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ARTICLE

# Vitamin D concentrations from neonatal dried blood spots and the risk of early-onset type 2 diabetes in the Danish D-tect case-cohort study

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## Abstract

**Aims/hypothesis** The aim of this study was to examine the influence of neonatal vitamin D concentration on the development of early-onset type 2 diabetes in a large population sample.

**Methods** We conducted a case-cohort study utilising data from the Danish biobank and registers. Neonatal vitamin D was assessed measuring 25-hydroxyvitamin $D_3$ [25(OH)$D_3$] concentrations on the dried blood spot samples from the Biological Specimen Bank for Neonatal Screening. Cases of type 2 diabetes ($n = 731$) were retrieved from the Danish National Patient Register for all individuals born in Denmark between 1 May 1981 and 31 December 1992. The sub-cohort ($n = 1765$) was randomly selected from all children born in the same period. We used a weighted Cox proportional hazard model assessing the hazard of first type 2 diabetes diagnoses by quintiles of 25(OH)$D_3$ and restricted cubic spline.

**Results** The median 25(OH)$D_3$ concentration (IQR) among cases was 21.3 nmol/l (13.3–34.1) and 23.9 nmol/l (13.7–35.7) in the sub-cohort. There was no indication of a potential lower risk of early-onset type 2 diabetes among individuals in the higher quintile of vitamin D concentration compared with the lowest ($HR_{crude}$ 0.97 [95% CI 0.71, 1.33] $p = 0.85$; $HR_{adjusted}$ 1.29 [95% CI 0.92, 1.83] $p = 0.14$).

**Conclusions/interpretation** The results of this study do not support the hypothesis that higher neonatal vitamin D concentrations are associated with a lower risk of early-onset type 2 diabetes in adulthood.

**Keywords** 25(OH)D · Case-cohort · Fetal programming · Neonatal · Type 2 diabetes · Vitamin D

**Abbreviations**

| | |
|---|---|
| 1,25(OH)2D | 1,25-dihydroxyvitamin D |
| 25(OH)D | 25-hydroxyvitamin D |
| CPR | Central Personal Register |
| DBSS | Dried blood spot samples |
| DCRS | Danish Civil Registration System |
| DMBR | Danish Medical Birth Register |
| DNPR | Danish National Patient Register |
| LLOQ | Lower limit of quantification |
| SSI | Statens Serum Institut |
| VDR | Vitamin D receptors |

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## Research in context

**What is already known about this subject?**

- The presence of nuclear vitamin D receptors and the vitamin D-activating 1-α-hydroxylase enzyme in the placenta suggest that vitamin D plays an important role during pregnancy and may influence fetal development
- Low fetal or infancy vitamin D may influence later risk of disease development, via so-called early programming

**What is the key question?**

- Is a higher neonatal vitamin D concentration associated with a lower risk of early-onset type 2 diabetes in adulthood?

**What are the new findings?**

- In this study, a higher neonatal vitamin D concentration was not associated with a lower risk of early-onset type 2 diabetes in adulthood

**How might this impact on clinical practice in the foreseeable future?**

- The results of this study suggest that focusing on vitamin D concentration during pregnancy in regard to diabetes development in the offspring might not be essential in clinical practice

## Introduction

Type 2 diabetes is the most common type of diabetes affecting 463 million people globally [1]. Although still considered to be an older-adult disease, it is now increasingly diagnosed among younger adults [2–6]. Furthermore, individuals with early-onset type 2 diabetes more commonly exhibit risk factors such as obesity, smoking and dyslipidaemia, subsequently increasing their risk of developing type 2 diabetes complications compared with elderly newly-diagnosed individuals [7]. Type 2 diabetes is a heterogeneous disease with a multifactorial aetiology occurring as a result of organ dysfunctions in multiple tissues [8]. Many factors, such as economic transition, ageing, urbanisation, unhealthy eating habits, sedentary lifestyle, obesity, (epi)genetic and intrauterine exposures, influence the rising epidemic of type 2 diabetes [9]. Type 2 diabetes is initially managed by lifestyle interventions including increasing exercise and dietary modifications. Among several nutritional factors potentially associated with the risk of developing type 2 diabetes, a number of epidemiological studies have suggested an association between the onset of type 2 diabetes and vitamin D deficiency. However, evidence from intervention studies conducted among adults has yielded inconsistent results [10–12].

Vitamin D is considered to be both a fat-soluble vitamin and a secosteroid hormone which is synthesised in the skin after ultraviolet B (UVB) exposure or is obtained from dietary intake (such as oily fish and dairy products or fortified food and supplements [$D_3$] or vegetables [$D_2$]). After hydroxylation in the liver into 25-hydroxyvitamin D [25(OH)D] and in the kidney into its active form 1,25-dihydroxyvitamin D [1,25(OH)2D], vitamin D can enter the cells, and subsequently bind to vitamin D receptors (VDR) and influence gene transcription [13]. Apart from its role in calcium and phosphate homeostasis, and notably bone mineralisation, vitamin D also has non-classical actions such as regulation of hormone secretions, immune modulating functions as well as influencing cellular proliferation and differentiation processes [14]. VDR have been identified in most organs and tissues such as the beta cells of the pancreas, and some other tissues are also capable of producing 1,25(OH)2D [15]. Low vitamin D levels may therefore play an important role in the susceptibility to many diseases.

