Exposure to vitamin D from fortified margarine during fetal life and later risk of pre-eclampsia: the D-tect Study

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Abstract

Objective: To examine if fetal exposure to a small dosage of extra vitamin D from food fortification was associated with a decrease in the risk of pre-eclampsia later in life.
Design: Cancellation of the mandatory vitamin D fortification of margarine in 1985 created a societal experiment, with entire adjacent birth cohorts exposed or unexposed to extra vitamin D during fetal development. The Danish national medical health registries allowed the identification of pre-eclampsia cases later in life among all exposed and unexposed female individuals.
Setting: Denmark.
Subjects: Women born between June 1983 and August 1988, who gave birth to their first child at age 14·5–27·5 years (*n* 32 621).
Results: OR (95 % CI) for pre-eclampsia among women exposed *v.* unexposed to extra vitamin D from fortification during fetal development was 0·86 (0·76, 0·97). Exposure to extra vitamin D was associated with further reduced odds of pre-eclampsia (0·49 (0·34, 0·72)) among current smokers, but not among former smokers and non-smokers.
Conclusions: Additional vitamin D in fetal life from food fortification seems to reduce the risk of pre-eclampsia later in life. The risk reduction may be particularly strong among women who smoke during pregnancy.

Keywords
Vitamin D
Fortification
Fetal life
Pregnancy
Pre-eclampsia

Hypertension is the most common medical problem in pregnancy, occurring in up to 15 % of all pregnancies[1]. Pre-eclampsia is a severe pregnancy-related hypertensive syndrome characterized by hypertension and proteinuria[2]. Pre-eclampsia develops from mid-pregnancy and affects 3–5 % of all pregnancies. The incidence rate is 1·5- to 2·0-fold higher among first-time pregnancies compared with subsequent pregnancies[3–5]. On rare occasions pre-eclampsia can develop into eclampsia or haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, and other severe complications[2,6]. Pre-eclampsia is associated with increased morbidity and mortality in both the pregnant women and the offspring, with long-term negative health consequences such as CVD and diabetes[3,7–11].

The aetiology and pathophysiology of pre-eclampsia have not been fully elucidated. Abnormal placentation, oxidative stress and thrombosis are all thought to contribute to the development of pre-eclampsia[12]. Young and old age, high BMI, multiple pregnancies, autoimmune and infectious diseases, family history of pre-eclampsia and male offspring, as well as low maternal socio-economic status[13], are described risk factors associated with pre-eclampsia[3,14–16]. Physical activity before and early in pregnancy, as well as smoking during pregnancy was shown to reduce the risk of pre-eclampsia[17,18]. Sufficient vitamin D intake during pregnancy has also been suggested to reduce the risk of pre-eclampsia[19,20].

Vitamin D is a complex of fat-soluble secosteroids involved in Ca and P homeostasis and in the development of the skeleton. In addition, vitamin D also has non-skeletal functions that include immunomodulatory and anti-inflammatory properties, and has also been found to affect hormone secretion, cardiovascular function and blood pressure[13].

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It is well established that the fetal environment plays an important role in later health and disease risk[21]. During pregnancy, growth and development of the fetus depend on the nutritional status of the pregnant mother and on the capability of the placenta to exchange nutrients, such as vitamin D, between the pregnant woman and fetus[22]. Vitamin D insufficiency is common among otherwise healthy pregnant women[23,24] and, as maternal and fetal vitamin D statuses are strongly correlated, there is increasing interest in maternal vitamin D insufficiency during pregnancy and its potential long-term health consequences for the offspring[25].

So far, only one previous study has examined if extra vitamin D from supplementation early in life influences the risk of developing pre-eclampsia later in life[26]. The study followed 2969 women in Northern Finland born in 1966 who, in their first year of life, regularly or irregularly, received up to 50 µg of vitamin D daily, as well as women who did not receive any supplementation. After adjusting for biological, social and medical characteristics, a 50 % risk reduction of pre-eclampsia was found among women who had regularly received vitamin D supplementation during infancy, compared with those who received irregular or no supplementation[26].

Studies examining the importance of fetal nutrition on health later in life are time-consuming and may involve substantial costs and logistic difficulties. Conducting 'societal experiments' (e.g. fetal undernutrition during famines) has been widely used in exploring early origins of adult diseases in a cost-effective manner[27,28]. The present study is based on a societal experiment around margarine fortification with vitamin D in Denmark. In June 1985, mandatory fortification of margarine with vitamin D, which started in the 1930s and supplied on average 13 % (3–29 %) of all dietary intake of vitamin D to adult Danes[29], was abandoned[30–33]. Consequently, individuals born before 1985 were exposed to vitamin D from fortified margarine during fetal development and individuals born after the cancellation were not.

The purpose of the present study was to examine if fetal exposure to extra vitamin D from fortified margarine lowered the risk of pre-eclampsia later in life.

