High Pregnancy, Cord Blood and Infant Vitamin D Concentrations May Predict Slower Infant Growth

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Abstract

Context The relationship of maternal and infant 25-hydroxyvitamin D concentration [25(OH)D] with infant growth is unclear.

Objective Our objective was to explore whether 25(OH)D in pregnancy, umbilical cord blood (UCB) or in infancy are associated with infant growth.

Design This study involved 798 healthy infants and their mothers in Finland. We assessed 25(OH)D during pregnancy, from UCB at birth and from the infant at the age of 12 months.

Main Outcome Measures Infant length, weight, length-adjusted weight, head circumference at 6 and 12 months and mid-upper-arm circumference at 12 months.

Results Of the mothers and infants 96% and 99% were vitamin D sufficient [25(OH)D ≥50 nmol/L]. Mothers with pregnancy 25(OH)D >125 nmol/L had the shortest, lightest (in weight) and thinnest (in length-adjusted weight) infants at 6 months (P for all <0.05). For each 10 nmol/L higher UCB 25(OH)D, the infants were 0.03 SD score (SDS) shorter at 6 months (95% confidence interval -0.05, -0.01), adjusted for birth size, infant 25(OH)D, parental height and body mass index, socioeconomic status and breastfeeding. Higher UCB 25(OH)D associated with smaller head circumference at 6 and 12 months (P for all <0.05) but attenuated after adjustments. Mothers with pregnancy 25(OH)D >125 nmol/L had the thinnest infants at 12 months (P=0.021). For each 10 nmol/L higher infant 25(OH)D, the infants were 0.03 SDS lighter (-0.05, -0.01) and 0.03 SDS thinner (-0.05, 0.00) at 12 months.

Conclusions Our results suggest that high pregnancy, cord blood and infant vitamin D concentration may have disadvantageous effects on infant growth.

Precis: We investigated the effect of 25-hydroxyvitamin D concentration in pregnancy, cord blood and infancy on postnatal growth, and found that higher vitamin D status predict slower infant growth.

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1. Introduction

Several studies have associated poor maternal vitamin D status with fetal growth restriction (1-3). The few studies examining whether and how maternal or infant vitamin D status affect postnatal growth in the infant show inconsistent results. Many observational studies have found no association between maternal vitamin D status and linear growth in infants or in older children (4-8). However, a randomized trial of maternal vitamin D supplementation in Bangladesh, with mean baseline 25-hydroxyvitamin D concentration [25(OH)D] of 42 nmol/L, resulted in greater offspring length at 12 months of age (9).

In children, severe vitamin D deficiency increases the risk of rickets, which can lead to growth impairment (10). Indeed, among low-birth-weight infants in India, among whom vitamin D deficiency was common, a weekly vitamin D supplementation of 35 µg improved infant linear growth compared with placebo (11). However, a Canadian vitamin D intervention study (with vitamin D doses of 10, 20, 30 and 40 µg) in 132 healthy infants showed no difference between the groups in growth at 11 months despite different 25(OH)D, although this study may have been underpowered (12).

Previous studies have largely lacked subjects with high 25(OH)D concentrations and have focused on the effects of very low vitamin D status or vitamin D supplementation in vitamin D deficient children on infant growth. In addition, studies have rarely included data for both maternal and infant 25(OH)D concentrations. In Finland, vitamin D supplementation is recommended for pregnant women and all children, and vitamin D food fortification is used in liquid dairy products and fat spreads (13). Our objective was to explore whether 25(OH)D in pregnancy, umbilical cord blood (UCB) or in infancy at 12 months of age are associated with growth parameters in 6 and 12 months old infants. This study was part of the Vitamin D Intervention in Infants (VIDI) study, a randomized trial on vitamin D supplementation with the standard (10 µg) or a higher dose (30 µg) of vitamin D_3 in infants.

2. Materials and Methods

Subjects

At Kätilöopisto Maternity Hospital, Helsinki, Finland, 987 families were recruited into the VIDI study between January 2013 and June 2014, during the mother's hospital stay after delivery. Written informed consent was obtained from the parents at recruitment. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (107/13/03/03/2012). The project protocol is registered in ClinicalTrials.gov (NCT01723852). According to the inclusion criteria, the mothers were of Northern European origin without regular medication and with singleton pregnancy. Exclusion criteria for the newborns were: nasal continuous positive airway pressure treatment or need for nasogastric tube > one day, intravenous glucose infusion, seizures, and duration of phototherapy > three days. The infants were born between 37 + 0 and 42 + 0 weeks of gestation with birth weights appropriate for gestational age (SD-score [SDS] between -2.0 and +2.0).

