

1 higher serum 25(OH)D level ⁽¹⁷⁾. The higher serum 25(OH)D levels in children 6-8y and boys
2 might be related to more skin synthesis after sun exposure. However, reporting a suntan and the
3 amount of hours of sun exposure in the recent week collected in the questionnaire could not
4 reflect fully the duration of sun exposure in the longer period and the timing of sun exposure
5 related to zenith angle of the sun.

6 Our study has several limitations. First, seasonal variation in serum 25(OH)D was assessed
7 using the data collected over 15 months with a lack of data on 25(OH)D in the summer months,
8 and a longer time series of 25(OH)D levels would improve the determination of the seasonal
9 variations of 25(OH)D. Second, our study had a limited sample size in elderly persons ≥ 65 y of
10 age and this reduced the precision of estimates in that age group. Third, the factors associated
11 with serum 25(OH)D level among children might not be the same for adults. Finally, we did not
12 select participants at random from the population of Hong Kong, and our estimates of 25(OH)D
13 levels might need adjustment before being used to infer the mean of serum 25(OH)D in the
14 population as a whole.

15 In conclusion, we identified seasonal variation in serum 25(OH)D in Hong Kong, peaking in
16 early autumn (September) and troughing in early spring (March). Children 6-17y, and girls and
17 women had lower serum 25(OH)D levels than adults, boys and men. For children 6-17y, More
18 sunlight exposure and more intake of fish and eggs could improve vitamin D status.

19
20

1 **Financial Support**

2 This study was supported by the Research Fund for the Control of Infectious Diseases of the
3 Health, Welfare and Food Bureau of the Hong Kong SAR Government (grant nos. CHP-CE-03
4 and 11100862), and the Area of Excellence Scheme of the Hong Kong University Grants
5 Committee (grant no. AoE/M-12/06). The funding bodies had no role in study design, data
6 collection and analysis, preparation of the manuscript, or the decision to publish.

7
8 **Conflicts of Interest**

9 D.K.M.I has received research funding from F. Hoffmann-La Roche Ltd. JSMP receives
10 research funding from Crucell NV. G.M.L has received consulting honoraria from Janssen
11 Pharmaceuticals. B.J.C has received research funding from MedImmune Inc. and Sanofi Pasteur,
12 and consults for Crucell NV. The authors report no other potential conflicts of interest.

13
14 **Authorship Contributions**

15 C.X. and B.J.C. contributed to the study conception and design. V.J.F., S.N., D.K.M.I., A.M.S.K.,
16 G.M.L. and B.J.C. collected data. R.A.P.M.P. and J.S.M.P. conducted laboratory tests. C.X. and
17 V.J.F. analysed data. C.X. wrote the first draft of the paper. All authors contributed to the
18 interpretation of data and approved the final manuscript.

19
20 **Acknowledgements**

21 We thank Chan Kit Man, Calvin Cheng, Lai-Ming Ho, Ho Yuk Ling, Nicole Huang, Lam Yiu
22 Pong, Lincoln Lau, Winnie Lim, Tom Lui, Tong Hok Leung, Loretta Mak, Eunice Shiu, Joey Sin,
23 Jessica Wong, Kevin Yau and Eileen Yeung for research support. We thank Susan Chiu for
24 helpful discussions.