Previous studies, showing heterogeneous results, have assessed the association between vitamin D and type 2 diabetes in adult populations only [10–12]. As proposed by Barker [16] and others, a critical window in prenatal development may exist, and hence, transient prenatal vitamin D deficiency restricted to gestation may induce physiological changes leaving the individual vulnerable to the development of vitamin D related disorders later in life [8, 17]. The aim of this case-cohort study was to examine the influence of neonatal vitamin D concentration on the development of early-onset type 2 diabetes. We hypothesised that individuals who developed early-onset type 2 diabetes had lower neonatal vitamin D concentrations than individuals who did not develop early-onset type 2 diabetes.

## Methods

This is a case-cohort study comparing 25(OH)D concentrations in neonatal dried blood spot samples (DBSS) among

individuals who developed early-onset type 2 diabetes and those who did not. The study adheres to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement [18].

## Data sources

In Denmark, all residents are given a unique 10-digit Central Personal Register (CPR) number at time of birth or immigration and are registered in the Danish Civil Registration System (DCRS). The CPR number can be used to identify individuals in other Danish registers and databases [19]. Cases of early-onset type 2 diabetes were identified using the Danish National Patient Register (DNPR), a nationwide registry containing information on all hospital admissions, including dates of admission and discharge diagnoses according to the international classification of diseases (ICD) system [20]. Since 1981, all newborns in Denmark have capillary blood samples taken by heel prick during their first days of life for routine screening of congenital disorders [21]. After screening, residual DBSS are stored at the Biological Specimen Bank for Neonatal Screening at the Statens Serum Institut (SSI) at −20°C in locked freezers. Neonatal 25(OH)D concentrations were measured from DBSS. Information on covariates (see list below) was obtained from the DCRS [19], the Danish Medical Birth Register (DMBR) [22] and Statistics Denmark.

## Study population

**Cases** All individuals born in Denmark between 1 May 1981 and 31 December 1992, alive without a previous type 2 diabetes diagnosis at their 23rd birthday, and followed from age 23 until 10 February 2016, were identified in the DNPR. Early-onset type 2 diabetes was defined based on ICD-10 code: E11 (https://icd.who.int/browse10/2016/en). A total of 736 cases were identified (Fig. 1). The lower age limit of 23 years was chosen to minimise the risk of including individuals with other diabetes (i.e type 1 diabetes) misdiagnosed as type 2 diabetes from the registers.

**Sub-cohort** The DCRS was used to identify all live born children between 1 May 1981 and 31 December 1992 ($N$ = 645,840) from which a sub-cohort of 2550 individuals was randomly selected. The random sub-cohort was sampled conditional on year of birth with more weight given to those born during 1981–1985 so more cases could be captured. These individuals are the basis for our sub-cohort (Fig. 1).

Individuals' CPR numbers were used to retrieve DBSS for vitamin D analysis; 2691 individuals had DBSS with sufficient material for analysis. A complete case analysis excluding individuals with information missing on any covariate ($n$ = 195) was performed. Thus, 731 cases and 1765 individuals from the sub-cohort were included in the final sample (Fig. 1).

Follow-up was available until 2016 only as this study is part of the D-tect project which ran from 2012 to 2016. More up-to-date data are available in the registers, however, it was not feasible to retrieve further data owing to economical and analytical constraints.

## Assessment of vitamin D concentrations

Neonatal serum 25(OH)$D_3$ and 25(OH)$D_2$ concentrations were measured in 3.2 mm punches of neonatal DBSS using a modified version of an LC-MS method [23] by laboratory technicians at the SSI blinded for the outcome and season of birth. Currently, no quality assurance programmes for 25(OH)D measurements from DBSS exist; however, the SSI laboratory participates in the Vitamin D External Quality Assessment Scheme with the equivalent serum analysis method [24]. It has been shown that 25(OH)D from DBSS and cord blood are highly correlated [23]. In addition, minimal inter-individual variation and deterioration of 25(OH)D concentrations from DBSS and serum frozen over a period of 22 to 40 years has been documented [25, 26]. 25(OH)$D_2$ was excluded from the analyses since 87% of the values were below the lower limit of quantification (LLOQ) of 3 nmol/l, whereas all measures of 25(OH)$D_3$, including those below the LLOQ of 4 nmol/l (17%), were included. The coefficient variability for intra-assay and inter-assay for 25(OH)$D_3$ was 7–12% and 7–20%, respectively. The following formula was used to correct 25(OH)$D_3$ concentrations from DBSS and reflect concentrations equivalent to serum concentrations: serum 25(OH)$D_3$ nmol/l = DBSS 25(OH)$D_3$ nmol/l× 1/[1–0.61], where 0.61 is the haematocrit fraction for capillary blood [23].

The $t_{1/2}$ of 25(OH)D is approximately 2–3 weeks [27] and the fetus is completely dependent on maternal 25(OH)D supply [28], therefore it is expected that 25(OH)D concentrations at birth reflect, as a minimum, fetal 25(OH)D exposure during the end of the third trimester of pregnancy.

