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Concentration of 25-hydroxyvitamin D from neonatal dried blood spots and the relation to gestational age, birth weight and Ponderal Index: the D-tect study

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Abstract

Studies have suggested that vitamin D status at birth may be associated with a range of neonatal outcomes. The aim of this study was to assess the association between neonatal 25-hydroxyvitamin D_3 (25(OH)D_3) concentration and gestational age, birth weight, Ponderal Index and size for gestational age. Neonatal capillary blood stored as dried blood spots was used to assess 25(OH)D_3 concentrations among 2686 subjects selected from a random population sub-sample of individuals, born in Denmark from 1 May 1981 to 31 December 2002. There was an inverse association between 25(OH)D_3 concentration and gestational age at birth of −0·006 (95 % CI −0·009, −0·003, $P < 0·001$) weeks of gestation per 1 nmol/l increase in 25(OH)D_3 concentration. An inverted U-shaped association between 25(OH)D_3 and birth weight and Ponderal Index ($P = 0·04$) was found, but no association with size for gestational age was shown. This study suggests that neonatal 25(OH)D_3 concentration is associated with anthropometric measures at birth known to be correlated with many subsequent health outcomes such as obesity and type 2 diabetes.

Key words: Vitamin D: Dried blood spots: Anthropometry: Neonatal outcomes

Nutritional factors during pregnancy influence fetal growth and neonatal outcomes. Furthermore, neonatal outcomes such as birth weight, size for gestational age or Ponderal Index affect perinatal morbidity, persistent short stature and are associated with fat mass and risk of metabolic syndrome later in life[1–4].

The fetus relies entirely on maternal vitamin D stores, and neonatal plasma concentration of 25-hydroxyvitamin D (25(OH)D) corresponds to 60–70 % of maternal stores[5,6]. As vitamin D deficiency is relatively common among women of child-bearing age, the prevalence of vitamin D deficiency in newborns is also relatively high[7]. Studies have shown that women with higher vitamin D status tend to give birth to heavier and longer babies and may be less likely to give birth to small-for-gestational-age (SGA) infants[8,9]. On the other hand, two recent cohort studies did not find any association between umbilical cord blood or maternal 25(OH)D concentrations and neonatal anthropometric measures such as birth weight and Ponderal Index[10,11]. Other studies have shown that vitamin D deficiency in pregnancy was associated with an increased risk of macrosomia and postnatal overweight[12], as well as increased fat mass at ages 4 and 6 years[13], whereas a recent study did not find

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; DBS, dried blood spots; DMBR, Danish medical birth register; LGA, large for gestational age; SGA, small for gestational age.

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any association between 25(OH)D concentration at birth and the risk of overweight at the age of 7 years[14].

Recent reviews and meta-analyses on the association between 25(OH)D during pregnancy and neonatal outcomes highlight heterogeneity between studies and conflicting results[15,16]. In addition, most previous studies assessed 25(OH)D status in maternal blood during pregnancy, and a few have used measurements of cord blood 25(OH)D at delivery in relation to neonatal outcomes[6,17–21]. No previous study has used newborns' 25(OH)D measurements of capillary blood from dried blood spots (DBS) to study the relation between vitamin D stores and neonate anthropometric outcomes. We had the opportunity to access comprehensive registers in combination with systematically collected biological material from neonates, which offered the possibility to study such associations in Denmark.

The aim of this study was therefore to examine the associations between neonatal 25(OH)D_3 concentration measured from DBS and gestational age at birth, birth weight, Ponderal Index and size for gestational age among a large random sample of neonates born in Denmark.

Methods

Population

Using the Danish Personal Civil Registration (CPR) numbers, a random sample of 2865 individuals born in Denmark from 1 May 1981 to 31 December 2002 was selected from the Danish Civil Registration System. In addition, using the CPR numbers, individuals in the sub-cohort were linked to the Biological Specimen Bank for Neonatal Screening at Statens Serum Institut (SSI) for retrieval of DBS.

The initial criteria for being included in the study were that the individuals were required to be born in Denmark, and have accessible and sufficient biological material available from the DBS for the vitamin D analyses. Children from multiple pregnancies were included.

A flow chart of the selection of the included subjects is presented in Fig. 1.

