The relationship between maternal 25-hydroxyvitamin D status in pregnancy and childhood adiposity and allergy: An observational study

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Short Title: Maternal vitamin D status and childhood adiposity

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Abstract

Background

Vitamin D insufficiency (defined as <75 nmol/L) is widespread amongst pregnant women around the world and has been proposed to influence offspring outcomes in childhood and into adult life, including adiposity and allergy. Disorders including asthma and eczema are on the rise amongst children. Our aim was to investigate the relationship between maternal 25-hydroxyvitamin D status in pregnancy and offspring adiposity, asthma and eczema in childhood.

Subjects and Methods

Maternal 25-hydroxyvitamin D concentrations were analysed in serum samples collected at 15 weeks' gestation from 1710 participants of the prospective Screening for Pregnancy Endpoints (SCOPE) cohort study. The offspring of 1208 mothers were followed up at age 5 - 6 years. Data collected included height, weight, percentage body fat (PBF, measured by bioimpedence) and history of asthma and eczema. Multivariable analysis controlled for maternal BMI, age and sex of the child and season of serum sampling.

Results

Complete data were available for 922 mother-child pairs. Each 10 nmol/L *increase* in maternal 25-hydroxyvitamin D concentration at 15 weeks' gestation was associated with a *decrease* in offspring PBF of 0.2% (95% CI 0.04 - 0.36% p=0.01) after adjustment for confounders, but was not related to child BMI z-score. Maternal

mean (±SD) 25-hydroxyvitamin D concentration was similar in children who did and did not have asthma (71.7 ± 26.1 vs 73.3 ± 27.1 nmol/L p = 0.5), severe asthma (68.6 ± 28.6 vs 73.3 ± 26.8 nmol/L p = 0.2) and eczema (71.9 ± 27.0 vs 73.2 ± 27.0 nmol/L p = 0.5).

Conclusions

The finding of a relationship between maternal vitamin D status and adiposity in childhood is important, particularly because vitamin D insufficiency in pregnancy is highly prevalent. The association between maternal vitamin D supplementation in pregnancy and adiposity in the offspring merits examination in randomised controlled trials.

Introduction

Many critical processes in fetal development occur at the end of the first trimester and beginning of the second trimester of pregnancy and these could be influenced by maternal vitamin D status. Between 10 and 17 weeks' gestation processes in immune cell development include negative selection to remove auto-reactive cells (1) and migration from primitive generation sites such as the fetal liver to the bone marrow and lymph tissue. It is at this time that the vitamin D activating enzyme, 1-α hydroxylase, is at the highest concentration in the placenta (2).

Many tissues besides kidney and intestine are able to activate and respond to vitamin D, including adipose precursor cells (3) and cells of the immune system (4, 5) . In tissues outside of the kidney, 1-α hydroxylase is not regulated by parathyroid hormone, and may therefore be more sensitive to local substrate concentrations.

Obesity in childhood is on the rise (6) coinciding with a re-emergence of vitamin D deficiency (7). Obese children will most likely stay obese into adulthood (8) and some will suffer from the consequences of obesity related disease, such as metabolic syndrome in childhood (9). We are beginning to understand how early life conditions predispose to obesity (10) and how adipose tissue is an active participant in the regulation of food intake and metabolic regulation (11). Vitamin D promotes adipocyte maturation and inhibits adipocyte proliferation (3). An *in utero* insult such as vitamin D deficiency may therefore set a life-course towards fat accumulation.

Asthma and eczema are also on the increase (12), and there are mechanisms by which vitamin D deficiency may play a role. Vitamin D has a number of immunomodulator effects (13) and promotes T helper 2 cell (T_h2) proliferation and maturation (14). T_h2 cells regulate eosinophils, basophils mast cells and IgE producing B cells (15). Vitamin D has been shown to reduce B cell proliferation and reduce IgG in adult autoimmune disease, alters the signalling between adult immune cells and can alter immune cell differentiation (16, 17). An association between maternal 25-hydroxyvitamin D concentration during pregnancy and the development of the autoimmune disease type 1 diabetes mellitus in childhood has been found (18). Exposure to vitamin D deficiency *in utero* may also predispose to allergic hypersensitivity in childhood.