## Covariates

The following covariates were selected a priori: offspring sex (female, male), season of birth (continuous effect using a periodic function), birthweight (continuous, grams), gestational age (preterm <37 weeks, term ≥37 weeks), Caesarean section (yes, no), maternal age at time of delivery (continuous, years), maternal ethnicity (European, non-European), maternal highest obtained education (school, high school, university), parity (primiparous, multiparous) and maternal smoking during pregnancy (yes, no). Information on child's sex and date of birth was obtained from DCRS [19]. Birthweight, gestational age, parity, Caesarean section, maternal age, and

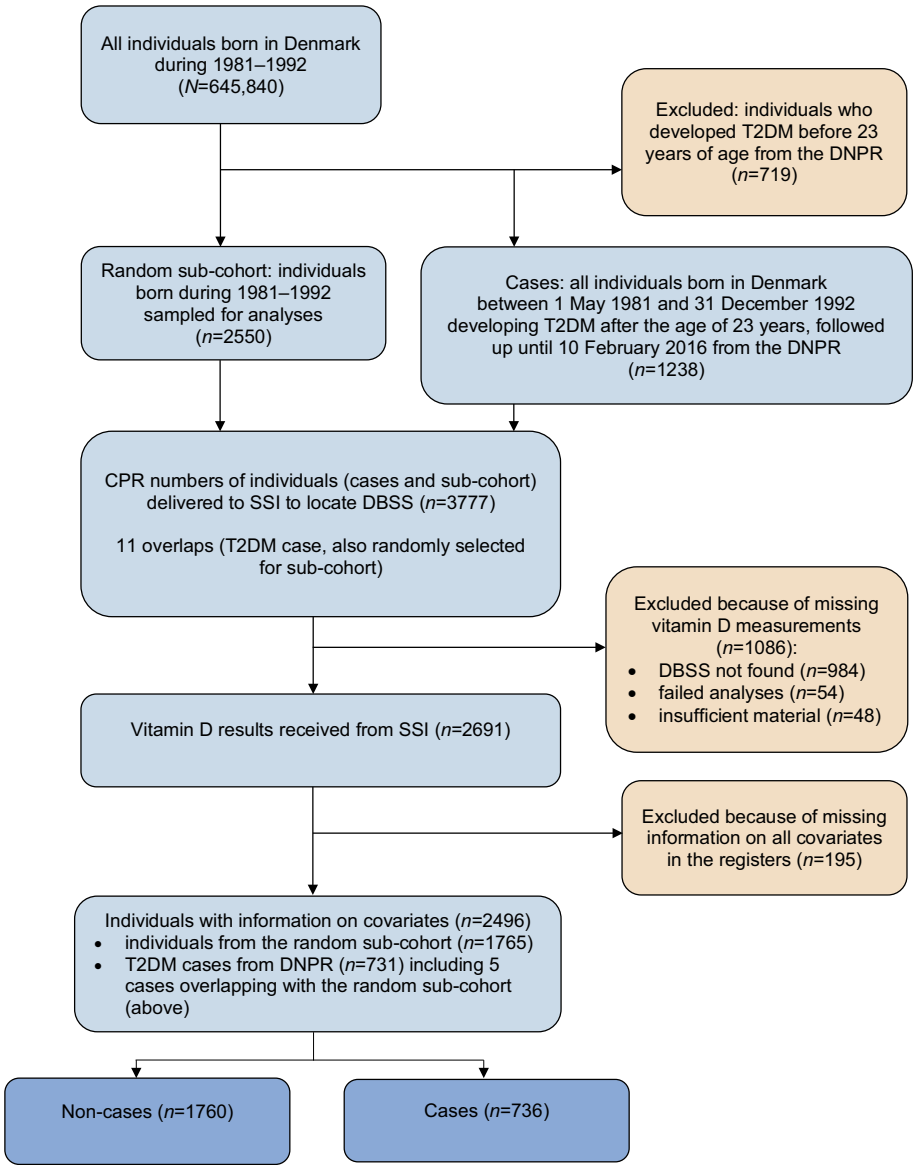


**Fig. 1** Flow chart of study population. T2DM, type 2 diabetes mellitus

smoking were retrieved from the DMBR [22]. Information on maternal education and ethnicity was obtained from Statistics Denmark.

## Statistical analysis

Characteristics of the study population for cases and sub-cohort are presented as number (n) and percentages (%) for categorical variables, and mean ± SD and median with IQR for continuous variables.

Cox regression model with the cases and non-cases weighted by their cohort specific inverse sampling probabilities was used [29] (electronic supplementary material [ESM] Table 1).

Using the Cox proportional hazard model with age as the underlying time variable, stratified by year of birth, we assessed the hazard of first early-onset type 2 diabetes diagnoses between ages 23 and 34 years and 8 months (34.7 years) by quintiles of $25(OH)D_3$, to capture a potential non-linear relationship, using the first quintile as reference. The results are presented as HR and 95% CI. In the adjusted model, we adjusted for potential confounders identified a priori using a directed acyclic graph (ESM Fig. 1). We tested for overall (no) association using Wald tests with 4 *df*. In addition, we conducted restricted cubic spline analysis with 3 knots at 7.5 nmol/l, 23.59 nmol/l and 50.23 nmol/l representing the 10th, 50th and 90th percentiles, respectively. In post hoc sensitivity analyses, we also assessed the hazard of first early-onset type 2 diabetes diagnoses between ages 23 and 34 years and 8 months (34.7 years) using standard cut-offs of vitamin D status (deficient: <50 nmol/l vs not deficient: ≥ 50 nmol/l).

