Determinants of serum 25-hydroxyvitamin D in Hong Kong

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ABSTRACT

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

Introduction

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

Humans acquire vitamin D from exposure to sunlight, from their diet, and from dietary supplements [1]. The main natural source of vitamin D is the sun, as vitamin D is synthesized in the skin after exposure to solar ultraviolet B radiation (wavelength 290–315 nm) [1]. A diet high in oily fish prevents vitamin D deficiency [11].Vitamin D from the skin and diet converts to 25-hydroxy-vitamin D (25(OH)D) in the liver and subsequently to 1,25-dihydroxy-vitamin D (1,25(OH)$_2$D) in the kidney, which is the active form of vitamin D [1]. 25(OH)D is the major form of vitamin D that circulates in the blood stream and can be used as a marker to determine vitamin D status [12].

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

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

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

Methods

Study participants

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

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

Ethics

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

Laboratory analysis

Blood from all household members were collected in tubes containing clot activator and held at 4-8°C from collection until receipt at the laboratory. At the laboratory, each specimen was centrifuged to extract the sera, which was then frozen at -80°C. The serum specimens were subsequently tested for 25(OH)D using the OCTEIA ELISA 25-hydroxyvitamin D immunoassay kit manufactured by Immunodiagnostic Systems (IDS) Ltd (Boldon, United Kingdom) (18). According to the package insert of the assay, the inter-assay Coefficient of Variation for the 25(OH)D assay was 4.6%-8.7%, and the intra-assay Coefficient of Variation was 5.3%-6.7%. In our own laboratory we found that the intra-assay Coefficient of Variation was 7.4%.

Statistical analysis

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

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

We used a generalized linear model to compare the mean of serum 25(OH) by age and sex in each season to estimate age-specific and sex-specific patterns in serum 25(OH)D levels. Since solar radiation can reflect climatic season, we fitted a random-effects linear regression model to obtain quantitative seasonality estimates of serum 25(OH)D based on the repeated measures of serum 25(OH)D, which included daily level of solar radiation as a predictive factor. Daily means of solar radiation were obtained from Hong Kong observatory, and were smoothed using Kernel density smoothing as a proxy measure for seasonal variation in the climate in Hong Kong [20]. In a separate secondary analysis, a random-effects sinusoidal linear regression model with annual periodicity was fitted to characterize the seasonal variation of serum 25(OH)D. In the two random-effects linear regression models used to estimate the seasonal variation of serum 25(OH)D, the associations of 25(OH)D with age, sex, educational attainment of the household head, vaccination and chronic conditions were adjusted for. The ratio of serum 25(OH)D levels between the peak season and the trough season in each age group was calculated to estimate the degree of seasonal variation in serum 25(OH)D levels.

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

Results

Characteristics of participants

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

Mean of serum25(OH)D by age and sex in different seasons

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

Seasonal variation of serum 25(OH)D

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

Factors that influence serum 25(OH)D among children

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

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

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

Discussion

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

Similar to the findings from several temperate locations [(19,35-40)], our study estimated that there is substantial seasonal fluctuation in serum 25(OH)D levels in Hong Kong. Previous studies in subtropical Taiwan, Florida and Hong Kong reported the differences in serum 25(OH)D level between summer (or autumn) and winter[32,41,42]. However our study with 15-month study duration was able to predict the year-round seasonal fluctuation by using a cyclic regression model, although there was a lack of data on summer levels of serum 25(OH)D in our study. Sun exposure and solar radiation are known to be a major determinant of vitamin D status [1] and the seasonal pattern of vitamin D in Hong Kong is consistent with seasonal variation in solar radiation. In Hong Kong, hours of sunlight (136 and 111 hours/month respectively) and solar radiation (10 and 12 megajoule (MJ)/m^2 respectively) in winter and spring are lower than those (182 and 182 hours/month; 16 and 14 MJ/m^2 respectively) in the summer and autumn [20]. The weather in winter and spring is suitable for outdoor activity in Hong Kong, while in the autumn temperatures are still high (22–27 °C) in Hong Kong so people also reduce outdoor activity in daytime. That previous study in Hong Kong in 1980's reported that the means of serum 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 58.5 nmol/L) in September and January respectively [41], which were higher than our study for the age group 18-44y at similar months.

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

We identified five factors associated with higher serum 25(OH)D levels among children 6-17y of age namely, younger age, male sex, reporting a suntan, having at least 1 serving of fish per week, and having at least 1 serving of egg per week. Only a limited number of foods naturally contain vitamin D. Oily fish and egg yolks are rich in both vitamin D3 and 25(OH)D3, which is consistent with more fish and egg ingestion helping to increase serum 25(OH)D3 levels [47,48]. A suntan reflect a large amount of cutaneous sun exposure so children reporting a suntan had higher serum 25(OH)D level [17]. The higher serum 25(OH)D levels in children 6-8y and boys might be related to more skin synthesis after sun exposure. However, reporting a suntan and the amount of hours of sun exposure in the recent week collected in the questionnaire could not reflect fully the duration of sun exposure in the longer period and the timing of sun exposure related to zenith angle of the sun.

