

**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**

Keller, Amélie; Händel, Mina N.; Frederiksen, Peder; Jacobsen, Ramune; Cohen, Arie S.; McGrath, John J.; Heitmann, Berit L.

Published in:
British Journal of Nutrition

DOI:
10.1017/S0007114518000879

Publication date:
2018

Document version:
Final published version

Citation for pulished version (APA):

Keller, A., Händel, M. N., Frederiksen, P., Jacobsen, R., Cohen, A. S., McGrath, J. J., & Heitmann, B. L. (2018). 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. *British Journal of Nutrition*, 119(12), 1416-1423.
<https://doi.org/10.1017/S0007114518000879>

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use

This work is brought to you by the University of Southern Denmark.
Unless otherwise specified it has been shared according to the terms for self-archiving.
If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim.
Please direct all enquiries to puresupport@bib.sdu.dk

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

Amélie Keller^{1*}, Mina N. Händel¹, Peder Frederiksen¹, Ramune Jacobsen^{1,2}, Arieh S. Cohen³, John J. McGrath^{4,5} and Berit L. Heitmann^{1,6,7,8}

¹Research Unit for Dietary Studies, The Parker Institute, Capital Region, Bispebjerg and Frederiksberg University Hospitals, 2000 Frederiksberg, Denmark

²Research Unit for Chronic Conditions, Department of Clinical Epidemiology, Capital Region, Bispebjerg and Frederiksberg University Hospitals, 2000 Frederiksberg, Denmark

³Department of Congenital Diseases, Biobanks and Biomarkers, Staten Serum Institute, 2300 Copenhagen, Denmark

⁴Queensland Centre for Mental Health Research, and Queensland Brain Institute, University of Queensland, St. Lucia, QLD 4072, Australia

⁵National Centre for Register-based Research, Aarhus BSS, Aarhus University, 8210 Aarhus, Denmark

⁶The Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, University of Sydney, Camperdown, NSW 2006, Australia

⁷The National Institute of Public Health, University of Southern Denmark, 1353 Copenhagen, Denmark

⁸The Department of Public Health, Section for General Medicine, University of Copenhagen, 1014 Copenhagen, Denmark

(Submitted 14 November 2017 – Final revision received 5 March 2018 – Accepted 8 March 2018 – First published online 25 April 2018)

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 D₃ (25(OH)D₃) 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)D₃ 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)D₃ 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)D₃ concentration. An inverted U-shaped association between 25(OH)D₃ 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)D₃ 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.

* **Corresponding author:** A. Keller, email Amelie.Cleo.Keller@regionh.dk

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)D₃ 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)D₂ and 25(OH)D₃ 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)D₂ ranged from 4 to 8% and 9 to 18%, respectively. For 25(OH)D₃, 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)D₂ 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)D₃ (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)D₂ and the fact that in Denmark vitamin 25(OH)D₂ supplements are not recommended (25(OH)D₃ supplements are recommended). To approximate sera values, the 25(OH)D₃ concentrations have been corrected using an algorithm that takes into account the neonatal haematocrit (Hct) fraction for capillary blood: serum 25(OH)D₃ (nmol/l) = DBS 25(OH)D₃ (nmol/l) × 1 / (1 – 0.61 (Hct fraction))^(23,25). Serum 25(OH)D₃ 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 cm³)). The mean Ponderal Index among term infants is usually about 2.5 × 100/cm³^(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)D₃ and gestational age at birth, birth weight and Ponderal Index. Multinomial regression analyses were used to assess the associations between neonatal 25(OH)D₃ 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)D₃.

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)D₃) measurements were available for a total of 2873 newborns. After exclusion of individuals born preterm, 2686 25(OH)D₃ measurements were available (Fig. 1). The mean concentration of 25(OH)D₃ 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.