**Table 1** Baseline characteristics of the study population

| | Cases ($n$=731) | Random sub-cohort ($n$=1765)[a] |
|---|---|---|
| Continuous variables | | |
| 25(OH)$D_3$, nmol/l; mean (SD) | 25.0 (17.0) | 26.7 (17.1) |
| 25(OH)$D_3$, nmol/l; median (IQR) | 21.3 (13.3, 34.1) | 23.9 (13.7, 35.7) |
| Birthweight, g; mean (SD) | 3319 (624) | 3442 (566) |
| Birthweight, g; median (IQR) | 3300 (2950, 3700) | 3450 (3100, 3800) |
| Maternal age, years; mean (SD) | 26.0 (5.0) | 27.4 (4.8) |
| Maternal age, years; median (IQR) | 26.0 (22.0, 29.0) | 27.0 (24.0, 30.0) |
| Age at follow-up, years; mean (SD) | 27.3 (2.7) | 28.9 (3.3) |
| Age at follow-up, years; median (IQR) | 26.8 (25.0, 29.0) | 28.9 (26.0, 31.7) |
| Categorical variables; n (%) | | |
| Quintiles of 25(OH)$D_3$, nmol/l | | |
| Q1 | 160 (22.0) | 339 (19.2) |
| Q2 | 154 (21.1) | 349 (19.8) |
| Q3 | 158 (21.6) | 362 (20.5) |
| Q4 | 119 (16.3) | 353 (20.0) |
| Q5 | 140 (19.2) | 362 (20.5) |
| Year of birth | | |
| 1981 | 53 (7.3) | 68 (3.9) |
| 1982 | 98 (13.4) | 156 (8.8) |
| 1983 | 109 (14.9) | 167 (9.5) |
| 1984 | 81 (11.1) | 168 (9.5) |
| 1985 | 88 (12.0) | 194 (11.0) |
| 1986 | 81 (11.1) | 127 (7.2) |
| 1987 | 76 (10.4) | 136 (7.7) |
| 1988 | 52 (7.1) | 167 (9.5) |
| 1989 | 30 (4.1) | 136 (7.7) |
| 1990 | 38 (5.2) | 191 (10.8) |
| 1991 | 23 (3.2) | 131 (7.4) |
| 1992 | 2 (0.3) | 124 (7.0) |
| Season of birth | | |
| Winter | 363 (49.7) | 877 (49.7) |
| Summer | 368 (50.3) | 888 (50.3) |
| Maternal education | | |
| Primary school | 450 (61.6) | 687 (38.9) |
| High school | 219 (30.0) | 736 (41.7) |
| University | 62 (8.5) | 342 (19.4) |
| Maternal ethnicity | | |
| European | 701 (95.9) | 1728 (97.9) |
| Non-European | 30 (4.1) | 37 (2.1) |
| Maternal smoking status[b] | | |
| Non-smoking | 11 (1.5) | 150 (8.5) |
| Smoking | 13 (1.8) | 80 (4.5) |
| Missing | 707 (96.7) | 1535 (87.0) |
| Parity | | |
| Primiparous | 335 (45.8) | 823 (46.6) |
| Multiparous | 396 (54.2) | 942 (53.4) |
| Offspring sex | | |
| Male | 338 (46.2) | 908 (51.4) |
| Female | 393 (53.8) | 857 (48.6) |
| Preterm | | |
| No | 685 (93.7) | 1690 (95.8) |
| Yes | 46 (6.3) | 75 (4.2) |
| Caesarean section | | |
| Yes | 24 (3.3) | 48 (2.7) |
| No | 707 (96.7) | 1717 (97.3) |

[a] Descriptive characteristics for all sub-cohort individuals, including five cases with early-onset type 2 diabetes

[b] Maternal smoking available from 1991 from the DMBR

We checked the difference between individuals included in the analysis and excluded because of missing data on covariates using $\chi^2$ test and t test. We did not adjust for smoking owing to the large percentage of missing data, as maternal smoking data were available from 1991 only, from the DMBR. We did not adjust for season of birth in our main

model, as our aim was to assess the association between neonatal vitamin D concentrations and early-onset type 2 diabetes, regardless of the source of vitamin D; however, we ran a sensitivity analysis where a potential effect of day of birth was modelled using a cosinor with a yearly period. We further tested sex and maternal education interactions with $25(OH)D_3$ quintiles using Wald tests and conducted stratified analyses by sex and maternal education. We also conducted analysis, excluding all individuals of non-European mothers, to see if the association was modified by ethnicity ($n = 67$, 2.7%). Furthermore, we excluded siblings and individuals with missing information about siblings ($n = 15$, 0.6%) to see if the potential violation of the independency assumption affected the SE of our estimates. In addition, analyses adjusted for maternal age, maternal ethnicity, maternal education, offspring sex and parity only, and excluding gestational age, birthweight and Caesarean section from the model, were also conducted as these three variables may be mediators rather than confounders. Post hoc restricted cubic spline analyses stratified by maternal education levels were also conducted.

