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Original Article

Vitamin D supplementation is associated with higher serum 25OHD in Asian and White infants living in Vancouver, Canada

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Abstract

To prevent rickets, the Health Canada and the American Academy of Pediatrics recommend that breastfed infants receive a daily vitamin D supplement of 10 μg d^{-1}. Compliance with this recommendation is variable and its effect on infant vitamin D status is unclear. We measured serum 25-hydroxyvitamin D (25OHD) in Asian immigrant ($n = 28$) and White ($n = 37$) mothers and their infants aged 2–4 months living in Vancouver (49°N). Mothers completed health and demographic questionnaires. All subjects were term infants who were primarily breastfed. Analysis of variance, χ^2, multiple regression and logistic regression analysis were performed as appropriate. Mean 25OHD of the infants was 31 (95% confidence interval 28–34) ng mL^{-1}. Only two infants had a 25OHD concentration indicative of deficiency, <10 ng mL^{-1}. Of the infants, 14% ($n = 9$) and 49% ($n = 32$) were vitamin D insufficient based on two commonly used cut-offs of 20 and 30 ng mL^{-1}, respectively. Fifty-eight (89%) infants had been given a vitamin D supplement. Mean 25OHD was 9.4 ng mL^{-1} higher in infants consuming ≥10 μg d^{-1} of vitamin D from supplements vs. those consuming less ($P = 0.003$). Mother's 25OHD, season, skin colour or ethnicity (Asian vs. White) did not influence infant 25OHD. The infants in our study, most of whom received vitamin D supplements, were generally protected against low 25OHD. The study was limited by sample size and the nature of the cross-sectional study design.

Keywords: vitamin D, infant, supplementation, 25-hydroxyvitamin D, Asian, White.

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Introduction

Lack of vitamin D during infancy leads to rickets (Holick 2006) and possibly other negative health outcomes such as type 1 diabetes (Hyppönen *et al*. 2001) and asthma (Camargo *et al*. 2007) later on. Exclusive breastfeeding is recommended for the first 6 months of life (World Health Organization 2002; American Academy of Pediatrics 2005); however, breast milk does not usually contain adequate amounts of vitamin D to meet infant needs (Health Canada 2004a). Indeed, rickets most frequently occurs in children who were unsupplemented and exclusively breastfed (Ward *et al*. 2007), and low circulating 25-hydroxyvitamin D (25OHD), the best indicator of vitamin D status (Heaney 2004), is common in breastfed infants (Gordon *et al*. 2008; Madar *et al*. 2009a). The evidence base for what defines optimal 25OHD concentration during infancy is lacking; however, the US Institute of Medicine (IOM) (2011) has recently recommended >20 ng mL^{-1} as a cut-off, whereas the Canadian Pediatric Society (First Nations, Inuit

and Métis Health Committee 2007) has endorsed >30 ng mL^{-1}.

Health Canada (2004a) and more recently the American Academy of Pediatrics (2005) have recommended that breastfed infants receive a daily vitamin D supplement of 10 μg d^{-1}. Compliance with this recommendation varies considerably; for example, only 10% of breastfed infants in the US Infant Feeding Practices Study II (2005–2007) were receiving supplemental vitamin D at 2 months of age (Perrine *et al*. 2010) compared with over 90% in Vancouver, Canada (Crocker *et al*. 2011). The efficacy of 10 μg d^{-1} vitamin D in preventing low 25OHD was recently demonstrated in a German study where infants randomized to 6.3 or 12.5 μg per day ($n = 20$ per group) at birth achieved mean [95% confidence interval (CI)] 25OHD of 56 (46–66) and 60 (50–70) ng mL^{-1}, respectively after 6 weeks (Siafarikas *et al*. 2011). While the efficacy of 10 μg d^{-1} has been demonstrated in controlled trial context, there is a lack of data on the effectiveness of the recommendation in preventing low infant 25OHD in observational settings. Even in the Vancouver study, only 67% of breastfed infants were receiving $\geq$10 μg d^{-1} and 19% were receiving less than 5 μg d^{-1}, mainly because of less than daily dosing (Crocker *et al*. 2011). Here we describe 25OHD concentration in infants of Asian immigrants, and those of White background living in Vancouver, BC, Canada, and the influence of vitamin D supplementation on 25OHD. Asian infants were chosen as prior studies have shown these groups, especially recent immigrants, are at greater risk of low 25OHD and rickets (Ford *et al*. 1973; Madar *et al*. 2009b). We also explore other determinants of 25OHD in these infants such as maternal 25OHD, skin colour and season.