Methods and materials

Data sources

Information about the study population was retrieved from the Danish Civil Registration System (CSR); information on the pregnant women and their newborns was retrieved from the Danish Medical Birth Registry (MBR); and the presence of pre-eclampsia in the pregnant women was identified from the Danish National Patient Registry (DNPR). Age of delivery was calculated based on information on maternal day of birth retrieved from the CSR and information on date of delivery retrieved from the MBR.

The CSR was established in April 1968 and has since registered all people alive and living in Denmark with a 10-digit civil person register (CPR) number. The CPR number enables linkage of individual information from different nationwide registers and large clinical databases[34]. The MBR was established in 1973 and contains information obtained during the antenatal care visits for all women with permanent residence in Denmark[35]. The DNPR was established in 1977, and records information on all patients discharged from Danish non-psychiatric hospitals since 1977 and emergency and outpatient departments since 1995[36].

Definition of outcome

Since 1994, the diseases collected into the DNPR have been coded according to the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10). The present study included the following ICD-10 codes: gestational hypertension (code O13.9); mild to moderate pre-eclampsia (code O14.0); severe pre-eclampsia (code O14.1); HELLP syndrome (code O14.2); pre-eclampsia, unspecified (code O14.9); eclampsia in pregnancy (code O15.0); eclampsia during delivery (code O15.2); and eclampsia, unspecified as to time period (O15.9). The included diagnoses were categorized into three groups (Table 1). In cases where a woman had been diagnosed with more than one of the included diagnoses during the included pregnancy, only the diagnosis indicating the most severe form of pre-eclampsia was assigned.

Study population

In Denmark, it was mandatory to fortify margarine with vitamin D (1·25 µg/100 g) until June 1985. A 15-month 'wash-out period', including the full 9 months of pregnancy and additional 6 months to secure that the fortified margarine was no longer available at home or in stores, was introduced from June 1985 to September 1986. Therefore, the cohort of women born in the two years immediately before June 1985, born from June 1983 to May 1985, was defined as exposed to extra vitamin D during fetal

Table 1 Description of gestational hypertension, pre-eclampsia and eclampsia by International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD10) code and grouping

Classification	ICD10 code with description
Group 1	Gestational hypertension (code O13.9)
Group 2	Mild to moderate pre-eclampsia (code O14.0)
	Pre-eclampsia, unspecified (code O14.9)
Group 3	Severe pre-eclampsia (code O14.1)
	HELLP syndrome (code O14.2)
	Eclampsia in pregnancy (code O15.0)
	Eclampsia in labour (code O15.2)
	Eclampsia, unspecified as to time period (O15.9)

HELLP, haemolysis, elevated liver enzymes, low platelets.

development and the cohort of women born after the washout period, born from September 1986 to August 1988, was defined as unexposed. From June 1983 to August 1988, 162 397 females were born in Denmark; among these females, 112 311 gave birth. Those born during the 'washout period' were excluded from the study (n 27 003), reducing the number of births to 85 308. In the present study, the number of births by women who, due to the mandatory margarine fortification, were exposed to additional vitamin D during fetal life was 52 873. The number of births by women unexposed to the extra vitamin D during fetal development was 32 435. Women in the exposed cohort were frequently older and more often multiparous; therefore, the number of births among the exposed women was higher compared with the unexposed. As incidence of pre-eclampsia is higher in first-time pregnancies, the study population was restricted to nulliparous women[3–5]. Based on maternal day of birth and date of delivery, the study population was restricted to women giving birth at age 14·5 to 27·5 years and delivering their offspring after gestational week 22 to ensure similar age in both cohorts. Newborns with birth weight of 0 g and very young women, as well as very young women with pre-pregnancy BMI below the established cut-offs for this age group[37], were also excluded, resulting in 16 081 births in the exposed group and 17 168 births in the unexposed group. Finally, those with missing information on singleton and multiple births, offspring birth weight or gender, smoking status or gestational age were also excluded. The final groups consisted of 15 733 exposed nulliparous women and 16 888 unexposed nulliparous women. A flowchart of the study population is illustrated in Fig. 1. The sampling is illustrated in Fig. 2. The nulliparous women exposed to the vitamin D fortification *in utero* gave birth between December 1997 and December 2012; nulliparous women unexposed to the vitamin D fortification *in utero* gave birth between March 2001 and March 2016.

Variables

Information on pre-pregnancy BMI, smoking status, singleton and multiple births and gender of the newborn was retrieved from the MBR.

Pre-pregnancy BMI has been included in the MBR since 2004. At the first antenatal visit, normally taking place during the first trimester, the woman's BMI is calculated based on self-reported information. Applying International Obesity Task Force standards for adolescent BMI[37,38], women with pre-pregnancy BMI below 15·46 kg/m² were excluded[37]. Obesity defined as BMI ≥ 30·0 kg/m² is an established risk factor for pre-eclampsia[39,40] and the association between pre-pregnancy BMI and pre-eclampsia may not be linear[41]. Therefore, we categorized pre-pregnancy BMI into the four following categories: <18·5 kg/m², 18·5–24·9 kg/m², 25·0–29·9 kg/m² and ≥30·0 kg/m².