Stata version 15 (StataCorp, College Station, TX, USA, www.stata.com) and R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria, www.R-project.org) was used to perform all statistical analyses. The statistical tests were two-sided at a 5% significance level.

## Ethical considerations

Permission to conduct the study was granted by the Ethical Committee of the Capital Region of Denmark (J. no. H-3-2011-126). The steering committee for scientific use of the Biological Specimen Bank for Neonatal Screening granted permission to access and analyse the DBSS. Permission to use register data was granted by the Danish Health Data Authority and Statistics Denmark. The Danish Data Protection Agency provided permission to process data (J. no. 2012-41-1156). The study is part of the D-tect project which is registered at www.clinicaltrials.gov (NCT03330301).

## Results

Characteristics of the 731 individuals with early-onset type 2 diabetes and the 1765 individuals from the random sub-cohort are presented in Table 1. Individuals were aged between 23 and 34.7 years. Overall, the $25(OH)D_3$ concentrations in our study were low, with a median $25(OH)D_3$ concentration of 21.3 nmol/l (IQR 13.3–34.1) among cases and 23.9 nmol/l (IQR 13.7–35.7) among individuals from the sub-cohort. The mean 25(OH)D concentration by covariates is presented in ESM Table 2. Characteristics of those included ($n = 2496$) in the analysis and those excluded ($n = 195$) from the analysis owing to missing information on covariates are presented in ESM Table 3. When comparing those included with those excluded, we found that the $25(OH)D_3$ concentration was lower among those excluded (15.9 nmol/l, IQR 7.9–30.5) than among those included (23.3 nmol/l, IQR 13.6–35.4) ($p < 0.001$), and especially among excluded cases (14.4 nmol/l, IQR 7.4–27.2).

There was no indication of a potential lower risk of early-onset type 2 diabetes among individuals in the higher quintile of vitamin D concentration compared with the lowest ($HR_{crude}$ 0.97 [95% CI 0.71, 1.33] $p = 0.85$; $HR_{adjusted}$ 1.29 [95% CI 0.92, 1.83] $p = 0.14$) (Table 2). Results from the restricted cubic spline analysis showed a slight inverted U-shaped to flat association between neonatal $25(OH)D_3$ concentration and HR of developing early-onset type 2 diabetes (Wald $df = 2$, $p = 0.68$) (Fig. 2). There were no significant interactions between categories of $25(OH)D_3$ concentration and sex (all $p > 0.28$) (data not shown). Consistently, in analyses stratified by sex, similar estimates to the main analyses were found. There were interactions between the 2nd ($p = 0.02$), the 4th ($p = 0.03$) and 5th ($p = 0.01$) quintiles of $25(OH)D_3$

**Table 2** Unadjusted and adjusted HR (95% CI) of early-onset type 2 diabetes among Danish adults (23–34.7 years), according to quintiles of neonatal $25(OH)D_3$ concentrations (nmol/l)

| $25(OH)D_3$ | Crude model ($n$=2496) | | | | Adjusted model[a] ($n$=2496) | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | SE | 95% CI | $p$ value | HR | SE | 95% CI | $p$ value |
| Q1 (0.0–12.0) | Ref | | | | Ref | | | |
| Q2 (12.1–19.1) | 0.96 | 0.15 | 0.71, 1.29 | 0.76 | 1.10 | 0.18 | 0.80, 1.52 | 0.55 |
| Q3 (19.2–27.5) | 0.92 | 0.14 | 0.69, 1.24 | 0.58 | 1.15 | 0.19 | 0.83, 1.58 | 0.40 |
| Q4 (27.6–38.9) | 0.73 | 0.12 | 0.53, 1.00 | 0.05 | 0.91 | 0.16 | 0.65, 1.28 | 0.59 |
| Q5 (39.0–199) | 0.97 | 0.16 | 0.71, 1.33 | 0.85 | 1.29 | 0.23 | 0.92, 1.83 | 0.14 |
| Wald test | | | | 0.30 | | | | 0.30 |

HR calculated by weighted Cox regression analysis

[a] Adjusted for maternal age, maternal ethnicity, maternal education, offspring sex, parity, gestational age in days, birthweight, Caesarean section

concentration and tertiary maternal education (university). Consistently, when stratifying by maternal education, a higher 25(OH)$D_3$ concentration among offspring of highly educated mothers (university) was associated with an increased risk of early-onset type 2 diabetes (5th quintile: $HR_{crude}$ 4.00 [95% CI 1.33, 12.03] $p = 0.01$; $HR_{adjusted}$ 6.53 [95% CI 1.98, 21.55] $p = 0.002$) compared with offspring of highly educated mothers in the lowest quintile of vitamin D (Table 3). Results from the stratified restricted cubic spline analyses supported these findings, although the results were not statistically significant (ESM Fig. 2 a,b,c).