Methods

Participants

A convenience sample of Asian Immigrants ($n = 28$) and White mothers ($n = 37$) and their infants aged 2–4 months were recruited in Metro Vancouver (49°N), Canada, between April 2010 and August 2011. A variety of recruitment methods was used, including newspaper advertisements, notices distributed in doctors' offices, prenatal classes, infant drop-in centres and word-of-mouth. Inclusion criteria specified that the Asian mothers must have moved to Canada from Asia and that the White mothers must have been born in Canada. For our purposes, Asian refers to women from Middle East, South Asia (India, Pakistan, Bangladesh and Sri Lanka), China and all countries in South East Asia. White mother refers to women of European background. The large majority of the Vancouver population is of European or Asian ancestry. All infants were healthy term infants who were predominantly breastfed since birth (<1 feeding of formula per day). The study was approved by the University of British Columbia Clinical Research Ethics Board and written informed consent was obtained from all mothers.

Procedures

Participants completed a lifestyle and demographic questionnaire that included questions on infant diet, nutrient supplementation, age, ethnicity, income and education. A non-fasting blood sample was collected from both the mother and infant via venipuncture. Serum was separated from whole blood and samples were stored at –80°C. Infant skin colour was measured by reflectance colorimetry using a hand-

Key messages

- Most predominantly breastfed infants were receiving vitamin D supplements and they were very well protected against 25OHD concentrations associated with development of rickets (<10 ng mL^{-1}).
- Nearly all infants, if receiving $\geq$10 μg d^{-1} vitamin D, had higher 25OHD than the latest IOM recommendation of 20 ng mL^{-1}.
- Maternal 25OHD, season, skin colour and ethnicity were not significant determinants of infant 25OHD, likely attributable to the overwhelming effect of infant supplementation.

held spectrophotometer (Konica Minolta Sensing CM-600d; Tokyo, Japan). Skin colour was measured at the upper inner arm, which receives little ultraviolet (UV) exposure so represents constitutive or genetically inherited skin colour (Rockell *et al*. 2008). This instrument assigns L^* and b^* values, which represent the relative brightness of colour (ranging from black to white) and degree of pigmentation, respectively (Westerhof 1995). Skin pigmentation is best described by the individual typology angle (ITA°): ITA° = arc tangent $[(L^*\text{-}50)/b^*)] \times 180/\pi$. Lower ITA° indicates darker skin colour (Piérard 1998).

Laboratory methods

Serum 25OHD was determined by BC Biomedical Laboratories Ltd. (Surrey, BC, Canada) using a *DiaSorin* LIAISON® 25-OH Vitamin D TOTAL Assay, a competitive chemiluminescence immunoassay used for the quantitative determination of both $25OHD_2$ and $25OHD_3$ metabolites (DiaSorin 2012). BC Biomedical Laboratories Ltd. participates in the Vitamin D External Quality Assessment Scheme, an external quality control program for 25OHD measurement (DEQAS 2012).

Data analyses

Statistical analyses were performed using SPSS Statistics 20.0 for Microsoft Windows (SPSS Inc., Chicago, IL, USA). Significance was set at a level of $P < 0.05$. Serum 25OHD concentrations were normally distributed based on visual inspection of a histogram and the Shapiro–Wilk Test ($P > 0.05$). We classified 25OHD below 10 ng mL^{-1} as vitamin D deficiency (Mulligan *et al*. 2009), and assessed two commonly used levels of vitamin D insufficiency, ≤20 (IOM 2011) and ≤30 ng mL^{-1} (Bischoff-Ferrari *et al*. 2006). Univariate comparisons between selected characteristics by infant 25OHD were made by analysis of variance and χ^2 as appropriate. Multiple regression analysis was used to examine the independent relationship between variables and infant serum 25OHD. Logistic regression was performed to obtain adjusted prevalence of insufficiency (25OHD <20 or 30 ng mL^{-1}). To estimate the effect of skin colour on 25OHD, we replaced 'group' in the model with ITA° measures.