Infants were randomized to receive daily vitamin D_3 supplementation with either 10 µg or 30 µg from age 2 weeks to 24 months. A detailed description of the recruitment and study protocol has been reported previously (14). Briefly, VIDI study included three study visits at the ages of 6, 12 and 24 months, and multiple retrospectively and prospectively collected questionnaires. Before study visit at 12 months, 112 infants (11%) dropped out from the study. For the present study, we included mother-infant-pairs with data on Infant 25(OH)D concentration, and body size measurements at 6 months and 12 months of age. Infants who did not meet the inclusion criteria (n=12) or were later diagnosed with a congenital disease influencing growth (n=1; Rieger syndrome) were excluded. Thus, the total number of subjects was 798. Of the infants, 51% (409/798) were girls. Number of subjects varies in different analyses due to partial missing data, and are reported in tables. As data on the infants' vitamin D supplemental dose were unavailable for this study, we adjusted the analyses for Infant 25(OH)D at 12 months as a marker of total vitamin D intake including supplements.

Biochemical analyses

25(OH)D measurements were obtained at three time points for each subject. Pregnancy serum samples were collected at prenatal clinics on average (SD) at gestational week 11 (2) between June 2012 and February 2014 as part of the mothers' normal follow-up [hereafter referred to as Pregnancy 25(OH)D]. Pregnancy samples were stored in the Finnish Maternity Cohort serum bank organized by the National Institute for Health and Welfare. UCB for 25(OH)D measurement was obtained at birth (gestational weeks 37 to 42) between January 2013 and June 2014 [hereafter referred to as UCB 25(OH)D]. At the 12-month follow-up visit, infant serum samples were obtained between December 2013 and June 2015, and analyzed for 25(OH)D [hereafter referred to as Infant 25(OH)D] and intact parathyroid hormone (PTH).

Pregnancy serum and UCB plasma 25(OH)D were analyzed simultaneously, and Infant serum 25(OH)D and PTH in a separate series using the IDS-iSYS fully automated immunoassay system with chemiluminescence detection (Immunodiagnostic Systems Ltd., Bolton, UK) with intra-assay variation <7% for Pregnancy 25(OH)D, Infant 25(OH)D and PTH, and <13% for UCB 25(OH)D. Detailed information on UCB plasma 25(OH)D (15) and Pregnancy 25(OH)D analysis (16) has been previously reported. The quality and accuracy of the 25(OH)D analyses are validated on an ongoing basis by participation in the vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, London, UK). The method showed a ≤8% positive bias against NIST Reference Measurement Procedure.

We defined vitamin D deficiency as 25(OH)D <50 nmol/L (17), and vitamin D sufficiency as 25(OH)D ≥50 nmol/L (18). Further, we divided subjects into groups based on 25(OH)D concentrations, using an additional cut-off values of 75 nmol/L, which has been considered a lower threshold value for bone health (17), and 125 nmol/L, above which values have been related to health risks (18).

Parental data

Parental data were obtained from a self-administered baseline questionnaire, filled out after delivery, and from medical records. Parental heights (cm) and weights (kg) before pregnancy and parity were collected primarily from the prenatal maternity card or from our baseline questionnaire. Parental heights were standardized into sex-specific z-scores. Prepregnancy body mass index (BMI) was calculated (kg/m^2). Duration of gestation was determined by first trimester ultrasound examination.

Parental education level was categorized into ‘lower’ and ‘higher’ education (lower =lower or upper secondary or post-secondary non-tertiary education/less than a bachelor degree, higher =first or second stage of tertiary education/at least a bachelor degree), according to the highest received degree of the parents. Parental smoking status was assessed before pregnancy and at infant age of 24 months, and applied as a merged previous and current smoking status. Family income level was enquired with a questionnaire completed at infant age of 24 months.

