Original Article

Predictors of vitamin D status in New Zealand preschool children

C.T. Cairncross^{*}, W. Stonehouse[†], C.A. Conlon^{*}, C.C. Grant^{‡,§}, B. McDonald[¶],
L.A. Houghton^{**}, D. Eyles^{‡,§,§§}, C.A. Camargo Jr^{¶¶}, J. Coad^{*} and P.R. von Hurst^{*}

^{*}School of Food and Nutrition, Massey University, Auckland, New Zealand, [†]CSIRO Food and Nutrition, Adelaide, Australia, [‡]Department of Paediatrics: Child and Youth Health, University of Auckland, Auckland, New Zealand, [§]General Paediatrics, Starship Children's Hospital, Auckland, New Zealand, [¶]Institute of Natural and Mathematical Sciences, Massey University, Auckland, New Zealand, ^{**}Department of Human Nutrition, University of Otago, Dunedin, New Zealand, ^{‡‡}Queensland Brain Institute, University of Queensland, Brisbane, Australia, ^{§§}Queensland Centre for Mental Health Research, Brisbane, Australia, and ^{¶¶}Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Abstract

Vitamin D deficiency has adverse health effects in young children. Our aims were to determine predictors of vitamin D status and then to use these factors to develop a practical tool to predict low 25(OH)D concentrations in preschool New Zealand children. A cross-sectional sample of 1329 children aged 2 to <5 years were enrolled from throughout New Zealand in late-winter to spring 2012. 25-Hydroxyvitamin D (25(OH)D) was measured on dried blood spot (DBS) samples collected using finger-prick sampling. Caregivers completed a questionnaire. Mean (SD) DBS 25(OH)D concentration was 52(19)nmol/L. 25(OH)D < 25 nmol/L was present in 86(7%), 25(OH)D < 50 nmol/L in 642(48%), 25(OH)D 50- < 75 nmol/L in 541(41%) and 25(OH)D > 75 nmol/L in 146(11%) of children. Factors independently associated with the risk of 25(OH)D < 25 nmol/L were female gender (OR 1.92, 95% CI 1.17–3.14), other non-European ethnicities (not including Māori or Pacific) (3.51, 1.89–6.50), had olive-dark skin colour (4.52, 2.22–9.16), did not take vitamin D supplements (2.56, 1.06–6.18), had mothers with less than secondary-school qualifications (5.00, 2.44–10.21) and lived in more deprived households (1.27, 1.06–1.53). Children who drank toddler milk (vitamin D fortified cow's milk formula marketed to young children) had a zero risk of 25(OH)D < 25 nmol/L. The predictive tool identified children at risk of 25(OH)D < 25 nmol/L with sensitivity 42%, specificity 97% and ROC area-under-curve 0.76(95% CI 0.67–0.86, $p < 0.001$). Predictors of low vitamin D status were consistent with those identified in previous studies of New Zealand children. The tool had insufficient predictive ability for use in clinical situations, and suggests a need to promote safe, inexpensive testing to determine vitamin D status in preschool children.

Keywords: vitamin D deficiency, child, questionnaire, status, New Zealand.

Correspondence to: PR von Hurst¹, School of Food and Nutrition, Massey University, Auckland, New Zealand. E-mail: p.r.vonhurst@massey.ac.nz

Introduction

Low vitamin D status has negative consequences for the skeletal development of young children, with vitamin D deficiency, in its severe form, being a cause of rickets. Low vitamin D status may have also have other adverse effects on health including allergic and respiratory diseases in which the role of vitamin D is currently being investigated (Goksugur et al. 2015; McNally et al. 2009; Uysalol et al. 2013).

Internationally there is a relative lack of data on the vitamin D status of preschool children. To date, vitamin

D status has been described in other age groups in New Zealand; newborns (Camargo et al. 2010), children aged 6 to 23 months (Grant et al. 2009; Houghton et al. 2010) and 5 to 14 years (Rockell et al. 2005) and adults aged ≥15 years (Rockell et al. 2006).

Identified risk factors for vitamin D deficiency in New Zealand infants and children include female gender (Grant et al. 2009; Houghton et al. 2010), non-European ethnicity (Grant et al. 2009; Houghton et al. 2010; Rockell et al. 2005), higher body mass index (BMI) (Rockell et al. 2005), not taking vitamin supplements (Grant et al. 2009), not drinking infant formula

(Grant *et al.* 2009), maternal education level (Houghton *et al.* 2010), residence in a more crowded household (Grant *et al.* 2009) and winter season of testing (Blok *et al.* 2000; Houghton *et al.* 2010; Rockell *et al.* 2005). The impact of these factors on the vitamin D status of preschool children is unknown. Likewise, an area that has yet to be explored and of specific relevance to this age group is the relationship between attendance at daycare or preschool education centres and 25(OH)D concentrations.

One issue that has contributed to vitamin D status being less well described in this preschool age group is the challenge posed by the collection of blood samples from this age group (Fradet *et al.* 1990). Parents of young children are reluctant to subject their children to venepuncture, negatively affecting participation in research studies (Shaddy & Denne 2010). Recently, sensitive methods for measuring 25-hydroxyvitamin D (25(OH)D) concentrations on dried blood spots have been developed with good agreement shown between that method and serum obtained by venepuncture (Eyles *et al.* 2009; McNally *et al.* 2008; Newman *et al.* 2009). This methodology allows for small volume capillary sampling and thus created the opportunity for measurement of 25(OH)D concentration in a large sample of preschool children.

Timely identification of young children with vitamin D deficiency may improve outcomes related to skeletal health. However, routine testing for serum 25(OH)D is expensive and not publically funded in New Zealand (Bolland *et al.*, 2012). Questionnaire based tools able to predict vitamin D deficiency or insufficiency are non-invasive and inexpensive and therefore have a potentially important role. A small number of questionnaires have been developed as predictors of risk of vitamin D deficiency for specific populations, including

children and adolescents (Absoud *et al.* 2011), young adults (Bolek-Berquist *et al.* 2009) and older adults (Lopes *et al.* 2014; Nabak *et al.* 2013; Sohl *et al.* 2014). To our knowledge there is currently no questionnaire for predicting vitamin D deficiency in any age group in New Zealand, nor specifically in preschool-aged children.