Table 1. Participant characteristics ($n = 65$)

Characteristic	*n*	%
Maternal age (year)	31.5 (30.0, 35.0)§	
Infant age (week)	12.0 (10.0, 16.0)§	
Season		
October to March	21	32.3
April to September	44	67.7
Group		
Asian immigrant*	28	43.1
White non-immigrant	37	56.9
Annual family income		
<$40 000	5	7.7
$40 000–<80 000	9	13.8
$80 000–<120 000	13	20.0
≥$120 000	14	21.5
Non-responders†	24	36.9
Education		
<High school	1	1.5
Some and completed high school	5	7.7
Some and completed trade/vocational training/college	9	13.8
Some and completed university	50	76.9
Infant vitamin D supplement (μg d^{-1})		
0–<10	23	35.4
≥10	42	64.6
Infant upper inner arm ITA°‡	50.6 (40.4, 55.2)§	

*Chinese ($n = 16$), Indian ($n = 2$), Pakistani ($n = 1$), Filipino ($n = 3$), Iranian ($n = 6$). †Do not know or do not want to say. ‡ITA° is classified as very light > 55° > light > 41° > intermediate > 28° > tanned > 10° > brown > –30° > dark. §Median (1, third quartile).

Results

Twenty-eight Asian immigrants and 37 White mothers (European) and their infants aged 2–4 months participated. Asian immigrant participants were from China ($n = 16$), India ($n = 2$), Pakistan ($n = 1$), Philippines ($n = 3$) and Iran ($n = 6$). Of the infants, 51 (78%) were exclusively breastfed, using the World Health Organization (2002) definition, 11 (15%) infants had received some formula or infant cereal since birth but were not receiving formula at the time of the study and 3 (5%) infants received less than one feeding of formula per day. Participant characteristics are given in Table 1. Mothers were generally well educated and

Table 2. Infant serum 25OHD concentration and prevalence of 25OHD insufficiency

Characteristic	*n*	Serum 25OHD (ng mL^{-1})		
		Mean (95% CI)	*n* (%)	
			<20 ng mL^{-1}	<30 ng mL^{-1}
All	65	31 (28–34)	9 (14)	32 (49)
Infant vitamin D supplement (μg d^{-1})				
0–<10	23	25 (20–30)	6 (26)	15 (65)
≥10	42	34 (31–38)†	3 (7)†	17 (41)
Group				
Asian immigrant*	28	32 (28–37)	3 (11)	11 (39)
White non-immigrant	37	30 (26–34)	6 (16)	21 (57)
Season				
October to March	21	34 (29–40)	2 (10)	7 (33)
April to September	44	30 (26–33)	7 (16)	25 (57)
Maternal 25OHD (ng mL^{-1})				
<20	6	27 (12–42)	2 (33)	3 (50)
≥20	59	32 (28–35)	7 (11)	29 (49)
<30	31	29 (24–34)	6 (19)	18 (58)
≥30	34	34 (30–37)	3 (9)	14 (41)

25OHD, 25-hydroxyvitamin D; CI, confidence interval. *Chinese ($n = 16$), Indian ($n = 2$), Pakistani ($n = 1$), Filipino ($n = 3$), Iranian ($n = 6$). †Significantly different from the 0–<10 μg d^{-1} category ($P < 0.05$).

of those who responded to the annual family income question ($n = 41$), 88% had an income ≥$40 000. Fifty-eight (89%) infants had been given a vitamin D supplement, in most cases providing 10 μg per dose. Of these infants, 69% received Ddrops® (a concentrated formula that provides 10 μg in a single drop; Ddrops®, Woodbridge, Ontario, Canada) and others received a vitamin D liquid supplement (a formula such as D-Vi-Sol® (Mead Johnson & Company, LLC, Glenview, IL, USA) that provides 10 μg per 1 mL). Most mothers reported covering up their infant when he/she was outside.

