FACTORS ASSOCIATED WITH VITAMIN D STATUS AMONG THAI CHILDREN AGED 3-13 YEARS

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Abstract. The impact of vitamin D status on bone health and other health conditions is receiving increasingly attention. We aimed to determine the association between environmental factors and vitamin D intake by examining serum 25-hydroxy vitamin D [25(OH)D] levels in Thai children. We conducted a cross sectional study among 477 Thai children from 4 regions in Thailand. Vitamin D intake was assessed using a self-administered questionnaire completed by the older children or by the parents for younger children. Dietary vitamin D intake was assessed using a semi-quantitative food frequency questionnaire. A serum 25(OH)D level was examined for each subject using a chemiluminescence immunoassay. We used structural equation modeling (SEM) to assess the association between studied factors and the serum 25(OH)D level. The final model showed a significant association between sunlight exposure and the serum 25(OH)D level (β=0.65, p<0.01), but not between dietary vitamin D intake and the serum 25(OH)D level (β=0.01, p=0.77), suggesting sunlight exposure is more important for the vitamin D status than dietary vitamin D intake.

Keywords: serum 25(OH)D, sunlight exposure, vitamin D intake, Thai children

INTRODUCTION

Vitamin D is an essential nutrient that plays a primarily role in calcium metabolism and maintenance of skeletal integrity in the human body (Holick, 2005). Vitamin D deficiency (VDD) is associated with rickets in growing children (Weisberg *et al*, 2004; Holick, 2006) and progressive bone loss and increased risk of bone fractures in adults and the elderly (Hwang *et al*, 2014). Deficiency may result from inadequate vitamin D intake from food, reduction of vitamin D synthesis in the skin due to aging or the use of sunscreen (Holick and Chen, 2008) or poor renal function may reduce the conversion of 25-hydroxy vitamin D [25(OH)D] to its active metabolite in the kidney, resulting low availability of active vitamin D in target tissues (Patel *et al*, 2007). A circulating serum 25(OH)D concentration is commonly used to assess the body vitamin D level. Adequate vitamin D intake is associated with a lower risk of colorectal and breast cancer (Garland *et al*, 2006; Lappe *et al*, 2007), auto-immune

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diseases; such as type 1 diabetes mellitus (Zipitis and Akobeng, 2008) and multiple sclerosis (Munger and Ascherio, 2011).

Vitamin D deficiency (VDD) and insufficiency are common worldwide with prevalences in developing countries ranging from 30% to 90% depending on the cut-off values used (Arabi *et al*, 2010). A study from China among children and adults found a prevalence of subclinical VDD [plasma 25(OH)D <12.5 nmol/l] of 45.2% during the winter and 6.7% during the summer (Du *et al*, 2001). The prevalence of VDD [serum 25(OH)D ≤37.5 nmol/l] among school children in Malaysia was reported to be 35.3% and vitamin D insufficiency [serum 25(OH)D >37.5 ≤50 nmol/l] was reported to be 37.1% (Khor *et al*, 2011). The results from one randomized controlled trial in rural school-aged children in northeastern Thailand found only 4% had a serum 25(OH)D concentration <50 nmol/l (Houghton *et al*, 2014). Another cross sectional study among school-aged children in Bangkok found a prevalence of 19.5% (Reesukumal *et al*, 2015). A survey conducted in 2011 among children aged 3-13 years from 4 regions of Thailand found a prevalence of vitamin D deficiency [serum 25(OH)D concentration < 50 nmol/l] of 45.6% among urban and 27.7% among rural children (Rojroongwasinkul *et al*, 2013).

VDD in children may be due to several factors, such as decreased vitamin D synthesis in the skin from the use of sun block, higher latitudes, low vitamin D intake or malabsorption of vitamin D in the gastrointestinal tract (Holick, 2007; Misra *et al*, 2008). Since unprocessed foods, except for oily fish, contain little vitamin D; most vitamin D is obtained from synthesis in the skin (Mason *et al*, 2011; Norman and Henry, 2012). Thailand is located in a tropical zone where sunlight is abundant but the prevalence of vitamin D deficiency is common among both urban and rural Thai children (Rojroongwasinkul *et al*, 2013).

The objective of this study was to investigate the relationship between sunlight exposure, socio-economic variables, and vitamin D intake on the vitamin D status of studied Thai children.