Our aims were to describe the vitamin D status in a self-selected sample of New Zealand children aged 2 to <5 years using 25(OH)D concentration measured on a dried blood spot, to identify factors associated with 25(OH)D concentration <25 nmol/L and <50 nmol/L in this age group, and to develop and assess the validity of predictive tools for the identification of children at risk of 25(OH)D <25 nmol/L and <50 nmol/L.

Method

Study design and participants

In this cross-sectional study, 1329 children aged 2 to <5 years were recruited from cities and towns from around New Zealand. Participants were recruited and tested during the 10-week period from August to October 2012 (late winter – early spring). Intensive advertising through early childhood education facilities was combined with the primary recruitment tool of purchased access to the email database of ReachMe (Reachme 2014), a company that provides products and information for parents of newborn and young children.

Ethical approval was granted by the Health and Disability Ethics Committee, Northern Region, reference number NTX/12/04/036, and a parent or caregiver of each subject provided written informed consent for the participation of their child in the study.

Key messages

- This is the first large scale study of vitamin D status in New Zealand preschool children, facilitated by use of dried blood spot collection.
- Factors associated with vitamin D deficiency in these preschool children reflected those found in other age groups in New Zealand.
- Predictive tools have insufficient accuracy to assess risk of low vitamin D status in clinical situations.
- Need for safe, inexpensive testing to determine vitamin D status in young children.

Data collection

To ensure national coverage, 49 geographically dispersed pharmacies volunteered to act as testing centres, enabling short travelling distances and reduced burden for participating families. Pharmacists were trained in blood collection via a fingerprick procedure by study team members. Children also visited the nutrition clinics of Massey University, Auckland, and the University of Otago, Dunedin, for testing.

A capillary blood sample from a fingerprick was obtained from each child by the trained testers using paediatric lancets. Blood was dropped onto a pre-stamped circle on Whatman 305 filter paper and allowed to air dry for a minimum of 1 h before the flap on the protective envelope was closed. Dried blood spot cards were stored at room temperature then couriered back to the Auckland study office. At the conclusion of the data collection period all cards were then delivered to the Queensland Brain Institute (Brisbane, Australia), for measurement of 25(OH)D concentration.

A 59-item questionnaire including questions regarding demographics, recent health history and risk factors for vitamin D deficiency was completed by a caregiver of each child. The questionnaire was offered in online (www.surveymonkey.com) and paper formats. Caregivers were asked to list all ethnicities they identified for their child. Ethnicity was then assigned using the following prioritisation; Māori, then Pacific, then Other, then European (Ministry of Health 2008). Maternal education was classified as no secondary qualifications, secondary qualifications or post-secondary qualifications. Parents were asked to specify who cared for their child during the day, and if attending formal preschool education facilities such as daycare, preschool or kindergarten, for how many hours per week. The effect of socioeconomic status was modelled on a location-wide basis, relating to the area census unit the subject resided in. Specifically, each household was allocated to a deprivation quintile 1–5 with 1 being the least deprived (affluent) 20% of households, and 5 being the most deprived (poorest) 20% of households, as defined by the NZ Index of Social Deprivation (Salmond et al. 2007), a census-based measure.

A six-point parental report of skin colour (very fair, fair, medium, olive, dark and very dark) was used. For

the purposes of analysis this was collapsed into three categories (very fair–fair, medium, olive dark–very dark). Questions regarding dietary sources of vitamin D were restricted to the type of milk the child consumes and current use of vitamin D containing supplements or cod liver oil. A list of common milks and those low-fat milks fortified with vitamin D was provided, along with a free-text option for other milks consumed, as there are few dietary sources of vitamin D in New Zealand.

Height and weight were measured without shoes by the trained testers using household non-calibrated scales and wall-mounted measuring devices. We calculated BMI adjusted-for-age, as described by Cole (Cole et al. 2000). Residential address was used to establish latitude, with the country then separated into three regions; northern (34–38°S), central (39–42°S) and southern (43–46°S) New Zealand.

Measurement of dried blood spot 25 (OH)D concentration

The analytical method for measurement of dried blood spot 25(OH)D concentration has been described elsewhere (Eyles et al. 2009). In brief, it involves a derivatising agent combined with liquid chromatography tandem mass spectrometry (Eyles et al. 2009). The assay measured both 25(OH)D D2 and D3. Results are described as total 25(OH)D.

Precise 3.2-mm whole blood punches were obtained from each dried blood spot. This is equivalent to 3.2 µL of whole blood. As 25(OH)D is excluded from erythrocytes, 25(OH)D concentration measured on the dried blood spot was corrected for the haematocrit fraction in serum of children this age (0.41 for girls and 0.45 for boys) (Bain 2011) using the following equation:

$$\text{Plasma}[25(\text{OH})\text{D}](\text{nM}) = \text{DBS}[25(\text{OH})\text{D}](\text{nM}) / (1 - \text{haematocrit fraction}).$$

Statistical analyses

We used G*Power 3 (Heinrich Heine University, Düsseldorf) to calculate that a total sample size of 1300 was needed for estimation of risk factors and validation of the predictive tool for 25(OH)D concentrations <25 nmol/L. A sample of 1000 would provide

>80% power to demonstrate statistical significance for a risk factor acting across the whole population for which there was a relative risk of 2.0 for the association of this factor with the presence of 25(OH)D <25 nmol/L (alpha=0.05 two-tailed, ratio unexposed: exposed=1:4, frequency of deficiency in unexposed=5%, odds ratio=2.1: minimum sample needed=929). For the tool validation, a further 300 subjects were required to allow estimation of the probability of correct classification and consequent misclassification rates.