The aim of this study was to investigate the relationship between vitamin D status in pregnancy at the beginning of the second trimester of pregnancy in a cohort of low risk first time mothers and the development of allergic disease and obesity in their children between 5 and 6 years of age.

Methods

Participants – Mothers

The Screening for Pregnancy Endpoints Study (SCOPE) is an international prospective cohort study which aimed to identify early pregnancy predictors of late pregnancy complications in women in their first ongoing pregnancy (19). Only SCOPE Auckland participants were included in this study. Auckland participants were

recruited between 2005 and 2008. The study was approved by the Health and Disability Ethics Committee (AKX/02/00/364).

Women were interviewed at the first SCOPE research visit at 15 weeks' gestation. Data collected included weight and height to the nearest kg and cm from which BMI was calculated. Non-fasting serum samples were also collected and stored at -80ºC for later processing. SCOPE study data collection has been described in detail elsewhere (19).

Participants - Children

Women were asked at the first visit whether they were happy to be contacted for a follow-up study (Children of SCOPE). These women were then contacted again 5-6 years postpartum to seek consent to participate in the follow up study of their children. Children aged between 5 and 6.25 years were eligible to participate if they did not meet any of the exclusion criteria. The exclusion criteria were major chromosomal abnormality such as Down's syndrome, a major congenital abnormality such as cyanotic heart disease or diaphragmatic hernia, or other chronic illness that interferes with growth such as type 1 diabetes mellitus or inflammatory bowel disease. The study was approved by the Health and Disability Ethics Committee (NTX/10/10/106).

The aim of the follow up study was to identify early life modifiable determinants of obesity and insulin resistance in children, with emphasis on factors occurring during pregnancy. The age of 5 years was identified as the follow-up period because, unlike at a younger age, parameters such as adiposity are predictive of long term health

outcomes (20). It is also early enough that health trajectories may be malleable to change with intervention. Data were collected at a single visit and entered into a secure internet-accessed auditable database (MedSciNet^{AB}, Stockholm, Sweden). Data is accessible following application to the SCOPE consortium (www.scopestudy.net). Parents were interviewed and children were examined.

Outcome variables

Children's height was measured by stadiometer (SECA 213) to the nearest 1mm. Weight was measured in light clothing without shoes to the nearest 0.1kg and BMI calculated (weight/height squared). BMI was converted to a z-score according to the LMS method (21). Lean body mass was estimated using bioelectrical impedance (ImpediMed Imp SFB7). As the equation used in the calculations in the SFB7 software is not validated on children we extracted the data from the 50Khz reading (as used in previous versions of the Impedimed machines) and calculated fat free mass (FFM) according to a validated equation (22). The equation used to calculate FFM was: $FFM = 0.65(height^2/impedance) + 0.68 \times age + 0.15$. Percentage body fat (PBF) was calculated using (weight-FFM/weight x 100).

Current wheezing was identified by a positive response to the question: "In the past 12 months, has your child/have you had wheezing or whistling in the chest?"(23). If the participant answered 'yes' further questions followed to determine the severity of asthma:

In the last 12 months ..

How many attacks of wheezing has your child had?

Has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

How often, on average, has your child's sleep been disturbed due to wheezing?

Severe asthma was defined by fulfilling one of the following criteria:

1. 4 or more attacks of wheezing in the past 12 months
2. Wheezing limiting speech to one or two words at a time between breaths in the past 12 months
3. Sleep disturbance due to wheezing, occurring one or more nights per week in the past 12 months

Current eczema was identified by a positive answer to each of the following questions:

1. "Has your child ever had an itchy rash, which was coming and going for at least 6 months?" and
2. "If yes, has your child had this itchy rash at any time in the past 12 months?" and
3. "If yes, has this itchy rash at any time affected any of the following places – the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?"

