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Ultra-violet radiation exposure and serum vitamin D levels in young children

Original article

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Running head: UV and Vitamin D in children

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ABSTRACT

Background: Health benefits of adequate vitamin D levels in the blood include better bone health and a reduced incidence of a range of chronic diseases and infections. Ultra-violet (UV) radiation exposure from the sun is the main source of vitamin D; however such exposure, especially from a young age, is also a potential risk factor for skin cancer. The current study examined the association of UV exposure with vitamin D production in young children to determine the period of weekly exposure prior to blood testing that affected serum 25-hydroxyvitamin D (25(OH)D) levels.

Methods: Between 2009 and 2011, healthy children aged three, six and nine years were recruited from the community for a cross-sectional study of nutritional factors and DNA damage. Parents of 464 children provided information on the children's average weekly sun exposure and level of sun protection during each of the 16 weeks before blood sample collection by a domiciliary phlebotomist.

Results: Serum 25(OH)D levels were best predicted from UV exposure during the week before blood collection for samples drawn in autumn, summer or spring. For samples drawn in winter, serum 25(OH)D levels were best predicted by UV exposure during the two weeks before blood collection.

Conclusions: Consistent weekly sun exposure may be beneficial for young children, especially in winter, to maintain healthy vitamin D levels in the blood. However, confirmation of these results is needed before their public health significance can be fully evaluated.

Keywords: sunlight; vitamin D; child, preschool; osteomalacia; rickets

INTRODUCTION

The health benefits of adequate vitamin D levels in the blood include better bone health, and possibly a reduced incidence of cardiovascular disease, hypertension, type I diabetes, cancer, and infections.¹ Insufficient vitamin D causes softening of bones (osteomalacia) in adults and rickets in children. The source of approximately 90% of vitamin D in humans is exposure to sunlight;² however, dietary vitamin D intake also contributes.^{1, 3}

Serum 25(OH)D is the most commonly used laboratory measure of vitamin D status.⁴ Deficiency at any age is defined as mild with a serum 25(OH)D level of 30-49 nmol/L, moderate with a level of 12.5-29 nmol/L, and severe with a level below 12.5 nmol/L.⁵ Despite the abundance of sunlight in Australia, a recent study indicated that approximately one third of all Australian adults have serum 25(OH)D levels below 50 nmol/L.⁶ In another study of children living in Tasmania, the southernmost Australian State, approximately 8% of 8-year olds and 68% of 16-year olds had levels below 50 nmol/L during winter and spring.⁷

Vitamin D deficiency is a problem that affects all stages of human growth and development; however, simply increasing sun exposure is not straightforward since high ultra-violet (UV) radiation exposure is a potential risk factor for skin cancer. So it is important to understand the impact of UV exposure on serum 25(OH)D levels. This is particularly important in children because of bodily demands for vitamin D for skeletal growth and development,^{8, 9} and their increased susceptibility to pre-cancerous cellular change with solar UV exposure.¹⁰

Many factors may affect the amount of serum 25(OH)D production from exposure to sunlight, including: age, amount of skin exposed,² BMI,¹¹ latitude, season, time of day, use of sunscreen, skin pigmentation,¹² air pollution, ozone and cloud cover.^{13, 14}

To date, studies examining the effects of UV exposure on serum 25(OH)D production have focussed primarily on adults,¹⁵⁻¹⁷ and there have been few studies of the impact of UV

exposure on serum 25(OH)D production in young children;¹⁸⁻²¹ none has assessed this association among children living in areas of very high solar irradiation. The current study aims to address this gap by determining the period of exposure prior to blood sampling that affects serum 25(OH)D levels in healthy young children living in Perth, Western Australia.