Descriptive statistics were used to report 25(OH)D concentrations and the prevalence of 25(OH)D <25 nmol/L and <50 nmol/L. Differences in mean 25(OH)D concentration were determined using Independent *t*-test or ANOVA. Post-hoc analysis for variables with more than two groups was completed using linear regression or Dunnett's test. Independent predictors of 25(OH)D <25 nmol/L and 50 nmol/L were identified using multivariable logistic regression and associations were described using adjusted odds ratios (OR) with 95% confidence intervals (CI).

For the predictive tool, two models were developed; for predicting 25(OH)D <25 nmol/L and for predicting 25(OH)D <50 nmol/L. For the validation of the predictive questionnaires, study participants were randomly allocated into two datasets, 70% in the 'development' and 30% in the 'validation' groups. An alternate prioritisation of ethnicity was used in the development of the predictive questionnaire to enable inclusion of specific ethnicities in which higher rates of vitamin D deficiency have been reported. Children were coded into six categories from the parental report of ethnicity in the prioritisation order: children of Indian/African > Pacific > Any European > Māori/Asian ethnicities.

None of the children who drank vitamin D fortified toddler milk had 25(OH)D concentrations <25 nmol/L. These children have a zero risk of vitamin D deficiency and thus were removed from the 'development' dataset for the model predicting 25(OH)D <25 nmol/L. All of the children in the 'validation' dataset were included in the validation process. For the model predicting 25(OH)D <50 nmol/L, all children in the 'development' dataset were included.

The predictors were fitted in a multivariable logistic regression, and any redundant variables eliminated, until the best-fitting model was obtained in which all variables were significantly associated with the dependent variable. Non-significant variables were then examined, and those of borderline significance were tested in the model and retained if they added to the model's performance. A $P < 0.05$ was used to define statistical significance.

A mathematical scoring system was developed to predict 25(OH)D <25 nmol/L or <50 nmol/L. The risk score associated with each predictor was weighted based on the regression coefficient values and an equation developed. Receiver Operating Characteristic (ROC) analysis was used to determine the cut-off criterion by choosing the best balance of sensitivity and specificity. For internal validation in the 25(OH)D <25 nmol/L model, a negative score of 8 (higher than the total score) was added to the equation for children who drank toddler milk so these children would not be below the cutoff for 25(OH)D <25 nmol/L. This modified equation was applied in ROC analysis of the 'validation' group ($n=400$). The equation developed for the model of 25(OH)D <50 nmol/L was applied in ROC analysis of the 'validation' group ($n=400$) for internal validation.

Performance of the predictive questionnaire was assessed by ROC analysis, using area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). All analyses were performed with the Statistical package for the Social Sciences (SPSS).

Results

Participant sample recruitment and characteristics

The 1329 children were recruited and tested in 17 cities and towns throughout New Zealand. The majority (49%) lived in the northern region with 28% and 23% in the central and southern regions, respectively.

The mean age was 41 months (range 24–60 months). The majority (70%) were of NZ European ethnicity. Most (83%) of the children had a BMI-adjusted-for-age that was within the normal range,

and most (86%) attended day care. At the time the questionnaire was completed 29% of children were taking a supplement containing vitamin D or cod liver oil, and toddler milk was being consumed by 6% of participants. Most (81%) mothers had post-secondary school qualifications, and 51% of children lived in affluent areas (the two least deprived quintiles of New Zealand households) (Table 1).

Univariate associations with dried blood spot 25(OH)D concentrations

The mean (SD) DBS 25(OH)D concentration was 52 (19) nmol/L, ranging from 5 to 173 nmol/L. Analyses of 25(OH)D concentrations by child characteristics are presented in Table 1. Distribution of 25(OH)D concentration is in the Supplementary Appendix, Fig. 1.

DBS 25(OH)D concentrations varied by ethnic group and maternal education qualifications, with the lowest in children of Pacific ethnicity and mothers without secondary school qualifications. Lower mean 25(OH)D concentrations were present in female compared with male children, and for children with olive-very dark skin colour compared with those with very fair-fair and medium skin colour.

Children not attending formal daycare had lower 25(OH)D concentrations than those attending daycare. Mean 25(OH)D concentrations were higher in children who took vitamin D containing supplements or who drank toddler milk. For those children who drank regular cow's milk, those who consumed non-fortified milk had lower 25(OH)D concentrations than those consuming fortified low-fat milk. Mean 25(OH)D concentration varied by residential latitude but there was not a latitudinal gradient.

Categorisation of 25(OH)D concentration

The prevalence of 25(OH)D concentrations <25 nmol/L was 7% (86/1329). Almost half (42%) of the children (556/1329) had 25(OH)D from 25 to <50 nmol/L, with 41% (541/1329) from 50 to <75 nmol/L and 11% (146/1329) had 25(OH)D ≥75 nmol/L. The highest prevalence of 25(OH)D <25 nmol/L was found in children of Pacific ethnicity (23%), or who had darker skin colouring (26%) or whose mothers did not have

secondary school qualifications (35%). None of the 80 (6%) children who drank toddler milk had 25(OH)D concentrations <25 nmol/L (Table 1).

Predictors of 25(OH)D <25 nmol/L

In a multivariable analysis which only included children who did not drink toddler milk, factors independently associated with the risk of 25(OH)D <25 nmol/L were female gender (OR = 1.92, 95% CI 1.17–3.14), other non-European ethnicities (not including Māori or Pacific) (3.51, 1.89–6.50) compared with NZ European, having olive-dark skin colour (4.52, 2.22–9.16), not taking vitamin D supplements (2.56, 1.06–6.18) and having a mother who did not have secondary school qualifications (5.00, 2.44–10.21). Analysed on a covariate basis, each quintile increase in deprivation index was associated with an increase in risk of vitamin D deficiency (1.27, 1.06–1.53). Factors not associated with an increased risk of vitamin D deficiency were age, BMI, attendance at daycare, consumption of low-fat milk fortified with vitamin D (not toddler milk) and latitude (Table 2).

