Predicted vitamin D status during pregnancy in relation to offspring forearm fractures in childhood: a study from the Danish National Birth Cohort

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Abstract

In a prospective cohort study, the association between maternal vitamin D status during pregnancy and offspring forearm fractures during childhood and adolescence was analysed in 30 132 mother and child pairs recruited to the Danish National Birth Cohort between 1996 and 2002. Data on characteristics, dietary factors and lifestyle factors were collected on several occasions during pregnancy. We analysed the association between predicted vitamin D status, based on a subsample with 25-hydroxyvitamin D (25(OH)D) biomarker measurements (*n* 1497) from gestation week 25, and first-time forearm fractures among offspring between birth and end of follow-up. Diagnoses were extracted from the Danish National Patient Register. Multivariable Cox regression models using age as the underlying time scale indicated no overall association between predicted vitamin D status (based on smoking, season, dietary and supplementary vitamin D intake, tanning bed use and outdoor physical activity) in pregnancy and offspring forearm fractures. Likewise, measured 25(OH)D, tanning bed use and dietary vitamin D intake were not associated with offspring forearm fractures. In mid-pregnancy, 91 % of the women reported intake of vitamin D from dietary supplements. Offspring of women who took >10 µg/d in mid-pregnancy had a significantly increased risk for fractures compared with the reference level of zero intake (hazard ratios (HR) 1·31; 95 % CI 1·06, 1·62), but this was solely among girls (HR 1·48; 95 % CI 1·10, 2·00). Supplement use in the peri-conceptional period exhibited similar pattern, although not statistically significant. In conclusion, our data indicated no protective effect of maternal vitamin D status with respect to offspring forearm fractures.

Key words: Vitamin D: Fractures: Epidemiology: Pregnancy: Fetal programming

Osteoporosis, characterised by the disruption of bone architecture and increased bone fracture risk, is a major public health issue and implies substantial costs to the society[1]. The disease may be prevented through increasing bone mass early in life to a degree that will have a biologically relevant effect in middle-aged and elderly people – for example, by the influence of diet, physical activity and endocrine status[2,3]. A growing body of evidence also suggests that adult bone mass may be influenced by factors operating as early as in fetal life[4], and especially vitamin D status during pregnancy has been hypothesised to play a role[5–9]. It is well documented that vitamin D is a key factor in the regulation of Ca homoeostasis[10], and children born to women with low vitamin D status tend, apart from being lighter and smaller, to have lower bone mineral content at birth[11]. However, it is unclear whether maternal vitamin D status during pregnancy impacts offspring bone development as studies using measures from dual-energy X-ray absorptiometry (DXA) scans in childhood have shown conflicting results[5–9].

Few studies have yet looked at the association between functional measures of bone health, such as bone fractures, in relation to prenatal vitamin D status. A meta-analysis from 2006 indicated an association between low bone mineral density (BMD) and bone fractures in children[12], and a review from 2010 concluded that there is consistent and convincing evidence for an association between BMD and childhood risk of forearm fractures[13]. Forearm fractures seem to be a good indicator of low BMD compared with other types of fractures[13,14], because they often occur as a consequence of falls on the same plane, and contrary to fractures caused by collisions or downhill falls, they do not require great forces to come into play.

In the large prospective Danish National Birth Cohort (DNBC), we had a unique opportunity to examine the association between predicted maternal vitamin D status and offspring forearm fractures through linkages to nationwide registries. Therefore, in the present study, we aimed at testing the hypothesis that a low maternal vitamin D status during pregnancy increases the risk of offspring forearm fractures during childhood and adolescence.

Methods

Study population

In the DNBC, a total of 101 042 pregnancies were recruited between 1996 and 2002 during the first antenatal visit to the general practitioner at approximately 6–10 weeks of gestation. At recruitment, women provided consent for themselves and their unborn child for participation and later data linkage to Danish health registers[15]. During the recruitment period, approximately 35 % of all deliveries in Denmark (predominantly only Caucasian mothers) were included in the cohort[15]. The initial data collection consisted of an enrolment form covering personal information as well as medication and dietary supplement use. The pregnant women were interviewed four times by telephone about lifestyle and diseases; two prenatal interviews were conducted during gestational weeks 12 and 30 and two postnatal interviews were conducted when the child was 6- and 18-months old. In addition, a validated semi-quantitative FFQ was mailed to the women during gestation week 25, covering the diet during the previous 4 weeks of completion[15,16]. The DNBC was conducted according to the guidelines laid down in the Declaration of Helsinki, and all the procedures involving study participants were approved by the National Committee on Health Research Ethics. Further, the study is registered by the Danish Data Protection Agency (J.nr. 2009-41-3332).

