Clinical Research Article

Maternal and Neonatal 3-epi-25-hydroxyvitamin D Concentration and Factors Influencing Their Concentrations

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Abstract

Background: Little is known about the presence of 3-epi-25 hydroxyvitamin D in maternal and neonatal circulation, the extent of its contribution to total 25 hydroxyvitamin D, or factors influencing its levels.

Methods: A total of 1502 and 1321 archived maternal and umbilical cord serum samples from the Hyperglycemia and Adverse Pregnancy Outcome Study cohort from Hong Kong were assayed for 25(OH)D2, 25(OH)D3, and isomeric form of 25(OH)D3 (3-epi-25(OH)D3) by a liquid chromatography-tandem mass spectrometry method.

Results: Vitamin D deficiency (total serum 25(OH)D level < 50 nmol/L) and severe vitamin D deficiency (total serum 25(OH)D level < 25 nmol/L) occurred in 590 (39.3%) and 25 (1.7%) mothers, respectively. 3-epi-25(OH)D3 could be detected in 94.5% of maternal and 92.1% of neonatal umbilical sera, with the highest 3-epi-25(OH)D3 levels contributing to 19.9% and 15.3% of the maternal and umbilical cord sera 25(OH)D3 levels, respectively. Pregnancy with a male fetus, ambient solar radiation, and maternal glycemia and 25(OH)D3 levels were

independent factors associated with maternal 3-epi-25(OH)D3 level. Advanced maternal age, multiparity, maternal gestational weight gain below the Institute of Medicine recommendation, maternal glycemic status, and earlier gestational age at delivery were significantly associated with higher umbilical cord serum 3-epi-25(OH)D3.

Conclusions: 3-epi-25(OH)D3 accounted for a significant portion of total 25(OH)D in maternal and neonatal circulations. Further study is needed to determine the possible mechanism underlying this observation.

Key Words: vitamin D, 3-epi-25(OH)D3, 25-hydroxyvitamin D, maternal, umbilical cord

Vitamin D is now recognized not only for its importance in maintaining bone health, but also for its role in a broader range of other chronic diseases including cardiovascular disease, autoimmune disease, and certain cancers. Observational studies suggest a link between low levels of vitamin D and adverse pregnancy outcomes, such as gestational diabetes, preeclampsia, infections, fetal growth restriction, and cesarean delivery [1]. Moreover, maternal vitamin D status could have long-term effects on a child's neurodevelopment, atopy, and bone health [2-7]. Because vitamin D levels in newborns are largely dependent on their mother's vitamin D status, infants born to vitamin D-deficient mothers are at risk of vitamin D deficiency. Strictly speaking, 1,25 hydroxyvitamin D, the active form in the body, acts as a hormone rather than a vitamin. Vitamin D comes in 2 main forms, namely, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol).

Vitamin D status can be evaluated by the measurement of serum concentrations of 1 major vitamin D metabolite, 25-hydroxyvitamin D (25(OH)D). The introduction of liquid chromatography-tandem mass spectrometry (LC-MS/MS) allows more precise measurement of 25(OH)D and quantitatively separates 25(OH)D2 and 25(OH)D3. Despite significant improvements in vitamin D assay standardization, inaccurate recovery of 25(OH)D2 remains a pitfall of immunoassays, whereas many LC-MS/MS methods do not separate the biologically inactive 3-epimer interferents [8]. The isomeric form of 25(OH)D3, 3-epi-25(OH)D3, has been shown to contribute significantly to the 25(OH)D3 measured by LC-MS/MS methods, particularly in infants [9, 10].

Meanwhile, little is known about the C-3 epimerization of 25(OH)D in utero and to what extent the presence and concentration of 3-epi-25(OH)D in the neonatal circulation precludes accurate measurement of maternal and neonatal vitamin D status. Studies from several cohorts of mother-child dyads reported that 3-epi-25(OH)D3 varied from 6.6% to 22.2% of total 25(OH)D and absolute concentrations were between 3 and 5 nmol/L in the neonatal circulation [11-15]. However, the biological role of 3-epi-25(OH)D3 in mothers and neonates remains largely unknown. In the present study, we aimed to assess factors that determine the presence and fractions of 3-epi-25(OH)D3 in newborns and their association with maternal vitamin D status.