Infant data

Birth size was measured by midwives according to standard procedure and we collected data from birth records. Infant weight (kg), length (cm) and head circumference (cm) were measured at 6- and 12-month follow-up visits by a pediatrician or a research nurse. In addition, at 12-month follow-up visit, mid-upper-arm circumference (MUAC) (mm) was measured. Length was measured with a tabletop meter in a supine position, and weight with an electronic scale (Seca®, Hamburg, Germany). Weight, length, length-adjusted weight and head circumference were expressed as SDS using age- and sex-specific Finnish references (19). Weight, length, length-adjusted weight and head circumference were considered normal when between -2.0 and +2.0 SDS. MUAC was standardized into sex-specific z-score within the present study population as no Finnish normative data exist. Duration of breastfeeding was determined based on repeated questionnaires in prospectively collected study diaries.

Statistical analysis

The normality of the variables was visually inspected. Infant and family characteristics were reported as means, standard deviations, and percentages. Independent variables were Pregnancy, UCB and Infant 25(OH)D concentrations, and outcome variables were infant size at 6 and 12 months of age. Covariates were chosen based on their statistically significant association with several outcome measures. To assess the independent effect of Pregnancy and UCB 25(OH)D on growth, we adjusted the analyses for Infant 25(OH)D concentration at 12 months of age as a marker of the postnatal supplemental vitamin D intake. Missing values of covariates were multiple imputed (5 imputations). In tables with infant and family characteristics (Tables 1-2) all values are non-imputed.

A change in infant growth (length, weight, length-adjusted weight and head circumference) between birth, 6 and 12 months of age was calculated by saving the residuals from linear regression models of body size SDS at each successive age versus the corresponding body size SDS at all earlier ages. These residuals were referred to as “conditional growth”. Using conditional growth indicators as outcomes enabled us to explore whether 25(OH)D had an effect on the growth rate between different growth periods. Univariate and multivariate linear regression analysis was used to explore associations between 25(OH)D and infant growth and conditional growth. We used three models; Model 1: crude, Model 2: adjusted for corresponding birth size SDS, maternal and paternal height z-scores, and Infant 25(OH)D (except in analyses of Infant 25(OH)D), and Model 3: adjusted for Model 2 covariates, and for maternal and paternal prepregnancy BMI, parental smoking status, parental education level, family income level, and duration of breastfeeding. The analyses on conditional growth was conducted in similar manner, but without adjusting for birth size. Quadratic associations were explored with linear regression by adding the corresponding 25(OH)D squared in the growth models.

Infant size was investigated in categories of 25(OH)D with ANCOVA adjusted for corresponding birth size SDS, maternal and paternal height z-scores and Infant 25(OH)D. Differences in infant size were compared between categories of 25(OH)D with linear regression, using the 25(OH)D category of 50-74.9 nmol/L as a reference group. Statistical significance was determined at $P < 0.05$. All statistical analyses were conducted using the IBM SPSS program for Windows, version 22 (IBM, Chicago, IL, USA).

3. Results

Infant and family characteristics are presented in Tables 1 and 2. Almost all infants had normal body size (weight and length between -2.0 and +2.0 SDS) at 6 and 12 months of age. Of the infants, 79% were partially breastfed at 6 months and 40% at 12 months of age. Of the mothers, 96% and, of the 12-month-old infants, 99% were vitamin D sufficient (25(OH)D ≥50 nmol/L). Altogether 2% of Pregnancy, 4% of UCB and 17% of Infant 25(OH)D values were above 125 nmol/L, ranging up to 189 nmol/L, 284 nmol/L and 241 nmol/L, respectively. Correlation coefficient between Pregnancy and UCB 25(OH)D was 0.27 ($P < 0.001$), between UCB and Infant at 12 months 25(OH)D 0.15 ($P < 0.001$), and between Pregnancy and Infant at 12

months 25(OH)D 0.07 (P =0.081). In 88% of the infants, PTH concentration at 12 months was normal (11.5-78.4 pg/mL), and declined with increasing Infant 25(OH)D concentration (Supplementary Table 5).