25
26
27
28

1 **References**

- 2
- 3 1. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266-281.
- 4 2. Giovannucci E, Liu Y, Rimm EB *et al.* (2006) Prospective study of predictors of vitamin D
- 5 status and cancer incidence and mortality in men. *J Natl Cancer Inst* **98**, 451-459.
- 6 3. Lappe JM, Travers-Gustafson D, Davies KM *et al.* (2007) Vitamin D and calcium
- 7 supplementation reduces cancer risk: results of a randomized trial. *The American journal of*
- 8 *clinical nutrition* **85**, 1586-1591.
- 9 4. Bergman P, Lindh AU, Bjorkhem-Bergman L *et al.* (2013) Vitamin D and Respiratory Tract
- 10 Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *PloS one*
- 11 **8**, e65835.
- 12 5. Ginde AA, Mansbach JM, Camargo CA, Jr. (2009) Association between serum 25-
- 13 hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and
- 14 Nutrition Examination Survey. *Arch Intern Med* **169**, 384-390.
- 15 6. Douglas AS, Ali S, Bakhshi SS (1998) Does vitamin D deficiency account for ethnic
- 16 differences in tuberculosis seasonality in the UK? *Ethn Health* **3**, 247-253.
- 17 7. Cannell JJ, Vieth R, Umhau JC *et al.* (2006) Epidemic influenza and vitamin D. *Epidemiology*
- 18 *and infection* **134**, 1129-1140.
- 19 8. Wang TJ, Pencina MJ, Booth SL *et al.* (2008) Vitamin D deficiency and risk of cardiovascular
- 20 disease. *Circulation* **117**, 503-511.
- 21 9. Wahl D, Cooper C, Ebeling P *et al.* (2012) A global representation of vitamin D status in
- 22 healthy populations. *Arch Osteoporos* **7**, 155-172.
- 23 10. Hilger J, Friedel A, Herr R *et al.* (2013) A systematic review of vitamin D status in
- 24 populations worldwide. *Br J Nutr*, 1-23.
- 25 11. Chen TC, Chimeh F, Lu Z *et al.* (2007) Factors that influence the cutaneous synthesis and
- 26 dietary sources of vitamin D. *Arch Biochem Biophys* **460**, 213-217.
- 27 12. Holick MF (2009) Vitamin D status: measurement, interpretation, and clinical application.
- 28 *Ann Epidemiol* **19**, 73-78.
- 29 13. Viljakainen HT, Palssa A, Kärkkäinen M *et al.* (2006) A seasonal variation of calcitropic
- 30 hormones, bone turnover and bone mineral density in early and mid-puberty girls—a cross-
- 31 sectional study. *Br J Nutr* **96**, 124-130.

- 1 14. Bhattoa H, Bettembuk P, Ganacharya S *et al.* (2004) Prevalence and seasonal variation of
2 hypovitaminosis D and its relationship to bone metabolism in community dwelling
3 postmenopausal Hungarian women. *Osteoporos Int* **15**, 447-451.
- 4 15. Cowling BJ, Ng S, Ma ES *et al.* (2012) Protective efficacy against pandemic influenza of
5 seasonal influenza vaccination in children in Hong Kong: a randomized controlled trial. *Clin*
6 *Infect Dis* **55**, 695-702.
- 7 16. Nucci AM, Russell CS, Luo R *et al.* (2013) The effectiveness of a short food frequency
8 questionnaire in determining vitamin D intake in children. *Dermatoendocrinol* **5**, 205-210.
- 9 17. Bolek-Berquist J, Elliott ME, Gangnon RE *et al.* (2009) Use of a questionnaire to assess
10 vitamin D status in young adults. *Public Health Nutr* **12**, 236-243.
- 11 18. Roth HJ, Schmidt-Gayk H, Weber H *et al.* (2008) Accuracy and clinical implications of
12 seven 25-hydroxyvitamin D methods compared with liquid chromatography–tandem mass
13 spectrometry as a reference. *Ann Clin Biochem* **45**, 153-159.
- 14 19. Ono Y, Suzuki A, Kotake M *et al.* (2005) Seasonal changes of serum 25-hydroxyvitamin D
15 and intact parathyroid hormone levels in a normal Japanese population. *J Bone Miner Metab* **23**,
16 147-151.
- 17 20. Observatory HK Extract of Meteorological Observations for Hong Kong.
18 <http://www.hko.gov.hk/wxinfo/pastwx/mwsc.htm> (accessed March 2 2014)
- 19 21. Khoo A-L KH, Chai LYA, *et al.* (2010) Seasonal variation in vitamin D₃ levels is paralleled
20 by changes in the peripheral blood human T cell compartment. *PloS one* **7**, e29250.
- 21 22. Zittermann A, Scheld K, Stehle P (1998) Seasonal variations in vitamin D status and calcium
22 absorption do not influence bone turnover in young women. *Eur J Clin Nutr* **52**, 501-506.
- 23 23. Carnevale V, Modoni S, Pileri M *et al.* (2001) Longitudinal evaluation of vitamin D status in
24 healthy subjects from southern Italy: seasonal and gender differences. *Osteoporos Int* **12**, 1026-
25 1030.
- 26 24. Ross AC, Manson JE, Abrams SA *et al.* (2011) The 2011 report on dietary reference intakes
27 for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin*
28 *Endocrinol Metab* **96**, 53-58.
- 29 25. Dawson-Hughes B, Mithal A, Bonjour J-P *et al.* (2010) IOF position statement: vitamin D
30 recommendations for older adults. *Osteoporos Int* **21**, 1151-1154.