Predictors of 25(OH)D <50 nmol/L

Independent risk factors associated with 25(OH)D concentrations <50 nmol/L differed to those for 25(OH)D <25 nmol/L. From multivariable analysis, independent predictors were female gender (OR = 1.55, 95% CI 1.24–1.95), Pacific (2.46, 1.25–4.84) and other non-NZ European ethnicities (2.21, 1.57–3.10), not attending a formal daycare centre (1.50, 1.08–2.07), not taking a vitamin D supplement or cod liver oil (2.05, 1.52–2.76), not drinking toddler milk (3.19, 1.85–5.48) and having mother who did not have secondary school qualifications (2.45, 1.34, 4.51) (Table 3).

Model for prediction of 25(OH)D <25 nmol/L

The mathematical equation to predict the risk of 25(OH)D <25 nmol/L was:

Total score = $0.59 \times \text{female} + 1.47 \times \text{Indian or African ethnicity} + 1.07 \times \text{Pacific ethnicity} + 1.26 \times \text{Maori or Asian ethnicity} + 1.28 \times \text{olive-dark skin colour} + 1.66 \times \text{mother with no secondary qualification}$.

Table 1. Vitamin D status

Variable	n (%)	Mean (S.D.*) 25(OH) D (nmol/ L)	25(OH)D categories (nmol/L) n (%)				P value Comparison of means ^a
			<25	25 to <50	50 to <75	>75	
Total	1329	52 (19)	86 (7)	556 (42)	541 (41)	146 (11)	—
Child demographics							
Age (years)							0.47
2 years old	496 (37)	52 (10)	30 (6)	223 (45)	183 (37)	60 (12)	
3 years old	448 (34)	53 (20)	25 (6)	180 (40)	194 (43)	49 (11)	
4 years old	385 (29)	51 (19)	31 (8)	153 (40)	164 (43)	37 (10)	
Gender							<0.001
Female	648 (49)	49 (18)	51 (8)	296 (46)	248 (38)	53 (8)	
Male	681 (51)	51 (20)	35 (5)	260 (38)	293 (43)	93 (14)	
Ethnicity							<0.001
NZ European	926 (70)	54 (19) ^a	32 (3)	378 (41)	401 (43)	115 (12)	
Māori	174 (13)	50 (20) ^a	16 (9)	74 (43)	66 (38)	18 (10)	
Pacific	44 (3)	40 (18) ^b	10 (23)	21 (48)	12 (27)	1 (2)	
Other non-European	185 (14)	46 (19) ^b	28 (15)	83 (45)	62 (34)	12 (7)	
Body mass index							
BMI, kg/m ² (n = 1315) ^b							0.08
Normal	1094 (83)	52 (19)	70 (6)	449 (41)	452 (41)	123 (11)	
Overweight	173 (13)	52 (19)	9 (5)	79 (46)	65 (38)	21 (12)	
Obese	48 (14)	46 (19)	7 (15)	22 (46)	16 (33)	3 (6)	
Sunlight exposure related variables							
Skin colour – parental report							<0.001
Very fair – fair	767 (58)	53 (18) ^a	27 (4)	339 (44)	313 (41)	88 (11)	
Medium	445 (33)	53 (20) ^a	29 (7)	172 (39)	193 (43)	51 (11)	
Olive – very dark	117 (9)	41 (20) ^b	30 (26)	45 (38)	35 (30)	7 (6)	
Attend formal daycare							0.01
Yes	1137 (86)	53 (19)	71 (6)	464 (41)	473 (42)	129 (11)	
No	192 (14)	49 (18)	15 (8)	92 (48)	68 (35)	17 (9)	
Hours attended formal daycare							0.27
1–10	257 (19)	54 (19)	12 (5)	110 (43)	100 (39)	35 (14)	
11–20	460 (35)	52 (21)	39 (9)	177 (38)	194 (42)	50 (11)	
21–30	234 (18)	54 (19)	9 (4)	96 (41)	102 (44)	27 (12)	
30+	186 (14)	51 (18)	11 (6)	81 (44)	77 (41)	17 (9)	
Dietary sources of vitamin D							
Vitamin D supplement use ^c							<0.001
Yes	253 (19)	58 (20)	6 (2)	78 (31)	131 (52)	38 (15)	
No	1076 (81)	51 (19)	80 (7)	478 (44)	410 (38)	108 (10)	
Drinks milk (n = 1250)							0.01
Non-fortified milk	1159 (93)	51 (19)	81 (7)	500 (43)	458 (40)	120 (10)	
Fortified low-fat milk	91 (7)	57 (18)	3 (3)	27 (30)	49 (54)	12 (13)	
Drinks toddler milk							<0.001
Yes	80 (6)	61 (16)	0 (0)	20 (25)	44 (55)	16 (20)	
No	1249 (94)	54 (19)	86 (7)	536 (43)	497 (40)	130 (10)	
Maternal and household characteristics							
Mother education qualifications							<0.001
No secondary	59 (4)	40 (24) ^e	20 (34)	23 (39)	13 (22)	3 (5)	
Secondary	198 (15)	51 (19) ^d	10 (5)	93 (47)	77 (39)	18 (9)	
Post secondary	1072 (81)	53 (19) ^d	56 (5)	440 (41)	451 (42)	125 (12)	

(Continues)

Variable	n (%)	Mean (S.D.*) 25(OH) D (nmol/ L)	25(OH)D categories (nmol/L) n (%)				<i>P</i> value
		Comparison of means ^a					
Household deprivation							0.21
Quintile 1	343 (26)	54 (19)	17 (5)	142 (42)	138 (40)	45 (13)	
Quintile 2	331 (25)	52 (19)	13 (4)	144 (44)	140 (42)	34 (10)	
Quintile 3	287 (22)	52 (19)	18 (6)	114 (40)	126 (44)	29 (10)	
Quintile 4	227 (17)	51 (20)	19 (8)	103 (45)	78 (34)	27 (12)	
Quintile 5	141 (11)	49 (19)	19 (13)	52 (37)	59 (42)	11 (8)	
Residential latitude							0.01
Northern: 35 to 39°S	651 (49)	53 (19) ^d	29 (4)	265 (41)	281 (41)	76 (12)	
Central: 40 to 42°S	378 (28)	50 (20) ^e	42 (11)	155 (41)	155 (41)	35 (9)	
Southern: 43 to 46°S	300 (23)	52 (19) ^d	15 (5)	136 (45)	136 (45)	35 (12)	