All linear and quadratic associations between 25(OH)D concentrations and infant growth are shown in Tables 3-4. Conditional growth results are shown in Supplementary Tables 1-2. We categorized 25(OH)D concentrations into four groups; <50 nmol/L, 50-74.9 nmol/L (reference group), 75-125 nmol/L and >125 nmol/L (Figures 1-2 and Supplementary Tables 3-4). Infant growth in the lowest and the two highest 25(OH)D groups was compared with the reference group of 50-74.9 nmol/L. Pregnancy 25(OH)D predicted length-adjusted weight at 6 and 12 months (Tables 3-4). At 6 months, mothers whose Pregnancy 25(OH)D was above 125 nmol/L had the shortest (in length) (mean difference 0.41 SDS, P =0.048), lightest (in weight) (mean difference 0.63 SDS, P =0.016) and thinnest (in length-adjusted weight) (mean difference 0.70 SDS, P =0.013) infants compared with the reference group (Figure 1, Supplementary Table 3). We found a quadratic association between Pregnancy 25(OH)D and length at 12 months (Table 4). We also observed an inverse association between Pregnancy 25(OH)D and MUAC but the association attenuated to non-significance after adjustments (Table 4). The 12 months' MUAC was smaller in the group with Pregnancy 25(OH)D at 75-125 nmol/L (mean difference 0.17 SDS, P =0.049) compared with the reference group (Figure 2, Supplementary Table 4). At 12 months of age, infants of mothers with the highest Pregnancy 25(OH)D were the thinnest (mean difference 0.60 SDS, P =0.021) compared with the reference group (Figure 2, Supplementary Table 4).

Infants with higher UCB 25(OH)D were shorter at 6 months, and they had a smaller head circumference at 6 and 12 months, although this attenuated to non-significance when adjusted (Tables 3-4). As demonstrated by conditional lengths, infants with higher UCB 25(OH)D grew more slowly between birth and 6 months but more rapidly between 6 and 12 months (Supplementary Tables 1-2). Newborns with UCB 25(OH)D below 50 nmol/L were the lightest at 6 months (mean difference 0.32 SDS, P =0.034) compared with the reference group (Figure 1, Supplementary Table 3).

A higher Infant 25(OH)D at 12 months was associated with smaller length, weight, length-adjusted weight and head circumference at 12 months, although the associations with length and head circumference attenuated with adjustments (Table 4). Further, infants with 25(OH)D above 125 nmol/L at 12 months had

217 the smallest weight (mean difference 0.25 SDS, P =0.022) and length-adjusted weight (mean difference 0.25
218 SDS, P =0.032) compared with the reference group (Figure 2, Supplementary Table 4).
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4. Discussion

We here demonstrate that high 25(OH)D in the mother and infant were associated with slower postnatal growth in a large prospective study of healthy full-term infants. This study is among the few to explore whether maternal and infant vitamin D status were associated with growth parameters in 6 and 12 months old infants. The study was carried out in a population of Northern latitude but where severe vitamin D deficiency is rare. Almost all mothers and infants were vitamin D sufficient [25(OH)D ≥50 nmol/L]. Our main finding was counterintuitive: high 25(OH)D in pregnancy, UCB and infancy associated with delayed growth in infants.

The main findings showed that mothers with Pregnancy 25(OH)D >125 nmol/L had the shortest (in length), lightest (in weight) and thinnest (in length-adjusted weight) infants at 6 months of age. A comparable trend persisted until 12 months of age. Similarly, infants with higher UCB 25(OH)D were shorter at 6 months of age, but those with 25(OH)D <50 nmol/L were the lightest. As for length, we observed a slow linear growth until the age of 6 months and an accelerated growth from 6 to 12 months of age in those with higher UCB 25(OH)D. Furthermore, infants with higher vitamin D status at 12 months had lower weight and length-adjusted weight at 12 months of age, with the lightest and thinnest infants having 25(OH)D >125 nmol/L. These results give new insight into how vitamin D status affects postnatal growth in healthy infants in a mostly vitamin D sufficient population.

Evidence has been inconsistent concerning associations between maternal vitamin D status and postnatal growth. The few studies in populations with relatively low 25(OH)D have found positive (20, 21), negative (21) or lacking (22) associations between maternal 25(OH)D and infant length or weight among 1-6 month-old infants. In Gambian mothers with relatively high mean pregnancy 25(OH)D (103-111 nmol/L), vitamin D status was unrelated to infant growth at 3 months of age (23). However, we found that higher Pregnancy and UCB 25(OH)D predicted slower infant growth until 6 months of age. In the Bangladeshi study with 134 infants, of which 26% were stunted during the follow-up, maternal supplementation of 875 µg/week resulted in divergent mean UCB 25(OH)D between supplemented and placebo-treated mothers (103 vs. 39 nmol/L) (9). Opposed to our findings, this study found that maternal supplementation increased infant length at 1 and 2 months but no longer at 4 and 6 months, and then again at 12 months of age (9). In older infants