- 1 26. Nakamura K, Nashimoto M, Hori Y *et al.* (1999) Serum 25-hydroxyvitamin D levels in
2 active women of middle and advanced age in a rural community in Japan. *Nutrition* **15**, 870-873.
- 3 27. Nakamura K, Nashimoto M, Yamamoto M (2001) Are the serum 25-hydroxyvitamin D
4 concentrations in winter associated with forearm bone mineral density in healthy elderly
5 Japanese women? *Int J Vitam Nutr Res* **71**, 25-29.
- 6 28. Suzuki T, Kwon J, Kim H *et al.* (2008) Low Serum 25 - Hydroxyvitamin D Levels
7 Associated With Falls Among Japanese Community - Dwelling Elderly. *J Bone Miner Res* **23**,
8 1309-1317.
- 9 29. Ho-Pham L, Nguyen N, Lai T *et al.* (2011) Vitamin D status and parathyroid hormone in a
10 urban population in Vietnam. *Osteoporos Int* **22**, 241-248.
- 11 30. Chailurkit L-o, Kruavit A, Rajatanavin R (2011) Vitamin D status and bone health in healthy
12 Thai elderly women. *Nutrition* **27**, 160-164.
- 13 31. Soontrapa S, Chailurkit L (2005) Difference in serum calcidiol and parathyroid hormone
14 levels between elderly urban vs suburban women. *J Med Assoc Thai* **88**, S17-20.
- 15 32. Tsai K, Hsu S, Cheng J *et al.* (1997) Vitamin D stores of urban women in Taipei: effect on
16 bone density and bone turnover, and seasonal variation. *Bone* **20**, 371-374.
- 17 33. Kelishadi R, Moeini R, Poursafa P *et al.* (2013) Independent association between air
18 pollutants and vitamin D deficiency in young children in Isfahan, Iran. *Paediatr Int Child Health*
19 **34**, 50-55.
- 20 34. Powe CE, Evans MK, Wenger J *et al.* (2013) Vitamin D–Binding Protein and Vitamin D
21 Status of Black Americans and White Americans. *N Engl J Med* **369**, 1991-2000.
- 22 35. Davies PS BC, Cole TJ, Prentice A, Clarke PC (1999) Vitamin D: seasonal and regional
23 differences in preschool children in Great Britain. *Eur J Clin Nutr* **53**, 1.
- 24 36. Klenk J, Rapp K, Denking MD *et al.* (2013) Seasonality of vitamin D status in older people
25 in Southern Germany: implications for assessment. *Age Ageing* **42**, 404-408.
- 26 37. Mavroeidi A, O'Neill F, Lee PA *et al.* (2010) Seasonal 25-hydroxyvitamin D changes in
27 British postmenopausal women at 57 degrees N and 51 degrees N: a longitudinal study. *J Steroid*
28 *Biochem Mol Biol* **121**, 459-461.
- 29 38. Nakamura K1 NM, Yamamoto M (2000) Summer/winter differences in the serum 25-
30 hydroxyvitamin D3 and parathyroid hormone levels of Japanese women. *Int J Biometeorol* **44**, 4.

