Environmental and individual predictors of 25-hydroxyvitamin D concentrations in Denmark measured from neonatal dried blood spots: the D-tect study

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Abstract

Environmental factors such as sunshine hours, temperature and UV radiation (UVR) are known to influence seasonal fluctuations in vitamin D concentrations. However, currently there is poor understanding regarding the environmental factors or individual characteristics that best predict neonatal 25-hydroxyvitamin D (25(OH)D) concentrations. The aims of this study were to (1) identify environmental and individual determinants of 25(OH)D concentrations in newborns and (2) investigate whether environmental factors and individual characteristics could be used as proxy measures for neonatal 25(OH)D concentrations. 25-Hydroxyvitamin D_3 (25(OH)D_3) was measured from neonatal dried blood spots (DBS) of 1182 individuals born between 1993 and 2002. Monthly aggregated data on daily number of sunshine hours, temperature and UVR, available from 1993, were retrieved from the Danish Meteorological Institute. The individual predictors were obtained from the Danish National Birth register, and Statistics Denmark. The optimal model to predict 25(OH)D_3 concentrations from neonatal DBS was the one including the following variables: UVR, temperature, maternal education, maternal smoking during pregnancy, gestational age at birth and parity. This model explained 30 % of the variation of 25(OH)D_3 in the neonatal DBS. Ambient UVR in the month before the birth month was the best single-item predictor of neonatal 25(OH)D_3, accounting for 24 % of its variance. Although this prediction model cannot substitute for actual blood measurements, it might prove useful in cohort studies ranking individuals in groups according to 25(OH)D_3 status.

Key words: Vitamin D: UV radiation: Dried blood spots: Neonates

The most well-known source of vitamin D is through skin synthesis induced by sun exposure. Studies from Northern Europe have highlighted a marked seasonal fluctuation in serum 25-hydroxyvitamin D (25(OH)D) concentrations among adults and newborns, with values being lowest in late winter and spring, because of less UV and skin-mediated vitamin D synthesis during these seasons[1].

During gestation, the vitamin D stores of the fetus depend entirely on those of its mother[2], and newborns' 25(OH)D concentrations are correlated with their mothers'[3]. Vitamin D insufficiency (25(OH)D <50 nmol/l) and deficiency (25(OH)D <25 nmol/l) are common among otherwise healthy pregnant women, especially in high-latitude countries such as Denmark (56° North) where UV radiation (UVR) from the sun (and thus vitamin D synthesis) is insufficient to produce vitamin D from October to March[4–7]. It has been estimated that 16–31 % of Danish women suffer from vitamin D insufficiency in early pregnancy, of which 3·5–6·3 % are vitamin D deficient[3,8–10]. Vitamin D insufficiency and deficiency during pregnancy have been linked to many pregnancy-related health complications and may also impair offspring health[11]. The most evident poor health outcome related to low vitamin D during fetal

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development and early life is the development of infancy and childhood rickets[12]. Apart from its role in Ca and phosphate homoeostasis and bone mineralisation[13], vitamin D also has extra-skeletal functions such as regulation of hormone secretion, immune function and cellular proliferation and differentiation[14,15]. Exposure to low vitamin D *in utero* may therefore predispose to a range of adverse health outcomes throughout life[16].