In sensitivity analyses dichotomising 25(OH)$D_3$ concentration < or ≥50 nmol/l, individuals with a neonatal vitamin D status ≥50 nmol/l tended to have a lower risk of developing early-onset diabetes compared with vitamin D deficient individuals ($HR_{crude}$ 0.75 [95% CI 0.53, 1.06] $p = 0.11$; $HR_{adjusted}$ 0.86 [95% CI 0.59, 1.25] $p = 0.42$), however, the results were not statistically significant (ESM Table 4). Sensitivity

**Table 3** Unadjusted and adjusted HR (95% CI) of early-onset type 2 diabetes among Danish adults (aged 23–34.7 years), according to quintiles of neonatal 25(OH)$D_3$ concentrations (nmol/l) stratified by sex and maternal education

| 25(OH)$D_3$ quintiles (nmol/l) | Crude model | | | | Adjusted model[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | SE | 95% CI | *p* value | HR | SE | 95% CI | *p* value |
| Men (*n*=1246)[b] | | | | | | | | |
| Q1 (0.0–11.7) | Ref | | | | Ref | | | |
| Q2 (11.8–18.7) | 1.07 | 0.24 | 0.70, 1.64 | 0.76 | 1.22 | 0.29 | 0.77, 1.95 | 0.39 |
| Q3 (18.8–27.0) | 0.94 | 0.22 | 0.60, 1.48 | 0.80 | 1.19 | 0.29 | 0.74, 1.91 | 0.48 |
| Q4 (27.1–38.9) | 0.72 | 0.17 | 0.45, 1.14 | 0.16 | 0.87 | 0.22 | 0.54, 1.42 | 0.58 |
| Q5 (39.0–114.9) | 1.06 | 0.25 | 0.67, 1.69 | 0.80 | 1.52 | 0.39 | 0.92, 2.51 | 0.11 |
| Women (*n*=1250)[b] | | | | | | | | |
| Q1 (0.0–11.2) | Ref | | | | Ref | | | |
| Q2 (11.3–18.1) | 0.82 | 0.18 | 0.53, 1.26 | 0.36 | 0.85 | 0.21 | 0.53, 1.37 | 0.51 |
| Q3 (18.2–26.6) | 0.97 | 0.21 | 0.63, 1.49 | 0.89 | 1.17 | 0.28 | 0.74, 1.86 | 0.50 |
| Q4 (26.7–37.4) | 0.67 | 0.15 | 0.42, 1.04 | 0.08 | 0.81 | 0.21 | 0.50, 1.34 | 0.42 |
| Q5 (37.5–199.0) | 0.89 | 0.20 | 0.57, 1.39 | 0.60 | 1.12 | 0.29 | 0.69, 1.85 | 0.63 |
| School (*n*=1137)[c] | | | | | | | | |
| Q1 (0.0–9.8) | Ref | | | | Ref | | | |
| Q2 (9.9–16.1) | 1.10 | 0.24 | 0.71, 1.69 | 0.67 | 1.12 | 0.26 | 0.71, 1.76 | 0.62 |
| Q3 (16.2–24.3) | 1.20 | 0.27 | 0.78, 1.87 | 0.41 | 1.35 | 0.35 | 0.85, 2.12 | 0.20 |
| Q4 (24.4–35.6) | 0.78 | 0.18 | 0.50, 1.21 | 0.27 | 0.92 | 0.22 | 0.58, 1.47 | 0.73 |
| Q5 (35.7–105.4) | 0.99 | 0.23 | 0.63, 1.56 | 0.98 | 1.21 | 0.31 | 0.74, 1.99 | 0.41 |
| High school (*n*=955)[c] | | | | | | | | |
| Q1 (0.0–13.2) | Ref | | | | Ref | | | |
| Q2 (13.3–20.7) | 1.02 | 0.27 | 0.60, 1.72 | 0.95 | 1.04 | 0.29 | 0.61, 1.81 | 0.87 |
| Q3 (20.8–28.7) | 0.82 | 0.23 | 0.47, 1.44 | 0.50 | 0.86 | 0.25 | 0.49, 1.52 | 0.60 |
| Q4 (28.8–39.6) | 1.21 | 0.34 | 0.70, 2.10 | 0.49 | 1.30 | 0.38 | 0.74, 2.30 | 0.36 |
| Q5 (39.7–199.0) | 1.21 | 0.34 | 0.69, 2.11 | 0.50 | 1.22 | 0.36 | 0.68, 2.19 | 0.51 |
| University (*n*=404)[c] | | | | | | | | |
| Q1 (1.8–15.5) | Ref | | | | Ref | | | |
| Q2 (15.6–23.6) | 3.81 | 2.09 | 1.30, 11.17 | 0.02 | 4.94 | 3.00 | 1.50, 16.25 | 0.01 |
| Q3 (23.7–31.6) | 2.80 | 1.72 | 0.85, 9.29 | 0.09 | 3.15 | 2.15 | 0.83, 11.99 | 0.09 |
| Q4 (31.7–44.1) | 2.72 | 1.54 | 0.90, 8.26 | 0.08 | 2.86 | 1.95 | 0.76, 10.86 | 0.12 |
| Q5 (44.2–114.8) | 4.00 | 2.25 | 1.33, 12.03 | 0.01 | 6.53 | 3.98 | 1.98, 21.55 | 0.002 |

HR calculated by weighted Cox regression analysis

[a] Adjusted for maternal age, maternal ethnicity, maternal education, offspring sex, parity, preterm birth, birthweight, Caesarean section