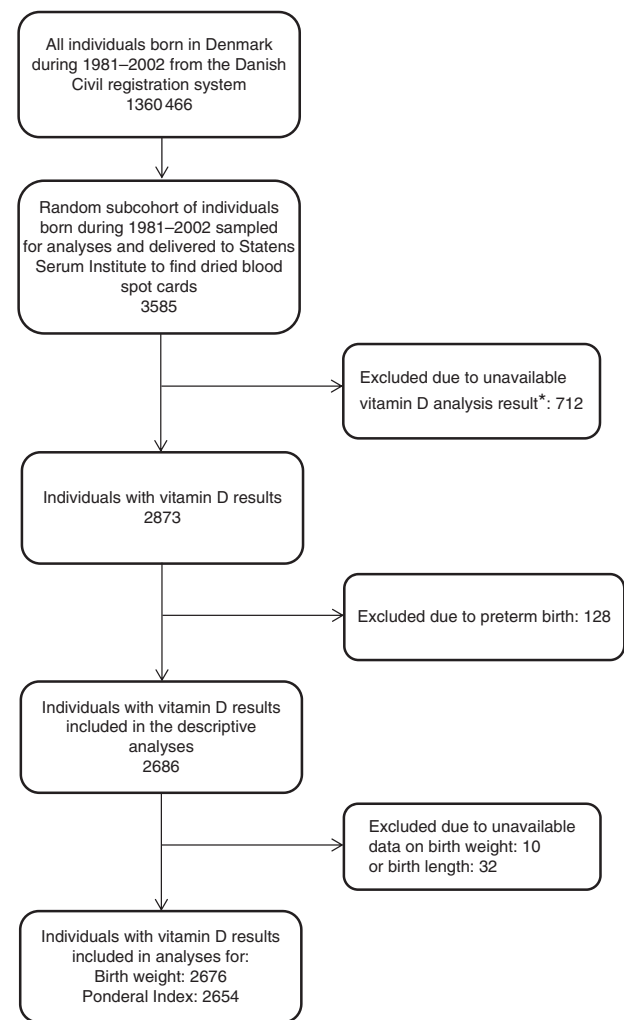


Fig. 1. Flow chart of the study population. *Dried blood spot cards were either not found, there was insufficient material for analysis or the analysis failed.

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

Continuous variables	<i>n</i>	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	<i>n</i>	%	
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.

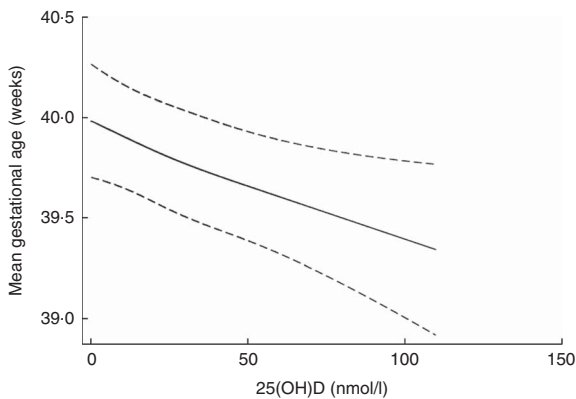


Fig. 2. Spline 25-hydroxyvitamin D₃ (25(OH)D₃) concentration (nmol/l) and gestational age (weeks) at birth after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (*n* 2686).

25-hydroxyvitamin D₃ 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 D₃ (25(OH)D₃) 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	<i>P</i>
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)D₃ 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)D₃ 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)D₃ 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)D₃ concentration (Table 2).

25-Hydroxyvitamin D₃ 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)D₃ 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 D₃ and Ponderal Index

The mean Ponderal Index was 2.5 g × 100/cm³ (SD 0.3), ranging from 1.8 to 3.5 g × 100/cm³ (*n* 2654). Assessment of the

fitted spline (Fig. 4) shows an inverted U-shaped association between 25(OH)D₃ 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 ($P=0.04$) and the likelihood test of non-linearity indicated that the spline regression model fitted the data better than the linear one ($P=0.01$).