1 39. Vecino-Vecino C, Gratton M, Kremer R *et al.* (2006) Seasonal variance in serum levels of
2 vitamin d determines a compensatory response by parathyroid hormone: study in an ambulatory
3 elderly population in Quebec. *Gerontology* **52**, 33-39.

4 40. Kasahara AK, Singh RJ, Noymer A (2013) Vitamin D (25OHD) Serum Seasonality in the
5 United States. *PloS one* **8**, e65785.

6 41. MacDonald D, Swaminathan R (1988) Seasonal Variation in 25-OH Vitamin D In Plasma of
7 Hong Kong Chinese. *Clin Chem* **34**, 1.

8 42. Levis S, Gomez A, Jimenez C *et al.* (2005) Vitamin D deficiency and seasonal variation in
9 an adult South Florida population. *J Clin Endocrinol Metab* **90**, 1557-1562.

10 43. Sherman SS, Hollis BW, Tobin JD (1990) Vitamin D Status and Related Parameters in a
11 Healthy Population: The Effects of Age, Sex, and Season. *J Clin Endocrinol Metab* **71**, 405-413.

12 44. Robien K, Butler LM, Wang R *et al.* (2013) Genetic and environmental predictors of serum
13 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore. *Br J*
14 *Nutr* **109**, 493-502.

15 45. Chailurkit L-o, Aekplakorn W, Ongphiphadhanakul B (2011) Regional variation and
16 determinants of vitamin D status in sunshine-abundant Thailand. *BMC Public Health* **11**, 853.

17 46. Choi HS, Oh HJ, Choi H *et al.* (2010) Vitamin D insufficiency in Korea—a greater threat to
18 younger generation: the Korea National Health and Nutrition Examination Survey (KNHANES)
19 2008. *J Clin Endocrinol Metab* **96**, 643-651.

20 47. Lamberg-Allardt C (2006) Vitamin D in foods and as supplements. *Prog Biophys Mol Biol*
21 **92**, 33-38.

22 48. Schmid A, Walther B (2013) Natural vitamin D content in animal products. *Adv Nutr* **4**, 453-
23 462.

24
25

1 **Table Legends**

2 Table 1. Demographic characteristics of participants in this study.

3

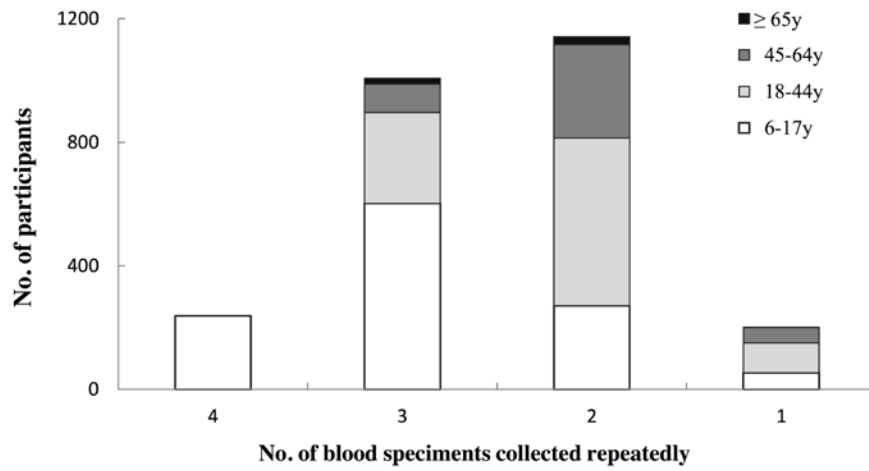
4 Table 2. Comparison of serum 25-hydroxyvitamin D (25(OH)D) levels (nmol/L) in each season
5 by age and sex using a generalized linear model

6

7 Table 3. The individual characteristics of sun seeking behaviors, diet and vitamin D supplements,
8 and their associations with serum 25-hydroxyvitamin D (25(OH)D) levels among children 6-17y
9 of age in Hong Kong, in April and May 2010.