*Standard deviation. ^aComparison using independent t-test or ANOVA. ^bCole *et al.*, 2000. Normal BMI: <25 kg/m²; Overweight BMI: 25–<30 kg/m²; Obese BMI: >30 kg/m². ^cIncludes cod liver oil. ^{d,e}Values with different superscript letters indicate significant differences (Dunnets test, $P < 0.05$).

When added, total scores range from 0.0 to 6.0. The best balance of sensitivity and specificity were determined at a cut-off of ≥ 1.86 in the ‘development group’.

When applied to the ‘validation’ group ($n = 400$), the predictive tool was able to identify children at risk for 25(OH)D <25 nmol/L with a sensitivity of 42%, a specificity of 97% and an ROC area under the curve (AUC) of 0.76 (95%CI 0.67–0.86, $P < 0.001$). Positive and negative predictive values were calculated to be PPV 52% (95%CI 31–72%) and NPV 95% (95%CI 93–97%). (Supplementary Appendix Table 1).

Model for prediction of 25(OH)D <50 nmol/L

The mathematical equation to predict risk of 25(OH)D <50 nmol/L was:

Total score = $0.51 \times \text{female} + 1.58 \times \text{Indian or African ethnicity} + 0.76 \times \text{Pacific ethnicity} + 1.03 \times \text{Maori or Asian ethnicity} + 0.79 \times \text{not taking vitamin D supplement} + 0.92 \times \text{not drinking toddler milk} + 0.83 \times \text{mother with no secondary qualification}$. Total scores range from 0.0 to 5.63. The best balance of sensitivity and specificity were determined at a cut-off of ≥ 2.21 in the ‘development group’.

When this equation and cut-off were applied to the ‘validation’ group ($n = 400$), the ROC AUC was 0.63 (95%CI 0.57–0.68, $P < 0.001$) with a sensitivity of 52% and a specificity of 66%. Positive and negative

predictive values were calculated to be PPV 58% (95%CI 50–65%) and NPV 60% (95%CI 53–66%) (Supplementary Appendix Table 2).

Discussion

In this cross-sectional study of >1300 New Zealand preschool aged children in whom 25(OH)D was measured in late winter, the prevalence of 25(OH)D <25 nmol/L was 7% and for 25(OH)D <50 nmol/L was 49%.

Predictors of 25(OH)D concentration <25 nmol/L in children who did not drink toddler milk were female gender, being of non-European (not Māori or Pacific) ethnicity, darker skin colouring, not taking a vitamin D supplement, having a mother with less than secondary school education level and living in a more deprived household. None of the children who drank toddler milk were vitamin D deficient. Predictors of 25(OH)D concentration <50 nmol/L were female gender, being of Pacific and other non-European ethnicity, not attending a formal daycare centre, not taking a vitamin D supplement, not drinking toddler milk and having a mother with less than secondary school education level. Predictive tools which utilised these risk factors lack sufficient sensitivity to be used to identify children with low vitamin D status.

Table 2. Factors independently associated with dried blood spot 25 (OH)D concentration <25 nmol/L for children who do not drink toddler milk ($n = 1249$)

Variable	<i>n</i>	Association with 25(OH)D concentration <25 nmol/L	
		Multivariable odds ratio (95% confidence intervals)	<i>P</i> value
Gender			
Male	35	1.00	
Female	51	1.92 (1.17, 3.14)	0.01
Ethnicity			
NZ European	32	1.00	
Maori	16	1.56 (0.77, 3.17)	0.22
Pacific	10	2.53 (0.96, 6.68)	0.06
Other	28	3.51 (1.89, 6.50)	<0.001
Skin colour			
Fair – very fair	27	1.00	
Medium	29	1.37 (0.77, 2.44)	0.29
Olive –very dark	30	4.52 (2.22, 9.16)	<0.001
Vitamin D supplements			
Yes	6	1.00	
No	80	2.56 (1.06, 6.18)	0.04
Maternal education level			
Post secondary qualification	56	1.00	
Secondary qualification	10	0.73 (0.34, 1.52)	0.40
No secondary qualification	20	5.00 (2.44, 10.21)	<0.001
Household deprivation¹			
Change of one quintile from less to more deprived		1.27 (1.06, 1.53)	0.01

Children who drink toddler milk, sample of probability of 25(OH)D <25 nmol/L is 0/80, odds of deficiency = 0. ¹Deprivation index covariate.

This is the first large-scale study in New Zealand to measure the vitamin D status of preschool-aged children. The DBS mean 25(OH)D concentration (52 nmol/L) found in the current study is similar to those of other age groups of children in New Zealand. The winter mean serum 25(OH)D concentrations of infants and schoolchildren ranged from 39 to 47 nmol/L (Grant et al. 2009; Houghton et al. 2010; Rockell et al. 2005). However, the prevalence of <25 nmol/L in the current study (7%) is lower than that seen in these previous studies of infants (10–16% with 25[OH]D <27.5 nmol/L) (Grant et al. 2009; Houghton et al. 2010) and higher than that reported for

Table 3. Factors independently associated with dried blood spot 25 (OH)D concentration <50 nmol/L