MATERIALS AND METHODS

A cross-sectional study of nutritional factors and DNA damage in children was conducted in Perth, Western Australia between 2009 and 2011. Healthy children aged three, six and nine years were recruited through childcare centres, schools and community-based advertisements. Letters, consent forms and reply-paid envelopes were given to parents; parents returned the consent forms directly to the research team if they wanted to take part. Children with asthma, diabetes, cancer, arthritis or epilepsy were not eligible to take part in the study. Parents of 464 children gave informed consent: comprising 155 three-year olds, 155 six-year olds and 154 nine-year olds. Assent of the nine-year olds was also required. The study was approved by the Department of Education and Training, and the University of Western Australia's Human Research Ethics committee.

Parents were mailed instructions about giving the child a light, non-cereal breakfast on the day of the scheduled blood collection. The phlebotomists visited the family's home and measured the child's height and weight. They then collected 18 mL of blood on either one or two days, depending on the child's size. After setting aside approximately 1 mL of whole blood for other measurements, the remaining blood was spun at 3000rpm for 20 min at 4°C and 3.8 mL of the plasma collected were used to measure a range of micronutrient and mineral levels. Serum 25(OH)D was measured using Enzyme ImmunoAssay (BEST 2000 Analyzer, Immuno Diagnostic Systems, Boldon, UK).

Parents completed a telephone interview, providing information about their level of education and household income, and the child's age, sex and ethnicity. Parents were also asked to rate how easily their child's unprotected skin would tan and/or burn if exposed to the sun in summer; and about their child's recent sun exposure. Specifically, they were asked to estimate the number of hours the child spent outdoors between 9am and 5pm on an average weekday, weekend day and school holiday during each of the 16 weeks prior to the scheduled blood collection date. The parent was also asked to rate the proportion of time sun protection such as clothing, hat, shade or sunscreen was used during the time spent outdoors on weekdays and weekend days, giving a Protection Rating classified as: 'None or hardly any of the time' (=1), 'Some of the time' (=2), 'About half the time' (=3), 'Most of the time' (=4) or 'All the time' (=5).

In Australia, the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) provides a standardised daily measure of ambient UV irradiance at a number of locations expressed in terms of standard erythemal doses (SEDs).²² One standard erythemal dose is defined by Ravnbak and Wulf²³ as "the UV dose that elicits just perceptible erythema in the most sensitive people in a group of very sun-sensitive but otherwise healthy individuals. One SED is defined as 100 J/m² at 298 nm using the CIE erythema action spectrum". We obtained ARPANSA ambient UV data for the 8-hour period from 9am to 5pm for each day from 1st January 2009 to 31st July 2011. We estimated the daily erythemal UV exposure for each child as follows:

$$\text{Estimated daily UV} = \left(\frac{\text{Average Hours}}{\text{Protection Rating}} \right) \times \left(\frac{\text{Daily UV}}{8 \text{ hours}} \right)$$

where Average Hours is the hours the child spent outdoors on each category of day (weekday, weekend, holiday) in each of the 16 weeks as estimated by their caregiver, and Protection Rating and Daily UV are as defined above. We then summed the estimated daily

UV for the seven days of each week to calculate the estimated weekly UV (EWUV) in SED units for each of the 16 weeks prior to blood collection.

Statistical analysis

A series of linear regression models were fitted to the natural logarithm of average serum 25(OH)D. In addition, each model included, as potentially explanatory variables, the season in which blood was taken (Blood Season), age, sex, BMI z-score, ethnicity and reported ability to tan ~~a parent rating of how much the child's skin would tan after spending short periods of time in the sun over summer~~ (Tan). The first model also included as explanatory variables, the EWUV for the week immediately before blood collection and its first order interactions with age, sex, BMI z-score, Blood Season, ethnicity and Tan. The second model included all terms in the first model plus EWUV for the second week before blood collection and its first order interactions as above. Each subsequent model included all terms in the previous model plus EWUV for the next week in sequence and its interactions as above. Initial analysis indicated there was no effect on average serum 25(OH)D of EWUV for weeks more than 8 weeks before blood collection. Thus the following 8 models were fitted:

$$y = \alpha_n + \sum_{i=1}^n [(\beta_i + \delta_i \text{Age} + \phi_i \text{Sex} + \varphi_i \text{BMI}(z\text{-score}) + \gamma_i \text{BloodSeason} + \lambda_i \text{Ethnicity} + \theta_i \text{Tan}) * x_i] + \varepsilon_n$$

for n=1,...,8

where $y = \log(\text{average serum 25(OH)D level})$,

$x_i = \text{EWUV for } i\text{th week}$,

$\alpha_n = \text{intercept for } n\text{th model}$,

$\beta_i, \delta_i, \phi_i, \varphi_i, \gamma_i, \lambda_i, \theta_i = \text{corresponding coefficients for each of the explanatory variables of the regression model}$

$\varepsilon_n = \text{residual for } n\text{th model}$

Likelihood ratio tests were used to sequentially compare the nested models. The models assume that the error terms are normally and independently distributed with zero mean and constant variance. The response data (y in the above equation) were transformed before analysis using the logarithmic transformation and scatter plots, histograms and normal probability plots of the residuals were examined to verify that the model assumptions were met. The data were analysed using R.²⁴

RESULTS

Data on serum 25(OH)D levels were available for 461 of the 464 children. The average heights and weights of the children were close to the CDC 50th centiles for age and sex (Table 1). Household income and parental education were higher than in the general Australian population. Regarding household income, 53% of study children lived in households with >\$100,000 income per year, compared with 13% of children in the Australian population.²⁵ Regarding parental education, 71% of children had at least one parent with a tertiary education, while 43% of Australian adults had a tertiary education.²⁶ Nearly 75% of children had at least 3 grandparents of Northern European descent, and therefore considered to be of ‘mostly European’ ancestry. Correspondingly, some 85% of parents said their child would get some degree of sunburn if exposed to sun for 30 mins for the first time in summer. Mean values for serum 25(OH)D levels varied little by sex or age when considered in combination with their associated standard deviations (Table 1). However, there were differences in serum 25(OH)D levels between seasons with highest levels present in summer, mean (SD) = 124.5 (46.0), followed by autumn, mean (SD) = 92.8 (33.8), spring, mean (SD) = 86.2 (24.1), and winter, mean (SD) = 82.4 (29.3).

EWUV varied most between subjects in autumn, summer and spring, and least in winter. This can be seen by comparing the boxplots in Fig 1 relating to the first week before blood collection for each of the seasons. Note that for blood collected in a given season, this first

week is not necessarily the same calendar week for each child. In addition, as the number of weeks before blood collection increases, the relevant week of sun exposure may not fall in the same season as that of blood collection.

On average, children spent between 2 and 4 hours outdoors per day throughout the year, with least variability among children seen for school days and weekends during summer (Fig 2).

On average, sun protection was reported to have been used most of the time spent outdoors, regardless of season or type of day (Fig 3). The greatest variability in sun protection use was in spring and winter.

Serum 25(OH)D was found to be best modelled by a linear function of age, sex, Blood Season, EWUV during each of the first two weeks before blood collection, and the interaction between Blood Season and EWUV for these weeks. Details of the fitted model are presented in Table 2. This model accounts for approximately 15% of the total variability in the serum 25(OH)D levels among children in this study.

Serum 25(OH)D was positively associated with age and, on average, levels were lower among females than males. No association was seen between BMI z-score and serum 25(OH)D, and there were no significant interactions of age or sex with any of the other variables included in the initial models.

After adjustment for age and sex, the EWUV that best predicted serum 25(OH)D levels was dependent on the Blood Season (Fig 4). For blood taken in either autumn, summer or spring, EWUV in the week before blood collection best predicted serum 25(OH)D level. However, for blood taken in winter, EWUV during the 2 weeks before blood collection gave the best predictions.