(9 to 18 months) the relation of maternal vitamin D status with infant growth has similarly been conflicting (5, 6, 8, 9, 20, 21, 23). In our study, the possibly disadvantageous effect of high Pregnancy and UCB 25(OH)D on several infant growth indicators remained to some extent until 12 months of age. Our finding is somewhat contrary to a Dutch multi-ethnic study, where slow fetal growth but enhanced postnatal growth was observed in infants of mothers with 25(OH)D <30 nmol/L compared with those with 25(OH)D >50 nmol/L (21). However, our results may suggest a similar catch-up growth but, in contrast, in infants of mothers with high 25(OH)D. Together these results may indicate an inverse U-shaped association between maternal 25(OH)D and infant growth, as has been proposed to exist between maternal vitamin D status and fetal growth (24, 25). We previously reported in this cohort an inverse relationship between UCB 25(OH)D and newborn head circumference (16). This finding was still apparent at 6 and 12 months but attenuated when adjusting for birth head circumference and parental height.

In addition to Pregnancy and UCB 25(OH)D, also high Infant 25(OH)D was negatively associated with postnatal growth; the strongest associations appeared with weight and length-adjusted weight. These findings are in line with a Danish study which found 25(OH)D (mean 77 nmol/L) at 9 months to associate negatively with length and BMI (26). However, two previous vitamin D supplementation trials in infants found no effect on growth between different dosages (12, 27). By contrast, in Indian low-birth-weight infants, the vitamin D supplementation from the age of 1 week until 6 months increased infant length and weight at 6 months but no longer at 3-6 years (11). In that study, vitamin D deficiency was highly prevalent in the placebo group (73%) (11). The inconsistent findings on vitamin D and infant postnatal growth may be due to ethnic, genetic, geographical and lifestyle differences between study populations (28-30), and above all, differences in 25(OH)D concentrations. However, this is difficult to prove as there is lack of study populations with both low and high 25(OH)D values. Further, previous studies have applied varying cut-off values for 25(OH)D (8, 31). Ong et al. suggested that severe vitamin D deficiency (<30 nmol/L) may impair child growth but beyond that there might not be any association between vitamin D status and early life growth (8). However, studies involving populations with especially high 25(OH)D are limited, and many studies have presented only restricted group-level data.

Taken together, our results suggest that an inverse U-shaped association may exist between 25(OH)D and postnatal growth, as slower growth was more evident in the group of 25(OH)D below 50

nmol/L and above 125 nmol/L in several growth parameters, although the limited number of subjects in both extreme ends of the 25(OH)D range constrained our analysis. The inverse U- or J-shaped relationship between vitamin D status and health is supported by observations in adults (31). In addition, our previous finding of a positive association between UCB 25(OH)D and inflammatory markers further support the possible adverse effect of high 25(OH)D on infant health (32). The rather high Pregnancy and UCB 25(OH)D in our cohort can be explained by the fact that almost all mothers (95%) in our study were taking vitamin D supplements during pregnancy with a mean daily intake of 16 µg (15). In addition, vitamin D fortified foods are commonly used in Finland (13). As regard to infants, based on the VIDI study protocol, infants received vitamin D supplements of 10 µg or 30 µg daily leading to relatively high 25(OH)D.

High vitamin D status may impair infant growth by affecting calcium and phosphate homeostasis. We have previously shown that Infant 25(OH)D correlated positively with ionized calcium concentration but no severe hypercalcemia developed (33). It is possible that high 25(OH)D leads to increased bone resorption via increased $1,25(OH)_2$ vitamin D [$1,25(OH)_2D$], thus possibly reducing infant growth by impairing bone growth and growth plate function. In genetically modified mice, high maternal $1,25(OH)_2D$ levels adversely affected the total amount of calcium stored in fetal bones (34). In current study, the mean PTH concentration was normal, although lower in the higher 25(OH)D category, indicating that there was no overt suppression of PTH secretion. Our previous study showed that urine calcium excretion was similar in 3-month-old infants supplemented with 10, 30 or 40 µg vitamin D_3, and that 25(OH)D did not correlate with bone parameters (27). The nature of our study did not allow us to explore potential mechanisms for adverse growth outcomes caused by high 25(OH)D concentrations.