The use of a proxy model may be of value when vitamin D measurements are lacking and thus need to be estimated. Environmental factors such as sunshine hours, temperature and UVR might explain seasonal fluctuations in vitamin D concentrations, but few studies have investigated the influence of these environmental factors during pregnancy on neonatal vitamin D concentrations. Most of these studies concerned season of birth in relation to cord blood measures of vitamin D[17,18]. In addition, some studies have used self-reported maternal intake of 25(OH)D or UVR exposure during pregnancy[19–21] as well as season of birth as proxies of maternal or offspring 25(OH)D status in relation to the development of diseases in infancy and adulthood[22–24]. However, currently there is poor understanding regarding the environmental factors or individual (maternal and offspring) characteristics that best contribute to determine neonatal 25(OH)D concentrations. Previous studies describing the determinants of neonatal 25(OH)D were based on either relative small samples <300 individuals or used cord blood 25(OH)D concentration[21,25–30]. Only one previous study used 25(OH)D from dried blood spots (DBS) obtained from 259 children[25]. Hence, there is a need for large cohort studies based on representative samples of the population using objective measurements of neonatal 25(OH)D. Furthermore, no previous studies have included measured bright sunshine hours and temperature as environmental proxies of 25(OH)D status among neonates. In Denmark, a relative low temperature might further prevent individuals from exposing their skin to the sun even when there is a high UV index and bright sunshine. Therefore, we hypothesised that UVR would be the main predictor of the neonatal 25-hydroxyvitamin D_3 (25(OH)D_3) variation and that temperature would be an additional important environmental determinant. We hypothesised that bright sunshine hours would be highly correlated to UVR and therefore be a minor additional environmental predictor. We also hypothesised that adding individual maternal and offspring characteristics would further improve the proxy model.

Therefore, using DBS from a large sample of Danish newborns, the aims of this study were to (1) identify environmental and individual determinants of 25(OH)D concentrations in newborns and (2) investigate whether these environmental factors and individual characteristics combined could be used as proxy measures for neonatal 25(OH)D concentrations.

Methods

25(OH)D_3 was measured from neonatal DBS from a random sub-sample of the Danish population of 2873 individuals, born between 1 May 1981 and 31 December 2002, retrieved from the Biological Specimen Bank for Neonatal Screening (BSBNS)-

Statens Serum Institute (SSI) in Denmark. Inclusion criteria were children born in Denmark, being alive 1 week after birth, and who had sufficient biological material from the DBS for the vitamin D analyses. Individuals were identified through the Danish Civil Registration system. No restriction on type of birth (multiple births) or gestational age was applied.

As part of a national routine screening programme for congenital disorders among neonates in Denmark, a heel prick is taken within 5–7 d after birth (until 2009 and within 48–72 h after birth thereafter). The blood samples were collected on filter paper and subsequently stored at –20°C in a locked freezer at SSI[31]. To assess the 25(OH)D status, one 3·2 mm punch was taken halfway from the centre of the blood spot. A modified version of Eyles and colleagues' method[32] of the highly sensitive liquid chromatography tandem MS method (LC-MS/MS) was used to measure the concentration of 25(OH)D. It has previously been reported that 25(OH)D measurements from archived DBS are highly correlated with cord blood among neonates as well as with sera or plasma in adults[33,34]. Sample degradation occurring during 13–34 years storage time is unlikely, as studies have shown that, regardless of temperature and light exposure, storage times of 25(OH)D for up to 40 years do not bias inter-individual variation in concentrations for a given birth cohort[32,35]. Natural dietary sources of 25(OH)D_2 are scarce and, in Denmark, vitamin D supplements come in the form of 25(OH)D_3. Accordingly, more than 90 % of 25(OH)D_2 measured in the DBS were below the detection limit of 3 nmol/l and were therefore excluded from these analyses. All measured concentration levels for intra-assay and inter-assay analyses had acceptable precision, as the variability coefficient for intra-assay and inter-assay variation for 25(OH)D_3 ranged 7–12 and 7–20 %, respectively. There was no exclusion of the results based on the detection limit for 25(OH)D_3, which was 4 nmol/l. The concentration of 25(OH)D_3 is reported in nmol/l and the following formula, which takes into account the neonatal haematocrit fraction for capillary blood, was used to approximate the sera values: serum (25(OH)D_3) nmol/l = DBS (25(OH)D_3) nmol/l × 1/(1 − 0·61 (the Hct fraction))[32,36]. The SSI laboratory participates in the vitamin D external quality assessment scheme with the equivalent-serum method[37].

Monthly aggregated data on daily number of bright sunshine hours recorded by Campbell–Stoke sunshine recorders[38], average temperature recorded from thermometers placed 2 m above ground level in a ventilated screen or in a thermometer cabin[39], and UVR recorded from Brewer spectrophotometer[40], available since 1993, were retrieved from the Danish Meteorological Institute[39]. The standard erythemal dose (SED, J/m²) was used as the UVR unit. SED is a weighted measure of ambient UVR using an erythemal response function to give biologically effective UVR and is independent of skin tone[41]. Temperature was measured in °C.