Neonatal vitamin D measures

In Denmark, every newborn undergoes heel-prick collection of capillary blood stored on filter paper (DBS card) in connection with a routine screening for congenital disease within 5–7 d after birth until 2009 and within 48–72 h after birth thereafter. The remaining blood on the DSB cards is then stored at the Biological Specimen Bank for Neonatal Screening at the SSI[22] at −20°C in a locked freezer. The concentrations of 25(OH)D_2 and 25(OH)D_3 were assessed from one 3·2-mm punch, taken half-way from the centre in the residual of the DBS card. Regardless of temperature and light exposure, storage times of the DBS cards for more than 20 years are not expected to bias inter-individual variation in concentrations[23].

The sample preparation and assays were performed by the SSI using a highly sensitive liquid chromatography tandem mass spectroscopy assay[23]. The CV% for intra-assay and inter-assay variation for 25(OH)D_2 ranged from 4 to 8 % and 9 to 18 %, respectively. For 25(OH)D_3, the intra-assay and inter-assay CV% ranged from 7 to 12 % and 7 to 20 %, respectively, which is considered an acceptable precision for intra-assay and inter-assay analyses[23]. Laboratory investigators were blinded to the season of birth, as well as the outcome measurements. Currently no quality-assurance programmes for 25(OH)D measures in DBS were found, but the SSI laboratory participates in the Vitamin D External Quality Assessment Scheme with the equivalent-serum method[24].

The majority (91 %) of 25(OH)D_2 concentrations were below the detection limit of 3 nmol/l, which led us to omit these analyses, and consequently only the concentrations of 25(OH)D_3 (detection limit of 4 nmol/l) are reported here. These results are probably owing to the very few natural dietary sources for vitamin 25(OH)D_2 and the fact that in Denmark vitamin 25(OH)D_2 supplements are not recommended (25(OH)D_3 supplements are recommended). To approximate sera values, the 25(OH)D_3 concentrations have been corrected using an algorithm that takes into account the neonatal haematocrit (HCt) fraction for capillary blood: serum 25(OH)D_3 (nmol/l) = DBS 25(OH)D_3 (nmol/l) × 1/(1 − 0·61 (HCt fraction))[23,25]. Serum 25(OH)D_3 concentration was expressed in nmol/l and used as a continuous variable.

Anthropometric variables

Information from each individual on gestational age and anthropometric measures at birth was retrieved from the Danish Medical Birth Register (DMBR) maintained by Denmark Statistics. Gestational age at birth was based on gestational weeks. Birth weight in grams was used.

Owing to possible multicollinearity between birth weight and length, the Ponderal Index was used as an indicator of fetal growth. The Ponderal Index compares birth weight with length and is an assessment of fetal growth. To calculate Ponderal Index, the following formula was used: ((body weight in grams × 100)/(birth length in cm^3)). The mean Ponderal Index among term infants is usually about 2·5 g × 100/cm^3[26,27].

Another clinically relevant assessment of fetal growth is the use of categories of size for gestational age at birth, which, unlike birth weight that is independent of gestational age, provide additional information about fetal growth adjusted for sex and gestational age at birth using population references. To classify infant size in relation to gestational age, at first, the relevant International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21) references were considered to be used. However, they were found to largely overestimate the number of infants born large for gestational age (LGA) in our data; hence, Danish references were preferred[28,29]. Those below the 10th percentile were considered SGA infants, and those above the 90th percentile of birth weight were considered LGA infants, adjusted for sex-specific gestational age using Danish references[29].

Covariates

The DMBR includes information on maternal demographics and pregnancy characteristics such as maternal place of origin, maternal smoking status during pregnancy, maternal age,

maternal education and parity. Maternal place of origin was dichotomised into European *v.* Non-European (according to maternal place of birth). Manual data collection regarding maternal smoking during pregnancy started in 1991, and women were categorised as either smoking or non-smoking during pregnancy. Since 1997, data were collected electronically and further categories were added: unknown, mother not smoking, mother smokes, mother stopped smoking in the first trimester, mother stopped smoking after the first trimester and mother smokes up to 5, 6–10, 11–20 or >20 cigarettes/d. Combining this information, maternal smoking was categorised into ever smoking (women who ever smoked during their pregnancy), never-smoking (women who never smoked during their pregnancy) or unknown. Maternal age at time of delivery was used as a continuous variable. Maternal education was categorised into Primary school, High school and University based on the highest education achievement level. On the basis of date of birth of the children, we defined season of birth as follows: Winter-born, between November and January; Spring-born, between February and April; Summer-born, between May and July; and Autumn-born, between August and October. Categorisation was based on the seasonal variation in serum 25(OH)D concentration among individuals from countries in northern latitudes[30,31]. The reporting of parity changed during the study period. From 1973 to 1996, the DMBR only included information on live and still births, and to estimate parity we used the summarised estimates of total births and the actual birth. From 1996, parity was dichotomised into primiparous and multiparous.