Information on smoking habits of the pregnant women has been collected into the MBR since 1997. This information was grouped into three categories: current smokers, former smokers (women who stopped smoking during or after the first trimester in the current pregnancy) and non-smokers.

The Danish national guidelines regarding late term definition were changed in 2009[42]. Therefore, to make cohorts comparable with regard to term of birth, we categorized gestational age at birth by weeks + days, rather than pre-term, full-term and post-term categories. The following seven categories were constructed: (i) <37, (ii) 37 + 0 to 37 + 6, (iii) 38 + 0 to 38 + 6, (iv) 39 + 0 to 39 + 6, (v) 40 + 0 to 40 + 6, (vi) 41 + 0 to 41 + 6 and (vii) ≥42 complete weeks of gestation.

Statistical analyses

Differences between exposed and unexposed women were tested by the χ^2 test for categorical data and the Mann–Whitney rank-sum test for skewed continuous data. The age restriction caused the age distribution to be skewed *a priori*; therefore, a formal statistical test of normality was unnecessary.

The association between exposure status and risk of pre-eclampsia later in life was examined by logistic regression, generating OR with 95 % CI. The following confounders were included into the multivariate regression models: woman's age at delivery, smoking habits, and singleton and multiple pregnancies. These confounders were hypothesized to be related to pre-eclampsia risk[3,16,17,43] or to vitamin D levels during gestation[20,26,44]. The main analyses were not adjusted for pre-pregnancy BMI; this information is available only from 2004 and forwards, thus adjustment would have made exposed and unexposed women incomparable in terms of age. To explore the impact of pre-pregnancy BMI, sensitivity analyses were conducted as sub-analyses including only those women who had information on pre-pregnancy BMI; the analyses in this subgroup were adjusted for pre-pregnancy BMI.

Earlier studies have found that smoking during pregnancy may protect against development of pre-eclampsia. Furthermore, smoking has previously been associated with vitamin D levels[45,46]. Interactions between smoking and vitamin D exposure status in relation to pre-eclampsia risk were analysed by conducting the models stratified by women's smoking status. Additionally, interactions between exposure status and women's age at delivery[3,15], as well as between exposure status and pre-pregnancy BMI[14], were tested using likelihood ratio tests.

Data were analysed using the statistical software package Stata version 13; P < 0·05 was considered statistically significant.