Exposure assessment

Vitamin D prediction scores. A vitamin D prediction score model was created based on biomarker assessment of 25-hydroxyvitamin D_2 (25(OH)D_2) and D_3 (25(OH)D_3) concentrations in 1497 maternal blood samples collected by the general practitioner at 25 weeks of gestation[17]. The samples were part of a previous case–control study of postpartum depression (893 non-cases and 605 cases), which showed no overall association between maternal status of vitamin D and the risk of postpartum depression[18]. Concentrations of plasma 25(OH)D_2 and 25(OH)D_3 were measured by the liquid chromatography-tandem MS method with a vitamin D kit from PerkinElmer, which is considered to be an accurate measure of vitamin D status[19]. The vitamin D prediction score was constructed by including factors previously associated with vitamin D status and examining their ability to predict measured vitamin D status in a regression model. The final prediction score included smoking, season, dietary and supplementary vitamin D intake, tanning bed use and outdoor physical activity level (n 36 977). Validation of the model in an independent

sample with vitamin D measurements showed good ability to rank individuals according to vitamin D status. The model explained 40 % of the variation in total 25-hydroxyvitamin D 25(OH)D levels, and it reflected fair agreement between measured concentrations of total 25(OH)D and predicted vitamin D status[17].

Dietary vitamin D. Information about dietary intake of vitamin D was available from the FFQ in mid-pregnancy with questions on frequencies of intake of approximately 360 different items of foods and beverages[16]. To estimate the intake of vitamin D, standard portion sizes and standard recipes were applied for all items in the questionnaire. Standard portion sizes were multiplied with the daily frequencies to estimate intake of each food item in grams and then coupled with the Danish Food Tables to estimate the daily intake of vitamin D[20]. Intake of vitamin D was energy-adjusted by the residual method described by Willett *et al.*[21]. The FFQ has been validated in a group of younger non-pregnant women[22], and also in a subsample from the DNBC for the intake of fruits, vegetables and pregnancy-relevant nutrients (folate, protein, retinol and *n*-3 fatty acids) by a 7-d weighed food diary and by biomarkers[23,24].

Supplementary vitamin D. Data on dietary supplements were available from the enrolment form and from the FFQ. Intake of vitamin D in the peri-conceptional period, defined as estimated daily intakes in weeks 0–8 of gestation, was extracted from the enrolment form, where women reported the name, manufacturer, period and dose of each supplement used since 4 weeks before the 1st day of the last menstruation period (LMP) until the day of enrolment (maximum 14 weeks after LMP). Intake of vitamin D during mid-pregnancy was extracted from the FFQ, where women wrote the name, manufacturer and dose of each supplement. To estimate daily vitamin D intake, information from the enrolment form and the FFQ, respectively, was linked to nutritional ingredient data for supplements sold in Denmark during the study period. Data were gathered from the Danish Medicines Agency and the Danish Veterinary and Food Administration.

Tanning bed use. We extracted data on tanning bed use in pregnancy from the telephone interview at 30 weeks of gestation, where the women answered a question about using tanning beds and during which gestational weeks they used them. Answers for gestational weeks 1–25 were summarised and divided into four exposure groups according to the number of weeks they used tanning beds (no use, 1–4, 4–8, >8 weeks). Further, the answers were summarised for the first trimester (gestation weeks 1–12) and the second trimester (gestation weeks 12–25), respectively.

Outcome

For our outcome, we extracted data from the Danish National Patient Register (DNPR) by means of the unique Danish personal identifier (CPR). The DNPR is a mandatory nationwide register established in 1977, recording information from all hospital admissions, including outpatient activities and

emergency room contacts[25]. In general, the register has a high precision of diagnoses[26], and also for fracture diagnoses[27]. Fracture diagnoses are registered along with cause of diagnosis, including data on involvement in a traffic accident. We defined our outcome as the first occurrence of any forearm fracture between birth and end of follow-up using the ICD-10 codes. The following codes for forearm fractures were used in the study: DS525, DS526, DS525A, DS525B and DS525C. For other fracture diagnoses, we used the following codes: DS020-DS029, DS120-DS129, DS220-DS229, DS302P, DS320-DS329, DS420-DS429, DS520-DS529, DS620-DS629, DS720-DS729, DS820-DS829, DS920-DS929 and DT020-DT029.