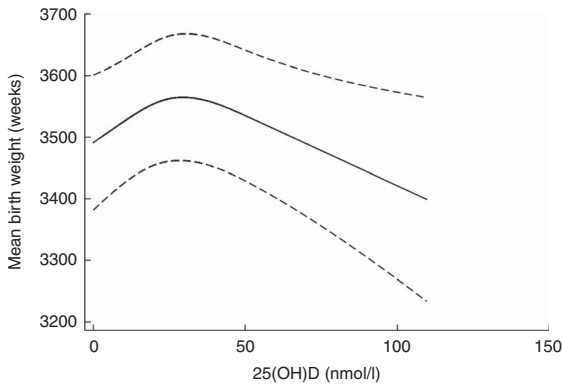


Fig. 3. Spline of 25-hydroxyvitamin D₃ (25(OH)D₃) (nmol/l) and birth weight (g) after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (n 2676).

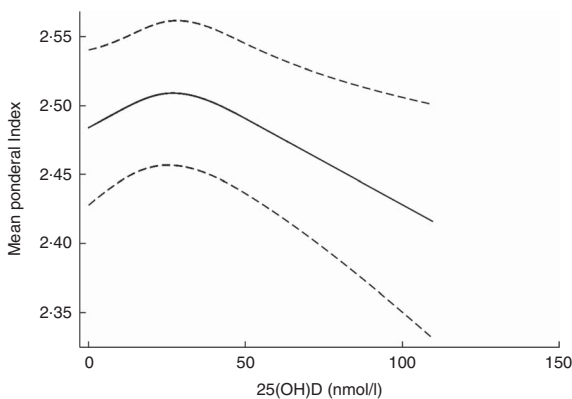


Fig. 4. Spline 25-hydroxyvitamin D₃ (25(OH)D₃) (nmol/l) and Ponderal Index ($g \times 100/cm^3$) after mutual adjustment for maternal place of origin, smoking, age, education, season and year of birth among a random sample of infants born at term (weeks 37–44) in the years between 1981 and 2002 in Denmark (n 2654).

25-Hydroxyvitamin D₃ 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)D₃ 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)D₃ concentration ($P=0.6$) (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)D₃ 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)D₃ 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 D₃ (25(OH)D₃) 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)

Categories of birth weight	Crude model				Adjusted model*			
	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)D₃ 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)D₃ and birth weight, as well as between neonatal 25(OH)D₃ 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)D₃ and the categories of size for gestational age. These results are consistent with our findings of an inverted U-shaped association between 25(OH)D₃ 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)D₃ 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)D₃ 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)D₃ 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.

Acknowledgements

The study was part of the 4-year project 'D-TECT' funded by the Programme Commission on Health, Food, and Welfare under the Danish Council for Strategic research (grant no. 0603-00453B). The Danish Diabetes Academy supported by the Novo Nordisk Foundation. The Lundbeck Foundation (grant no. R170-2014-643). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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>