25-hydroxyvitamin D analysis

25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 were analysed by liquid chromatography tandem mass spectrometry (Finnigan TSQ

Quantumn Ultra AM triple quadrupole mass spectrometer, Thermo Electron Corporation, San Jose, CA, USA). Samples were prepared using Phree phospholipid removal plates (Phenomenex, Torrance, CA, USA). Chromatographic separation of the 25-hydroxyvitamin D_3 stereoisomers was achieved with a pentaflurophenyl (PFP) 100 x 3mm 2.6 µm Kinetex HPLC column (Phenomenex, Torrance, CA, USA). The intra- and inter-assay coefficients of variation were 7.3% and 4.9% for 25-hydroxyvitamin D_3 and 8.2% and 5.8% for 25-hydroxyvitamin D_2, respectively. Quality control was through participation in the DEQAS program operating out of Charing Cross Hospital, London (24) and the use of commercially available control materials of known vitamin D concentration.

Statistical analysis

Continuous variables are reported as mean ± standard deviation. Total 25-hydroxyvitamin D was analysed as a continuous and categorical variable. Cut-offs for vitamin D as a categorical variable were 50nmol/L and 75nmol/L based upon the Institute of Medicine and the Endocrine Society clinical practice guideline definitions of vitamin D deficiency and insufficiency, respectively (25, 26).

T-tests were performed to investigate the relationship between maternal 25-hydroxyvitamin D concentration as a continuous variable and asthma and eczema, and between vitamin D as a categorical variable and child (PBF) and child BMI z-scores. Linear regression analysis was performed to investigate the relationship between 25-hydroxyvitamin D as a continuous variable and i) child PBF and ii) child BMI z-score. Multivariable linear regression was

performed to control for maternal BMI at 15 weeks' gestation, ethnicity, maternal smoking during pregnancy, socioeconomic status, sex of the child and age at sampling, and season of sample collection. There were a large number of potential confounding variables available to us. Traditional approaches to adjustment for large numbers of confounders can lead to unrecognised conditional associations and bias. We used directed acyclic graphs (DAGs) (27) to construct theoretical causal pathways between maternal vitamin D status and childhood outcomes, eliminating from our analysis covariates that were on the same causal path, arriving at the reduced list presented in the Results below.

Results

The SCOPE study was completed by 2065 Auckland women. Of these, 4% were excluded due to loss to follow up (n=23), fetal or neonatal death (n=27) or major congenital abnormalities (n=34). The remaining 1981 children were eligible for the Children of SCOPE follow-up study. From this group 1208 children were followed up at 5 to 6 years of age (58%). Maternal serum samples were available for 25-hydroxyvitamin D analysis from 1715 women, child BMI z-scores from 1207 children and PBF from 1176 children (figure 1). SCOPE participants, whose children participated in Children of SCOPE, compared to SCOPE participants did not participate in follow-up, were older by an average of 1.6 years (p=0.001), a smaller proportion were smokers (9.4% vs 15.9% p = <0.0001) and a greater proportion were NZ European (87.5% vs 78.8% p = 0.001) (table 1). The mother's BMI at 15 weeks' gestation was similar in those who were and were not followed up (24.7 ± 4.2 and 24.9 ± 4.3 kg/m^2, p =

0.46). Infant birth weight was higher in children followed up than in those who were not (3445 ± 533 vs 3345 ± 728 g p = 0.0004) but customised birth centile was similar (49.0 ± 28.1 vs 48.2 ± 30.3 p = 0.55) (table 2).

Mean PBF was higher in the children with maternal 25-hydroxyvitamin D concentration < 50 nmol/L compared to ≥ 50 nmol/L (23.9% ± 6.8 vs 22.7% ± 6.3 vs p=0.02). It was similar between children with maternal 25-hydroxyvitamin D concentration < 75 nmol/L and ≥ 75 nmol/L (23.3% ± 6.6 vs 22.6% ± 6.3 p=0.09). In multivariate linear regression analysis, adjusting for maternal BMI, season of sample collection, child's age and sex, each 10 nmol/L decrease in maternal 25-hydroxyvitamin D was associated with an increase in child PBF of 0.18% p = 0.02 (Table 3). Similarly, this association was seen with body fat (70g increase per 10nmol/L decrease in maternal 25-hydroxyvitamin D concentration) but there were no associations with lean mass, weight, height or BMI z-score (Table 3). After adjustment for ethnicity this effect was no longer significant (10 nmol/L decrease in maternal 25-hydroxyvitamin D associated with a 0.12% increase in child PBF 95% CI 0.28% to -0.05% corresponding to a 5g increase in body fat for a 6 year old weighing 20kg). Maternal smoking during pregnancy and socioeconomic status were not independently associated with child PBF (p = 0.2 and p = 0.4 respectively) and were not included in the multivariable analysis.