MATERIALS AND METHODS

Subjects

This cross sectional study was conducted as part of the Southeast Asian Nutritional Survey (SEANUTS), Thailand (Rojroongwasinkul *et al*, 2013) and was carried out from January 2011 to August 2011. A multi-stage cluster sampling method was used to select children aged 3-13 years from all four regions of Thailand. The representative provinces for the regions were Bangkok and Lopburi for the central region, Chiang Mai for the northern region, Kalasin and Si Sa Ket for the northeastern region and Phang-Nga for the southern region. Information about SEANUTS is described elsewhere (Rojroongwasinkul *et al*, 2013; Schaafsma *et al*, 2013). The study was conducted according to the guidelines of the Helsinki Declaration for experiments in humans. Ethical approval for this study was obtained from the committee on human rights related to research involving human subjects of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (MURA 2010/467), Thailand. Written informed consent was obtained from the parents or guardians of each study subject.

Data collection

Data were collected at health centers in each district. Structured questionnaires were completed by the parents or guardians of the study subjects for children aged 3-9.9 years and by the subjects themselves for children aged 10-13 years.

Socio-demographic variables

Age, gender, religion, family income and area of residence were recorded. If the family income was not known by the child, the parents were asked by phone.

Anthropometric measurements

The body weight of each subject was measured using a digital weighing scale (Seca, Model 882 GmbH, Humburg, Germany) to the nearest 0.1 kg in light indoor clothing. The body height of each subject was measured in bare feet with a microtoise (Stanley-Mabo, Besançon, France) to the nearest 0.1 cm. The body mass index (BMI, kg/m^2) was calculated for each subject as the weight in kilograms divided by the height in meters squared. Obesity was defined as a BMI-for-age Z-score (BAZ) >3 SD above the median for children aged 3-5.9 years (WHO, 2006) and as a BAZ >2 SD above the median for children aged 6-13 years (de Onis *et al*, 2007).

Sunlight exposure

The duration of sunlight exposure (SLE) was assessed using a structured questionnaire. The questionnaire asked about how much time the subject spent outdoors during weekday and the weekends. The results were categorized into: <30 minutes, 30 minutes to 2 hours and >2 hours. History of sunburn or a suntan was also obtained.

Screen time

Children were asked about the amount of time spent in front of a computer, tablet or phone during the weekdays and on the weekends.

Vitamin D intake

Dietary vitamin D intake was assessed using a semi-quantitative food frequency questionnaire (semi-FFQ) for the previous month. Pictorial food models were used to estimate serving size. Total daily vitamin D intake was calculated from the United States Department of Agriculture Standard Reference Database (USDA, 2009-2013) and the Australian Food, Supplement and Nutrient Database (AUSNUT, 2007).

Determination of serum 25(OH)D levels

A fasting blood sample was obtained from the antecubital vein of each subject. The serum was separated and analyzed for total 25(OH)D concentration using a chemiluminescence immunoassay (LIAISON®; Diasorin, Stillwater, MN). The intra-assay and inter-assay coefficient of variation for serum 25(OH)D were 3.9 and 5.5%, respectively. VDD was defined for our study as a serum 25(OH)D concentration <50 nmol/l (Holick, 2007).

Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS, version 20; IBM, Armonk, NY). Demographic information is presented using descriptive statistics. The proportions of children with low and adequate serum 25(OH)D concentrations were compared using the chi-square test and significance was set at $p<0.05$. Structural equation modeling (SEM) was used to determine the relationship between the serum 25(OH)D level and sunlight exposure and between the serum 25(OH)D level and vitamin D intake. SEM provided estimates of the relationships between measured and latent variables and also indicated whether the inclusion of one factor resulted in a better fit for the data than a model without that factor (Kline, 2011). A latent variable was a variable that could not be measured but was determined by a number of other variables (Bollen, 2002; Kline, 2011). For the initial conceptual model, SLE was a latent variable, indicated by an ellipse and various measurable variables were indicated by a square (Fig 1). The model's goodness of