Materials and Methods

The participants were ethnic Chinese mothers who participated in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study at the Hong Kong study center between 2000 and 2006. Details of the HAPO Study were described previously [16]. All pregnant women booked for antenatal care in the study center were eligible to participate unless they had 1 or more of the following exclusion criteria: age younger than 18 years, a plan to undergo delivery at another hospital, an uncertain date of last menstrual period and no ultrasonographic estimation between 6 and 24 weeks of gestational age, inability to complete the oral glucose tolerance test (OGTT) within 32 weeks of gestation, multiple pregnancy, conception by means of gonadotropin ovulation induction or in vitro fertilization, glucose testing before recruitment or a diagnosis of diabetes during the current pregnancy, diagnosis of diabetes before the current pregnancy and requiring treatment with medication, participation in another study that could interfere with the HAPO study, or infection with HIV or hepatitis B or C virus. If glucose measurements were made outside the setting of the HAPO Study after initial enrollment, the participants were excluded.

All participants underwent a standard 75-g OGTT between 24 and 32 weeks of gestation and umbilical cord blood samples were collected at delivery. A blood specimen was collected between 34 and 37 weeks of gestation for evaluation of a random plasma glucose level as a safety measure to identify cases with hyperglycemia above a predefined threshold. The OGTT data were unblinded and subjects were excluded if the 2-hour plasma glucose level was diagnostic of diabetes (ie, > 11.1 mmol/L) or the fasting plasma glucose level exceeded 5.8 mmol/L, if the random plasma glucose level was ≥ 8.9 mmol/L, or if any plasma glucose level was < 2.5 mmol/L. Maternal fasting serum samples collected at the time of the OGTT and umbilical cord serum samples collected at delivery, which had been

stored at -80°C at the Northwestern University Feinberg School of Medicine (Chicago, IL), were shipped back on dry ice to Hong Kong for the 25(OH)D assays.

Laboratory Assay

Serum 25(OH)D2, 25(OH)D3, and 3-epi-25(OH)D3 were extracted from the archived sera and measured by an LC-MS/MS method at the Biomedical Mass Spectrometry Unit, Department of Chemical Pathology, the Chinese University of Hong Kong, as previously described [17]. The LC-MS/MS method was modified to separate 3-epi-25(OH)D3 from 25(OH)D3. Deuterium-labelled 25(OH)D2 (d_3-25(OH)D2) and 25(OH)D3 (d_6-25(OH)D3) were used as internal standards. Quantitation of the metabolites was on a Waters UPLC Xevo TQS Micro System (Waters Corporation, Milford, MA, USA). 25(OH)D3 and 25(OH)D2 were separated on a Waters Acquity UPLC CSH Fluoro-Phenyl Column (1.7 μm, 2.1 × 100 mm) by gradient programming of mobile phase solutions containing methanol in 2 mM ammonium acetate (flow rate, 200 μL/min). Injection to injection time was 12 minutes. Under electrospray-positive ionization, peak areas of 25(OH)D3 and 25(OH)D2 and their internal standards were measured by monitoring specific multiple reaction monitoring (MRM) transitions. The calibrators for serum 25(OH)D2 and 25(OH)D3 were from Chromsystems (3 PLUS 1 Multilevel serum calibrator set 25-OH-Vitamin D3/D2, Munich, Germany). The calibrators for serum 3-epi-25(OH)D3 were prepared in-house by spiking different amounts of pure standard into horse serum, which did not have detectable 3-epi-25(OH)D3 and was supplied by the local Racing Laboratory. Quantitation of 25(OH)D3 and 25(OH)D2 was performed by the TargetLynx Manager in the Waters MassLynx 4.1 software by linear regression of peak area ratios: 25(OH)D2/d_3-25(OH)D2, 25(OH)D3/d_6-25(OH)D3, and 3-epi-25(OH)D3/d_3-25(OH)D2, against the calibrator concentrations with 1/× weighting. The difference between the results of the confirmation MRMs and the quantitation MRMs was required to be < 20%. The lower limits of detection (LOD) and quantitation were 0.4 and 1.0 nmol/L, respectively. The analytical measurement ranges were 1 to 750 nmol/L for serum 25(OH)D2 and 25(OH)D3, and 1 to 250 nmol/L for 3-epi-25(OH)D3. Between-batch coefficients of variation of 25(OH)D2 and 25(OH)D3 were < 3.8% and that for 3-epi-25(OH)D3 was < 7.6%. Accuracy performance was monitored regularly by measuring proficiency testing samples from the UK Vitamin D External Quality Assessment Scheme. An earlier study showed that 25(OH)D remained stable in serum stored below -20°C up to 24 years [18]. Our experience also showed that serum 25(OH)D levels remained unchanged after 3 freeze-thaw cycles.