Ethics

The study was conducted in accordance with Danish law and approved by the Danish Data Protection Agency (journal number 2012-41-1156) providing permission to access the relevant registries. The study was based on
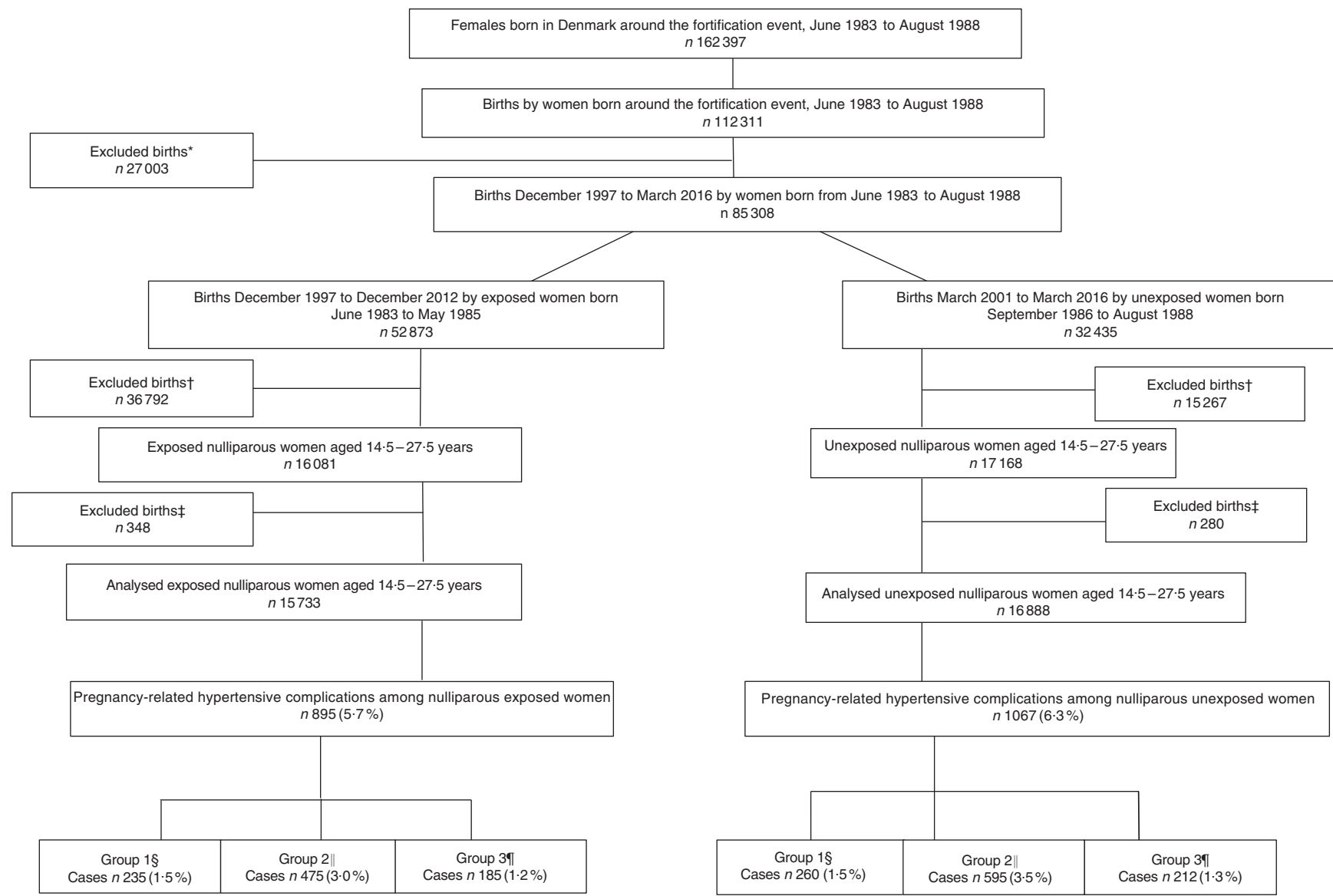
Fig. 1 Flowchart of the study population of women born between June 1983 and August 1988, who gave birth to their first child at age 14·5 to 27·5 years. *Women excluded because they gave birth during the 15-month wash-out period from June 1985 to September 1986. †Births where the woman was either below 14·5 or above 27·5 years of age, the offspring's birth weight was misclassified, gestational weeks was <22 weeks or pre-pregnancy BMI was <15·46 kg/m². ‡Excluded due to missing information on age at delivery, smoking habits, singleton and multiple births, gestational age at delivery or offspring gender. §Group 1, gestational hypertension; ICD-10 code: O13.9. ‖Group 2, mild to moderate and unspecified pre-eclampsia; ICD-10 codes: O14.0 and O14.9. ¶Group 3, severe pre-eclampsia, HELLP syndrome and eclampsia; ICD-10 codes: O14.1, O14.2 and O15.0 (ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision; HELLP, haemolysis, elevated liver enzymes, low platelets)
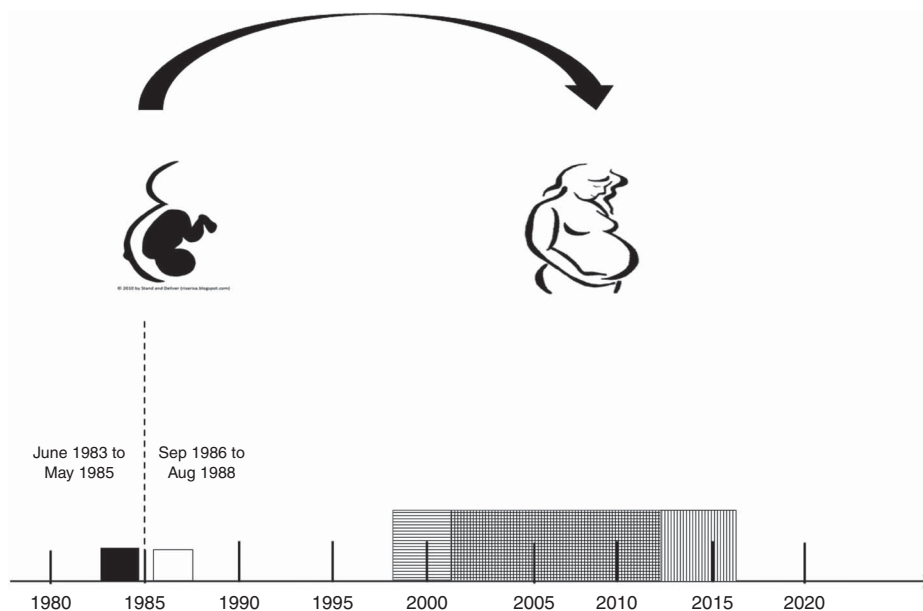
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Fig. 2 Sampling of the study population of women born between June 1983 and August 1988, who gave birth to their first child at age 14·5 to 27·5 years. (■), exposed women born between June 1983 and May 1985; (□), unexposed women born between September 1986 to August 1988; (– – – – –), the 'wash-out' period from June 1985 to September 1986. The exposed women gave birth between December 1997 and December 2012 (▤); the unexposed women gave birth from March 2001 to March 2016 (▥)

already collected data, the use of which does not require ethical approval.