A strength and a limitation of this study is in the recruitment, which took place in a single hospital; this enabled standardized data collection but a multi-centre study might have resulted in a more diverse study population. A limitation was the inability to use data on infant vitamin D supplementation because of the ongoing double-blind intervention. However, we applied the attained 25(OH)D concentration as a reflection of the total vitamin D intake. Furthermore, the limited number of subjects with low 25(OH)D concentration may have restrained our analyses. The strength of our study is the varied and in-depth approach to analyze the relationships between Pregnancy, UCB and Infant 25(OH)D on infant growth. In

addition, we were able to adjust our analyses for several potential confounders in order to identify the independent role of vitamin D in growth regulation.

5. Conclusion

In our large cohort, high 25(OH)D concentrations in pregnancy, UCB and infancy were associated with slower growth in infants at 6 and 12 months of age. Overall, our findings suggest an inverse U-shaped association between vitamin D status and infant postnatal growth. Although direct clinical implications remain to be further explored, our results indicate that in Finland an adequate maternal and infant vitamin D status has been achieved with a daily vitamin D supplementation of 10 µg and with vitamin D food fortification. We urge caution in aiming for a higher maternal or infant 25(OH)D concentration with excessive supplementation, as this might have unexpected and possibly disadvantageous effects on infant growth.

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Author contributions

The authors' contributions are as follows: SA, OM, OMH, EHS and HTV initialized the study; HHH, OM and EK planned the work; HHH, EMH, MEC, JR, SMV, HTV, TKH and OMH participated in acquisition of the data; HHH analyzed the data; HHH wrote the first draft of the manuscript; all authors participated in interpretation of the results, writing the manuscript and approved the final version.

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Table 1 Infant characteristics (n=798)

	At birth	At 6 months	At 12 months
Gestational age, week	40.2 (1.1)	-	-
Age, months	-	6.0 (0.2)	12.0 (0.4)
Length, cm	50.4 (1.7)	67.5 (2.2)	75.3 (2.5)
Length, SDS	-0.19 (0.88)	-0.47 (0.97)	-0.54 (1.01)
Weight, kg	3.5 (0.4)	8.0 (0.9)	9.8 (1.1)
Weight, SDS	-0.26 (0.79)	0.21 (1.07)	-0.24 (1.01)
Length-adjusted weight, SDS	0.07 (0.94)	0.15 (1.11)	0.02 (1.02)
Head circumference[1], cm	35.2 (1.4)	43.6 (1.2)	46.5 (1.2)
Head circumference[1], SDS	-0.16 (0.97)	-0.30 (0.94)	-0.42 (0.94)
Mid-upper-arm circumference [2], cm	-	-	15.3 (1.2)
Blood 25(OH)D, nmol/L [3] [range]	82.5 (25.8) [36.7-283.7]	-	98.9 (29.0) [23.0-241.0]
Blood 25(OH)D ≥50 nmol/L, % [3]	96	-	99
Normal length SDS (-2.0-2.0), %	98	94	93
Normal weight SDS (-2.0-2.0), %	100	94	96
Normal length-adjusted weight SDS (-2.0-2.0), %	96	94	96
Normal head circumference SDS (-2.0-2.0), %[1]	95	96	96
Breastfed [4], %	-	79	40
Sex, female, %		51	

Values are means (SD) unless stated otherwise, a dash indicate no data available.
SDS, SD-score, which is based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, 25-hydroxyvitamin D concentration.
[1] At birth 2 values are missing, at 6 months follow-up 21 values are missing, and at 12 months follow-up 5 values are missing.
[2] 39 values are missing.
[3] Samples at birth are from umbilical cord blood and 18 values are missing. Samples at 12 months are from infant serum blood.
[4] 13 values are missing.

Table 2 Family characteristics

	n	Mean (SD)
Maternal age, year	798	31.7 (4.3)
Paternal age, year	759	33.6 (5.3)
Maternal height, cm	798	166.4 (6.0)
Paternal height, cm	778	180.3 (6.6)
Maternal prepregnancy BMI	794	23.2 (3.6)
Paternal prepregnancy BMI	771	25.7 (3.4)
Pregnancy 25(OH)D, nmol/L [range]	671	82.4 (20.3) [24.8-189.2]
Pregnancy sampling, gestational week	671	11.3 (2.2)
Pregnancy 25(OH)D ≥50 nmol/L, %	671	96
Parity, primipara, %	797	63
Maternal smoking[1], yes, %	793	16
Paternal smoking[1], yes, %	784	26
Parental education, higher[2], %	789	81
Family income level	689	
<40 000 €/year, %		19
40 000-89 000 €/year, %		60
>90 000 €/year, %		21

Values are means (SD) unless stated otherwise.
BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D concentration.
[1] Previous and current smoking status.
[2] Higher education reflects at least a bachelor level education.