Mean BMI z-score was similar in children with maternal 25-hydroxyvitamin D concentrations above and below 50 nmol/L (0.12

± 0.90 vs 0.27 ± 1.13 p = 0.06) and likewise for concentrations above and below 75 nmol/L (0.11 ± 0.10 vs 0.19 ± 1.00 p = 0.2). Linear regression analysis showed no significant association between BMI Z-score and maternal total 25-hydroxyvitamin D analysed as a continuous variable (Table 2).

Mean maternal 25-hydroxyvitamin D was similar between children who had asthma and those who did not (71.7 ± 26.1 vs 73.3 ± 27.1 nmol/L p = 0.5) and between those with severe asthma and those who did not have severe asthma (68.6 ± 28.6 vs 73.3 ± 26.8 nmol/L p = 0.2). Likewise, mean maternal 25-hydroxyvitamin D concentration was not significantly different between children who had eczema and those who did not (71.9 ± 27.0 vs 73.2 ± 27.0 nmol/L p = 0.5)

Discussion

Adjusting for maternal BMI, and child age and sex, we found that lower maternal 25-hydroxyvitamin D concentration at 15 weeks' gestation was associated with higher PBF in children aged 6 years. PBF in childhood predicts BMI and metabolic risk in adulthood (8) and it is widely acknowledged that small changes at an individual level can result in important changes in disease incidence at a population level (28). From 1988-1994 to 2013-2014 the mean BMI for a 6 year old in the United States rose from 15kg/m^2 to 16kg/m^2. This corresponds to a 1.32kg increase in weight (of which approximately 20% or 264g is adipose) for a 6 year old on the 50th centile for height (29). This has been associated with a large increase in type 2 diabetes mellitus amongst children and adolescents. In the

early 1990's 1-4% of youth with diabetes mellitus had type 2, now up to 50% (30, 31). We found no relationship between maternal vitamin D status and asthma or eczema in their children.

Our findings are consistent with others who have found an association between lower maternal 25-hydroxyvitamin D concentrations and greater fat mass in childhood (32, 33). Our study differs from Crozier *et al* in that we analysed our maternal samples at 15 weeks' gestation, 6 months before term delivery, while they analysed 25-hydroxyvitamin D at 34 weeks' gestation. This finding suggests that 25-hydroxyvitamin D concentration throughout pregnancy may be important in relation to childhood adiposity. While others have found an association between maternal vitamin D deficiency in the third trimester and greater adiposity at one year, this relationship did not remain significant at 4 years of age (34).

Vitamin D is best known for its role in increasing intestinal calcium absorption and the effects of vitamin D on percentage body fat (PBF) may be mediated through calcium. Studies in rat models of adult obesity have shown that high calcium diets reduce PBF (35, 36). Randomised trials of calcium supplementation in pregnancy have not reported neonatal or childhood adiposity; however the findings of a Cochrane systematic review found on pooled analysis of 19 trials an increase in birth weight with supplementation (mean difference 64.66 g 95% CI 15.75 to 113.58 g) (37). Furthermore, it is uncertain whether vitamin D facilitates calcium transport across the placenta and vitamin D may be having a direct effect upon adipose tissue. The active form of vitamin D, 1,25 dihydroxyvitamin D has been

shown to prevent adipogenesis in vitro by suppressing adipogenic factors such as PPARγ (38) and lowering lipid accumulation (39, 40).