Statistical methods

The study sample for our analyses consisted of 30 132 mother–child pairs who had available information on predicted vitamin D status, except for the analysis on peri-conceptional use of vitamin D that consisted of 18 635 mother–child pairs with completed information on the use of dietary supplements in the enrolment form (Fig. 1). We analysed the association between different measures of vitamin D status in pregnancy and offspring forearm fractures using Cox proportional hazard models and calculated hazard ratios (HR) and 95 % CI, using age in days as the underlying time scale. Kaplan–Meier estimates were used to visualise the associations. We restricted our study sample to live births, singletons and offspring with a validated CPR number. All exposure measures were analysed both as continuous and categorical variables as follows: vitamin D prediction scores (quintiles), dietary vitamin D intake (quintiles), total 25(OH)D concentration (<25, 25 to <50, 50–75 and >75 nmol/l, according to guidelines from the Danish National Board of Health and the American Institute of Medicine[28,29]), peri-conceptional supplementary vitamin D intake (0, 0–5, >5–10 µg/d) and mid-pregnancy supplementary vitamin D intake (0, 0–5, 5–10 and >10 µg/d). Season of birth
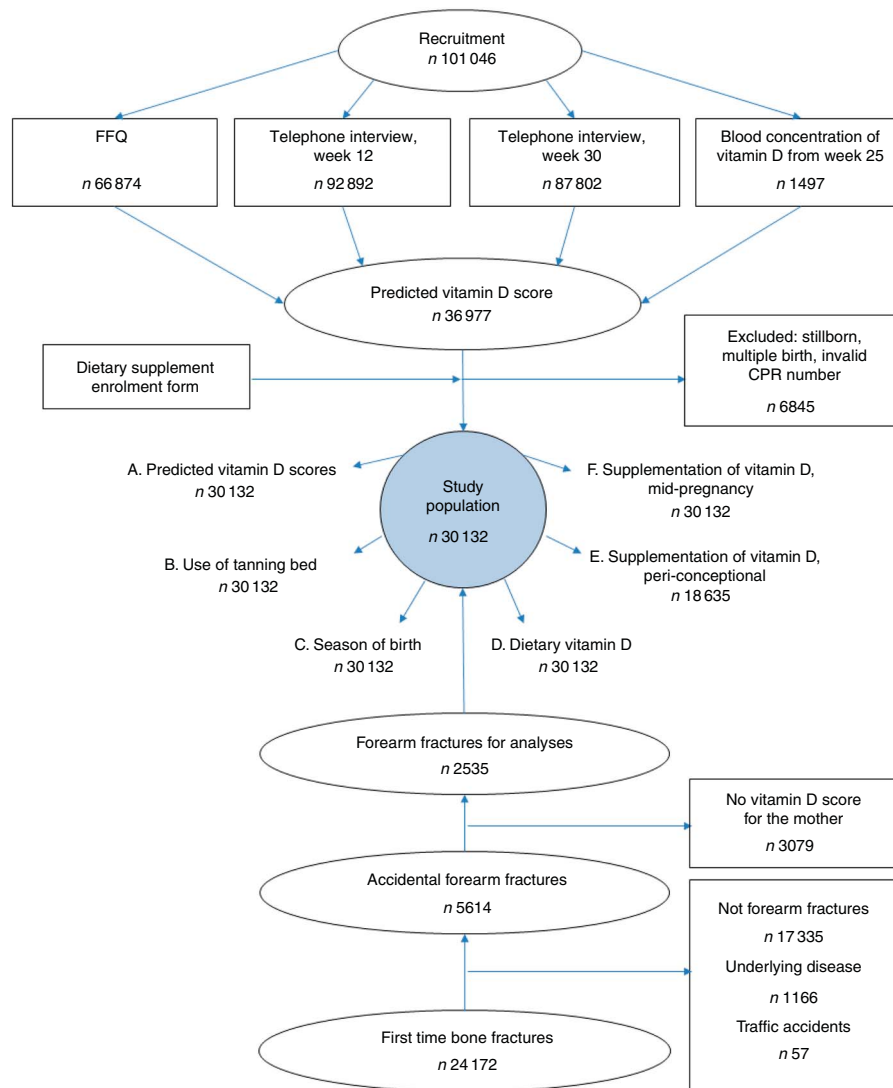

Fig. 1. Flow chart of the data collection in the Danish National Birth Cohort, and elucidation of the analyses conducted for the association between maternal vitamin D status during pregnancy and offspring forearm fracture risk. CPR, Danish personal identifier.

was analysed as a categorical variable (Mar/Apr/May, Jun/Jul/Aug, Sep/Oct/Nov and Dec/Jan/Feb). Visual inspection of cumulative residual plots did not indicate violations to the assumption of proportional hazards.