Statistical analysis

We defined maternal vitamin D deficiency as total serum 25(OH)D (the sum of serum 25(OH)D2 and 25(OH)D3) concentration below 50 nmol/L and severe deficiency as total serum 25(OH)D < 25 nmol/L, according to the Endocrine Society definition [19]. Most publications also define neonatal vitamin D and severe vitamin D deficiency using the same cutoffs for umbilical cord total serum 25(OH)D level [20-22]. However, some authors and position statements have defined neonatal vitamin D deficiency and severe vitamin D deficiency as umbilical cord serum 25(OH)D < 30 nmol/L and < 12.5 nmol/L, respectively [23-25]. The monthly global solar radiation recorded at the Hong Kong Observatory during the study period (supplementary Table 1) [26] was available from the public domain at the website http://www.hko.gov.hk/en/index.html. In Hong Kong, May to October are the warm season months with stronger solar radiation, whereas November to April are the cold season months, with lower solar radiation.

The concentrations of all 3 vitamin D metabolites were assessed for normality by observing the histograms. The overall distribution of maternal and umbilical serum vitamin D metabolites at or above the LOD and the 3 epi-25 (OH)D3 to 25(OH)D3 ratio were expressed as median (IQR) and ranges. Other continuous data were expressed as mean ± SD unless otherwise specified, whereas categorical data were expressed as counts (percentage). The maternal and neonatal characteristics were compared according to different maternal and umbilical cord serum 3 epi-25(OH)3 levels, categorized as ≤ 1.00, 1.01 to 1.50, 1.51 to 2.00, 2.01 to 3.00, and > 3.0 nmol/L, using analysis of variance with continuous variables, and χ^2 tests with categorical variables.

Multiple regression analyses with log-transformation of 3-epi-25(OH)D3 levels were used to show the factors and covariates that may be associated with the maternal and umbilical cord serum 3-epi-25(OH)D3 levels. 3-epi-25(OH)D3 levels below the LOD were replaced by 0 and removed from multiple regression analyses.

Pearson correlation was used to display the relationship between maternal and umbilical cord serum 25(OH)D3 levels and 3-epi-25(OH)D3 levels. $P < 0.05$ was used to indicate significance for 2-tailed statistical test results. Statistical analyses were performed using IBM SPSS 26.0 software (SPSS Inc., Chicago, IL, USA).