Variable	<i>n</i>	Association with 25(OH)D concentration <50 nmol/L	
		Multivariable odds ratio (95% confidence intervals)	<i>P</i> value
Gender			
Male	295	1.00	
Female	347	1.55 (1.24, 1.95)	<0.001
Ethnicity			
NZ European	410	1.00	
Maori	90	1.22 (0.87, 1.71)	0.24
Pacific	31	2.46 (1.25, 4.84)	0.01
Other	111	2.21 (1.57, 3.10)	<0.001
Daycare attendance			
Yes	535	1.00	
No	107	1.50 (1.08, 2.07)	0.02
Vitamin D supplements			
Yes	84	1.00	
No	558	2.05 (1.52, 2.76)	<0.001
Toddler milk			
Yes	20	1.00	
No	622	3.19 (1.85, 5.48)	<0.001
Maternal education level			
Post secondary qualification	496	1.00	
Secondary qualification	103	1.19 (0.87, 1.63)	0.28
No secondary qualification	43	2.45 (1.34, 4.51)	0.01

schoolchildren (4% with 25[OH]D <17.5 nmol/L) (Rockell et al. 2005). Measurement of vitamin D concentration in these studies was completed throughout the year compared with the winter–spring sampling done in the present study.

The risk factors for low vitamin D status in the preschool age group are consistent with those found in previous studies of New Zealand children; female gender (Rockell et al. 2005), non-European ethnicity (Grant et al. 2009; Houghton et al. 2010; Ministry of Health 2012; Rockell et al. 2005), not taking supplements containing vitamin D (Grant et al. 2009), not drinking infant formula or toddler milk (Grant et al. 2009), low maternal education level (Houghton et al. 2010) and lower socioeconomic status (Grant et al. 2009). Skin colour was not a risk factor for vitamin D deficiency

in New Zealand infants or in school children (Grant et al. 2009; Houghton et al. 2010; Rockell et al. 2005). This is in contrast to the finding of this current study where those children with darker skin colouring were at increased risk of vitamin D deficiency.

We identified an association between consumption of toddler milk and vitamin D status in these preschool aged children. The proportion drinking this milk (6%) may be reflective of the study sample being of higher socio-economic status. Our study was unable to determine if the absence of 25(OH)D concentration < 25 nmol/L among those drinking toddler milk was because of the consumption of this milk or because of the other characteristics of toddler milk drinkers that were protective against 25(OH)D concentrations < 25 nmol/L. A limitation of this study is the omission of specific toddler milk products in the list of milks consumed; thus, some caregiver responses in the free-text box may have been missed. Future studies of this age group should include toddler milk as a specific dietary consumption product. Currently, there is no mandatory fortification of milk in New Zealand and voluntary vitamin D fortification of food products is limited. Future studies should explore the relationship between consumption patterns of toddler milk in the preschool population and 25(OH)D concentrations as this, alongside mandatory vitamin D fortification of milk, may be potential strategies for preventing vitamin D deficiency in this age group in this country.

A second unexpected finding of this study was that the 25(OH)D concentration was higher in those children who attended formal daycare than those who did not (53 vs 49 nmol/L, $P = 0.01$). With strict sun protection policies in formal preschool establishments, we hypothesised that hours of attendance of formal daycare would be inversely associated with 25(OH)D concentrations. It may be that those children who do not attend childcare have more indoor lifestyles or caregivers who are more vigilant in sun protection, with both of these factors having a negative impact on vitamin D status. No association was found between the number of hours of daycare attendance by the children and mean 25(OH)D concentrations. This lack of association may be because of variation in adherence to the sun protection policies between centres and between different centre staff.

No latitudinal gradient was evident in the multivariable adjusted comparison of dried blood spot 25(OH)D concentrations from samples obtained in the northern versus southern regions of New Zealand, a finding that was consistent with that observed in the national surveys for children and adults (Ministry of Health 2003; Ministry of Health 2012). This lack of association of vitamin D status with latitude in New Zealand has positive implications for policymakers, as it helps to simplify any future public health recommendations being made on a nationwide basis.

No New Zealand studies of infants or school children have reported the prevalence of 25(OH)D < 50 nmol/L to date. The Institute of Medicine and New Zealand guidelines recommend an annual average 25(OH)D concentration 50 nmol/L or higher for bone health (Diamond et al. 2005; Institute of Medicine (US) 2010; Ministry of Health and Cancer Society of New Zealand 2012). The implication of low vitamin D status during winter is unknown, and the definition of optimal levels, in terms of human health as well as disease prevention, continues to be debated (Holick 2009; Heaney & Holick 2011). There is currently no vitamin D preparation suitable for young children in New Zealand, which raises questions as to how best to increase 25(OH)D concentrations in children who have inadequate vitamin D status. These are important issues for further research and policy development.

To our knowledge, no prediction tool has been developed for assessment of vitamin D status in preschool aged children. Comparison with previously published predictive tools is complicated by the differing definitions used. No other study has developed a prediction tool for 25(OH)D < 25 nmol/L, with the closest a study of older Dutch adults using 25(OH)D < 30 nmol/L (Sohl et al. 2014). The performance of the questionnaire developed in the current study is inferior in terms of AUC (0.76 vs 0.80, respectively) and sensitivity (42% vs 61%), but superior in terms of specificity (97% vs 84%). The lower performance may be because of diverse range of ethnicities in the current study compared with the homogeneous group of Dutch adults, the lack of variables measuring sun exposure and the different 25(OH)D cut-off points used. There was also a lower prevalence of vitamin D deficiency in the preschool population (7%) compared with that of the

Dutch adults (17%). Despite our best efforts, and data from a sample of 1329 participants, we cannot accurately predict those at risk of 25(OH)D concentrations <25 nmol/L. To obtain this information a simple, safe and inexpensive blood test for 25(OH)D is needed for this age group. Currently in New Zealand health professionals/clinicians are discouraged from requesting blood tests for vitamin D, so this should be a priority for health policy.