Maternal characteristics age at delivery, education level, parity and smoking status during pregnancy; and offspring characteristics birth date, birth weight, gestational age at birth and sex were obtained from the Danish Medical Birth Register (DMBR)[42] via Statistics Denmark.

From 1991, binary (smokers or non-smokers) categorisation of maternal smoking status during pregnancy was started from

manually collected data. Electronic data collection started in 1997 and further categories were added as follows: unknown, mother not smoking, mother smokes, mother stopped smoking in the first trimester, mother stopped smoking after the first trimester, mother smoked up to 5, 6–10, 11–20 or >20 cigarettes/d. Combining this information, maternal smoking was dichotomised into ever smoking (women who ever smoked during their pregnancy) v. never smoking (women who did not smoke during their pregnancy) or unknown. Ethnicity was defined based on the mother's place of birth and was dichotomised into European and non-European origin. Hence, children with fathers with non-European origin were considered European if mothers were of European origin. Maternal education was categorised into school, high school and university as the highest education achievement level. Parity was dichotomised into primiparous and multiparous. Estimation of parity was based on summarised estimates of total births and the actual birth, as the DMBR only included information on live and still births before 1996. Maternal age at time of delivery was categorised as follows: <20, 20–24·9, 25–29·9, 30–34·9, 35–39·9 and >40. Season of birth was defined based on the seasonal variation in serum 25(OH)D concentration among individuals from countries in northern latitudes[43,44]: winter – born between November and January; spring – born between February and April; summer – born between May and July; autumn – born between August and October. Year of birth included 1993–2002. Gestational age at birth was based on gestational weeks (37–44 weeks). Birth weight in grams was used and categorised as follows: <2500, 2500–4000 and >4000.

Ethics

The Danish National Committee on Biomedical Research Ethics and Steering committee of the BSBNS granted permissions to access and analyse the DBS samples from the BSBNS (J. no. H-3-2011-126). Permission from the Danish Data Protection Agency was granted to merge biobank information with the other individual-level information from the Danish Administrative registers. (J. no. 2012-41-116).

Statistical methods

Forward selection of covariates based on the mean squared error (MSE) and subsequent cross-validation (CV) was used to determine the best predictive model based on the available data. Hence, each covariate was included by forward selection and at each selection step the covariate giving the highest reduction in the MSE of the previous model was included. The models resulting from the forward selection process were compared by estimating their out-of-sample performance using ten rounds of 5-fold CV for each model, and the model with the lowest CV error was chosen as the final model[45].

The base model was a linear mixed model, with partition of the residual variance into a between-group component (GC) and a within-GC, with individuals grouped by year of birth and in the month before the month of birth. UVR in the month before the month of birth was used in this study as the half-life of 25(OH)D during pregnancy is around 21 d[46]. To allow for a possible non-linear relationship between SED and 25(OH)D$_3$,

the forward selection algorithm had both a linear model and a restricted cubic spline model, with a single knot at the median value of UVR (210 SED) among the candidate models.

As 25(OH)D$_3$ standard deviations increased proportionally with the mean (online Supplementary Fig. S1a and b), a generalised linear model with γ distribution (where standard deviations are proportional to the mean) was used as sensitivity analysis.

We used R version 3.5.0[47]. To generate the descriptive tables, we used the 'Hmisc' package[48]; and to fit the mixed models, we used the 'lme4' package[49].