Statistics

Analyses were restricted to children born at term, defined as between 37 and 44 weeks of gestation.

Restricted cubic spline regression models and likelihood ratio tests were used to assess possibility of non-linear associations between $25(OH)D_3$ and gestational age at birth, birth weight and Ponderal Index. Multinomial regression analyses were used to assess the associations between neonatal $25(OH)D_3$ and categories of size for gestational age (SGA and LGA). All regression models were adjusted for maternal place of origin, maternal smoking, maternal age, maternal education, year of birth and season of birth based on theoretical and biological plausibility. Sensitivity analyses defining gestational age based on gestational week −1 were run, as data on gestational age in the DMBR were previously found to be overestimated by 1 week (systematic error) compared with medical records[32]. The associations between parity and neonatal vitamin D, gestational age at birth, birth weight and Ponderal Index is not yet well established. However, parity could potentially be a confounder; hence, sensitivity analyses adding parity to the model were performed.

The analyses and the graphics were produced using the statistical software package Stata 13 (2013, StataCorp LP; www.stata.com). Bases for the splines were computed by the 'mkspline' command. All splines had three knots, and the locations of the knots were at the 10th (concentration: 7·6 nmol/l), 50th (concentration: 23·7 nmol/l) and 90th (concentration: 51·8 nmol/l) percentiles of $25(OH)D_3$.

The significance level for all tests was set at 0·05.

The Danish National Committee on Biomedical Research Ethics Permission, as well as the steering committee from the biobank, gave permission to access and analyse the DBS samples from the Biological Specimen Bank for Neonatal Screening (J. no. H-3-2011-126). Permission from the Danish Data Protection Agency to merge biobank information with the information from the registers was granted (J. no.: 2012-41-116).

This study is part of the D-tect project, which is registered on clinicaltrials.gov: NCT03330301

Results

Vitamin D ($25(OH)D_3$) measurements were available for a total of 2873 newborns. After exclusion of individuals born preterm, 2686 $25(OH)D_3$ measurements were available (Fig. 1). The mean concentration of $25(OH)D_3$ was 27·2 (SD 18·3) nmol/l (ranging from 0·4 to 110 nmol/l).

The baseline characteristics of the study population are presented in Table 1.