Overall, the mean serum 25OHD concentration of the infants was 31 (95% CI 28–34) ng mL^{-1} (Table 2). Only 2 (3%) infants had a 25OHD concentration indicative of deficiency (<10 ng mL^{-1}). Of these two infants, one did not receive any vitamin D, whereas the other received only 3.6 μg d^{-1}. Nine (14%) and 32 (49%) infants were vitamin D insufficient based on the two cut-offs of 20 and 30 ng mL^{-1}, respectively. Infants who received ≥10 μg of vitamin D had significantly higher 25OHD concentrations than those who received less ($P = 0.003$). Vitamin D supplement use of <10 μg was associated with a higher prevalence of having a 25OHD concentration <20 ng mL^{-1} ($P = 0.046$), and there was a tendency ($P = 0.06$) for prevalence of 25OHD <30 ng mL^{-1} to be higher. In multivariate analysis, the only variable associated with infant 25OHD was supplement use. Mean 25OHD was 9.4 ng mL^{-1} higher in infants consuming ≥10 μg of vitamin D from supplements vs. those consuming less ($P = 0.003$). Group, season, maternal 25OHD, infant age and ITA° had no significant impact on infant 25OHD (Table 3).

Discussion

In this sample of infants, most of whom were receiving vitamin D supplements, low serum 25OHD was uncommon. Only 9 (14%) infants had a serum 25OHD less than 20 ng mL^{-1}, the cut-off recently recommended by the US IOM (2011), and only two infants had serum 25OHD less than 10 ng mL^{-1}, the cut-off set by Health Canada (2004a). At serum 25OHD >10 ng mL^{-1} the risk of rickets is thought to be low. Our mean 25OHD of almost 32 ng mL^{-1} is generally much higher than that reported for breastfed infants in other studies who were not supplemented. For example, Greer & Marshall (1989) reported that White infants living in Wisconsin had a

Table 3. Multivariable model for infant serum 25OHD concentrations and adjusted prevalence of 25OHD insufficiency

Characteristic	*n*	β ng mL^{-1} (95% CI)	Adjusted prevalence % (95% CI)	
			<20 ng mL^{-1} ($n = 9$)	<30 ng mL^{-1} ($n = 32$)
Infant vitamin D supplement (μg d^{-1})[†]				
0–<10	23	Referent	22 (8–47)	61(38–80)
≥10	42	9.4 (3.3, 15.5)[§¶]	5 (1–18)[§]	36 (22–53)
Group[†]				
Asian immigrant*	28	Referent	9 (2–30)	43 (24–64)
White non-immigrant	37	0.2 (–6.2, 6.5)	13 (4–33)	54 (35–72)
Season[†]				
October to March	21	Referent	9 (2–33)	38 (19–61)
April to September	44	–4.6 (–11.0, 1.9)	12 (4–30)	59 (41–75)
Maternal 25OHD, per 1 ng mL^{-1} increase[†]	65	0.12 (–0.04, 0.28)	–	–
Infant age (week)[†]	65	0.04 (–0.72, 0.76)	–	–
Infant upper inner arm ITA°, per 10° increase[‡]	65	0.16 (–0.12, 0.48)	–	–

25OHD, 25-hydroxyvitamin D; CI, confidence interval. *Chinese ($n = 16$), Indian ($n = 2$), Pakistani ($n = 1$), Filipino ($n = 3$), Iranian ($n = 6$). [†]Adjusted for variables other than infant upper inner arm ITA°. [‡]Adjusted for variables other than Group. [§]Significantly different from the 0–<10 μg d^{-1} category ($P < 0.05$). [¶]Mean (95% CI) 25OHD of infants supplemented with ≥10 and <10 μg d^{-1} were 35.2 (31.5–39.0) and 25.9 (21.0–30.8) ng mL^{-1}, respectively.

mean 25OHD of 16 ng mL^{-1} at 3 months. More recently, Ziegler *et al*. (2006) found that the mean 25OHD of 9-month-old unsupplemented mainly White infants ($n = 35$) living in Iowa was 22 ng mL^{-1}.