Results

UVR data were available from February 1993 only, therefore the sample was reduced from 2873 individuals to 1387 individuals born between February 1993 and December 2002. Individuals born preterm (<37 gestational weeks) were further excluded as preterm births might be due to underlying causes that may influence maternal and fetal pregnancy outcomes and vitamin D concentrations (n 50) (Fig. 1). In addition, no seasonal pattern could be seen among neonates of non-European origin (defined according to maternal place of birth in the DMBR), therefore they were excluded from the analyses (n 139) (Fig. 1). After further exclusion of sixteen individuals with missing information on some of the covariates, a total of 1182 individuals were included in the analyses (Fig. 1). The mean 25(OH)D$_3$ concentration was 30·1 (SD 19·8) nmol/l and median 25·6 (range 0·5–110) nmol/l when corrected for the haematocrit fraction. Maternal and offspring characteristics are presented in Table 1.

Vitamin D concentrations were higher among individuals born in summer and autumn and lower in winter and spring months $P < 0.001$ (Fig. 2). Month of birth explained 24% of neonatal 25(OH)D$_3$. The greatest correlation was between UVR and sunshine hours (r 0·93), followed by UVR and temperature (r 0·86) and sunshine hours and temperature (r 0·80).

Assessment of the fitted spline (Fig. 3) showed that association between UVR and 25(OH)D$_3$ was not linear and suggested a continuous increase in neonatal 25(OH)D$_3$ with increased UVR.

Based on the available data and according to re-sampling method for model selection, the preferred model to predict 25(OH)D$_3$ concentrations from neonatal DBS was the one including the group-level variables UVR (as a cubic spline) and temperature, together with the following individual-level variables: maternal education, maternal smoking during pregnancy, gestational age at birth and parity. This model explained 30% of the variation of 25(OH)D$_3$ in the neonatal DBS with ambient UVR in the month before the month of birth, accounting for 24·5% of the neonatal 25(OH)D$_3$ variance (Tables 2, 3 and Fig. 4).

The model was better at predicting between-group variation of neonatal 25(OH)D$_3$ compared with within-group variation (grouped by year of birth and the month before the month of birth). Group-level determinants, UVR and temperature, explained more than 60% of the between group-level variation (standard deviation basal model: 10·6 nmol/l to standard deviation prediction model (model 6): 4·3 nmol/l), while individual-level determinants (maternal education, maternal smoking during pregnancy, gestational age at birth and parity) explained <0·5%.
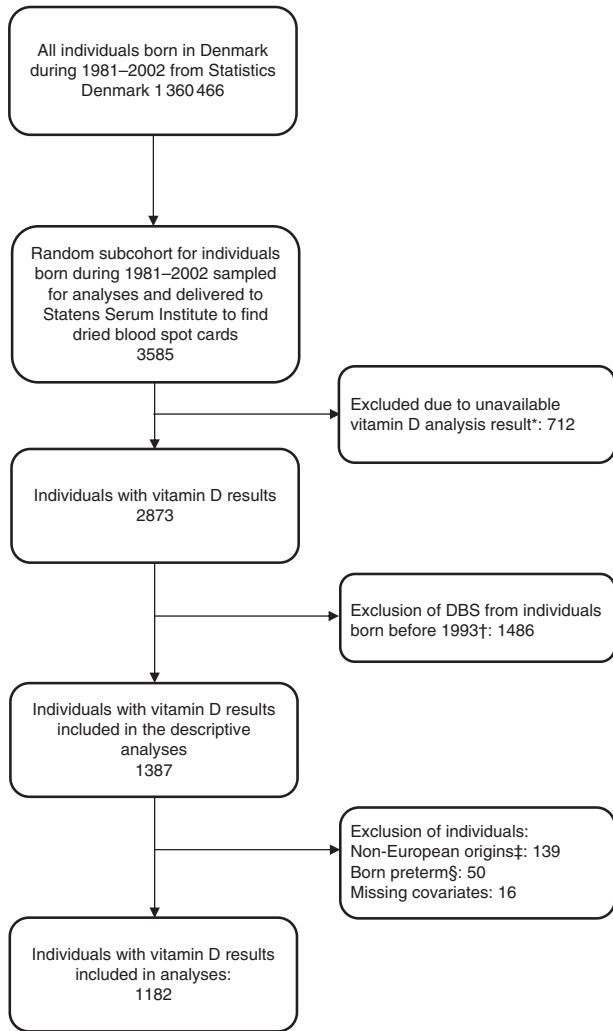
Fig. 1. Flow chart of study population. * Dried blood spot (DBS) cards were either not found, there was insufficient material for analysis or the analysis failed. † UV radiation data were available from February 1993 from the Danish Meteorological Institute. ‡ No seasonal pattern could be seen among offspring of non-European individuals. § Individuals born preterm (<37 gestational weeks) were excluded as preterm births might be due to underlying causes that may influence maternal and fetal pregnancy outcomes and vitamin D concentrations.