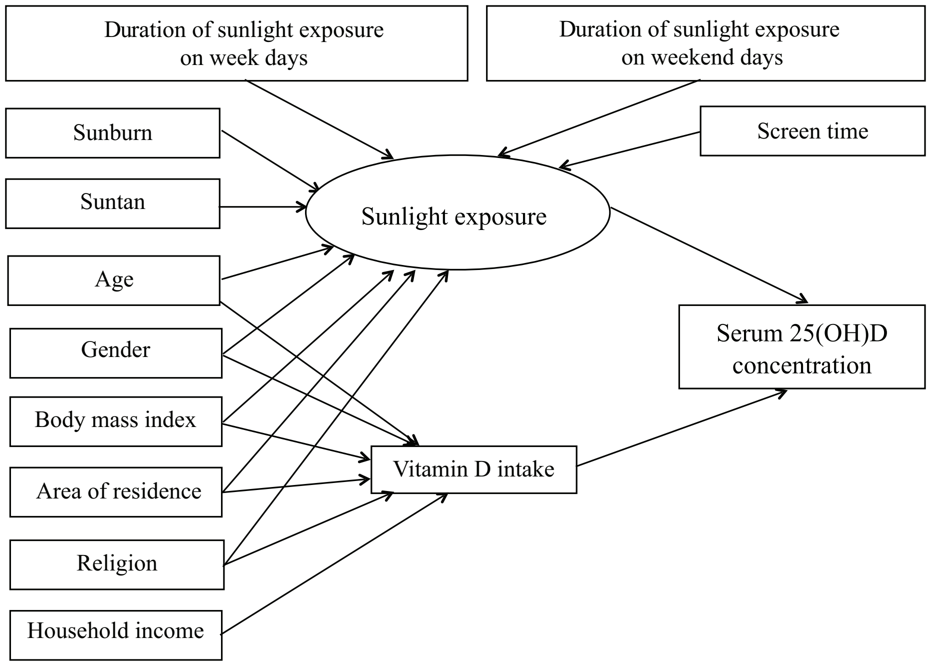

Fig1–Proposed model to determine factors influencing vitamin D status among study subjects.

fit was assessed by 4 indexes: the relative chi-square (χ^2/df), the comparative fit index (CFI), the Tucker-Lewis index (TLI), and the root mean square error of approximation (RMSEA). The chi-square test (χ^2/df) was used to determine the difference between the observed correlation or covariance matrix and the model correlation or covariance matrix, with a p-value of ≥ 0.05 indicating a good model fit. A model with a CFI ≥ 0.95, a TLI ≥ 0.90, and a RMSEA ≤0.08 was considered a model with good fit (Hu and Bentler, 1999). The R^2 (coefficient of determination) indicated how much of the variability in the response variable was explained by the explanatory variables (Byrne, 2001). SEM analysis was performed using SPSS Amos 20 software.

RESULTS

Four hundred seventy-seven subjects were included in the study; Table 1 shows various subject demographic characteristics. Eighty-eight point five percent of subjects were Buddhist and 68.8% were from a rural area. Fifty-one point eight percent of subjects had 30 minutes to 2 hours of SLE on weekdays and 42.5% at on weekend days. Ninety-six point four percent of subjects had no history of sunburn; 81.3% had no history of suntan. The mean (SD) age of the subjects was 7.8 ± 2.9 years; there was no difference in age between boys and girls. The average BMI among subjects was 16.6 ± 3.7 kg/m^2. The mean daily vitamin D intake was 4.5 ± 5.0 μg/d. The mean screen time was 2.2 ± 1.5 hours/day.

Table 2 shows selected variables among subjects with a normal serum 25(OH)D concentration and with VDD. Significantly more female subjects had VDD than male subjects (females: 38.5%; males: 25.2%). Fifty-five point six percent of Muslim children had VDD compared to 29.4% of non-Muslim children. There was

Table 1
Characteristics of study subjects (*N*=477).

		n	%
Gender	Males/females	238/239	49.9/50.1
Residential area	Urban/rural	149/328	31.2/68.8
Religion	Buddhist	422	88.5
	Islam	45	9.4
	Christian	10	2.1
Sun exposure per week day	< 30 minutes	129	27.0
	30 minutes to 2 hours	247	51.8
	> 2 hours	101	21.2
Sun exposure per weekend day	< 30 minutes	100	21.0
	30 minutes to 2 hours	203	42.5
	> 2 hours	174	36.5
History of sunburn	No	460	96.4
	Yes	17	3.6
History of suntan	No	388	81.3
	Yes	89	18.7
Household income in Baht/month[a]	< 6,000 (< USD 166)[b]	158	33.1
	6,000 - 14,000 (USD 166 - 386)	147	30.8
	>14,000 (> USD 386)	172	36.1
Serum vitamin D concentration	< 37.5 nmol/l	51	10.7
	37.5 nmol/l - <50 nmol/l	101	21.2
	≥ 50 nmol/l	325	68.1
Mean age in years (SD)		7.8 (2.9)	
Mean BMI in kg/m^2 (SD)		16.6 (3.7)	
Mean vitamin D intake in μg/d (SD)		4.5 (5.0)	
Mean serum vitamin D concentration in nmol/l (SD)		60.1 (20.0)	
Mean screen time in hours/day (SD)		2.2 (1.5)	

[a]Categorized by tertiles; [b]USD1=THB 36.24.

no significant difference in the percentage of subjects with VDD between urban and rural residences. Fifty percent of obese children had VDD versus 29.8% of non-obese children. Children with less SLE tended to have lower serum 25(OH)D concentrations compared to those with greater SLE.