10

1 Figure Legends

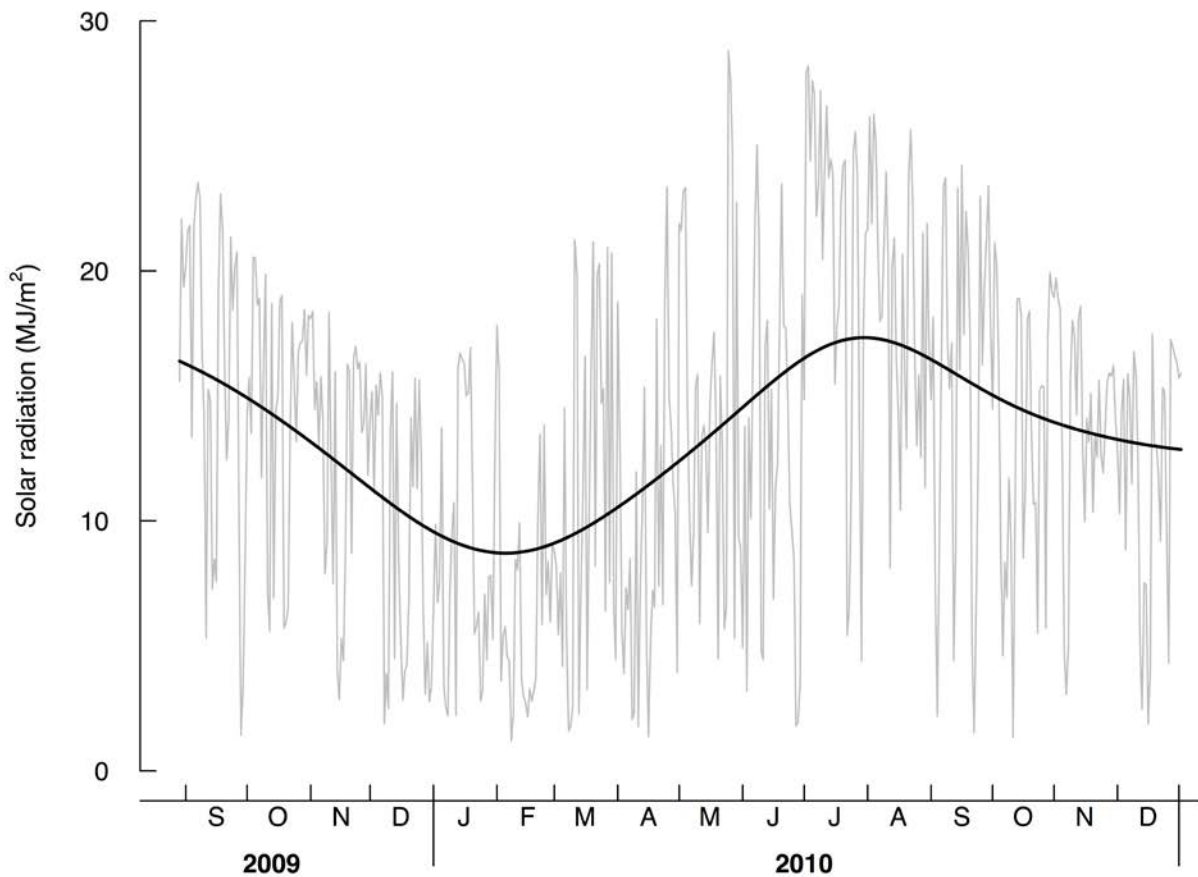


2

3

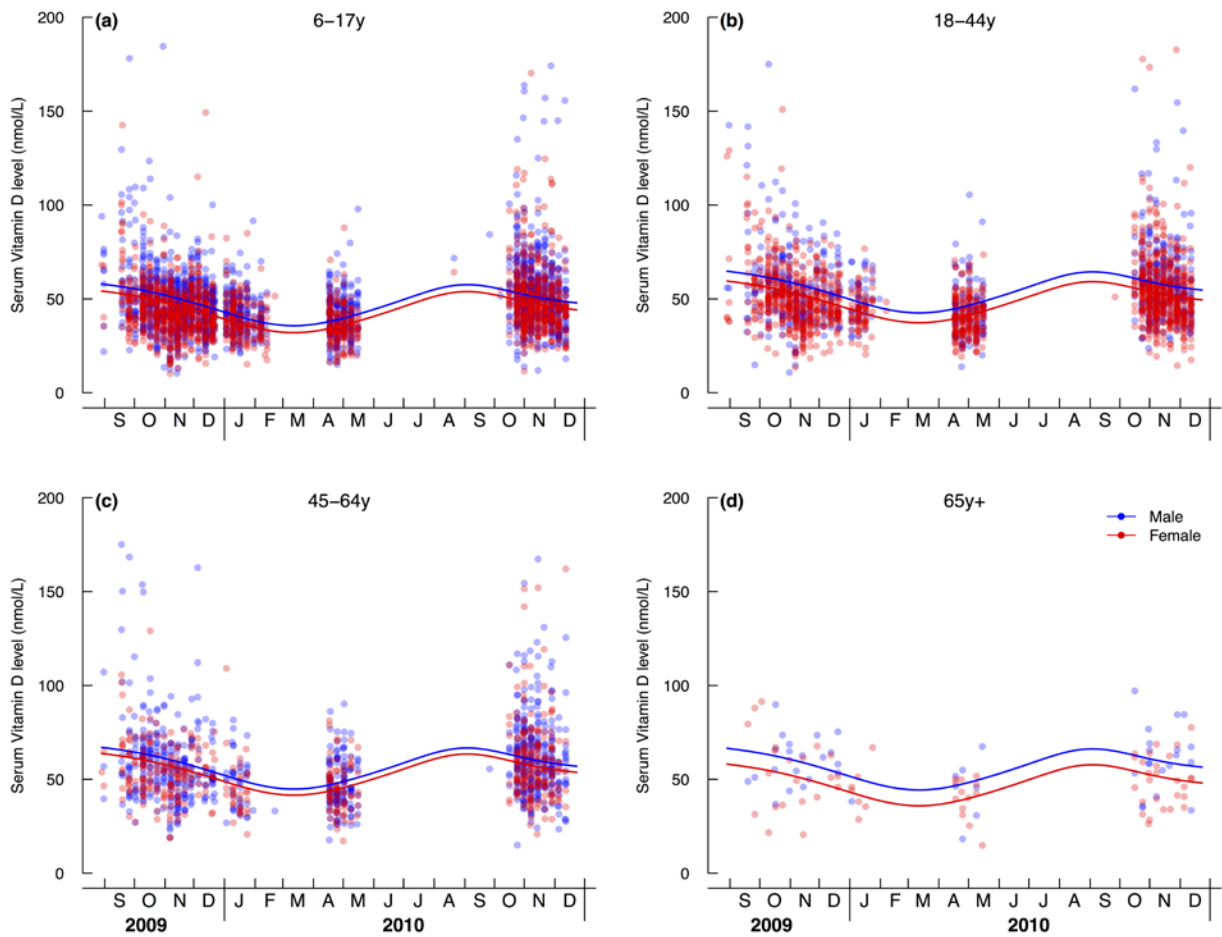
4 Figure 1. The number of serum specimens collected repeatedly in four age groups (6-17y, 18-44y,

5 45-64y and $\geq 65y$).