The within-group variation only reduced by 0·6 % when both group-level and individual-level determinants were included in the model (standard deviation basal model: 16·5 to standard deviation prediction model (model 6): 15·9 nmol/l) (Table 2). As 25(OH)D$_3$ standard deviations increased proportionally with the mean (online Supplementary Fig. S1a and b), this suggests that our prediction model might be better at predicting 25(OH)D$_3$ during winter and spring compared with summer and autumn. However, sensitivity analysis using generalised linear model with γ distribution showed similar results (data not shown).

Discussion

This study examined the environmental and individual predictors of 25(OH)D concentrations measured from neonatal DBS among a representative sample of neonates born in Denmark. Our findings showed as expected a seasonal pattern of vitamin D concentration, with higher values in summer and autumn and lower values in winter and spring. Ambient UVR in the month before the month of birth was the best predictor of neonatal 25(OH)D$_3$, accounting for 24 % of its variance. Neonatal 25(OH)D$_3$ concentration was further determined by other factors such as ambient temperature, maternal education, maternal smoking during pregnancy, gestational age at birth and parity. The final model explained 30 % of neonatal 25(OH)D$_3$ concentration from DBS. The model best predicted between-group (grouped by year of birth and in the month before the month of birth) variation of neonatal 25(OH)D$_3$ compared with within-group variation, and individual-level determinants explained only a small proportion of the between group-level variation. Our findings might be explained by the natural yearly and monthly variation in ambient UVR and temperature. Furthermore, our findings suggest that the individual-level determinants included in the model might only have a marginal influence on neonatal 25(OH)D$_3$ concentration. Our findings suggest that less than a third of neonatal 25(OH)D$_3$ concentration could be predicted by our model. Hence, this model might prove useful in cohort studies assessing the association between neonatal vitamin D and disease outcomes for ranking individuals in groups according to 25(OH)D$_3$ status. However, it cannot substitute actual blood measurements as a tool for evaluating individual vitamin D status, as only a proportion of the variation in 25(OH)D$_3$ could be appraised[20].

Previous prediction models of maternal or neonatal 25(OH)D concentrations found higher prediction than ours, explaining between 40[20] and 43 %[25] of the total 25(OH)D variance. This difference might relate to the different variables included in the prediction models, distinct study population characteristics, differences in sample sizes and potentially different analytical methods used.

The strengths of the present study include the large sample of individuals randomly selected among the entire Danish population and measurements of neonatal biological 25(OH)D$_3$ from DBS as well as the use of the high-quality register data from the DMBR. The use of the resampling method with MSE and CV guaranteed, to some degree, that the best model could be selected preventing overfitting. Furthermore, 25(OH)D$_3$ concentrations were measured using the validated modified version of Eyles and colleagues' method[32] of the highly sensitive LC–MS/MS.

The study also has limitations. Data on sunshine hours, temperature and UVR in the month before the month of birth obtained from the DMI were group-level determinants aggregated monthly; therefore, within-month variation including inter- and intra-individual exposure variations could not be assessed. Furthermore, pure UVB measurements, which are responsible for vitamin D skin synthesis, were only available from 2015. Therefore, it was only possible to include UVR in our model. However, as UVR measurements correlate very closely to vitamin D synthesis, it is not expected that the use of UVB instead of UVR would have greatly influenced our predictive model[50].