References

- Whincup PH, Kaye S, Owen C, *et al.* (2008) Birth weight and risk of type 2 diabetes – a systematic review. *JAMA* **300**, 2886–2897.
- Barker DJ (1998) *In utero* programming of chronic disease. *Clin Sci (Lond)* **95**, 115–128.
- Cho WK & Suh B-K (2016) Catch-up growth and catch-up fat in children born small for gestational age. *Korean J Pediatr* **59**, 1.
- Roth CL & DiVall S (2016) Consequences of early life programming by genetic and environmental influences: a synthesis regarding pubertal timing. *Endocr Dev* **29**, 134–152.
- Streym S, Moller UK, Rejnmark L, *et al.* (2013) Maternal and infant vitamin D status during the first 9 months of infant life – a cohort study. *Eur J Clin Nutr* **67**, 1022–1028.
- Weinert LS & Silveiro SP (2015) Maternal-fetal impact of vitamin D deficiency: a critical review. *Matern Child Health J* **19**, 94–101.
- Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
- Gernand AD, Simhan HN, Klebanoff MA, *et al.* (2013) Maternal serum 25-hydroxyvitamin D and measures of newborn and placental weight in a U.S. multicenter cohort study. *J Clin Endocrinol Metab* **98**, 398–404.
- Leffelaar ER, Vrijkotte TGM & van Eijsden M (2010) Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. *Br J Nutr* **104**, 108–117.
- Dalgård C, Petersen M & Steuerwald U (2016) Umbilical cord serum 25-hydroxyvitamin D concentrations and relation to birthweight, head circumference and infant length at age 14 days. *Paediatr Perinat Epidemiol* **30**, 238–245.
- Kiely M, O'Donovan SM, Kenny LC, *et al.* (2017) Vitamin D metabolite concentrations in umbilical cord blood serum and associations with clinical characteristics in a large prospective mother-infant cohort in Ireland. *J Steroid Biochem Mol Biol* **167**, 162–168.
- Morales E, Rodriguez A, Valvi D, *et al.* (2015) Deficit of vitamin D in pregnancy and growth and overweight in the offspring. *Int J Obes (Lond)* **39**, 61–68.
- Crozier SR, Harvey NC, Inskip HM, *et al.* (2012) Maternal vitamin D status in pregnancy is associated with adiposity in the offspring: findings from the Southampton Women's Survey. *Am J Clin Nutr* **96**, 57–63.
- Jensen CB, Lundqvist M, Sørensen TIA, *et al.* (2017) Neonatal vitamin D levels in relation to risk of overweight at 7 years in the Danish D-TECT case-cohort study. *Obes Facts* **10**, 273–283.
- Kiely M, O'Donovan SM, Kenny LC, *et al.* (2017) Vitamin D metabolite concentrations in umbilical cord blood serum and associations with clinical characteristics in a large prospective mother-infant cohort in Ireland. *J Steroid Biochem Mol Biol* **167**, 162–168.
- Karras SN, Anagnostis P, Naughton D, *et al.* (2015) Vitamin D during pregnancy: why observational studies suggest deficiency and interventional studies show no improvement in clinical outcomes? A narrative review. *J Endocrinol Invest* **38**, 1265–1275.
- Pérez-López FR, Pasupuleti V, Mezones-holguin E, *et al.* (2015) Effect of vitamin D supplementation during pregnancy on maternal and neonatal outcomes: a systematic review and meta-analysis of randomized controlled trials. *Fertil Steril* **103**, 1278–1288.
- De-Regil LM, Palacios C, Lombardo LK, *et al.* (2016) Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev*, issue 1, CD008873.
- Harvey NC, Holroyd C, Ntani G, *et al.* (2014) Vitamin D supplementation in pregnancy: a systematic review. *Health Technol Assess* **18**, 1–19.
- Aghajafari F, Field CJ, Rabi D, *et al.* (2016) Plasma 3-epi-25-hydroxycholecalciferol can alter the assessment of vitamin D status using the current reference ranges for pregnant women and Their newborns. *J Nutr* **146**, 70–75.
- Zhu P, Tong S, Hu W, *et al.* (2015) Cord blood 25-hydroxyvitamin D and fetal growth in the China – Anhui Birth Cohort Study. *Sci Reports Nat Publ Gr* **5**, 1–9.
- Nørgaard-Pedersen B & Hougaard DM (2007) Storage policies and use of the Danish Newborn Screening Biobank. *J Inherit Metab Dis* **30**, 530–536.
- Eyles D, Anderson C, Ko P, *et al.* (2009) A sensitive LC/MS/MS assay of 25OH vitamin D₃ and 25OH vitamin D₂ in dried blood spots. *Clin Chim Acta* **403**, 145–151.
- Carter GD, Berry J, Durazo-Arvizu R, *et al.* (2018) Hydroxyvitamin D assays: an historical perspective from DEQAS. *J Steroid Biochem Mol Biol* **177**, 30–35.
- Kayiran SM, Ozbek N, Turan M, *et al.* (2003) Significant differences between capillary and venous complete blood counts in the neonatal period. *Clin Lab Haematol* **25**, 9–16.
- Roje D, Banovic I, Tadin I, *et al.* (2004) Gestational age – the most important factor of neonatal Ponderal Index. *Yonsei Medial J* **45**, 273–280.
- Juhl M, Olsen J, Andersen PK, *et al.* (2010) Physical exercise during pregnancy and fetal growth measures: a study within the Danish National Birth Cohort. *Am J Obstet Gynecol* **202**, 63.e1–63.e8.
- Villar J, Ismail LC, Victora CG, *et al.* (2014) International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* **384**, 857–868.
- Marsal K, Persson P, Larsen T, *et al.* (1996) Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr* **85**, 843–848.
- Godang K, Frosli KF, Henriksen T, *et al.* (2014) Seasonal variation in maternal and umbilical cord 25(OH) vitamin D