Results

The proportion of births with pre-eclampsia, including cases of eclampsia (group 2 and 3; Table 1), among the nulliparous women exposed or unexposed to vitamin D fortification during fetal life was 4·2 % (660/15 733) and 4·8 % (807/16 888), respectively. Despite the age restrictions, the age at delivery was slightly higher for the exposed women compared with unexposed; the median (5th–95th percentile) age being 24·9 (19·5–27·3) and 24·7 (19·4–27·3) years, respectively ($P < 0.0001$). Furthermore, exposed compared with unexposed women were more often current smokers (18·9 *v.* 15·8 %, respectively) and more often gave birth at late gestational age (6·1 *v.* 3·6 %, respectively). Moreover, exposed compared with unexposed women more often had a BMI < 18·5 kg/m^2 (4·4 *v.* 5·2 %, respectively) and less often had a BMI ≥ 30·0 kg/m^2 (13·6 *v.* 13·9 %, respectively). Other characteristics were not different between exposed and unexposed women (Table 2).

Overall, there was a lower risk of pregnancy-related hypertensive complications among women who were exposed to extra vitamin D in fetal development compared with the unexposed women (Table 3). However, in the analyses stratified by outcome severity, the exposure effect was observed only among women diagnosed with mild to moderate and unspecified pre-eclampsia. No significant associations were found for the diagnoses of gestational hypertension, severe pre-eclampsia, HELLP

syndrome and eclampsia. Adjusting for women's age at delivery, smoking habits, singleton and multiple births and offspring gender gave essentially similar results (Table 3). The estimates for the covariates are listed in the online supplementary material, Supplemental Table 1.

The interaction between exposure to vitamin D and smoking status was significant ($\chi^2 = 11.82$; df = 2; $P = 0.003$) and stratifying analyses on smoking status revealed that the strongest association between *in utero* vitamin D exposure status and pre-eclampsia risk was seen among current smokers, where the risk of all types of pre-eclampsia was halved or more for the exposed women compared with unexposed ones (Table 4).

Pre-pregnancy BMI measurements were available only for a sub-population of 13 640 in the exposed group and a sub-population of 14 871 in the unexposed group. Sensitivity analyses in these sub-populations were restricted to women of similar age (age 20–27 years) and were adjusted for pre-pregnancy BMI; the results of these analyses (Table 5) were similar to the main findings.

Interactions between exposure status and women's age at delivery ($\chi^2 = 3.58$; df = 4; $P = 0.47$), as well as between exposure status and pre-pregnancy BMI, were not significant ($\chi^2 = 0.77$; df = 3; $P = 0.86$).

Discussion

In accordance with the incidence of pre-eclampsia worldwide, approximately 4·5 % of the women in our sample were diagnosed with pre-eclampsia of different severity[3]. The present study found that the risk of mild to

Table 2 Characteristics of women and their offspring, according to whether the woman was exposed to extra vitamin D from food fortification in fetal life or not; women born between June 1983 and August 1988, who gave birth to their first child at age 14·5 to 27·5 years

Characteristic	Exposed			Unexposed			P value
	N	Median	5th–95th percentile	N	Median	5th–95th percentile	
Age at delivery* (years)	15 733	24·9	19·5–27·3	16 888	24·7	19·4–27·3	< 0·0001
		n	%		n	%	
Outcome†	15 733			16 888			
Gestational hypertension‡		235	1·5		260	1·5	0·7
Mild to moderate and unspecified pre-eclampsia§		475	3·0		595	3·5	0·01
Severe pre-eclampsia, HELLP syndrome and eclampsia¶		185	1·2		212	1·3	0·5
All above		895	5·7		1067	6·3	0·02
Pre-pregnancy BMI†,‖	13 640			14 781			0·02
<18·5 kg/m^2		609	4·4		772	5·2	
18·5–24·9 kg/m^2		8251	60·5		8821	59·7	
25·5–29·9 kg/m^2		2930	21·5		3127	21·2	
≥30·0 kg/m^2		1850	13·6		2031	13·9	
Smoking status during pregnancy†	15 733			16 888			<0·0001
Current smoker		2968	18·9		2670	15·8	
Former smoker		799	5·1		969	5·7	
Non-smoker		11 966	76·0		13 249	78·5	
Singleton or multiple pregnancy†	15 733			16 888			0·38
Singleton		15 547	98·8		16 670	98·7	
Multiple		186	1·2		218	1·3	
Gestational age at delivery (weeks)†	15 733			16 888			<0·0001
<37		1120	7·1		1185	7·0	
37 + 0 to 37 + 6		822	5·2		866	5·1	
38 + 0 to 38 + 6		1950	12·4		2026	12·0	
39 + 0 to 39 + 6		3362	21·4		3538	21·0	
40 + 0 to 40 + 6		4419	28·1		4946	29·3	
41 + 0 to 41 + 6		3092	19·7		3712	22·0	
≥42		968	6·1		615	3·6	
Offspring's gender†	15 733			16 888			0·05
Female		7742	49·2		8151	48·3	
Male		7991	50·8		8737	51·7	

HELLP, haemolysis, elevated liver enzymes, low platelets; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.
*Tested by the Mann–Whitney rank-sum test.
†Tested by the χ^2 test.
‡ICD-10 code: O13.9.
§ICD-10 codes: O14.0 and O14.9.
¶ICD-10 codes: O14.1, O14.2 and O15.0.
‖Information available only from 2004 and forwards.