It has been reported that breastfed infants are at an increased risk of vitamin D deficiency compared with formula-fed infants[51]. As DBS were collected within 7 d after birth, the

Table 1. Maternal and offspring characteristics

Variables		(n 1232)	Mean 25(OH)D$_3$ (nmol/l)	SD	P
Offspring sex	Male	611	30·6	20·0	0·33
	Female	571	29·5	20·0	
Offspring month of birth	January	85	21·9	12·4	<0·001
	February	100	20·6	15·6	
	March	102	21·0	15·1	
	April	93	18·6	13·5	
	May	116	28·3	16·6	
	June	96	37·6	18·1	
	July	115	46·7	23·1	
	August	111	44·9	19·4	
	September	102	36·9	19·4	
	October	84	28·8	17·4	
	November	104	24·3	15·3	
	December	74	23·8	18·0	
Offspring year of birth	1993	97	30·2	20·9	<0·001
	1994	146	30·7	20·3	
	1995	136	34·2	19·8	
	1996	119	26·5	16·3	
	1997	124	25·6	17·8	
	1998	116	22·9	13·3	
	1999	124	30·0	19·3	
	2000	111	32·2	19·0	
	2001	119	36·5	24·1	
	2002	90	31·8	22·3	
Offspring birth weight (g)	<2500	19	32·9	23·4	0·78
	2500–3999	889	29·9	19·8	
	>4000	274	30·5	19·5	
Offspring gestational age at birth (weeks)	37	23	35·5	25·4	0·08
	38	85	28·2	16·0	
	39	202	31·1	20·3	
	40	310	31·9	20·5	
	41	305	30·1	20·6	
	42	187	27·0	17·4	
	43>	70	27·3	18·5	
Maternal age at time of delivery (years)	<25	170	24·6	18·0	<0·001
	25–29·9	450	31·4	20·2	
	30–34·9	393	32·0	20·5	
	>35	169	27·7	17·3	
Maternal education	School	248	24·7	17·9	<0·001
	High school	610	31·0	20·0	
	University	308	32·3	19·9	
	Unknown	16	31·9	22·7	
Maternal smoking during pregnancy	Yes	272	25·1	18·2	<0·001
	No	858	31·8	20·1	
	Unknown	52	27·6	18·5	
Parity	Primiparous	507	31·7	19·8	0·01
	Multiparous	675	28·8	19·7	

25(OH)D$_3$, 25-hydroxyvitamin D$_3$.

concentration of 25(OH)D$_3$ between breastfed and formula-fed infants could differ. In the present study, data about breast-feeding were not available; however, in Denmark <5 % of infants are not being breastfed at the time of hospital discharge[52]. Therefore, as our sample is representative of the Danish population, we expect similar rates in our cohort. Furthermore, supplementation with vitamin D is first recommended from 2 weeks of age[53]; therefore, 25(OH)D concentration from neonatal DBS is not expected to be affected by supplementation.

Vitamin D dietary intake from supplements and dairy products has previously been identified as a determinant of vitamin D status in pregnant women and neonates[28]; therefore, including these variables in the model would potentially have added to the explanation of a bigger proportion of the neonatal 25(OH)D variation. Furthermore,

maternal body weight has been identified as a determinant of vitamin D status in pregnant women and neonates in some[28] but not all studies[20] (pregnant women only). Therefore, it is unclear whether including this variable would have influenced our prediction model.

The influence on the prediction of factors such as outdoor activity, tanning beds use, sun exposure habits, skin colour, use of sunscreen and clothing habits known to influence maternal vitamin D skin synthesis could not be assessed in this study as such data were not available. Results from two studies from the UK have suggested that fair-skinned individuals need 9 min of daily sunlight at lunchtime from March to September, for 25(OH)D concentrations to remain >25 nmol/l throughout the winter and without causing erythema (sun burnt), while dark-skinned individuals need approximately 25 min[54,55]. In the study by Smith

et al.[25], maternal clothing and sun exposure habits explained 4 % of neonatal 25(OH)D variance; and in another study, the use of sunscreen and skin pigmentation were described as possible determinants of maternal 25(OH)D deficiency (defined as

<50 nmol/l)[21]. Therefore, these variables may be expected to explain a small proportion of neonatal 25(OH)D$_3$ concentration and inclusion in our prediction model would have strengthened our model.