[b] As main model, but excluding adjustment for offspring sex in the adjusted model

[c] As main model, but excluding adjustment for maternal education in the adjusted model

Q, quintile

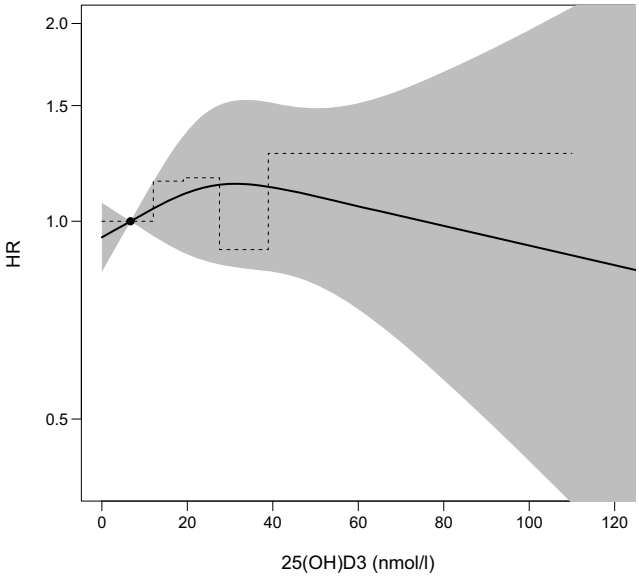


**Fig. 2** Cubic spline model of the adjusted HR (95% CI) of developing early-onset type 2 diabetes and neonatal 25(OH)$D_3$ concentrations. Adjusted for maternal age, maternal ethnicity, maternal education, offspring sex, parity, gestational age in days, birthweight and Caesarean section. Dashed line represents 25(OH)$D_3$ quintiles

analyses adjusted for season of birth or excluding gestational age, birthweight and Caesarean section from the adjusted model showed similar estimates to the main analyses (ESM Table 5). Excluding siblings and children whose mothers had non-European ethnicity from the analyses gave similar estimates to the main analyses (ESM Table 6).

## Discussion

To our knowledge, this study is the first to report on the long-term association between neonatal 25(OH)D concentration and the risk of developing early-onset type 2 diabetes in adulthood. Our results do not support the hypothesis that higher neonatal vitamin D concentration is associated with a lower risk of early-onset type 2 diabetes in adulthood.

One previous longitudinal study conducted in India has reported an association between low concentrations of 25(OH)D in pregnant mothers and higher fasting insulin concentrations and insulin resistance in their children at 9.5 years [30]. As glucose homeostasis variables tend to track from childhood to adulthood [31], 25(OH)D concentration during fetal life might influence glucose homeostasis variables in childhood and predict the development of type 2 diabetes in adulthood. However, our results were not in support of this.

The question remains whether vitamin D is significant to the risk of type 2 diabetes development. Previous studies on the association between vitamin D measured in adulthood and type 2 diabetes have shown contradictive findings. Indeed, while multiple longitudinal studies have shown an association between vitamin D deficiency and risk of type 2 diabetes, most prospective RCTs in adults did not show an effect of vitamin D supplementation on type 2 diabetes development [32–34]. In regard to diabetes treatment, evidence from meta-analyses of RCTs suggests that, among diabetic individuals, vitamin D supplementation has beneficial effects on different biomarkers of diabetes such as insulin resistance [11, 35], fasting glucose [35] and $HbA_{1C}$ [36]. However, from a recent systematic review of meta-analyses and RCTs, it was concluded that vitamin D supplementation did not show any benefit on glucose metabolism biomarkers or on diabetes progression, regardless of individuals' 25(OH)D status at baseline [37]. While observational studies may suffer from confounding bias, most RCTs were of short duration, with small numbers of patients and small doses of vitamin D supplementation, which may have impaired their results. Alternatively, it has been argued that null results from RCTs might be obtained because irreversible insult may have occurred during a critical period or over a long period of deprivation, before initiation of the trial [38]. However, in regard to neonatal vitamin D concentration or vitamin D status during pregnancy and offspring's risk of type 1 diabetes development, two recent case-cohort studies concluded that vitamin D concentrations around the time of birth were not associated with later type 1 diabetes [39, 40]; while another cohort study found that both a higher maternal vitamin D binding protein level and 25(OH)D concentration at delivery were associated with a lower risk of offspring type 1 diabetes risk, depending on VDR genotype [41]. In addition, two systematic reviews and meta-analyses of observational studies have concluded that vitamin D intake in early childhood may offer protection against the development of type 1 diabetes [42, 43].

In the present study, in sub-analysis of the offspring of highly educated women we found that a higher vitamin D concentration at birth may be associated with an increased risk of early-onset type 2 diabetes in adulthood. This latter finding is counterintuitive as offspring of mothers with a higher educational achievement had a lower risk of developing early-onset type 2 diabetes (data not shown) and the mean neonatal 25(OH)D concentration increased with higher maternal educational achievement (ESM Table 2). Also, there is an inverse association between educational level and incident type 2 diabetes in Europe [44], suggesting that this sub-result was a chance finding.

Hence, the role of low vitamin D in early life in the development of diabetes in adulthood remains unclear, and more studies on the effect of pre- and neonatal vitamin D for the development of type 2 diabetes are warranted.