DISCUSSION

Out of a limited set of potentially explanatory variables, only age, sex and the interaction of Blood Season with EWUV in the one or two weeks before blood collection were found to predict serum 25(OH)D levels in 3-9 year old children. The multiple linear regression model developed in this study explained approximately 15% of the variability in serum vitamin D levels of these young children. There may be other factors associated with these serum vitamin D levels that have not been included in this model. The actual relationships between serum vitamin D and these or other explanatory variables may be more complex than represented by this model: while the current cohort showed no evidence of nonlinear relationships, these (if they exist) may become apparent in a larger, or otherwise different, cohort. Nevertheless our approach allows for the simultaneous examination of a large number of potential explanatory variables which could be associated with serum vitamin D levels, as well as the comparison of many alternative models.

Several of our findings replicate those of previous studies. Rockell, et al.²¹ found that, in a national sample of New Zealand children aged 5-14 years, serum vitamin D concentrations were higher in summer than in winter. Sentongo, et al.²⁷ found that among children with Crohn's disease, serum vitamin D was higher in autumn than in winter. Misra *et al*²⁸ state in a review article that "children of all ages are more susceptible to low vitamin D levels during winter compared to the summer months" (p407).

Seasonal variation in serum vitamin D may be related to both the prevailing climatic conditions and clothing cover during the weeks leading up to blood collection. Since the questions regarding clothing cover were specifically asked in relation to sun protection, parents may have inadvertently underestimated their children's sun protection during late autumn and winter, when clothing is worn mainly for warmth. If this occurred, the calculated EWUV would overestimate the actual UV dose received in winter. As a result, it may be that *more than* two weeks of UV exposure (as suggested by our modelling) would best predict

children's blood vitamin D levels during winter. Our finding of more accurate estimation of vitamin D levels with 2 weeks of sun exposure only in winter could also be related to an increase during late autumn and winter of intra-individual variation in time spent outside and UV exposure, thus indicating that more weeks of exposure are necessary to obtain a stable estimate of serum 25(OH)D.²⁹

Comparable studies of adults have suggested that serum 25(OH)D levels were best predicted by the 4-8 weeks of UV exposure prior to blood collection.³⁰⁻³³ Our findings may therefore suggest that vitamin D produced from UV exposure is utilised in the bodies of young children more quickly than it is in adults. This may be explained by the greater need for vitamin D in younger bodies for the development and maintenance of bone and muscle tissue.^{34, 35} So, more consistent weekly sun exposure may be required in young children than in adults to maintain adequate 25(OH)D levels in the blood, particularly given their smaller body surface area.

There was no evidence of an association between BMI and serum vitamin D levels among children in this study. This differs from previous studies of both adults and children. BMI was found to be inversely associated with serum 25(OH)D levels in many studies world-wide: Malaysian school boys aged 7-12 years;³⁶ adults from a private clinic in Norway which specialises in weight loss counselling and obesity related disorders;³⁷ Spanish school children aged 9-13 years;³⁸ obese but otherwise healthy children and adolescents aged between 6 and 18 years from USA;³⁹ and healthy children below 16 years from Qatar.⁴⁰ One possible explanation for our findings is that very few children in our study were overweight or obese.⁴¹

Similarly, neither ethnicity nor skin sensitivity to the sun was found in this study to be associated with serum 25(OH)D levels, in contrast to a study which found that increased skin pigmentation reduces vitamin D synthesis.⁴² However, as most of our study children were of

European ethnicity, variation in skin pigmentation was likely to have been comparatively low. Furthermore, UV exposure for most of the 461 children was plentiful, with fewer than 10% of children having serum vitamin D levels less than 50 nmol/L (data not shown in tables). This is comparable with the Tasmanian study in which 8% of eight-year-olds had levels below 50 nmol/L,⁷ despite the difference in latitudes (Perth ~ 31.5 degrees S; Tasmania ~ 41.5 - 43.5 degrees S).

Along with the strength and abundance of Western Australian sunshine, the characteristics of children in this study may limit generalisation of our findings to European populations living in similar climatic conditions. Since this appears to be the first study on the effects of erythemal UV exposure on serum 25(OH)D levels in young children, confirmatory studies are needed before conclusions regarding their public health significance can be drawn.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Figure Legends

Fig 1: Boxplots showing distribution of estimated weekly UV across seasons.