Fig 1 shows the initial conceptual model describing the effect of SLE and vitamin D intake on serum 25(OH)D levels. The proposed influencing factors were time spent outdoors on a weekday and on a weekend, screen time, history of sunburn or suntan, age, gender, BMI, area of residence (urban/rural), religion and household income. The SEM showed some variables (time spent outdoors during a week day, screen time, history of sunburn or suntan and household income) did not fit the model.

Fig 2 shows the final SEM for factors associated with the serum 25(OH)D level. The latent variable SLE was found to be positively associated with gender (β=0.27, p<0.01) and time spent outdoors on the weekend (β=0.35, p<0.01). A negative as-

Table 2
Association between various factors and serum vitamin D levels.

Variable	Serum 25(OH)D levels < 50 nmol/l n (%)	Serum 25(OH)D levels ≥ 50 nmol/l n (%)	p-value
Gender			
Male	60 (25.2)	178 (74.8)	0.002
Female	92 (38.5)	147 (61.5)	
Area of residence			
Urban	55 (36.9)	94 (63.1)	0.111
Rural	97 (29.6)	231 (70.4)	
Religion			
Non-Islam	127 (29.4)	305 (70.6)	<0.001
Islam	25 (55.6)	20 (44.4)	
Weight status [a]			
Non-obese	128 (29.8)	301 (70.2)	0.003
Obese	24 (50.0)	24 (50.0)	
Exposure to sunlight each week day			
< 30 minutes	51 (39.5)	78 (60.5)	0.029
≥ 30 minutes	101 (29.0)	247 (71.0)	
Exposure to sunlight each weekend day			
< 30 minutes	47 (47.0)	53 (53.0)	<0.001
≥ 30 minutes	105 (27.9)	272 (72.1)	

[a]Obesity was defined as a BMI-for-age Z-score >3 SD above median for children aged 3-5.9 years (2006 WHO Growth Reference) and a BMI-for-age Z-score >2 SD above median for children aged 6-13 years (2007 WHO Growth Reference).

sociation was seen between SLE and the child's age (β=-0.38, p<0.01), religion (β= -0.27, p<0.01) and BMI (β=-0.38, p<0.01). The latent variable SLE had a standardized regression weight for serum 25(OH)D concentration of 0.65 (p<0.01), whereas the standardized regression weight of vitamin D intake for serum 25(OH)D concentration was only 0.01 (p=0.77). All fit indexes (Fig 2) indicate the model had a good fit. The coefficient of determination or explained variance (R^2) for the prediction of serum 25(OH)D was 0.43.

DISCUSSION

Several recent studies reported relatively high prevalences of vitamin D deficiency among Thai children (Rojroongwasinkul *et al*, 2013; Reesukumal *et al*, 2015) and Thai adults (Chailurkit *et al*, 2011). The present study aimed to explore the association between several factors and serum 25(OH)D levels among Thai children. Vitamin D status has been known to be affected by SLE and dietary vitamin D intake (Holick, 1995; Burgaz *et al*, 2007). In the present study, among Thai children, vitamin D status was associated with SLE but not dietary vitamin D intake. SLE was also associated with the child's age, gender, religion and BMI. The SLE associated with vitamin D levels was on the weekend, not the weekdays.