Children in the study sample were followed-up from their date of birth until the age of first forearm fracture, other censoring event or the defined end of follow-up, which was 10 November 2013. In total, 1891 children (2 %) in the study sample were censored due to other reasons, including death or emigration, according to data from the Danish Civil Registration System.

Information on covariates used in the multivariable Cox regression analyses was gathered from the enrolment form, the FFQ and the prenatal telephone interviews. Based on accessibility and previous evidence about vitamin D and fracture risk, the following variables were included as covariates: mother's age (continuous), parity (0, 1, 2, 3+), sex of the child (boy/girl), cohabitation (couple/single), pre-pregnancy BMI (continuous), smoking (non-smoker, occasional smoker, <15, >15 cigarettes/d), occupational status (high, medium, skilled, student, unskilled, unemployed) and physical activity (0, 1–44, 45–75, 75–149, ≥150 min/week). We adjusted for season of birth in all analyses, except for those in which season of birth was the exposure. In the vitamin D score analysis, we did not adjust for smoking, season and physical activity, because the variables were components of the prediction score model.

The proportion of missing values varied from 0·05 % (maternal age) to 1·8 % (pre-pregnancy BMI), and we substituted missing values by the median/mode for the variable in question. All the analyses were carried out using SAS statistical software (version 9.4; SAS Institute). In sensitivity analyses, we adjusted for offspring birth weight and gestational age as continuous variables. When we analysed the association between mid-pregnancy supplementary intake of vitamin D, we adjusted for the supplementary intake of Ca as a continuous variable in the sensitivity analysis.

Results

In total, 24 172 children from the DNBC were registered with at least one bone fracture in the DNPR. Of the fractures caused by accidents (86 %), the main locations of fracture were the elbow and forearm (41 %), and the remaining fractures were in the hand, wrist or fingers (18 %), shoulder or upper arm (17 %), lower leg or ankle (9 %), feet or toes (10 %), skull or face (4 %), thigh and hip (1 %) or other areas of the body (0·3 %). In total, 5614 first-time forearm fractures were recorded when we restricted to forearm fractures from accidents and excluded fractures from traffic accidents. This provided us with 2216 forearm fractures for the analyses in the final study sample of 30 132 mother–child pairs (30 % of the population) with a predicted vitamin D score from the pregnancy (Fig. 1). Mean fracture age among the children was 7·9 (sd 3·2) years (range 0·5–14·1). Background characteristics of the study population are shown in Table 1, along with the distribution of pregnant women according to characteristics and

Table 1. Background characteristics of participants in the Danish National Birth Cohort according to offspring forearm fractures (n 30 132)

	Forearm fractures		
	Yes	No	P*
Maternal age (mean, years)	30·5	30·4	0·77
Pre-pregnancy BMI (mean, kg/m²)	23·8	23·6	0·004
Cohabitation status (%)			0·41
Couple	98·7	98·5	
Single	1·3	1·5	
Occupational status (%)			0·23
High	24·1	23·5	
Medium	31·1	32·3	
Skilled	28·7	27·1	
Student	5·0	5·4	
Unskilled	9·3	10·3	
Unemployed	1·7	1·4	
Physical activity (min/week)			0·061
0	51·0	50·8	
1–44	17·0	16·6	
45–75	11·9	12·0	
75–149	11·3	13·0	
≥150	8·8	7·6	
Maternal smoking (%)			0·81
Non-smoker	77·3	76·6	
Occasional smoker (cigarettes/d)	12·0	12·4	
<15	9·0	9·4	
>15	1·7	1·6	
Parity (%)			<0·001
0	46·5	50·3	
1	39·0	35·1	
2	12·3	12·0	
3+	2·2	2·6	
Child sex (%)			0·92
Boy	50·4	50·5	
Girl	49·6	49·5	
Gestational age (mean, weeks)	280·7	280·5	0·52
Birth weight (mean, g)	3627	3593	0·005

* Overall χ^2 test of effect.

offspring forearm fractures. Women whose offspring experienced forearm fractures were found to be of higher parity, have higher pre-pregnancy BMI and offspring with higher birth weight.