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

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

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Conflicts of Interest

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

Authorship Contributions

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., 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 V.J.F. analysed data. C.X. wrote the first draft of the paper. All authors contributed to the interpretation of data and approved the final manuscript.

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Table Legends

Table 1. Demographic characteristics of participants in this study.

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

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) levels among children 6-17y of age in Hong Kong, in April and May 2010.

Figure Legends

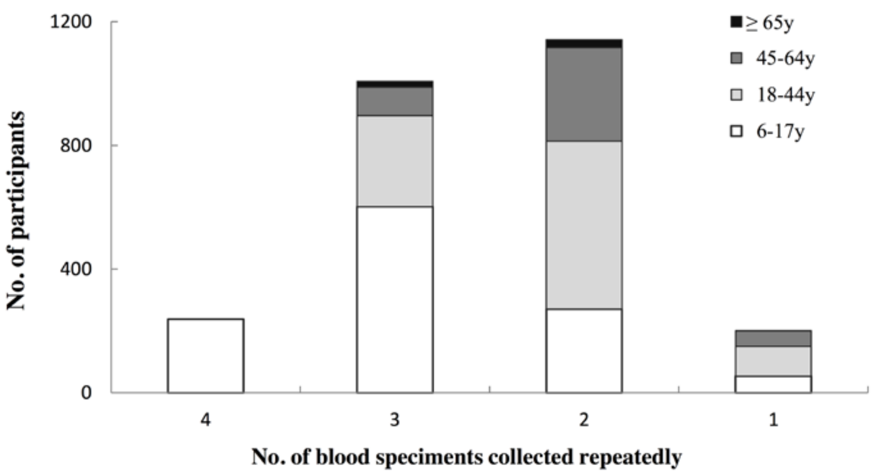

Figure 1. The number of serum specimens collected repeatedly in four age groups (6-17y, 18-44y, 45-64y and ⩾65y).