1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 2. Daily levels of solar radiation (megajoule (MJ)/m²) that were obtained based on daily means of solar radiation from Hong Kong observatory using Kernel density smoothing as a proxy measure for meteorological season. (Grey line: daily means of solar radiation, black line: daily level of solar radiation).



1
2
3
4
5
6
7
8
9
10
11

Figure 3. Serum 25-hydroxyvitamin D (25(OH)D) levels (nmol/L) from each individual and a random-effects linear regression model of serum 25(OH)D level fitted to daily level of solar radiation as a covariate, adjusting for age groups and sex. Four panels display the vitamin D levels in subjects 6-17y, 18-44y, 45-64y and ≥ 65 y, and solid lines in each panel indicate the mean levels of serum vitamin D for men and women in the fitted model (blue dot/line: male, and red dot/line: female).

Table Legends

Table 1. Demographic characteristics of participants in this study.

Characteristic	Subjects with ≥ 1 blood sample tested		p-Value
	for Vitamin D level (N = 2694)		
	No.	%	
Age group, years			0.11
0-5	0	0	
6-17	1165	43	
18-44	933	35	
45-64	544	20	
65+	51	2	
Sex, Men	1251	46	0.99
Educational attainment of household head:			0.98
Primary or below	205	8	
Secondary	1842	68	
Tertiary	647	24	
Receipt of 2009-10 seasonal influenza vaccine			0.91
No	2136	79	
Yes	557	21	
Any chronic condition			0.96
No	2264	84	
Yes	429	16	

Table 2. Comparison of serum 25-hydroxyvitamin D (25(OH)D) levels (nmol/L) in each season by age and sex using a generalized linear model

	2009 Autumn (Sep-Nov)			2009-10 Winter (Dec-Feb)			2010 Spring (Mar-May)			2010 Autumn (Sep-Nov)		
	No. of subjects	Mean (95% CI)	p-Value	No. of subjects	Mean (95% CI)	p-Value	No. of subjects	Mean (95% CI)	p- Value	No. of subjects	Mean (95% CI)	p-Value
Age, years												
6-17	893	47 (46, 48)	<0.001	484	42 (41, 43)	<0.001	399	39 (37, 40)	<0.001	1072	53 (51, 54)	<0.001
18-45	800	53 (52, 55)	Ref.	111	48 (47, 50)	Ref.	323	42 (41, 43)	Ref.	829	57 (56, 58)	Ref.
46-64	458	58 (56, 60)	<0.01	69	53 (50, 57)	0.23	203	47 (45, 49)	<0.001	500	63 (60, 65)	<0.001
65+	42	56 (48, 63)	0.96	7	51 (45, 57)	0.18	20	41 (35, 48)	0.96	46	54 (48, 60)	0.36
Gender												
Male	1007	54 (53, 55)	<0.001	338	47 (46, 48)	<0.001	447	43 (42, 44)	<0.001	1133	58 (57, 60)	<0.001
Female	1187	49 (48, 50)	Ref.	333	44 (43, 45)	Ref.	496	40 (39, 41)	Ref.	1321	54 (53, 55)	Ref.

Ref. represents reference group.

P-Value denote the P value for comparing serum 25(OH)D levels in different age/sex groups with referent age/sex group in each season.

Table 3. The individual characteristics of sun seeking behaviors, diet and vitamin D supplements, and their associations with serum 25-hydroxyvitamin D (25(OH)D) (nmol/ L) levels among children 6-17y of age in Hong Kong, in April and May 2010.