Estimated vitamin D status by prediction scores was not associated with offspring risk of forearm fractures in our data (Table 2), and adjustment for potential confounders did not change this result, just as stratification by sex did not indicate significant differences between boys and girls. When we used the measured vitamin D status in the restricted sample of women with available biomarker measurements, we observed similar results: 25(OH)D concentration was not associated with offspring forearm fractures. Further adjustment for gestational age and birth weight in sensitivity analyses did not change these results (data not shown).

Tanning bed use in pregnancy was reported by 18·3 % of the study population, and 7·8 % used tanning beds in both the first and the second trimester. No associations were found between the use of tanning beds and offspring risk for forearm fractures (Table 2). The results were unchanged when we analysed the use during the first and second trimester separately and when we stratified by season (data not shown). With regard to season of birth, there was a borderline significant increased risk of

Table 2. Hazard ratios (HR) of offspring forearm fractures in Danish National Birth Cohort according to different sources of vitamin D during pregnancy
(Hazard ratios and 95 % confidence intervals; mean values and standard deviations)

	n	Cases	Crude		Adjusted		Boys		Girls	
			HR	95 % CI	HR*	95 % CI	HR*	95 % CI	HR*	95 % CI
Vitamin D scores (µg/d)										
Q1 (39·1 (SD 31·2))	6030	435	1·00		1·00		1·00		1·00	
Q2 (51·1 (SD 2·2))	5990	444	1·03	0·90, 1·17	1·03	0·91, 1·18	1·10	0·91, 1·32	0·97	0·80, 1·17
Q3 (58·6 (SD 2·2))	6064	469	1·07	0·94, 1·22	1·08	0·95, 1·23	1·13	0·94, 1·36	1·03	0·86, 1·24
Q4 (66·7 (SD 2·7))	6036	415	0·96	0·84, 1·10	0·97	0·85, 1·12	1·07	0·88, 1·29	0·88	0·73, 1·07
Q5 (81·3 (SD 8·2))	6012	453	1·07	0·94, 1·22	1·09	0·96, 1·25	1·03	0·85, 1·25	1·15	0·96, 1·38
P (class)†			0·42		0·35		0·70		0·090	
P (cont)‡			0·28		0·15		0·47		0·21	
Tanning bed use										
No use	24 602	1798	1·00		1·00		1·00		1·00	
<4 weeks	2852	223	1·08	0·94, 1·24	1·10	0·96, 1·27	1·05	0·86, 1·28	1·17	0·96, 1·42
4–12 weeks	1556	120	1·06	0·88, 1·27	1·08	0·89, 1·29	0·99	0·76, 1·30	1·15	0·89, 1·49
>12 weeks	1122	75	0·91	0·73, 1·15	0·93	0·74, 1·18	0·84	0·59, 1·19	1·02	0·75, 1·40
P (class)†			0·56		0·42		0·73		0·35	
P (cont)‡			0·36		0·46		0·35		0·90	
Season of birth										
Dec/Jan/Feb	7088	511	1·04	0·92, 1·17	1·04	0·92, 1·18	1·00	0·84, 1·19	1·08	0·91, 1·29
Mar/Apr/May	7056	492	1·00		1·00		1·00		1·00	
Jun/Jul/Aug	7887	624	1·12	0·99, 1·26	1·12	1·00, 1·26	1·17	0·99, 1·39	1·07	0·90, 1·27
Sep/Oct/Nov	8101	589	1·03	0·92, 1·17	1·04	0·92, 1·17	1·10	0·93, 1·31	0·98	0·82, 1·16
P (class)†			0·28		0·27		0·15		0·54	
Dietary vitamin D (µg/d)										
Q1 (1·6 (SD 0·6))	5986	422	1·00		1·00		1·00		1·00	
Q2 (2·1 (SD 0·1))	6132	440	1·01	0·88, 1·15	1·00	0·88, 1·15	1·23	1·02, 1·49	0·82	0·68, 0·99
Q3 (2·7 (SD 0·2))	5894	434	1·02	0·89, 1·17	1·02	0·89, 1·17	1·17	0·96, 1·42	0·89	0·75, 1·08
Q4 (3·6 (SD 0·3))	6064	474	1·10	0·96, 1·25	1·10	0·97, 1·26	1·30	1·08, 1·58	0·94	0·78, 1·13
Q5 (6·1 (SD 2·2))	5945	439	1·05	0·91, 1·19	1·06	0·92, 1·21	1·13	0·93, 1·38	1·00	0·83, 1·21
P (class)†			0·62		0·54		0·078		0·39	
P (cont)‡			0·39		0·28		0·53		0·37	
Supplementation of vitamin D, peri-conceptional (µg/d)										
0	1685	102	1·00		1·00		1·00		1·00	
0–5	11 654	852	1·21	0·98, 1·48	1·20	0·98, 1·48	1·21	0·90, 1·63	1·20	0·90, 1·60
>5	5296	392	1·23	0·99, 1·54	1·23	0·99, 1·53	1·16	0·84, 1·59	1·30	0·96, 1·77
P (class)†			0·15		0·17		0·43		0·21	
P (cont)‡			0·19		0·22		0·51		0·30	
Supplementation of vitamin D, mid-pregnancy (µg/d)										
0	2576	173	1·00		1·00		1·00		1·00	
>0–5	10 159	737	1·08	0·91, 1·27	1·08	0·91, 1·28	1·09	0·87, 1·37	1·08	0·84, 1·37
>5–10	13 478	988	1·10	0·94, 1·29	1·11	0·95, 1·31	1·02	0·81, 1·27	1·22	0·96, 1·54
>10	2028	172	1·30	1·05, 1·60	1·31	1·06, 1·62	1·16	0·86, 1·56	1·48	1·10, 2·00
P (class)†			0·086		0·071		0·59		0·17	
P (cont)‡			0·029		0·019		0·45		0·013	