- and their associations with neonatal adiposity. *Eur J Endocrinol* **170**, 609–617.
31. Hyppönen E & Power C (2007) Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* **85**, 860–868.
 32. Kristensen J, Langhoff-Roos J, Skovgaard LT, *et al.* (1996) Validation of the Danish Birth Registration. *J Clin Epidemiol* **49**, 893–897.
 33. World Health Organization (2016) Preterm birth. Fact sheet. <http://www.who.int/mediacentre/factsheets/fs363/en/> (accessed October 2017).
 34. Hossain N, Hussain-kanani F, Shah T, *et al.* (2011) High prevalence of vitamin D deficiency in Pakistani mothers and their newborns. *Int J Gynecol Obstet* **112**, 229–233.
 35. Milman N, Hvas A & Bergholt T (2011) Vitamin D status during normal pregnancy and postpartum. A longitudinal study in 141 Danish women. *J Perinat Med* **40**, 57–61.
 36. Kachkache M, Rebut-Bonneton C, Demignon J, *et al.* (1993) Uterine cells other than stromal decidual cells are required for 1,25-dihydroxyvitamin D₃ production during early human pregnancy. *FEBS Lett* **333**, 83–88.
 37. Specker B (2004) Vitamin D requirements during pregnancy. *Am J Clin Nutr* **80**, 1740S–1747S.
 38. Figueiredo ACC, Cocate PG, Adegboye ARA, *et al.* (2018) Changes in plasma concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D during pregnancy: a Brazilian cohort. *Eur J Nutr* **57**, 1059–1072.
 39. Borodulin KM, Evenson KR, Wen F, *et al.* (2008) Physical activity patterns during pregnancy. *Med Sci Sports Exerc* **40**, 1901–1908.
 40. Nguyen TPH, Yong HEJ, Chollangi T, *et al.* (2015) Placental vitamin D receptor expression is decreased in human idiopathic fetal growth restriction. *J Mol Med* **93**, 795–805.
 41. Salle BL, Delvin EE, Lapillonne A, *et al.* (2000) Perinatal metabolism of vitamin D. *Am J Clin Nutr* **71**, 5 Suppl., 1317S–1324S.
 42. Shor DB, Barzel J, Tauber E, *et al.* (2015) The effects of maternal vitamin D on neonatal growth parameters. *Eur J Pediatr* **174**, 1169–1174.
 43. Eggemoen ÅR, Jennum AK, Mdala I, *et al.* (2017) Vitamin D levels during pregnancy and associations with birth weight and body composition of the newborn: a longitudinal multiethnic population-based study. *Br J Nutr* **117**, 985–993.
 44. Lykkedegn S, Beck-Nielsen SS, Sorensen GL, *et al.* (2017) Vitamin D supplementation, cord 25-hydroxyvitamin D and birth weight: findings from the Odense Child Cohort. *Clin Nutr* **36**, 1621–1627.
 45. Corkins MR, Lewis P, Cruse W, *et al.* (2002) Accuracy of infant admission lengths. *Pediatrics* **109**, 1108–1111.
 46. Karras SN, Anagnostis P, Petroczi A, *et al.* (2015) Maternal vitamin D status in pregnancy: a critical appraisal of current analytical data on maternal and neonatal outcomes. *Hormones (Athens)* **14**, 224–231.
 47. Theodoratou E, Tzoulaki I, Zgaga L, *et al.* (2014) Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* **348**, g2035.
 48. Chen Y, Zhu B, Wu X, *et al.* (2017) Association between maternal vitamin D deficiency and small for gestational age: evidence from a meta-analysis of prospective cohort studies. *BMJ Open* **7**, e016404.
 49. Schack-Nielsen L, Mølgaard C, Sørensen TIA, *et al.* (2006) Secular change in size at birth from 1973 to 2003: National Data from Denmark. *Obesity (Silver Spring)* **14**, 1257–1263.