We did not find a significant association between maternal vitamin D status and BMI z-score. The likely explanation for this is that there was a relationship between maternal 25-hydroxyvitamin D and child fat mass but not lean body mass. Lean body mass makes up between 70 - 80% of the weight of a 7 year old child. The contribution of lean body mass to total weight, blunted the relationship between maternal vitamin D status and BMI and BMI Z-score. BMI is dynamic through childhood (41) and while standardising for age by calculating BMI z-scores improves applicability, PBF is a more direct measure of childhood adiposity (42). The limitations of BMI as a measure of adiposity in children is well known (43). PBF as determined by DEXA is the gold standard for measuring adiposity (44). Cost, machine availability and acceptability to participants (a significant proportion of parents decline DEXA because of the exposure to X-rays) considerations led us to measure body fat using bioimpedance. In children, body fat and percentage body fat as determined by bioimpedance has been shown to correlate more closely with body fat and percentage body fat as determined by DEXA than BMI and BMI z-score (45).

We found no relationship between maternal vitamin D status and asthma. This is consistent with follow up studies from 2 recent randomised controlled trials. Participants of the Vitamin D Antenatal Asthma Reduction Trial (VDAART) were given either 4400 IU of vitamin D daily or 400 IU daily from 10 to 18 weeks' gestation through pregnancy (46). In a trial in Copenhagen, women were

randomised to receive 2800 IU of vitamin D or 400 IU daily from 24 weeks' gestation (47). Between these two studies 1391 children were followed up at three years of age. The prevalence of asthma or wheeze in these children was not statistically significant between those who received the higher and lower doses of vitamin D in either of those studies. A recent meta-analysis of observational studies investigating the relationship between maternal vitamin D status in pregnancy and childhood asthma and wheeze at 5 to 6 years of age found no relationship in combined analysis (pooled OR 0.98 95% CI 0.94 - 1.02 and Pooled OR 1.00 95% CI 0.98 – 1.01) (48). An earlier observational study not included in the meta-analysis found associations between maternal vitamin D intake during pregnancy and childhood wheezing at 5 years but not at 2 years (49).

The same meta-analysis reviewed studies investigating the relationship between maternal vitamin D status and offspring eczema (48). They found a reduced likelihood of childhood eczema for each unit increase in maternal 25-hydroxyvitamin D in children assessed between 1 and 5 years old (Pooled OR 0.944 95% CI = 0.831 – 0.983). We found no such relationship.

Findings from recent observational studies not included in the meta-analysis are also conflicting. Participants in a UK study who had a maternal 25-hydroxyvitamin D concentrations > 75nmol/L during pregnancy were more likely to have infants who had eczema at 9 months age compared to mothers with concentrations < 30nmol/L (OR 3.26, 95% CI 1.15-9.29) (50). The Generation R study found no correlation between maternal 25-hydroxyvitamin D concentration mid gestation and cord 25-hydroxyvitamin D and the development of

eczema before age 4 (51). Another study found in children with a family history of allergic disease however a higher cord blood 25-hydroxyvitamin D concentration was associated with reduced eczema in childhood (52). And finally, maternal dietary vitamin D intake during the first and second trimester of pregnancy was associated with a reduction in allergic rhinitis in school age children but there was no association between maternal 25-hydroxyvitamin D serum concentration or maternal vitamin D supplementation and allergic rhinitis (53).

The major limitation of this study is that we have no data to adjust for some possible additional confounding variables. These include maternal exercise, maternal diet and sunlight exposure during pregnancy. Additional factors known or suggested to affect childhood adiposity include the child's own exercise levels and dietary patterns. Participants in SCOPE were self-selected and were not representative of all pregnant women in Auckland. While not all of the mothers and children from the SCOPE study were followed up, because responders were more likely to be older, better educated, non-smokers we believe the findings are more likely to be conservative and likely to be at least as relevant to those who did not take part. Participants were also more likely to be NZ European (87%). Ethnicity influences vitamin D status in both skin pigmentation and cultural practice around sun exposure as well as influencing adiposity. Given the small numbers of individuals making up the remaining ethnic groups, and the diverse ways in which ethnicity interacts with vitamin D status and adiposity makes interpretation difficult. Ideally for the diagnosis of asthma and

eczema in this study, all participants would have lung function tests and assessment by a physician. This is not always practical in large studies and because signs maybe transient. On the other hand self-reports and questionnaires have been shown to correlate well with formal examination for example, in a comparison to a respiratory physician diagnosis the ISAAC questionnaire had a sensitivity of 0.85 (95% CI 0.73, 0.93) and specificity of 0.81 (95% CI 0.76, 0.86) in children (54).