### Strengths and limitations

The main strengths of our study lie in its large sample size, with the inclusion of children randomly selected from the

entire population born in Denmark from 1981 to 1992, and the use of a measured biomarker of vitamin D concentrations. Use of DBSS instead of plasma or sera has been shown to be an accurate, valid and reliable alternative to measuring 25(OH)D concentrations [45]. Furthermore, we adjusted for several potential confounders available in the Danish registers. However, we cannot exclude residual confounding from unknown or unmeasured confounders, e.g. maternal obesity (available from 2003 onwards) or parental diabetes (data not available). Obese individuals have greater risk of vitamin D deficiency [46] and offspring of obese and or diabetic mothers have greater risk of developing diabetes [47]. Nevertheless, it is unlikely that adjusting for maternal obesity would have greatly influenced our findings, as the prevalence of obesity among middle-aged women in Denmark was around 5% between 1981 and 1992 [48]. In addition, it cannot be excluded that vitamin D concentrations and other lifestyle and personal characteristics of offspring throughout the life-course may have mediated the association between 25(OH)D concentration and the risk of developing early-onset type 2 diabetes in adulthood.

Type 2 diabetes is largely under-diagnosed and it is estimated that only about half of the true cases can be found in the Danish registries [49]. In addition, the use of ICD codes may not be fully medically accurate for diagnosing type 2 diabetes and, owing to the clinical overlap between the phenotypes of type 1 diabetes and type 2 diabetes, the probability of misclassification of type 2 diabetes needs to be acknowledged. This is particularly relevant as the included population is young, and in Denmark, type 2 diabetes is mainly a mid- or late-adulthood disease. Therefore, it is likely that undiagnosed cases may form part of our sub-cohort, hence attenuating the association between neonatal vitamin D and early-onset type 2 diabetes. Another factor which may have attenuated the observed association (or lack thereof) was the lower concentration of $25(OH)D_3$ among excluded individuals in the cases and sub-cohort groups compared with those included in the cases and sub-cohort groups, respectively.

In general, measured concentrations of $25(OH)D_3$ were considerably lower than concentrations measured from neonatal DBSS in two previous studies [50, 51]. Several hypotheses can be drawn to explain the reasons for the generally low concentrations observed in our study. First, sample degradation could be brought forward, however, the explanation is unlikely to explain the low $25(OH)D_3$ concentration in the included DBSS, as studies have shown that, regardless of temperature and light exposure, storage times of DBSS for up to 40 years do not bias inter-individual variation in 25(OH)D concentrations for a given birth cohort [25, 26]. Furthermore, such bias would have similarly affected DBSS in the studies by McGrath et al. [50] and Nielsen et al. [51]. Second, intervariability between laboratories is a well-known factor for vitamin D measurement heterogeneity [24]; however, it is unlikely to explain the differences between our results and those from Nielsen et al. [51], as assays were both conducted at the SSI. Third, repeated freeze-thawing cycles of DBSS may have occurred in relation to multiple punches being taken for other research projects. The number of freeze-thawing cycles of the included DBSS has not been documented; however, it has been reported that 25(OH)D is not affected by repeated freeze-thaw cycles [52]. Fourth, systematic differences in punch location from the DBSS might explain the difference in $25(OH)D_3$ concentrations between our study and the two others, as higher $25(OH)D_3$ concentrations in the periphery of the DBSS compared with the centre of the DBSS have previously been reported [53]. However, punch location of the included DBSS has not been documented. As seasonal variations in $25(OH)D_3$ concentration from DBSS were well captured in our analyses, it is unlikely that the general low $25(OH)D_3$ values would have impaired the ranking of individuals into quintiles of $25(OH)D_3$ concentrations, and hence it is unlikely that the general low values would have affected the associations examined in the present study.

Our findings might only be generalised to populations of European descent from high-income countries with high living standards and living at high latitudes such as Denmark. In addition, our findings are based on populations born in the 1980s and early 1990s and might not be extrapolated to more recent birth cohorts, as a result of, but not limited to, the significant change in BMI, maternal age at delivery or use of assisted reproductive technology. Nevertheless, our findings based on an older birth cohort are useful to provide insights into early origins of adult diseases such as type 2 diabetes, which cannot yet be assessed using more recent birth cohorts. In addition, external validity of our findings might be impaired owing to the relatively young age at onset of type 2 diabetes (maximum 34.7 years), which is not representative of all individuals developing type 2 diabetes.

## Conclusion

The results of this case-cohort study conducted among the Danish population do not support the hypothesis that higher neonatal vitamin D concentrations are associated with a lower risk of early-onset type 2 diabetes in adulthood.

**Supplementary Information** The online version contains peer-reviewed but unedited supplementary material available at https://doi.org/10.1007/s00125-021-05450-2.

**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Contribution statement** BLH, RJ, AK, FT, IC, MS and AV designed the research. AK, FT, IC, MS and PF conducted the research. ASC provided essential materials and performed the 25(OH)D analysis. AK, FT and PF analysed the data or performed statistical analysis. AK and FT wrote the paper with contributions from all authors. AK is responsible for the integrity of the work as a whole. All authors have read and approved the final version of the manuscript.

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