The performance of the tool predicting 25(OH)D <50 nmol/L in the current study as measured by AUC (AUC 0.63) was lower than other questionnaires developed in studies of children and adolescents in Great Britain (Absoud et al. 2011), and older adults in Brazil (Lopes et al. 2014), the USA (Nabak et al. 2013) and The Netherlands (Sohl et al. 2014). Despite 25(OH)D concentrations <50 nmol/L being prevalent in the current study sample, the diagnostic performance of the developed predictive questionnaire is relatively poor. Conducting year-round testing and measurement of sun exposure in preschool children (e.g. physical activity, screen time, UV readings) has the potential to improve the performance of these predictive tools. Development of tools that are able to more accurately identify low vitamin D status would be very helpful to clinicians and public health practitioners.

The participation of 1329 children in a short 10-week recruitment/testing period was facilitated by the use of dried blood spots to measure 25(OH)D concentrations. Overall, we found parents were accepting of their child undergoing a finger prick test to obtain blood with the vast majority of children experiencing minimal trauma during testing. This reflects the positive experience of primary school subjects in a pilot study of dried blood spot testing in London (Griffiths et al. 2014). There may be limitations in comparing the 25(OH)D values obtained here from dried blood spots with previous studies of serum samples using a variety of assay techniques in other laboratories. In this study, the 25(OH)D in the dried blood spot was extracted using a methodology validated for paediatric population and analysed using the LC-MS/MS detection method (Eyles et al. 2009).

An advantage of this study was that the participants were free-living healthy children. The limitations of this study include the cross-sectional nature, which can

report associations but limits causal inferences, and the measurement of nadir vitamin D levels in late winter/early spring so the effect of seasonality was not explored. Our recruitment strategy, which was based upon self-selection and required access to a computer, will have introduced bias and limited the generalisability of our findings to the entire preschool-age population. Compared with national statistics, the study group had higher overall maternal education levels (Statistics New Zealand 2013), subjects lived in more affluent households (Salmond et al. 2007) and had lower BMI levels (Ministry of Health 2015), all of which have been associated with better vitamin D status (Houghton et al. 2010; Ministry of Health 2012). This suggests vitamin D status in the entire preschool-age population may be poorer than that observed in this study.

In conclusion, 25(OH)D concentrations <25 nmol/L during late winter were present in 7% of this cohort of preschool children, while 49% had 25(OH)D concentrations below 50 nmol/L. Predictors of 25(OH)D <25 nmol/L were consistent with those from previous New Zealand studies of children, while a new predictor identified of 25(OH)D <50 nmol/L was not attending a formal daycare centre. Despite having a large sample size, the performance of the predictive tools precludes use in clinical settings. The determination of 25(OH)D concentrations that are optimal for health and of affordable and acceptable methods to measure 25(OH)D concentration should remain research priorities.

Acknowledgment

We would like to thank the 1329 participants and their families, Dr Annie Judkins and Claudine Harvey (Newton Health Services), Waitemata DHB nurses, preschool nurses from Te Puna Haora and Jenny McKenzie (University of Otago) for their assistance in testing the children, study office and testers Cherie Wong, Peter Cairncross and Sherina Holland (Massey University), and Dr Daniel Exeter and Dr Jinfeng Zhao (University of Auckland) for their geocode and deprivation index work. We particularly wish to acknowledge the generosity of the pharmacists and pharmacy staff; without their donation of time, resources and goodwill this study could not have taken place.

Source of funding

Funding for the study was provided by the Health Research Council of New Zealand, who had no influence over the design, collection, analysis and interpretation of the data, writing of the manuscript or decision to submit the manuscript for publication.

Conflict of interests

The authors declare that they have no conflict of interest.

Contributions

CTC obtained ethics approval, project leader, recruited pharmacies and participants, designed testing protocols with advice from DE, carried out fingerprick tests, did statistical analysis, drafted the manuscript. BMCD supervised statistical analysis. LH recruited pharmacies and tested children. CC tested children. DE analysed dried blood spots for 25(OH)D concentration. PvH principal investigator, conceived research project and obtained funding.

All authors were involved in the design of the questionnaire. All authors were involved in revising the manuscript and all read and approved the final manuscript.

Abbreviations: BMI, body mass index; LC-MS/MS, liquid chromatography with tandem mass-spectrometer; DBS, dried blood spot; 25 (OH)D, 25-hydroxyvitamin D