Conclusion

The finding that lower maternal 25-hydroxyvitamin D concentration is associated with higher PBF in children is important at a time when obesity is so prevalent. A small reduction in adiposity of the individuals has the potential for large population benefits. Our findings support the inclusion of formal measurements of adiposity in the children of participants of randomised trials of vitamin D supplementation in pregnancy (55).

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Figure 1. Flow chart of participant's included in the Children of SCOPE study with data on maternal vitamin D status

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Table 1. Characteristics comparing mothers whose children undertook the Children of SCOPE assessment compared to those not taking part in the follow-up study

		Participants of Children of SCOPE	Those not participating in follow-up	P value
Mothers	Age at child's birth (years ± SD)	31.0 ± 4.4	29.4 ± 5.0	<0.001
	Ethnicity *n* (%)			<0.0001
	- Asian	47 (3.9)	60 (7.3)	
	- NZ European	1058 (87.5)	649 (78.8)	
	- Maori	17 (1.4)	15 (1.8)	
	- African/other	34 (2.8)	33 (4.0)	
	- Indian	40 (3.3)	37 (4.5)	
	- Pacific Island	12 (1.0)	30 (3.6)	
	BMI at 15 weeks (kg/m^2 ± SD)	24.7 ± 4.2	24.9 ± 4.3	0.46
	Smoke in pregnancy *n* (%)	113 (9.4)	131 (15.9	<0.0001
	Multi-vitamin supplementation during pregnancy *n* (%)	681 (56.9)	426 (52.2)	0.036
	Mean 25-hydroxyvitamin D concentration at 15 weeks (nmol/L ±SD)	73.0 ± 26.9	70.1 ± 27.1	0.036

Missing data. Smoking n=2, multivitamin supplementation n=20, maternal 25-hydroxyvitamin D n= 483

Table 2. Characteristics of Children of SCOPE participants for mothers with and without data on maternal vitamin D status

Children		Maternal vitamin D data n = 921	No maternal vitamin D data n-284	P value
	Birth weight (g ± SD)	3445 ± 533	3345 ± 728	0.0004
	Customised birth weight percentile (SD)	49.0 ± 28.1	48.2 ± 30.0	0.55
	Age (years ± SD)	5.96 (0.19)	5.94 ± 0.19)	0.15
	BMI z-score (±SD)	0.15 ± 0.97	0.03 ± 0.88	0.07
	Percentage body fat (% ± SD	23.0 ± 6.5	22.1 ± 6.5	0.06
	Asthma n (%)	160 (17.4)	50 (17.5)	0.94
	Severe asthma n (%)	54 (5.9)	19 (6.7)	0.62
	Eczema n (%)	118 (12.7)	36 (12.6)	0.94

Table 3. Univariate and multivariate analyses of maternal vitamin D at 15 weeks' gestation and child anthropometry at 6 years of age represented as the change in child parameters for a 10 nmol/L increase in maternal 25-hydroxyvitamin D concentration

	Univariate (95% CI)	p value	Multivariate* (95% CI)	P value
Percentage body fat (n=900)	-0.18 (-0.33, -0.02)	0.03	-0.2 (-0.36, -0.04)	0.01
Body fat (kg) (n=900)	-0.07 (-0.12, -0.02)	0.01	-0.07 (-0.12, -0.02)	0.01
Lean mass (kg) (n=900)	-0.01 (-0.05, -0.04)	0.79	0.01 (-0.03, 0.06)	0.55
BMI z-score (n=922) (n=922)	-0.01 (-0.04, 0.01)	0.23	-0.01 (-0.01, 0.01)	0.41

*Multivariable model controls for maternal BMI at 15 weeks' gestation, child age and sex, and season of serum sampling