In our sample, infants receiving 10 μg d^{-1} or more had higher 25OHD concentrations than those who had less (adjusted means, 34 vs. 25 ng mL^{-1}) and were less likely to be below 20 ng mL^{-1}. This confirms the effectiveness of infant supplementation in preventing low 25OHD. In the Iowa study, infants at 9 months who were being supplemented with vitamin D ($n = 49$) had significantly higher 25OHD than those who were not ($n = 35$), 28 vs. 22 ng mL^{-1}, respectively ($P = 0.012$). It was surprising in our study that despite receiving 10 μg d^{-1} or greater vitamin D, 5% and 36% of these infants did not achieve 25OHD concentrations of 20 and 30 ng mL^{-1}, respectively. This is at odds with the recent clinical trial evidence where infants given as little as 6.3 μg d^{-1} from birth had a mean 25OHD of 56 ng mL^{-1} by 6 weeks (Siafarikas *et al*. 2011). Infants in that trial were younger and presumably smaller than our study so that on a per kg basis, the infants in our study would have been receiving less vitamin D, but this would not entirely explain the discrepancy. Mothers in our study may have overestimated their compliance with daily dosing compared to the controlled study.

Maternal vitamin 25OHD, season, skin colour and ethnicity were not significant determinants of infant 25OHD in our study. The lack of association in our study is likely attributable to the overwhelming effect of infant supplementation. In the absence of supplementation, the effects of other 25OHD determinants may become more apparent. With regard to the effect of maternal 25OHD status, it is known that at birth, there is a strong correlation between mother and infant 25OHD concentrations (Wieland *et al*. 1980). Although this association falls as the infant gets older, maternal 25OHD still explained 36% of the variance in 25OHD concentrations of infants aged 10–14 weeks in Northern India (Jain *et al*. 2011). Among those infants, however, only a third were supplemented, and received an average of only 3.1 μg d^{-1} of vitamin D_2. The larger proportion of supplemented infants and the higher level of supplementation in our study likely overrode any impact of maternal 25 OHD concentrations. Sunlight exposure is another known determinant of 25OHD. In the Iowa study (Ziegler *et al*. 2006), season was a significant determinant of 25OHD in unsupplemented infants at 9 months of age. These infants had mean values of 8 and 27 ng mL^{-1} in winter and summer, respectively, and thus appeared to benefit from UV exposure during the summer months. Conversely, among supple-

mented infants in the same study, mean values were not significantly different between winter and summer, corresponding to our findings and again demonstrating the major impact of supplementation. Moreover, Health Canada (2004b) recommends that children under 1 year be kept out of direct sunlight, and most women in our study reported following this recommendation, further contributing to the lack of a seasonal effect. Finally, infants from darker skinned ethnic groups have been reported to have lower 25OHD concentrations. For example, South Asian and Arabic infants 1 to 4 months living in the United Arab Emirates had a serum 25OHD of only 5 ng mL^{-1} (Dawodu *et al*. 2003). In our study, supplementation and the apparent lack of infant exposure to sunlight likely explain the absence of an effect of ethic group or skin colour on infant 25OHD.

This study was limited by sample size, which may have reduced our ability to detect differences for some vitamin D determinants. In terms of our ability to detect effects of ethnicity and skin colour on 25OHD, we were somewhat limited as we had no African-American infants, but this is reflective of the Vancouver population. We were also limited in our ability to assess the impact of vitamin D supplementation vs. no supplementation on 25OHD, as only a few infants were not supplemented with at least some vitamin D. Also, our study was cross-sectional, which further limits our ability to infer that vitamin D supplementation protected against low 25OHD. However, the randomized trial evidence with vitamin D supplements (Madar *et al*. 2009a; Siafarikas *et al*. 2011) would support that it does. The high level of compliance with supplements seen in our study may be associated with the higher than average educational and socio-economic status of most mothers in our sample. Whether this level of compliance remains with other demographic groups is not known. We had a convenience sample and thus our results cannot be generalized to the Canadian population or even to Vancouver. In conclusion, the sample of infants we studied was very well protected against 25OHD concentrations associated with development of rickets (<10 ng mL^{-1}). Nearly all infants, if receiving ≥10 μg d^{-1} vitamin D, were higher than the latest IOM recommendation of 20 ng mL^{-1}. If new evidence becomes available confirming the need for higher 25OHD concentrations during infancy, vitamin D supplementation greater than the 10 μg currently recommended may be needed.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

GEC was responsible for the conception and design of the study, obtaining funding and management of the project. TJG and SIB contributed to the study design and WL and MJ had a role in the acquisition of data. TJG, WL, SIB and GEC interpreted the data and drafted the initial manuscript. All authors participated in manuscript preparation and critically reviewed all sections of the text for important intellectual content.

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