References

- Absoud M., Cummins C., Lim M.J., Wassmer E. & Shaw N. (2011) Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. *PLoS One* **6**, e22179.
- Bain B. (2011) *Dacie and Lewis Practical Haematology*. Churchill Livingstone: London.
- Blok B.H., Grant C.C., Mcneil A.R. & Reid I.R. (2000) Characteristics of children with florid vitamin D deficient rickets in the Auckland region in 1998. *New Zealand Medical Journal* **113**, 374–6.
- Bolek-Berquist J., Elliott M.E., Gangnon R.E., Gemar D., Engelke J., Lawrence S.J. *et al.* (2009) Use of a questionnaire to assess vitamin D status in young adults. *Public Health Nutrition* **12**, 236–243.
- Bolland M.J., Grey A., Davidson J.S., Cundy T. & Reid I.R. (2012) Should measurement of vitamin D and treatment of vitamin D insufficiency be routine in New Zealand? *New Zealand Medical Journal* **125** (1349), 83–91.
- Camargo C.A., Ingham T., Wickens K., Thadhani R.I., Silvers K.M., Epton M. *et al.* (2010) Vitamin D status of newborns in New Zealand. *British Journal of Nutrition* **104**, 1051–1057.
- Cole T.J., Bellizzi M.C., Flegal K.M. & Dietz W.H. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* **320**, 1240–1245.
- Diamond T.H., Eisman J.A., Mason R.S., Nowson C.A., Pasco J.A., Sambrook P.N. *et al.* (2005) Vitamin D and adult bone health in Australia and New Zealand: a position statement. *Medical Journal of Australia* **182**, 281–285.
- Eyles D., Anderson C., Ko P., Jones A., Thomas A., Burne T. *et al.* (2009) A sensitive LC/MS/MS assay of 25OH vitamin D3 and 25OH vitamin D2 in dried blood spots. *Clinica Chimica Acta* **403**, 145–151.
- Fradet C., Mcgrath P.J., Kay J., Adams S. & Luke B. (1990) A prospective survey of reactions to blood tests by children and adolescents. *Pain* **40**, 53–60.
- Goksugur S.B., Erdurmus M., Bekdas M., Erkocoglu M., Agca S., Tosun M. *et al.* (2015) Tear and serum vitamin D levels in children with allergic rhinoconjunctivitis. *Allergologia et Immunopathologia*. DOI: 10.1016/j.aller.2014.10.004.
- Grant C.C., Wall C.R., Crengle S. & Scragg R. (2009) Vitamin D deficiency in early childhood: prevalent in the sunny South Pacific. *Public Health Nutrition* **12**, 1893–1901.
- Griffiths C.J., Wood H., Cross L. & Smithers H. (2014) Feasibility and acceptability of assessing vitamin D status in primaryschool children using a finger-prick dried capillary blood spot method. *Vitamin D and Human Health: From the Gamete to the Grave*. London, UK.
- Heaney R.P. & Holick M.F. (2011) Why the IOM recommendations for vitamin D are deficient. *Journal of Bone and Mineral Research* **26**, 455–457.
- Holick M.F. (2009) Vitamin D status: measurement, interpretation, and clinical application. *Annals of Epidemiology* **19**, 73–78.
- Houghton L.A., Szymlek-Gay E.A., Gray A.R., Ferguson E.L., Deng X. & Heath A.-L.M. (2010) Predictors of vitamin D status and its association with parathyroid hormone in young New Zealand children. *American Journal of Clinical Nutrition* **92**, 69–76.
- Institute of Medicine (US) (2010) *Dietary Reference Intakes for Calcium and Vitamin D*. The National Academies Press: Washington DC.
- Lopes J.B., Fernandes G.H., Takayama L., Figueiredo C.P. & Pereira R.M.R. (2014) A predictive model of vitamin D insufficiency in older community people: from the São Paulo Aging & Health Study (SPA). *Maturitas* **78**, 335–340.

- McNally D.J., Matheson L.A., Sankaran K. & Rosenberg A.M. (2008) Capillary blood sampling as an alternative to venipuncture in the assessment of serum 25 hydroxyvitamin D levels. *Journal of Steroid Biochemistry and Molecular Biology* **112**, 164–168.
- McNally J.D., Leis K., Matheson L.A., Karuananyake C., Sankaran K. & Rosenberg A.M. (2009) Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediatric Pulmonology* **44**, 981–988.
- Ministry of Health (2003) *NZ Food NZ Children: Key Results of the 2002 National Children's Nutrition Survey*. Ministry of Health: Wellington.
- Ministry of Health (2008) *Presenting Ethnicity: Comparing Prioritised and Total Response Ethnicity in Descriptive Analyses of New Zealand Health Monitor Surveys*. Ministry of Health: Wellington.
- Ministry of Health (2012) *Vitamin D Status of New Zealand Adults: Findings from the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.
- Ministry of Health (2015) *Understanding Excess Body Weight: New Zealand Health Survey*. Ministry of Health: Wellington.
- Ministry of Health and Cancer Society of New Zealand (2012) *Consensus Statement on Vitamin D and Sun Exposure in New Zealand*. Ministry of Health: Wellington.
- Nabak A.C., Johnson R.E., Keuler N.S. & Hansen K.E. (2013) Can a questionnaire predict vitamin D status in postmenopausal women? *Public Health Nutrition* **17**, 739–746.
- Newman M.S., Brandon T.R., Groves M.N., Gregory W.L., Kapur S. & Zava D.T. (2009) A liquid chromatography/tandem mass spectrometry method for determination of 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 in dried blood spots: a potential adjunct to diabetes and cardiometabolic risk screening. *Journal of Diabetes Science and Technology* **3**, 156–162.
- Reachme. (2014). ReachMe [Online]. Auckland. Available: <http://www.reachme.co.nz/> [Accessed 15/8/2014].
- Rockell J., Green T.J., Skeaff C.M., Whiting S.J., Taylor R.W., Williams S.M. *et al.* (2005) Season and ethnicity are determinants of serum 25-hydroxyvitamin D concentrations in New Zealand children aged 5–14 y. *Journal of Nutrition* **135**, 2602–2608.
- Rockell J., Skeaff C., Williams S. & Green T. (2006) Serum 25-hydroxyvitamin D concentrations of New Zealanders aged 15 years and older. *Osteoporosis International* **17**, 1382–1389.
- Salmond C.E., Crampton P. & Atkinson J. (2007) *NZDep2006 Index of Deprivation*. Department of Public Health, University of Otago: Wellington.
- Shaddy R.E. & Denne S.C. (2010) Guidelines for the ethical conduct of studies to evaluate drugs in pediatric populations. *Pediatrics* **125**, 850–860.
- Sohl E., Heymans M.W., De Jongh R.T., Den Heijer M., Visser M., Merlijn T. *et al.* (2014) Prediction of vitamin D deficiency by simple patient characteristics. *American Journal of Clinical Nutrition* **99**, 1089–1095.
- Statistics New Zealand. (2013). 2013 Census [Online]. Wellington. Available: <http://www.stats.govt.nz/Census/2013-census.aspx> [Accessed 27/07/2014].
- Uysalol M., Mutlu L.C., Saracoglu G.V., Karasu E., Guzel S., Kayaoglu S. *et al.* (2013) Childhood asthma and vitamin D deficiency in Turkey: is there cause and effect relationship between them? *Italian Journal of Pediatrics* **39**, 78–86.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table 1. Internal validation: performance of model equation in identifying preschool children with 25 (OH)D <25 nmol/L – ‘validation’ dataset ($n = 400$)

Table 2. Internal validation: performance of model equation in identifying preschool children with 25 (OH)D <50 nmol/L – ‘validation’ subset ($n = 400$)

Figure 1. Distribution of 25(OH)D scores