Q, quintile.
* Adjusted for maternal age, parity, sex of the child, cohabitation, pre-pregnancy BMI, smoking, occupational status, season and physical activity. (Analyses on vitamin D scores are not adjusted for smoking, season and physical activity; analyses on season of birth are not adjusted for season; boys and girls, respectively, are not adjusted for sex of the child.)
† Overall χ^2 test of effect.
‡ Continuous values of vitamin D status in the Cox regression model.

forearm fractures for offspring born in Jun/Jul/Aug *v.* Mar/Apr/May. However, stratification by sex revealed that the association was present only for boys (Table 2).

Dietary intake of vitamin D in mid-pregnancy was not associated with the risk of offspring forearm fractures (Table 2). However, stratification by sex indicated that boys in the second and fourth quintile had a significantly higher HR compared with boys in the first quintile, although when data were analysed as continuous no significant association was found ($P = 0.53$). According to our data, the dietary intake of vitamin D during pregnancy was relatively low (median 2·66 (5th–95th percentile 1·43 – 6·67) µg/d).

Data on supplement use in the peri-conceptional period was available for 62 % of the study population, of whom 91 % took at least one dietary supplement with vitamin D, which provided a median of 5·0 (5th–95th percentile 1·25 – 10·00) µg/d of vitamin D. In both the crude ($P = 0.057$) and the adjusted analysis ($P = 0.063$), there was a borderline significant association between intakes >5·0 µg/d *v.* zero intake and the risk of offspring forearm fractures (Table 2), so that supplementation with vitamin D in the peri-conceptional period increased the risk for fractures. Stratification by sex did not indicate sex-specific effects, but daughters of women with an intake above 5·0 µg/d had a tendency for increased risk of fractures

($P = 0.084$). However, when analysed as a continuous variable, there was no significant association between supplemental vitamin D intake in the peri-conceptional period and fracture risk.

In mid-pregnancy, 91 % of women with dietary supplement information available took one or more dietary supplements with vitamin D, which provided a daily median intake of 10 (5th–95th percentile $4.46 – 15.00$) μg/d among supplement users. Mid-pregnancy supplemental vitamin D intake was significantly associated with the risk of offspring forearm fractures (Table 2). Offspring of women who took >10 μg of vitamin D/d had a significant higher risk of forearm fractures compared with the reference level of zero intake (HR 1·31; 95 % CI 1·06, 1·62). Stratifying by sex revealed that the significant association was present only for girls (HR 1·48; 95 % CI 1·10, 2·00), whereas no significant association was found for boys (HR 1·16; 95 % CI 0·86, 1·56). When we further adjusted for intake of Ca from dietary supplements, the risk estimate increased for girls (HR 1·67; 95 % CI 1·20, 2·32), which indicated that the association was independent of the supplementary intake of Ca. When we stratified for season in the analysis, we found that the higher risk of forearm fractures was present only in girls born during winter (HR 2·06; 95 % CI 1·14, 3·74), whereas no association was found for girls born during summer (HR 1·15; 95 % CI 0·67, 1·96). For boys, no significant associations where found between zero intake and a supplemental intake >10 μg of vitamin D/d, both during summer (HR 1·04; 95 % CI 0·61, 1·79) and winter (HR 1·18; 95 % CI 0·58, 2·40).