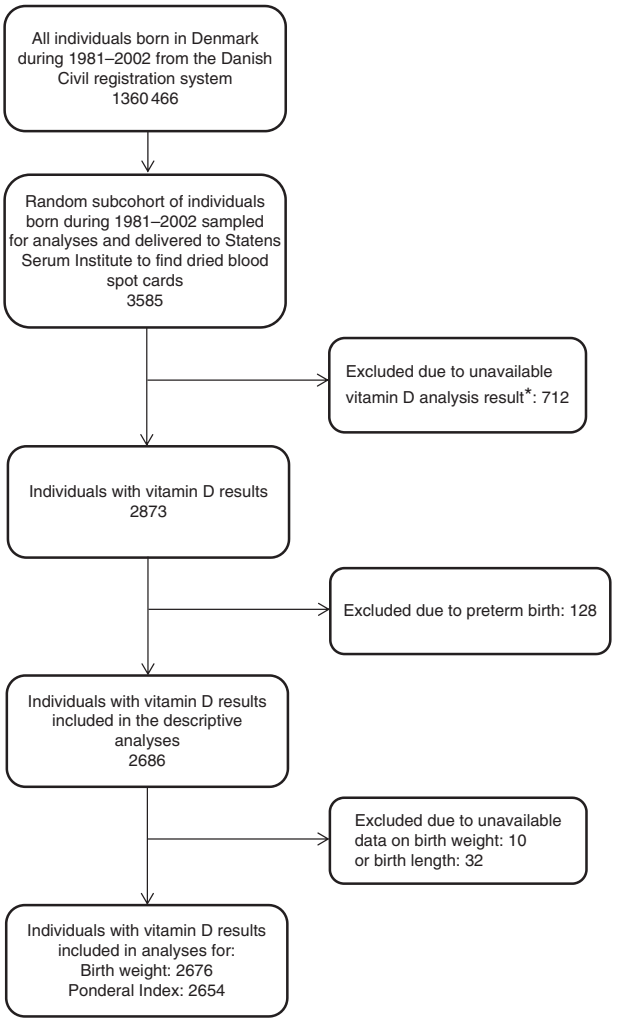

Fig. 1. Flow chart of the study population. *Dried blood spot cards were either not found, there was insufficient material for analysis or the analysis failed.

Table 1. Maternal and offspring characteristics
(Mean values and standard deviations; numbers and percentages)

Continuous variables	*n*	Mean	SD
Neonatal 25(OH)D_3 (nmol/l)	2686	27·2	18·3
Gestational age at birth (weeks)	2686	39·8	1·3
Birth weight (g)	2676	3532	506·9
Ponderal Index (g × 100/cm^3)	2654	2·5	0·3
Maternal age (years)	2686	28·3	4·8
Categorical variables		*n*	%
Size for gestational age			
AGA		2046	77·0
SGA		330	12·4
LGA		280	10·5
Offspring sex			
Male		1381	51·4
Female		1305	48·6
Maternal place of origin			
European		2497	93·0
Non-European		189	7·0
Maternal smoking			
Smoker		369	13·7
Non-smoker		1137	42·3
Unknown		1180	43·9
Maternal education			
Primary School		798	29·7
High school		1240	46·2
University		564	21·0
Unknown		84	3·1
Season of birth			
Winter		630	23·5
Spring		647	25·1
Summer		680	25·3
Autumn		702	26·1
Years of birth			
1981–1990		1092	40·7
1991–2002		1594	59·3

AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age.

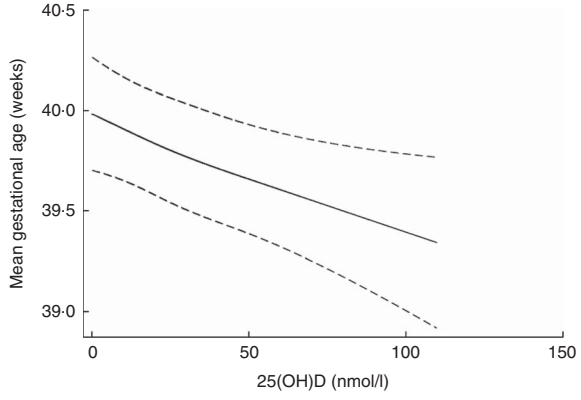

Fig. 2. Spline 25-hydroxyvitamin D_3 (25(OH)D_3) concentration (nmol/l) and gestational age (weeks) at birth after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (*n* 2686).

25-hydroxyvitamin D_3 and gestational age at birth

The mean gestational age at birth was 39·8 (SD 1·3) weeks, ranging from 37 to 44 (*n* 2686).

Table 2. Multivariate linear regression analyses of the association between 25-hydroxyvitamin D_3 (25(OH)D_3) concentration (nmol/l) and gestational age at birth in weeks among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (*n* 2686)*
(Coefficients with their standard errors and 95 % confidence intervals)

Gestational age at birth	Coefficient	SE	95 % CI	*P*
25(OH)D_3 (nmol/l) (continuous)	−0·006	0·002	−0·009, −0·003	<0·001
Maternal education				
School	Ref.			
Unknown	0·08	0·15	−0·22, 0·38	0·62
High school	0·15	0·06	0·03, 0·27	0·01
University	0·22	0·01	0·07, 0·37	0·004
Maternal age (years) (continuous)	−0·01	0·01	−0·02, 0·01	0·30
Maternal smoking				
Smoking	Ref.			
Unknown	−0·09	0·10	−0·29, 0·10	0·35
Non-smoking	0·01	0·08	−0·14, 0·17	0·86
Maternal place of origin				
European	Ref.			
Non-European	−0·17	0·11	−0·38, 0·04	0·11
Season				
Winter	Ref.			
Spring	0·14	0·07	−0·002, 0·28	0·05
Summer	0·23	0·07	0·09, 0·37	0·002
Autumn	0·22	0·07	0·08, 0·36	0·003
Year of birth (continuous)	−0·02	0·01	−0·03, −0·001	0·04

Ref., referent values.
* Analyses were performed after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth.