A recent genome-wide association study meta-analysis of serum 25(OH)D among European individuals validated three 25(OH)D risk loci (GC, 7-dehydrocholesterol reductase (NADSYN1/DHCR7) and CYP2R1) previously identified, and identified two new loci (Sec23 homolog A, coat protein complex II component and amidohydrolase domain containing 1). The study reported that there was a modest overall heritability in serum 25(OH)D among European individuals due to the common genome-wide SNP of 7·5 %[56]. In the Danish population, the 25(OH)D risk loci in GC and 25-hydroxylase (CYP2R1) were associated with serum 25(OH)D concentrations[57]; and in Hungary, selected SNP (NADSYN1, DHCR7, GC, CYP2R1 and CYP24A1) accounted for 13·1 % of the
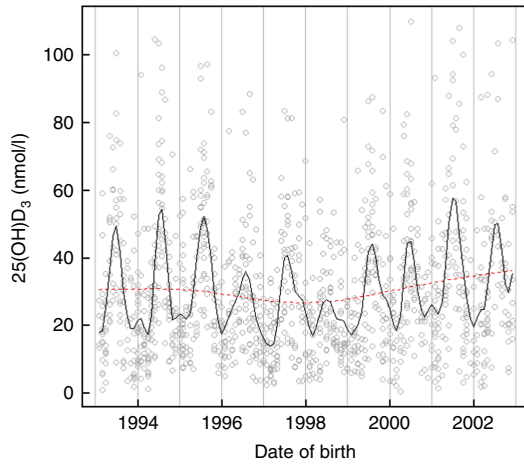

Fig. 2. Neonatal 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) variations by month and year of birth among a random sample of infants born between 1993 and 2002 in Denmark.
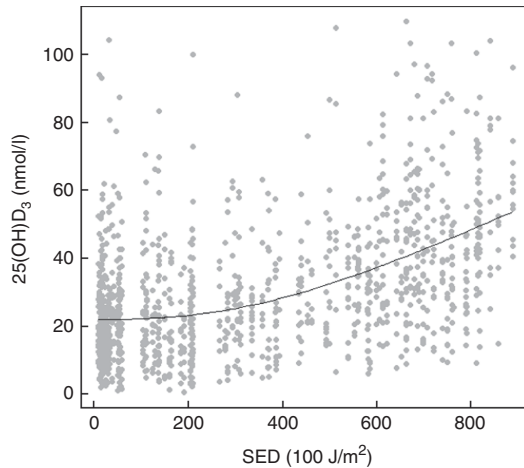

Fig. 3. UV radiation (standard erythemal dose (SED, J/m^2)) and neonatal 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) among a random sample of infants born between 1993 and 2002 in Denmark.

Table 3. Multivariable model of factors predicting 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) (nmol/l) concentration from neonatal dried blood spots

Variables		Estimate
Intercept		25·3
Maternal smoking during pregnancy	No	Ref.
	Yes	−4·7
	Unknown	−3·5
Maternal education	School	Ref.
	High school	5·6
	University	7·2
	Unknown	4·8
Parity	Primiparous	Ref.
	Multiparous	−2·9
Gestational age at birth (weeks)	37	Ref.
	38	−4·9
	39	−4·2
	40	−3·5
	41	−4·7
	42	−7·3
	43>	−8·8
Temperature residuals (°C)	Continuous	2·1*
UVR spline term 1 (SED)	Continuous	17·1†
UVR spline term 2 (SED)	Continuous	32·7†

Ref., referent values; UVR, UV radiation; SED, standard erythemal dose.
* Standardised to mean zero and standard deviation one: one standard deviation = 3·1°C.
† The spline basis was bases on SED values standardised to mean zero and standard deviation one. One standard deviation = 291·4 SED.