Table 3 Crude and adjusted odds for pre-eclampsia among women exposed to extra vitamin D in fetal life; women born between June 1983 and August 1988, who gave birth to their first child at age 14·5 to 27·5 years

Outcome	N$_{cases}$	Crude model		Adjusted model*	
		OR	95 % CI	OR	95 % CI
Gestational hypertension†	495	0·97	0·81, 1·16	0·96	0·81, 1·15
Mild to moderate and unspecified pre-eclampsia‡	1070	0·85	0·75, 0·96	0·86	0·76, 0·97
Severe pre-eclampsia, HELLP syndrome and eclampsia¶	397	0·94	0·77, 1·14	0·95	0·78, 1·16
All above	1962	0·89	0·82, 0·98	0·90	0·82, 0·99

HELLP, haemolysis, elevated liver enzymes, low platelets; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.
*Adjusted for age at delivery, maternal smoking status, and singleton or multiple births.
†ICD-10 code: O13.9.
‡ICD-10 codes: O14.0 and O14.9.
§ICD-10 codes: O14.1, O14.2 and O15.0.

moderate and unspecified pre-eclampsia was lower in those nulliparous women who were exposed to small amounts of additional vitamin D from food fortification during their fetal development, suggesting that vitamin D may have exerted programming effects on the risk of pregnancy complications later in life. Our results also indicate that the effects of vitamin D on the risk of pre-eclampsia were most obvious among women who smoked during pregnancy.

Table 4 Crude and adjusted odds for pre-eclampsia among smoking and non-smoking women exposed to extra vitamin D from fortification during fetal life; women born between June 1983 and August 1988, who gave birth to their first child at age 14·5 to 27·5 years

	Non-smokers					Former smokers					Current smokers				
		Crude model		Adjusted model*			Crude model		Adjusted model*			Crude model		Adjusted model*	
Outcome	N_{cases}	OR	95 % CI	OR	95 % CI	N_{cases}	OR	95 % CI	OR	95 % CI	N_{cases}	OR	95 % CI	OR	95 % CI
Gestational hypertension†	401	0·99	0·81, 1·20	0·97	0·80, 1·19	36	0·86	0·44, 1·69	0·84	0·43, 1·65	58	1·00	0·60, 1·67	0·97	0·59, 1·64
Mild to moderate and unspecified pre-eclampsia‡	892	0·93	0·81, 1·06	0·92	0·81, 1·05	62	0·87	0·52, 1·46	0·89	0·53, 1·50	116	0·50	0·34, 0·74	0·49	0·34, 0·72
Severe pre-eclampsia, HELLP syndrome and eclampsia§	335	1·04	0·84, 1·29	1·04	0·84, 1·29	17	1·37	0·52, 3·56	1·40	0·53, 3·65	45	0·40	0·21, 0·76	0·41	0·22, 0·76
All above	1628	0·96	0·87, 1·07	0·96	0·87, 1·06	115	0·93	0·63, 1·36	0·93	0·64, 1·37	219	0·58	0·44, 0·76	0·56	0·43, 0·74

HELLP, haemolysis, elevated liver enzymes, low platelets; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.
*Adjusted for age at delivery, and singleton and multiple births.
†ICD-10 code: O13.9.
‡ICD-10 codes: O14.0 and O14.9.
§ICD-10 codes: O14.1, O14.2 and O15.0.

Table 5 Crude and adjusted odds for pre-eclampsia among women exposed v. unexposed to extra vitamin D from fortification during fetal life, restricted to include only those women with information on pre-pregnancy BMI; women born between June 1983 and August 1988, who gave birth to their first child at age 14·5 to 27·5 years

		Crude model		Adjusted model*	
Outcome	N_{cases}	OR	95 % CI	OR	95 % CI
Gestational hypertension†	464	0·99	0·83, 1·19	0·99	0·82, 1·19
Mild to moderate and unspecified pre-eclampsia‡	972	0·86	0·76, 0·98	0·86	0·76, 0·99
Severe pre-eclampsia, HELLP syndrome and eclampsia§	355	0·95	0·77, 1·17	0·96	0·78, 1·19
All above	1791	0·91	0·82, 1·00	0·91	0·83, 1·01

HELLP, haemolysis, elevated liver enzymes, low platelets; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.
*Adjusted for age at delivery, maternal smoking status, pre-pregnancy BMI, and singleton or multiple births.
†ICD-10 code: O13.9.
‡ICD-10 codes: O14.0 and O14.9.
§ICD-10 codes: O14.1, O14.2 and O15.0.