Assessment of the fitted spline (Fig. 2) suggested an inverse linear association between 25(OH)D_3 and gestational age at birth, which was confirmed by the statistical tests. The likelihood ratio test of an overall association indicated that the null hypothesis (the model including 25(OH)D_3 is not better than the null model) could be rejected ($P = 0·003$), and the likelihood ratio test of non-linearity indicated that the spline regression model did not fit the data better than the linear one ($P = 0·71$). The linear model resulted in an inverse association between 25(OH)D_3 concentration and gestational age at birth, with a decrease of 0·006 (95 % CI − 0·009, −0·003, $P < 0·001$) weeks of gestation per 1 nmol/l increase in 25(OH)D_3 concentration (Table 2).

25-Hydroxyvitamin D_3 and birth weight

The mean birth weight was 3532 (SD 507) g (*n* 2676). Boys had a higher mean birth weight than girls, 3612 (SD 512) g and 3448 (SD 487) g, respectively ($P < 0·001$). Assessment of the fitted spline (Fig. 3) showed an inverted U-shaped association between 25(OH)D_3 and birth weight, which was confirmed by the statistical tests. The likelihood ratio test of an overall association indicated that the null hypothesis could be rejected ($P = 0·04$) and the likelihood ratio test of non-linearity indicated that the spline regression model fitted the data better than the linear one ($P = 0·01$).

25-Hydroxyvitamin D_3 and Ponderal Index

The mean Ponderal Index was 2·5 g × 100/cm^3 (SD 0·3), ranging from 1·8 to 3·5 g × 100/cm^3 (*n* 2654). Assessment of the

fitted spline (Fig. 4) shows an inverted U-shaped association between 25(OH)D_3 and Ponderal Index, which was confirmed by the statistical tests. The likelihood ratio test of an overall association indicated that the null hypothesis could be rejected ($P=0{\cdot}04$) and the likelihood test of non-linearity indicated that the spline regression model fitted the data better than the linear one ($P=0{\cdot}01$).

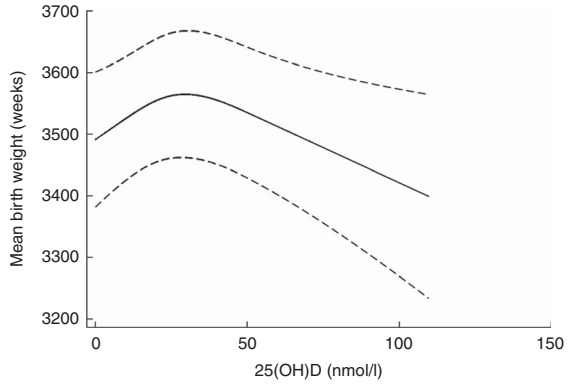

Fig. 3. Spline of 25-hydroxyvitamin D_3 (25(OH)D_3) (nmol/l) and birth weight (g) after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (*n* 2676).

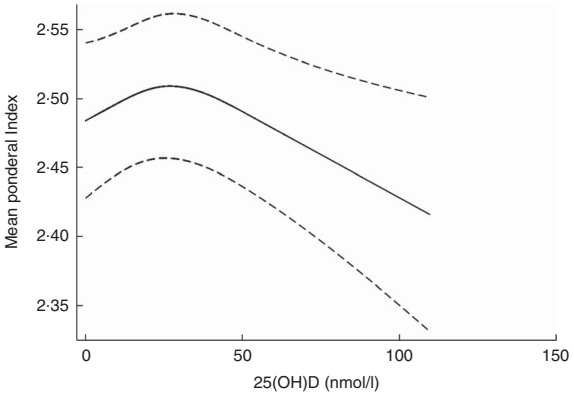

Fig. 4. Spline 25-hydroxyvitamin D_3 (25(OH)D_3) (nmol/l) and Ponderal Index ($g \times 100/cm^3$) after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (*n* 2654).