Fig 2: Distribution of time spent outdoors each day for each child across seasons

Fig 3: Distribution of sun protection for each child across seasons

Fig 4: Comparison of actual with predicted serum 25(OH)D levels by Blood Season after adjusting for both age and sex

Table 1: Descriptive statistics of participants

	Boys	Girls	3-year olds	6-year olds	9-year olds	All
N	234	230	155	155	154	464
N with Vitamin D	231	230	154	153	154	461
N boys (%)	—	—	75 (48.4)	86 (55.5)	73 (47.4)	234 (50.0)
Weight (kg): Mean (SD)	24.0 (7.8)	24.3 (9.0)	16.1 (2.0)	23.2 (3.7)	33.2 (6.9)	24.2 (8.4)
Height (cm): Mean (SD)	120.2 (16.2)	119.1 (17.2)	99.9 (4.3)	121.2 (5.2)	137.9 (8.0)	119.7 (16.7)
BMI (kg/m ²): Mean (SD)	16.2 (1.8)	16.6 (3.2)	16.1 (1.2)	15.7 (1.7)	17.4 (3.7)	16.41 (2.6)
BMI z-score: Mean (SD)	-0.35 (0.86)	-0.21 (1.25)	-0.39 (0.73)	-0.29 (1.14)	-0.17 (1.27)	-0.28 (1.07)
Serum 25(OH)D (nmol/L):						
Mean (SD)	91.4 (29.1)	85.5 (34.4)	82.1 (25.5)	92.4 (35.6)	90.8 (33.1)	88.5 (32.0)
(range)	(24-220)	(19-286)	(24-146)	(33-286)	(19-267)	(19-286)

Table 2: Regression coefficients for prediction of ln(serum vitamin D levels)

Term:	Estimate [†]	95 % CI	p value
(Intercept)	4.611	[4.259, 4.963]	< 0.001
Age (per year)	0.011	[-0.002, 0.023]	0.094
Female	-0.094	[-0.157, -0.032]	0.003
Autumn	0.054	[-0.044, 0.153]	0.271
Spring	-0.015	[-0.123, 0.092]	0.777
Summer	0.269	[0.029, 0.509]	0.026
‡EWUV.Wk1 (SED [§])	-0.021	[-0.036, -0.005]	0.010
EWUV.Wk2 (SED)	0.024	[0.007, 0.041]	0.004
¶Autumn × EWUV.Wk1	0.029	[0.009, 0.048]	0.004
¶Spring × EWUV.Wk1	0.022	[0.005, 0.038]	0.008
¶Summer × EWUV.Wk1	0.020	[0.001, 0.039]	0.038
¶Autumn × EWUV.Wk2	-0.023	[-0.044, -0.003]	0.024
¶Spring × EWUV.Wk2	-0.025	[-0.043, -0.007]	0.005
¶Summer × EWUV.Wk2	-0.021	[-0.040, -0.002]	0.025

[†]Change in natural logarithm of serum Vitamin D (nmol/L) per unit change in explanatory variable

[‡]Estimated Weekly Ultra-Violet in [§]Standard Erythemal Doses

¶The × symbol represents an interaction effect between the terms i.e. the additional change in serum Vitamin D (nmol/L) per EWUV in the respective season. Thus for example, for a 3 year old female with EWUV in the two weeks before blood collection of 10.3 and 9.7 respectively for winter, 40.5 and 48.5 respectively for summer, the predicted serum vitamin D for:

$$\text{winter} = \exp(4.611 + (0.011 * 3 + 0.094) - 0.021 * 10.3 + 0.024 * 9.7) = 72.9 \text{ nmol/L};$$

$$\text{summer} = \exp(4.611 + (0.011 * 3 + 0.094) + (-0.021 + 0.020) * 40.5 + (0.024 - 0.021) * 48.5) = 126.8 \text{ nmol/L}$$

Fig 1

Fig 2

Fig 3

Fig 4