Our findings are in accordance with those of Hypponen et al.[26], who found that the risk of pre-eclampsia was halved among the women who regularly received vitamin D supplementation in their first year of life. The supplemented dose in the study by Hypponen et al.[26] corresponded to 50 µg vitamin D/d. In our study, the extra daily vitamin D doses delivered with fortification were much smaller. Based on our calculations, the dosage could have been about 0·5–0·6 µg of extra vitamin D daily for the women; for the fetus, the dosages probably were even lower[29,47]. It is important to note that while we examined the influence of vitamin D exposure during fetal life, Hypponen et al.[26] examined the influence of vitamin D supplementation during the child's first year of life. Other previously conducted studies, including one from our group, showed that vitamin D supplementation dosage and timing were important to prevent type 1 diabetes, where supplementation in infancy with high dosages was shown to have a protective effect[44] while exposure to low-extra dosages from fortification throughout fetal development was not[48]. The dose and timing of the extra vitamin D seem to be of less importance in relation to prevention of pre-eclampsia[26].

Notably, our study and the study by Hypponen et al.[26] utilized different study designs. Hypponen et al.[26] conducted a classical cohort study, where information on vitamin D supplementation during the child's first years of life was collected. The female offspring were followed for pre-eclampsia outcome in their own pregnancies. Such a study takes a long time, many resources and is difficult logistically. The societal experiment introducing a termination of the mandatory vitamin D fortification programme in Denmark provided us with a unique opportunity to investigate research questions similar to those of Hypponen et al.[26] quickly and cost-effectively, and without the necessity to address each woman's individual vitamin D intake. However, the societal experiment design is prone to various biases, which are outlined below. On the other hand, in the study by Hypponen et al.[26], vitamin D intake is prone to recall bias. Further research confirming our and Hypponen et al.'s[26] findings is needed. Specifically, a randomized controlled trial would be needed to firmly conclude on causal relationships between fetal vitamin D exposure and pre-eclampsia risk later in life. Such a trial, however, would have to track offspring from quite large groups of in utero vitamin D-supplemented and

non-supplemented women for two or three decades after the supplementation actually took place. We would recommend that, before conducting a randomized controlled trial, an effort is made to identify already existing cohorts, similar to the one used in the study by Hypponen *et al.*[26], with information on fetal exposure to extra vitamin D. An alternative solution could be an observational study, with objective vitamin D biomarker measurement in fetal or neonatal blood, coupled with register information on pre-eclampsia incidence later in life. In fact, such a study is currently being conducted by our research group.

In Denmark, since 1981 neonatal blood has been routinely collected from all newborns 48–72 h after birth to screen for phenylketonuria. The remaining blood left after screening is stored on dried blood spots (DBS charts) in the Danish Neonatal Screening Biobank. Contemporary vitamin D analyses techniques can detect vitamin D concentration on the DBS charts[49]. Currently, our research group is conducting a case–control study which measures vitamin D levels in the blood on the DBS charts in a large population-based sample of women diagnosed with pre-eclampsia, and randomly selected control women. Results of this study will be used to validate the findings of the present study. If our findings are confirmed by more robust study designs, this will have important public health implications because it will demonstrate a possibility of pre-eclampsia prevention by simple and affordable food fortification. Efforts to prevent pre-eclampsia are important, as the disease bears health risks for the mother and the offspring, and the only definite cure known so far is delivery[3].

The biological mechanisms linking fetal vitamin D and later pre-eclampsia risk are most probably related to the maturity of the immune system. T-cell development and maintenance may be of importance in forming the adult immune system. A balance between helper T1 cytokines (Th1) and helper T2 cytokines (Th2) is necessary for an optimal immune system[50]. Vitamin D receptors are found on most immune cells, making hormonal vitamin D an important immune regulator stimulating Th2 expression and inhibiting Th1 expression[26,51]. An overexpression of Th1 response may be involved in the development of pre-eclampsia[43,52].

Several studies have reported that smoking in pregnancy reduces the risk of pre-eclampsia[53,54]. Smoking may interact with other risk and/or protective factors, including prenatal vitamin D level. Therefore, analyses differentiated by smoking status were used to investigate if small doses of extra vitamin D from food fortification affected pre-eclampsia risk. Indeed, we found that prenatal vitamin D intake seemed to be a protective factor in currently smoking pregnant women (i.e. women who smoked during pregnancy benefited most from extra vitamin D intake during their own gestation). The fact that the women exposed to extra vitamin D during fetal life were more often smokers should not bias our results, as they smoked while being pregnant, and exposure to

vitamin D occurred during the pregnant women's own fetal development. Exposure to smoking during fetal life (i.e. smoking status of the mothers of the studied women) was not recorded. Importantly, although smoking is associated with a lower risk of pre-eclampsia[45,46,55], smoking is also associated with several adverse perinatal outcomes and should never be promoted to reduce the risk of pre-eclampsia.