25-Hydroxyvitamin D_3 and size for gestational age

Most infants were adequate for gestational age (AGA) (*n* 2046), 330 were SGA (12 %) and 280 were LGA (11 %). 25(OH)D_3 concentrations differed between SGA (26·2 (SD 17·9)), AGA (27·2 (SD 18·5)) and LGA (29·2 (SD 17·9)) infants. However, the results from the multinomial regression analyses showed that the relative risk ratio of being SGA or LGA compared with AGA was not associated with 25(OH)D_3 concentration ($P=0{\cdot}6$) (Table 3).

Results from sensitivity analyses defining gestational age based on gestational week −1, and from including parity as a confounder, were essentially similar (online Supplementary Material).

Discussion

Using a large and randomly selected population-based sample of infants born in Denmark, we found that neonatal 25(OH)D_3 concentration was significantly associated with gestational age at birth, birth weight and Ponderal Index, but not associated with size for gestational age. Notably, our analyses were restricted to infants born at term, as the underlying causes of preterm births such as infections, diabetes, high blood pressure, stress or nutritional status may influence maternal and fetal pregnancy outcomes and vitamin D concentrations[33].

Gestational age

Our results suggested an inverse linear association between fetal 25(OH)D_3 and gestational age, indicating that lower vitamin D does not seem to be related to a suspension of gestation in babies born at term. Our findings are supported by results from previous studies. For instance, Hossain *et al.*[34] found higher vitamin D concentrations from cord blood among Pakistani neonates born before 37 weeks of gestation than among neonates born at later gestation. In addition, the authors reported a decrease in maternal serum concentrations of 25(OH)D with longer duration of gestation. Another longitudinal study among 141 Danish women reported that 25(OH)D concentration increased between weeks 18 and 32 and decreased from 32 to 8 weeks postpartum among healthy pregnant women[35]. As maternal and fetal 25(OH)D concentrations are correlated[5,6], it is likely that both maternal and fetal vitamin D concentrations fluctuate by gestational weeks following a similar pattern. Decreasing concentrations of

Table 3. Multinomial regression analyses of 25-hydroxyvitamin D_3 (25(OH)D_3) concentration (nmol/l) and size for gestational age among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (*n* 2656) (Relative risk ratios (RRR) with their standard errors and 95 % confidence intervals)

	Crude model				Adjusted model*			
Categories of birth weight	RRR	SE	95 % CI	*P*	RRR	SE	95 % CI	*P*
SGA	1·00	0·003	0·99, 1·00	0·38	1·00	0·004	0·99, 1·01	0·60
AGA	Ref.				Ref.			
LGA	1·01	0·003	1·00, 1·01	0·08	1·00	0·004	1·00, 1·01	0·55

SGA, small for gestational age; AGA, appropriate for gestational age; Ref., referent value; LGA, large for gestational age.
* Adjusted for maternal place of origin, smoking, age, education, season and year of birth.

neonatal vitamin D with longer duration of gestation might be explained by a decreasing efficiency of the decidua to transfer $25(OH)D_3$ from the mother to the fetus. This fluctuation might also be owing to maternal physiological variation in vitamin D concentrations during pregnancy, because of changes in maternal plasma vitamin D binding protein and variations in intestinal absorption of vitamin D[34–36]. In addition, the concentration of the active form of vitamin D (1,25-dihydroxycholecalciferol (1,25(OH)D)) increases during gestation[37], and as 25(OH)D has a shorter half-life when 1,25(OH)D concentration increases, 25(OH)D concentration tends to decrease. However, the aetiology behind the changes in 25(OH)D during pregnancy remains controversial[38]. Furthermore, in late gestation, mothers seem to have less outdoor activities and therefore potentially less skin synthesis of vitamin D through sun exposure[39].

The association between 25(OH)D and gestational age at birth might be bidirectional. Indeed, higher neonatal 25(OH)D concentrations may shorten gestational length among infants born at term, but longer gestational length may also lower neonatal 25(OH)D concentrations. In the present study, establishing a causal pathway was not possible.

Further basic studies on maternal–fetal vitamin D variations and placental transfer at different stages of gestation are needed to clarify the underlying mechanisms involved, as they still remain unclear[40]. However, at present, the restricted possibility to access direct information on vitamin D status of the human fetus limits our possibility for understanding fetal physiology[41].