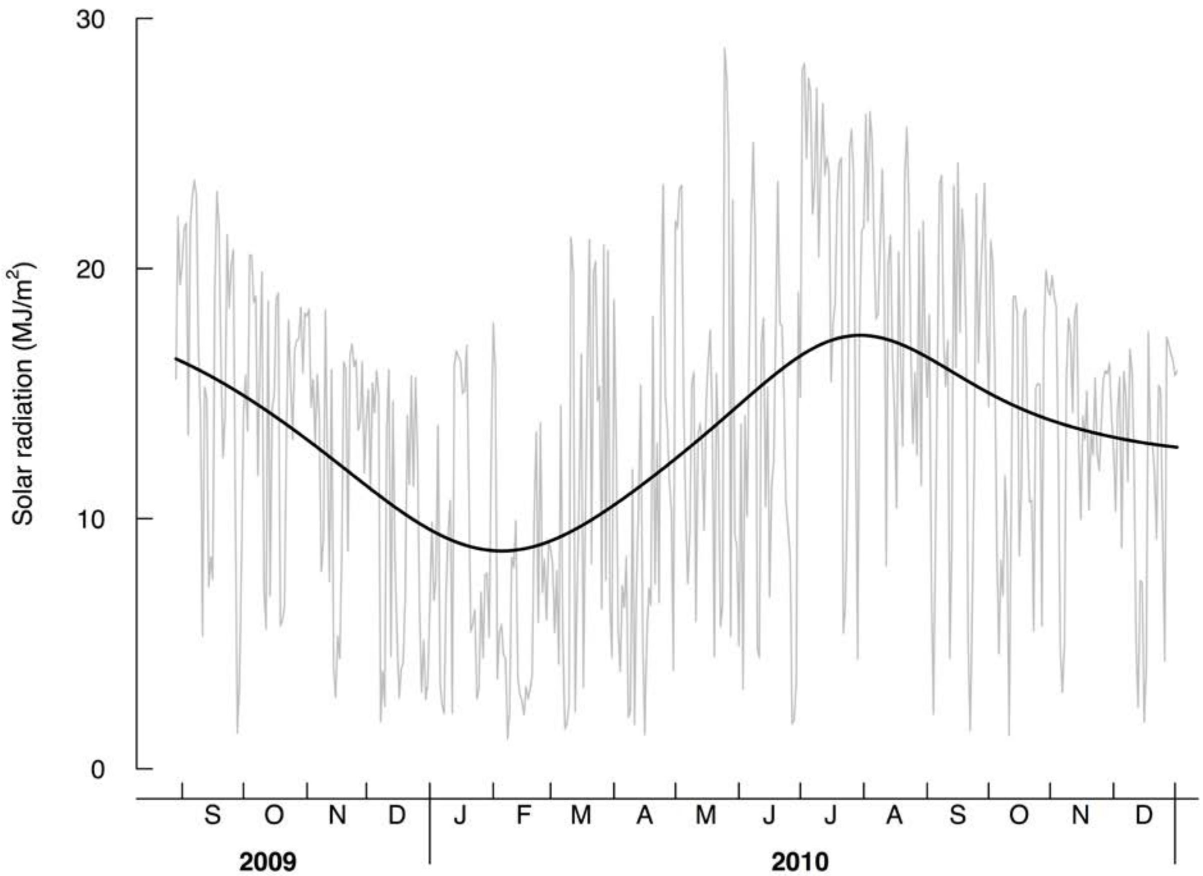

Figure 2. Daily levels of solar radiation (megajoule (MJ)/m^2) that were obtained based on daily means of solar radiation from Hong Kong observatory using Kernel density smoothing as a proxy measure for meteorological season. (Grey line: daily means of solar radiation, black line: daily level of solar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

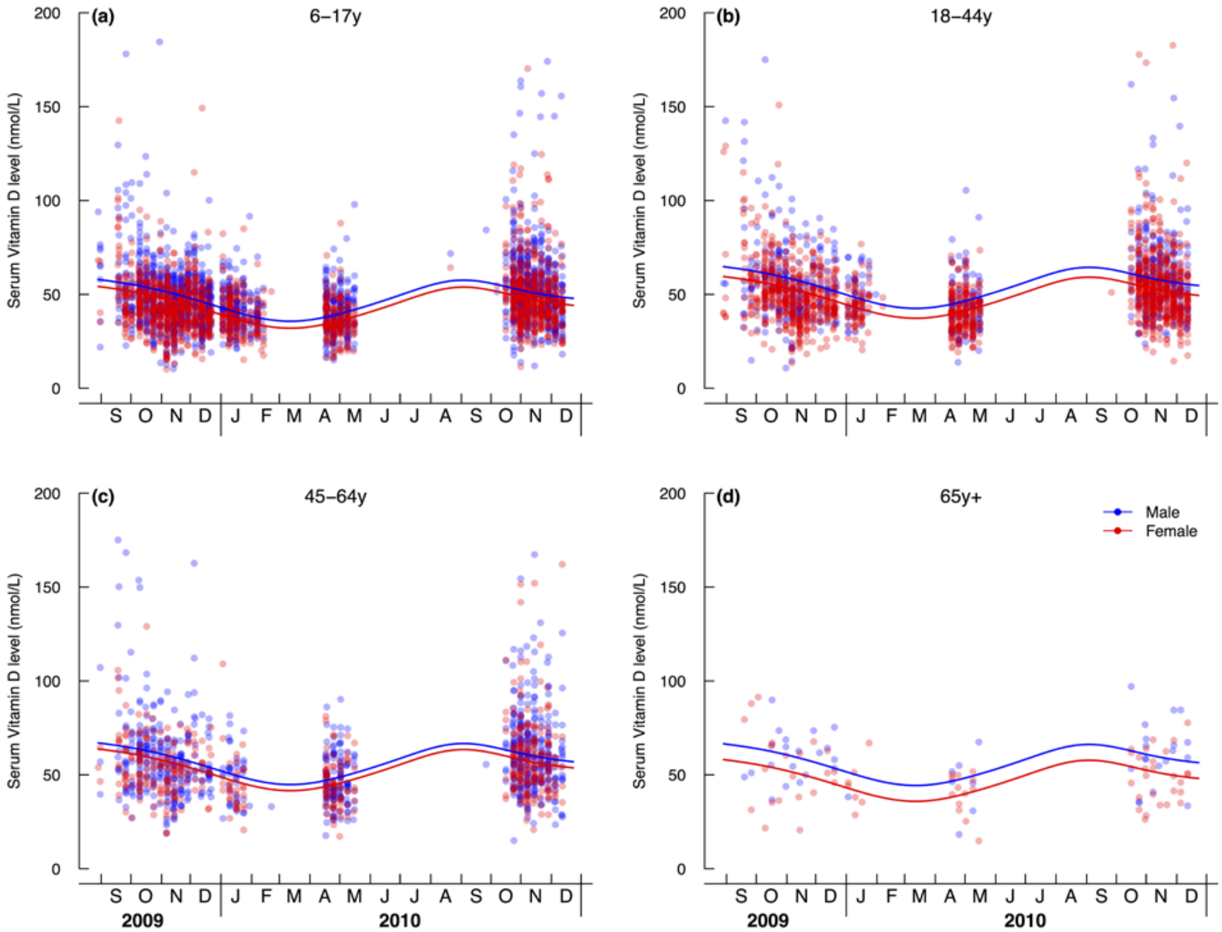

Figure 3. Serum 25-hydroxyvitamin D (25(OH)D) levels (nmol/L) from each individual and a random-effects linear regression model of serum 25(OH)D level fitted to daily level of solar radiation as a covariate, adjusting for age groups and sex. Four panels display the vitamin D levels in subjects 6-17y, 18-44y, 45-64y and ≥65y, 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 for Vitamin D level (N = 2694)		p-Value
	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)/m^2) 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.