Table 3 Associations between 25(OH)D concentrations and infant growth at 6 months follow-up

	SDS at 6 months' follow-up			
Pregnancy 25(OH)D, 10 nmol/L, n=671	Length	Weight	Length-adjusted weight	Head circumference[3]
Model 1, crude	-0.02 (-0.06, 0.01)	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)‡	-0.02 (-0.05, 0.02)
Model 2, adjusted[1]	-0.02 (-0.05, 0.01)	-0.03 (-0.07, 0.00)	-0.03 (-0.07, 0.01)‡	-0.02 (-0.05, 0.01)
Model 3, adjusted[2]	-0.02 (-0.05, 0.01)	-0.03 (-0.06, 0.01)	-0.03 (-0.07, 0.02)‡	-0.02 (-0.05, 0.01)
UCB 25(OH)D, 10 nmol/L, n=780				
Model 1, crude	-0.04 (-0.06, -0.01)*	-0.01 (-0.04, 0.02)	0.00 (-0.03, 0.03)	-0.03 (-0.06, -0.01)*
Model 2, adjusted[1]	-0.03 (-0.05, -0.01)*	0.00 (-0.01, 0.02)	0.01 (-0.02, 0.04)	-0.02 (-0.03, 0.00)
Model 3, adjusted[2]	-0.03 (-0.05, -0.01)*	0.00 (-0.02, 0.03)	0.01 (-0.02, 0.04)	-0.02 (-0.04, 0.01)

Values are B coefficients (95% CI) per 10 nmol/L higher in 25(OH)D concentration based on linear regression.
* Statistically significant linear association, P <0.05; ‡ Statistically significant quadratic association, P <0.05.
SDS, SD score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; UCB, umbilical cord blood.
[1] Model 2 is adjusted for the corresponding birth size SDS, maternal and paternal height z-scores, and Infant 25(OH)D which served as a marker of infant supplemental vitamin D intake (except for Infant 25(OH)D).
[2] Model 3 is adjusted for model 2 covariates, and in addition maternal and paternal prepregnancy BMI, smoking status of the parents, parental education, family income level, and duration of breastfeeding until 6 months of age.
[3] Number of subjects vary in analyses due to missing values of head circumferences; for Pregnancy 25(OH)D in Model 1: n=650, in Model 2 and 3: n=649; for UCB 25(OH)D in Model 1: n=759, in Model 2 and 3: n=757.

Table 4 Associations between 25(OH)D concentrations and infant growth at 12 months follow-up

	SDS				z-score
Pregnancy 25(OH)D, 10 nmol/L, n=671	Length	Weight	Length-adjusted weight	Head circumference[3]	MUAC[4]
Model 1, crude	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.01)	-0.03 (-0.07, 0.01)‡	-0.02 (-0.05, 0.02)	-0.04 (-0.08, 0.00)*
Model 2, adjusted[1]	-0.01 (-0.05, 0.02)‡	-0.03 (-0.06, 0.01)	-0.03 (-0.07, 0.01)‡	-0.02 (-0.05, 0.02)	-0.04 (-0.08, 0.00)*
Model 3, adjusted[2]	-0.01 (-0.04, 0.02)‡	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.02)‡	-0.02 (-0.05, 0.02)	-0.03 (-0.07, 0.01)
UCB 25(OH)D, 10 nmol/L, n=780					
Model 1, crude	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.01)	-0.02 (-0.04, 0.01)	-0.03 (-0.05, 0.00)*	-0.02 (-0.04, 0.01)
Model 2, adjusted[1]	0.00 (-0.03, 0.02)	0.00 (-0.02, 0.01)	-0.01 (-0.04, 0.02)	-0.01 (-0.02, 0.00)	-0.01 (-0.04, 0.01)
Model 3, adjusted[2]	0.00 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.01)	-0.01 (-0.04, 0.01)
Infant 25(OH)D, 10 nmol/L, n=798					
Model 1, crude	-0.03 (-0.05, 0.00)*	-0.03 (-0.06, -0.01)*‡	-0.02 (-0.05, 0.00)*	-0.02 (-0.05, 0.00)*	-0.02 (-0.04, 0.01)
Model 2, adjusted[1]	-0.02 (-0.04, 0.00)	-0.03 (-0.05, -0.01)*	-0.03 (-0.05, 0.00)*‡	-0.02 (-0.04, 0.01)	-0.02 (-0.04, 0.01)
Model 3, adjusted[2]	-0.02 (-0.04, 0.00)	-0.03 (-0.05, -0.01)*	-0.03 (-0.05, 0.00)*	-0.02 (-0.04, 0.01)	-0.02 (-0.04, 0.01)