Table 2. Prediction model selection*

Model	df	MSE	CV error	Between-group SD	Within-group SD	R^2 (%)
Base	1179	390·7	391·4	10·6	16·5	0
1	1177	294·8	296·8	4·7	16·5	24·5
2	1174	285·7	289·4	4·7	16·2	26·8
3	1173	281·3	285·3	4·2	16·2	27·9
4	1171	277·9	282·4	4·4	16·1	28·8
5	1165	274·9	283·2	4·3	16·0	29·6
6	1164	273·0	281·2	4·3	15·9	30·1
7	1161	270·6	282·1	4·2	15·9	30·7
8	1159	270·3	283·2	4·3	15·9	30·8
9	1158	270·0	283·1	4·2	15·9	30·8

MSE, mean square error; CV, cross-validation; R^2, explained variation without conditioning on group level parameters.
* Base model by year of birth and in the month before the month of birth; model 1: model 0 + UVR; model 2: model 1 + education; model 3: model 2 + temperature; model 4: model 3 + maternal smoking during pregnancy; model 5: model 4 + gestational age at birth; model 6: model 5 + parity; model 7: model 6 + maternal age at delivery; model 8: model 7 + birth weight; model 9: model 8 + sex.
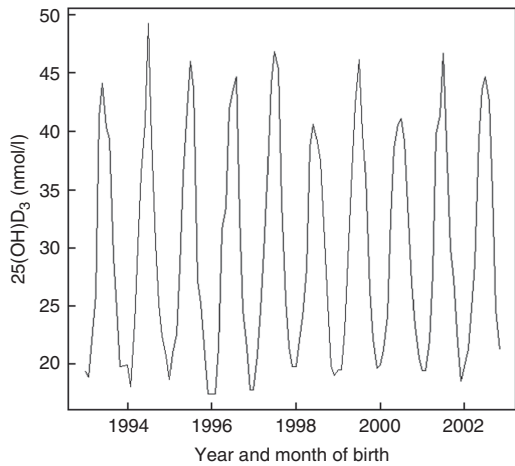
Fig. 4. Prediction of neonatal 25-hydroxyvitamin D_3 (25(OH)D_3) from the multilevel analysis based on a random sample of infants born between 1993 and 2002 in Denmark.

variance of total 25(OH)D levels[58]. In Australia, the study by Smith et al.[25] reported that 5·2 % of the predicted variance in neonatal 25(OH)D from DBS could be explained by infant genetic variants GC (rs2282679) and DHCR7 (rs12785878). Therefore, including information on genetic variance would also have most likely improved our model.

Information about maternal smoking status was missing for more than 40 % of individuals and reporting bias may have occurred. Therefore, the true effect of smoking on neonatal 25(OH)D concentration might have been underestimated. However, results from different studies suggest a non-significant inverse association between maternal smoking and maternal and neonatal 25(OH)D concentrations[21,28,59]. Therefore, maternal smoking during pregnancy might only marginally predict offspring 25(OH)D concentration at birth.

A high correlation ($r \geq 0.83$) between maternal and neonatal vitamin D concentrations has been previously reported, with maternal vitamin D concentration explaining between 69 and 77 % of the neonatal one[3,60]. Maternal 25(OH)D was not available in this study. However, the environmental factors and individual characteristics (UVR, temperature, parity, gestational age at birth, maternal education and maternal smoking during pregnancy) included in this study can be thought of as both maternal and offspring's proxies of 25(OH)D concentrations. As our model explained 30 % of neonatal 25(OH)D_3 concentration, determinants other than the ones included in our model must play a significant role in both maternal and neonatal 25(OH)D_3 concentrations.

Finally, the present results might only be generalised to populations living at high latitudes (56° North) and with fair skin.

Conclusion

Ambient UVR in the month before the month of birth was the best predictor of neonatal 25(OH)D_3, accounting for 24 % of its variance. Neonatal 25(OH)D_3 concentration was further determined by maternal and offspring characteristics, but the final model explained an additional 6 % of neonatal 25(OH)D_3 only,

leaving 70 % of the variation to be explained by other genetic and environmental factors and individual characteristics. Hence, although this model cannot substitute actual blood measurements, it might prove useful in cohort studies for ranking individuals in groups according to 25(OH)D_3 status.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518003604

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