Characteristics	No. (%)	Unadjusted β (95% CI)	p-Value	Adjusted β^* (95% CI)	p-Value
Age, years					
6-8	70(22)	Ref.		Ref.	
9-11	109(34)	-4.62 (-7.90, -1.35)	<0.01	-4.69(-8.04, -1.34)	<0.01
12-17	142(44)	-6.76 (-9.88 -3.63)	<0.001	--3.96(-7.11, 0.81)	<0.05
Male sex	171(53)	3.803 (1.402, 6.204)	<0.001	3.92(1.48, 6.36)	<0.01
Reporting suntan in the past year	260 (86)	5.31 (1.75, 8.86)	<0.01	4.06 (0.57, 7.55)	<0.015
Sunscreen used	124(39)	2.072 (-0.437, 4.582)	0.11		
Sun exposure in the past week					
< 1 hours per week	33(11)		Ref.		
1-6 hours per week	213(69)	2.95 (-1.14,7.03)	0.16		
≥ 7 hours per week	61(20)	1.00 (-3.72, 5.73)	0.68		
Meal of fish per week					
< 1 meal of fish per week	10(3)	Ref.		Ref.	
1-6 meals of fish per week	240(75)	11.08 (4.07, 18.10)	<0.01	11.38 (4.70, 18.06)	<0.001
≥ 7 meals of fish per week	68(21)	12.83 (5.71, 19.95)	<0.001	11.78 (5.00, 18.55)	<0.001
Cups of milk per week					
< 1 average cup of milk per week	78(25)	Ref.			
1-6 cups of milk per week	139(45)	3.02 (-0.06, 6.10)	0.06		
≥ 1 cups of milk per day	93(30)	4.91 (1.57, 8.26)	<0.01		
Number of egg per week					
<1 eggs per week	10(3)	Ref.		Ref.	
1-6 eggs per day	188(59)	4.26 (-2.78, 11.31)	0.24	7.26 (0.55, 13.98)	<0.05
≥ 7 eggs per week	119(38)	7.01 (-0.38, 14.41)	0.07	9.25 (2.11, 16.39)	<0.05
Vitamin D supplement	30(9)	0.92 (-3.29, 5.13)	0.68		
Multivitamin	20(6)	1.95 (-3.12, 7.03)	0.45		
Intake cod liver oil or fish oil	64(20)	3.425 (0.401, 6.450)	<0.015	2.99 (-0.12, 6.11)	0.06
Skin color compared with classmates					
Much darker	9(3)	Ref.		Ref.	
Darker	67(21)	-3.53 (-11.25, 4.20)	0.37	-1.68 (-9.06, 5.71)	0.66
Similar	183(57)	5.88 (-13.31, 1.55)	0.12	-5.32 (-12.38, 1.74)	0.14
Lighter	54(17)	-6.22 (-14.05, 1.62)	0.12	-5.54 (-12.98, 1.90)	0.15
Much lighter	7(2)	3.56 (-7.40, 14.53)	0.52	2.67 (-7.59, 12.94)	0.61
Diseases of Digestive System	2(1)	1.08 (-14.46, 16.62)	0.89		

Diarrhea in past 2 weeks	18(6)	-0.30 (-5.63, 5.02)	0.91
--------------------------	-------	---------------------	------

Ref. represents reference group.

A multiple linear model with backward selection was used. Only the factors with p-values <0.2 were included in the final model.

Supplementary Material

Supplementary Table 1. The association of daily levels of solar radiation (megajoule (MJ)/m²) and time-varying serum vitamin D level (nmol/L) using the random-effects linear regression model.

Characteristic	Adjusted β	p-Value
Solar radiation	0.46 (0.40, 0.52)	<0.01
Age group, years		
6-17	-6.06 (-7.45, -4.67)	<0.01
18-44	Ref.	-
45-64	3.57 (1.98, 5.16)	<0.01
65+	-0.50 (-4.72, 3.72)	0.82
Sex		
Female	Ref.	-
Male	4.13 (3.01, 5.25)	<0.01
Educational attainment of household head		
Primary or below	1.77 (-0.27, 3.82)	0.09
Secondary	Ref.	-
Tertiary	0.53 (-0.77, 1.82)	0.42
Receipt of 2009-10 seasonal influenza vaccine		
No	Ref.	-
Yes	-0.36 (-1.84, 1.12)	0.63
Any chronic condition		
No	Ref.	-
Yes	0.73 (-0.81, 2.27)	0.35

Ref. represents reference group.