Birth weight and Ponderal Index

A significant non-linear association was found between neonatal $25(OH)D_3$ and birth weight, as well as between neonatal $25(OH)D_3$ and Ponderal Index. These results are in accordance with results from a study including 1491 Chinese neonates, also finding an inverted U-shaped association between 25(OH)D from cord blood and birth weight[21] and another study from the USA finding a non-linear association between maternal 25(OH)D and birth weight[8], thus suggesting that higher vitamin D concentrations do not seem to benefit fetal growth, or may have dual biological effects on fetal growth[21]. However, contrary to our findings, as well as to those of Zhu *et al.* and Gernand *et al.*, Shor *et al.* and Eggemoen *et al.* did not find an association between maternal vitamin D and birth weight. However, in these two latter studies, analyses were limited to using linear models[8,21,42,43]. Furthermore, another Danish study[44] found a U-shaped association between cord 25(OH)D and birth weight. Multiple causes might explain the inconsistent findings, such as difference in birth period (1981–2002 *v.* 2010–2012), possible lifestyle changes between 1981 and 2012, sample selection (representative sample *v.* healthy women), 25(OH)D concentrations (27·2 (SD 18·3) *v.* 47·0 (SD 21·7) nmol/l) and methodological differences (DBS *v.* cord blood; adjustment for different confounders; residual confounding). In regard to Ponderal Index, no associations were found in the studies by Eggemoen *et al.* and Gernand *et al.*[8,43]. These discrepancies might be owing to different study designs, analytic methods and lack of power, but may also relate to the relative difficulty of measuring the crown–heel length of newborns. As cubic length is used to calculate Ponderal Index, even small measurement errors can have substantial influence on the accuracy of the Ponderal Index[45].

Size for gestational age

There was no association between $25(OH)D_3$ and the categories of size for gestational age. These results are consistent with our findings of an inverted U-shaped association between $25(OH)D_3$ and birth weight, which indicates that vitamin D effects on fetal growth might be dual. Conflicting findings on this association are, however, present in the literature. Narrative and systematic reviews and meta-analyses indicate that an association between 25(OH)D and the risk of SGA from observational studies exist, but not from randomised trials[20,46–48]. The discrepant results might be owing to differences in study designs, exposure and/or outcome definitions and measurements, as well as study populations.

Strengths and limitations

The strengths of this study lie in the large sample of individuals randomly selected among the entire Danish population with neonatal biological measurements of $25(OH)D_3$ from DBS, as well as the use of the high-quality register data from the DMBR, where demographic data, birth weight and parity have shown good precision compared with medical records[32]. Data on gestational age in DMBR, compared with medical records, were found to overestimate actual gestational age by 1 week[32], and to account for this, sensitivity analyses were run. In addition, another strength of this study is that multinomial and spline regression analyses with adjustment for a large number of confounders, including year of birth to account for secular changes in birth weight in Denmark between 1981 and 2002[49], were performed. The study has also limitations. First, measures of 25(OH)D, birth weight and gestational age were collected within 7 d after birth; hence, a cause–effect direction could not be established. Second, information on maternal vitamin D concentrations, breast-feeding, maternal weight or BMI, outdoor activity and dietary intake were not available; hence, they could not be included as covariates in our statistical models. Third, adjustment for smoking during pregnancy might not be comprehensive, as information about maternal smoking status was missing for more than 40 % of individuals and reporting bias may have occurred. The current results might only be generalised to populations with similar characteristics as the Danish population.

Conclusion

Overall, this large study conducted in a random subset of the Danish population suggests that neonatal $25(OH)D_3$ concentration is inversely associated with gestational age at birth; the later the gestational age, the lower the concentrations. In addition, our results suggest that neonatal $25(OH)D_3$ concentration has an inverted U-shaped association with birth weight and Ponderal Index, which are known to be correlated with many subsequent health outcomes such as obesity and type 2 diabetes, and is not associated with size for gestational age (SGA and LGA).

Randomised clinical trials are needed to draw firm conclusions regarding the causal direction of the associations found.

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B. L. H. designed the study. A. K., P. F. and B. L. H. formulated the research question. R. J., J. M. G. and A. C. provided feedback on the study design. A. K. and P. F. analysed the data. A. K. and M. N. H. wrote the manuscript. All authors read and provided written feedback on the manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518000879

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