Discussion

Overall, there was no association between maternal vitamin D status and offspring risk of forearm fractures during childhood in the DNBC. In the supplementary analyses, we analysed the role of vitamin D sources from the prediction score model, but again we found no indication of a protective effect of vitamin D-related exposures on offspring forearm fracture risk; neither maternal use of tanning bed nor dietary or supplementary intake of vitamin D decreased the risk of offspring forearm fractures. However, contrary to our hypothesis, we found a positive association between maternal vitamin D supplementation and offspring forearm fracture risk.

Strengths and limitations

The strengths of the present study include the large study sample and the availability of data on several maternal sources of vitamin D. In our study, we examined both the overall predicted status of vitamin D, use of a tanning bed and intake from diet and dietary supplements. Furthermore, for our outcome measure, we used data on fractures with high validity[26,27]. A major limitation of the study is the lack of information about offspring weight and height, which may be related both to maternal vitamin D status and to offspring bone mass. Likewise, we do not have information about offspring vitamin D status, physical activity level, sunlight exposure or

diet during childhood, thus our results may be confounded when those data are missing.

We cannot preclude misclassification of vitamin D status in our analyses, as we used predicted levels of vitamin D status and not measured levels of 25(OH)D. However, a study examining only the association with 25(OH)D would for one be very costly and second would use highly valuable and limited biomaterial, making it an unrealistic goal. The predicted status of vitamin D allows us to conduct a study with the present purpose on a large study sample, and still maintain a high data quality. In an exposure–disease approach using predicted vitamin D scores instead of biomarker measurements, we must expect random measurement error to attenuate the measure of association; however, the potential loss in precision may be recovered by the larger study sample available when using predicted vitamin D status[30]. Other studies defining prediction models of vitamin D status showed 25(OH)D concentrations to be predicted quite accurately by a range of demographic and lifestyle factors[30,33]. Several studies have been published using predicted vitamin D scores when testing exposure–disease hypotheses[30,34–37], and the method seems to be broadly accepted for an epidemiological approach in large cohort studies. In general, the experiences all indicate that, although the prediction scores are not appropriate for use on an individual level, they are suitable for ranking groups with respect to high- and low-exposure categories[30]. On this basis, the prediction scores in the present study were analysed as quintiles and not as defined status groups according to guidelines from health agencies.

Possible confounding in relation to vitamin D supplementation

Contrary to our hypothesis, we found that supplement intake of vitamin D in mid-pregnancy was associated with higher forearm fracture risk, but stratification by sex and season revealed that the increased risk was only present for girls born during winter. The analysis on supplement intake of vitamin D from the peri-conceptional period indicated a similar pattern: girls had a borderline increased risk for fractures if the mother took >5 μg/d of vitamin D in the peri-conceptional period; however, there was no linear trend. Information on supplement use in the peri-conceptional period was not available for the entire study sample. The highest intake during the peri-conceptional period was only >5 μg/d, whereas it was >10 μg/d in mid-pregnancy, and this discrepancy may have affected our results. Almost all women who reported taking a dietary supplement took mixed supplements with other nutrients, such as multivitamins. During the recruitment period, dietary supplements sold on the Danish market and intended for pregnant women mostly contained 5 or 10 μg of vitamin D. If a woman took >10 μg/d of vitamin D, we believe it to be a reflection of her having doubled or tripled the recommended dose, meaning that she was likely to have also increased her dose of other vitamins and minerals to very high intake levels. Therefore, our results may reflect the effect of other nutrients – for example, vitamin A – which seems to be associated with increased risk of bone fractures in adults, although the results are conflicting[38].