Values are B coefficients (95% CI) per 10 nmol/L higher in 25(OH)D concentration based on linear regression.
* Statistically significant linear association, P <0.05; ‡ Statistically significant quadratic association, P <0.05.
SDS, SD score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; UCB, umbilical cord blood.
[1] Model 2 is adjusted for the corresponding birth size SDS (except for MUAC; the covariate was length-adjusted birth weight), maternal and paternal height z-scores, and Infant 25(OH)D which was served as a marker of infant supplemental vitamin D intake (except when Infant 25(OH)D as dependent).
[2] Model 3 is adjusted for model 2 covariates, and in addition maternal and paternal prepregnancy BMI, smoking status of the parents, parental education, family income level, and duration of breastfeeding until 12 months of age.
[3] Number of subjects vary in analyses due to missing values of head circumferences; for Pregnancy 25(OH)D in Model 1: n=666, in Model 2 and 3: n=665; for UCB 25(OH)D in Model 1: n=775, in Model 2 and 3: n=773; for Infant 25(OH)D in Model 1: n=793, in Model 2 and 3: n=791.
[4] Number of subjects vary in analyses due to missing values of MUAC; for Pregnancy 25(OH)D: n=644; for UCB 25(OH)D: n=742; for Infant 25(OH)D: n=760.

Figure 1 The effect of Pregnancy **(a)** (n=671) and umbilical cord blood (UCB) **(b)** (n=780) 25-hydroxyvitamin concentration (25(OH)D) on infant growth parameters at the age of 6 months. 25(OH)D concentration is expressed in categories of **1** (<50 nmol/L), **2** (50-74.9 nmol/L (reference group)), **3** (75-125 nmol/L) and **4** (>125 nmol/L). Values are means with 95% confidence intervals adjusted for corresponding birth size SD score (SDS), maternal and paternal height z-scores, and Infant 25(OH)D which served as a marker of infant supplemental vitamin D intake. ***** indicates a statistically significant association between the reference group and other groups ($P < 0.05$). SDS, standard deviation score. Number of subjects in Pregnancy 25(OH)D categories: 1, n=25; 2, n=218; 3, n=412; 4, n=16, and in UCB 25(OHD: 1, n=29; 2, n=294; 3, n=425; 4, n=32.

Figure 2 The effect of Pregnancy **(a)** (n=671), umbilical cord blood (UCB) **(b)** (n=780) and infant **(c)** (n=798) 25-hydroxyvitamin concentration (25(OH)D) on infant growth parameters at the age of 12 months. 25(OH)D concentration is expressed in categories of **1** (<50 nmol/L), **2** (50-74.9 nmol/L (reference group)), **3** (75-125 nmol/L) and **4** (>125 nmol/L). Values are means with 95% confidence intervals adjusted for corresponding birth size SD score (SDS) (except for mid-upper-arm circumference (MUAC); the covariate was length-adjusted birth weight), maternal and paternal height z-scores, and Infant 25(OH)D which served as a marker of infant supplemental vitamin D intake (except when Infant 25(OH)D as dependent). ***** indicates a statistically significant association between the reference group and other groups ($P < 0.05$). SDS, standard deviation score. Number of subjects in Pregnancy 25(OH)D categories: 1, n=25; 2, n=218; 3, n=412; 4, n=16, in UCB 25(OHD: 1, n=29; 2, n=294; 3, n=425; 4, n=32, and in Infant 25(OH)D: 1, n=10; 2, n=160; 3, n=493; 4, n=135.