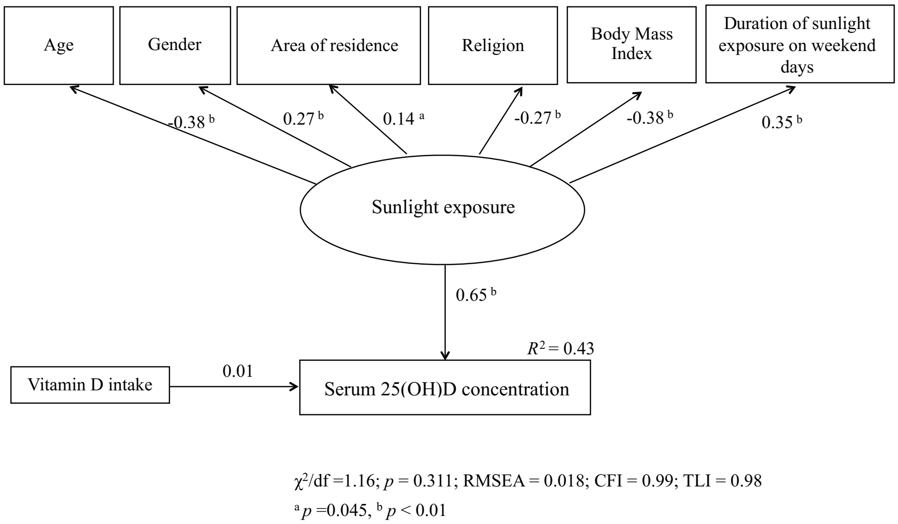

Fig 2–Diagram showing relationship between various factors and serum 25(OH)D levels.

A negative association between age and SLE has been reported in other studies among children (Rovner and O'Brien, 2008; Zhu *et al*, 2012; Houghton *et al*, 2014). Older children are more likely to engage in indoor activities, such as time spent in school, doing homework and screen time.

Our finding of the association between gender and vitamin D levels may be explained by the SLE difference due to dress and sunscreen use. The use of sun protection was more common among female subjects (56.5%) than male subjects (44.3%) (data not shown). This is consistent with a study of female adolescents that found they had a greater use of sun protection cream and umbrellas and stayed in the shade more often than their male counterparts (Tempark *et al*, 2012). Mishal (2001) found lower vitamin D levels among females than males due to differences in dress.

With the SEM, the association between SLE and area of residence was weak (β=0.14, p=0.045). This could be because the lifestyles and outdoor activities of the studied rural children was not much different from those of the studies urban children. This issue needs further study. Previous studies among adults (Nurbazlin *et al*, 2013) and children (Nichols *et al*, 2015) found urban populations were less likely to have SLE. However, some studies found living in an urban area was associated with VDD because of more time spent indoors (Harinarayan *et al*, 2007). Atmospheric pollution in cities has also been shown to affect vitamin D status (Agarwal *et al*, 2002). VDD is more prevalent in winter months (El-Hajj Fuleihan *et al*, 2001; Sullivan *et al*, 2005) and in more northern latitudes (Huotari and Herzig, 2008). VDD was significantly associated with being Muslim in our study; this is likely due to body-covering dress habits (Moy, 2011).

In our study we found an inverse association between BMI-for-age and SLE in children. Some studies have found VDD is more common among obese than non-obese children (Poomthavorn *et al*, 2012; Turer *et al*, 2013). VDD in obesity is likely due to decreased vitamin D synthesis in the skin due to inadequate SLE and vitamin D deposition in the adipose tissue of the body (Wortsman *et al*, 2000).

In our study, we found no significant association between dietary vitamin D intake and serum 25(OH)D levels. The amount of vitamin D found in natural food is generally low (Schmid and Walter, 2013); most foods are not fortified with vitamin D. In Thailand, Thai food composition tables contain no information about vitamin D content, the estimated intake in our study was obtained using US and Australian databases (AUSNUT, 2007; USDA, 2009-2013), which might not be accurate for Thailand. Since the Thai diet is predominantly composed of rice, vegetables and some meats, and is relatively low in milk and dairy products, Thais may have fewer food sources containing vitamin D. Vitamin D fortified food may be an important health benefit for improving vitamin D status in Thai children.

Our study found time spent outdoors on the weekend was positively associated with SLE. SLE on the arms and legs for 5 to 30 minutes, depending on the time of day, season and skin pigmentation, between 10:00 AM and 3:00 PM, twice weekly can result in an adequate vitamin D status (Holick, 2007). However, to ensure the adequacy of vitamin D status in Thai children, there is a need to further investigate the appropriate duration of SLE, since other factors, such as lifestyles, clothing type, season and latitude in Thailand may differ from other countries.

In summary, our study found a significant association between SLE and serum 25(OH)D levels but did not find a significant association between estimated vitamin D intake and serum 25(OH)D levels among Thai children. Further studies are needed to investigate the appropriate duration of SLE to optimize vitamin D status in Thai children.

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