Strengths and limitations

The use of a societal experiment design to study an association between exposure to extra vitamin D during fetal life and later risk of pre-eclampsia has both strengths and limitations. The design determined the sampling, exposure measurement and possible confounding. In our design, period of birth approximated prenatal exposure to extra vitamin D from the fortified margarine. As the fortification programme was cancelled in 1985, we analysed women from adjacent birth cohorts born around 1985. We knew the exact date of the cancellation of the fortification programme and therefore we could define precise exposure and non-exposure periods for the adjacent national birth cohorts. As the birth cohorts were very close to each other and covered a narrow time period, we considered all the characteristics of our study population, except for prenatal exposure to vitamin D fortification, to be randomly distributed between exposed and unexposed individuals; thus, confounding was not expected. To exemplify our reasoning in regard to a low social status as a potential confounder for pre-eclampsia[56], the following arguments can be provided. The study population covers all births, i.e. from all social strata, retrieved from the national Danish medical registries including all Danes. Maternity care in Denmark is free of charge and all pregnant women, i.e. irrespective of social status, are followed by a standardized protocol of prenatal care and are consequently registered into the national Danish medical registries. Thus, we assume that all social strata are equally represented in both groups.

The confounding in our design, however, could occur if secular changes in alternative exposure sources (i.e. vitamin D intake via food or exposure to bright sunshine), outcomes (i.e. pre-eclampsia incidence) and potential confounders (e.g. mother's socio-economic status and smoking, mother's exposure to infections and physical activity) were taking place in Denmark during 1983–1988, a narrow time period covering our exposed and unexposed birth cohorts. Consequently, the limitation of the societal experiment design is that it assumes that in the period analysed, there were no other changes except the one of interest. We could not identify such secular changes in our study. Nevertheless, we acknowledge that some secular changes where evidence is difficult or impossible to document may have occurred. For example, women born in 1983–1985 may have been slightly less exposed to

maternal smoking (or exposure to smoking in fetal life), compared with women born in 1986–1988, as since the 1970s all pregnant women in Denmark were advised to stop smoking[57] and studies suggest that smoking during pregnancy is declining[58,59]. We were unable to get information on smoking habits of the mothers of our study population, as smoking habits were not recorded into the MBR until 1997[60]. Moreover, in the period 1987–1988 changes occurred in the Danish fiscal policy aiming at reducing household expenditure[61]. A change towards a healthier self-reported diet in the Danish population during 1985–2001 was also noted[62,63]. Thus, there could have been confounding which we did not consider.

We did, however, consider alternative exposures to vitamin D around 1985: changes in fortified margarine intake in 1983–1988; changes in vitamin D supplement recommendations for pregnant women; and women's exposure to bright sunshine hours during the same period. Food disappearance statistics showed that the change in margarine consumption in the period analysed was minimal: from 16·9 kg/capita in 1983 to 16·2 kg/capita in 1985 and 17·0 kg/capita in 1988[47]. Vitamin D supplementation during pregnancy recommendations did not change during 1983–1988. Therefore, we assume that the overall alternative fetal exposure to vitamin D from foods was comparable in both groups. Further, differences in monthly bright sunshine hours per year during 1983–1988 were not significant. Amounts of bright sunshine hours during gestation, compared between the individuals in exposed and unexposed cohorts, were however significantly different, and ideally should have been adjusted for in our analyses[64,65].

The main strength of the present large-scale study is the use of data from the Danish nationwide medical registries. The Danish national administrative and medical registers are considered to have high completeness and good validity. A high degree of validity of the ICD-10 pre-eclampsia diagnoses in the DNPR has been confirmed by comparing diagnoses of pre-eclampsia from the registry with standardized phone interviews in a study carried out between 1998 and 2002 that included a total of 3039 pregnancies[66]. However, the coding of the diagnoses gestational hypertension and pre-eclampsia included other serious diseases and may have introduced non-differential misclassification errors causing a disappearance of the observed association[67]. The lower gestational age at delivery observed in older women supports the validity of the data from the MBR, as it reflects national guidelines for earlier induction of labour in post-term pregnancies in Denmark in 2009[42]. As the present study is based on information retrieved from the Danish national health registries collected independently of the study, selection and information bias are considered to be negligible. The registers, however, contain no information on the smoking status of the mothers of the women in our study population, which may be considered a potential limitation in our analyses.

Conclusion

The results of our study suggest that small extra doses of vitamin D from food fortification during fetal development may decrease the risk of developing pre-eclampsia during first pregnancy in adulthood. The beneficial effect of the extra vitamin D during fetal development appears to be particularly protective against pre-eclampsia for women who smoke during pregnancy.

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Supplementary material

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