In sensitivity analyses, we adjusted for mid-pregnancy intake of Ca from dietary supplements, which if anything tended to strengthen an association for vitamin D intake, indicating that our finding of a direct association between supplemental intake of vitamin D in mid-pregnancy and risk of offspring forearm fractures was not caused or biased by the intake of Ca. Review of the literature has concluded that there are no effects of vitamin D supplementation on bone fracture risk among adults, when the vitamin is taken as a single vitamin without Ca[39]. A double-blinded placebo-controlled trial on older women aged 70+ years actually showed an increased risk of falls and fractures when women received each year, for 3–5 years (in autumn or winter), an annual high dose (12·5 mg) of oral vitamin D as a single vitamin without Ca[40]. The results are not comparable with our results in young people, but they indicate that there might be mechanisms and effects of vitamin D supplementation, which still need to be examined. Randomised controlled trials (RCT) on vitamin D supplementation alone are limited, and especially in pregnancy. Further studies are required to evaluate the role of vitamin D supplementation on fetal and offspring health[41].

Our finding of a direct association between maternal supplemental vitamin D in mid-pregnancy and offspring bone fractures may be confounded by the underlying differences between women who reported high doses of dietary supplements *v.* women who did not report use of dietary supplements during pregnancy. A previous study from the DNBC (n 68 447) showed that women taking low *v.* high doses were different with respect to several characteristics[42]. Women with the highest dietary intake of vitamin D were also those with the highest supplementary intake. The determinants for a sufficient intake ($\geq 10\mu g/d$) were high maternal age, nulliparity and non-smoking, all variables that we adjusted for in our analyses.

Hypotheses for a negative effect of vitamin D supplementation during pregnancy

At Northern latitudes, everyday life during summer is quite different from that of winter, because of major changes in weather and sunlight exposure. The seasonal variation seems for instance to influence the levels of both melatonin, vitamin D, serotonin and cortisol in humans[43–47]. It has been suggested that supplementation with vitamin D can bring a 'message of light' to the pineal gland and consequently decrease melatonin synthesis during night[48]. Melatonin crosses the placenta freely without being altered[49], and we can therefore speculate that supplementation with vitamin D during sun-deprived periods may disturb melatonin synthesis. It has been hypothesised that interactions between melatonin, vitamin D, serotonin and cortisol during pregnancy may be important in early offspring developmental aetiology of different diseases via their effect on many organs and tissues[50]. Schizophrenia and autoimmune diseases have been the main foci of this hypothesis, but it is likely that melatonin, vitamin D and serotonin also effect offspring bone heath, as they appear to influence skeletal bones in adults[51–54].

Interestingly, a recently published RCT including 446 postmenopausal women found that vitamin D supplementation (27·5 µg/d) for at least 12 months changed the DNA methylation of some of the CYP family genes[55]. The 25(OH)D-1α-hydroxylase enzyme (*CYP27B1*), responsible for conversion of 25(OH)D to 1,25-dihydroxyvitamin D (1,25(OH)$_2$D), the active form of the vitamin, was not part of the examined genes; however, if the gene can also be regulated by vitamin D supplementation during pregnancy, this may influence the fetal level of 1·25(OH)$_2$D, as 1,25(OH)$_2$D does not cross the placenta. Only 25(OH)D crosses the placenta and is converted to 1·25(OH)$_2$D in the kidneys of the fetus. In the concept of fetal programming, expression of genes during pregnancy may be affected by the influence of dietary and environmental factors, with lasting effects on human health[56,57]. We can speculate that *CYP27B1* may be down-regulated during pregnancy to suppress high levels of 1,25(OH)$_2$D if the 25(OH)D status is highly elevated as a consequence of vitamin D supplementation. If this hypothesis is true, the expression of *CYP27B1* in the offspring later during childhood may be down-regulated, and lead to lower bone mass accrual as a consequence of low 1·25(OH)$_2$D levels. This somewhat speculative hypothesis could explain a direct association between vitamin D supplementation during pregnancy and offspring bone fractures.

Concluding remarks

In conclusion, our study showed no association between predicted maternal status of vitamin D in mid-pregnancy and offspring forearm fracture risk. However, contrary to our hypothesis, dietary supplement use during mid-pregnancy, with a vitamin D dose >10 µg/d *v.* zero intake, was associated with increased forearm fracture risk in offspring, and especially among girls. Further investigation into this field is highly needed to clarify whether dietary supplements with vitamin D during pregnancy may have a non-beneficial impact on offspring bone development and health.

management. M. S. supervised the study design, data analysis, interpretation and contributed to writing of the manuscript. All the authors had full access to study data.

None of the authors has any conflicts of interest.

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