Results

Among 1611 eligible participants, 1502 maternal and 1321 umbilical cord serum samples (1237 with paired samples) were available for the assay of 25(OH)D2, 25(OH)D3, and 3-epi-25(OH)D3; 25(OH)D2, 25(OH)D3 and

3-epi-25(OH)D3 were detectable in 641 (42.7%), 1502 (100%), and 1419 (94.5%) of the maternal sera and in 140 (10.6%), 1321 (100%), and 1217 (92.1%) of umbilical cord sera, respectively. The median total 25(OH)D, 25(OH)D2, 25(OH)D3, and 3-epi-25(OH)D3 in the maternal and umbilical cord sera were 54.8, 1.3, 53.0, and 1.9 and 40.7, 1.4, 39.6, and 1.7 nmol/L, respectively (Table 1). Among the serum samples with detectable 3-epi-25(OH)D3, the level was 0.5% to 19.9% and 0.7% to 15.3% of the maternal and umbilical cord serum 25(OH)D3 levels, respectively. The frequency distributions of maternal and umbilical cord serum total 25(OH)D, 25(OH)D2, 25(OH)D3, and 3-epi-25(OH)D3 are shown in supplementary Fig. 1 [26].

A total of 590 (39.3%) mothers were classified as vitamin D deficient (total serum 25(OH)D level < 50 nmol/L) and 25 (1.7%) as severe vitamin D deficient (total serum 25(OH)D level < 25 nmol/L). If an assay method unable to distinguish 3-epi-25(OH)D3 from 25(OH)D3 was used to quantify vitamin D deficiency, 544 (36.2%) mothers (ie, 3.1% less) would be classified as deficient and 18 (1.2%) (ie, 0.5% less) as severely deficient. Significant monthly variations (based on the month of serum sample collection) of maternal and umbilical cord blood serum total 25(OH)D concentrations were observed (Fig. 1). The median maternal serum total 25(OH)D levels were lowest in the quarter from February to April (46.4-49.0 nmol/L), whereas the median umbilical cord serum total 25(OH)D levels were lowest in the quarter from January to March (36.1-37.4 nmol/L). The rate of maternal vitamin D deficiency was highest (59.2%) in February and lowest (27.8%) in August (supplementary Fig. 2) [26]. Neonatal vitamin D deficiency was present in 952 (72.1%) newborns using the criteria of umbilical cord serum total 25(OH)D level < 50 nmol/L, whereas severe deficiency occurred in 116 (8.8%) or 1 (0.1%) newborn as defined by umbilical cord serum total 25(OH)D level < 25 or < 12.5 nmol/L, respectively.

Table 2 showed that mothers who had higher serum 3-epi-25(OH)D3 levels were significantly older, exposed to greater ambient solar radiation at the month of blood collection, and had higher maternal 25(OH)D3 levels, higher glucose levels (represented by the area under the curve of maternal glucose levels at the OGTT [AUC_{glu}]) and higher rate of carrying a male fetus in a univariate analysis.

Table 3 compared maternal characteristics and pregnancy outcomes across different umbilical cord serum 3-epi-25(OH)D3 levels. Mothers whose newborns had higher umbilical serum 3-epi-25(OH)D3 levels were older, exposed to greater ambient solar radiation at the month of delivery, and had lower body mass index, higher rate of multiparity, higher glycemic levels (AUC_{glu}), and less weight gain in pregnancy, whereas their offspring were born at an earlier gestational age and had higher maternal 25(OH)D3 levels.

In a multivariate regression model, ambient solar radiation, maternal glycemic level and maternal 25(OH)D3 level were independent factors associated with maternal 3-epi-25(OH)D3 levels, whereas maternal glycemic status, earlier gestational age at delivery, ambient solar radiation, and maternal and umbilical cord sera 25(OH)D3 levels were independent factors associated with higher umbilical cord 3-epi-25(OH)D3 levels (supplementary Tables 2 and 3) [26]. The coefficients (β) ranged from -0.004 to 0.02. Hence, the overall effect of this combination of factors on the absolute serum 3-epi-25(OH)3 levels was minimal. We also found a moderate correlation (Pearson correlation coefficient R = 0.47;

Table 1. The median (IQR) and range of maternal and umbilical cord sera total 25(OH)D, 25(OH)D2, 25(OH)D3, and 3-epi-25(OH)D3 among samples ≥limit of detection (0.4 nmol/L) as well as their respective 3-epi-25(OH)D3 to 25(OH)D3 ratios

	n	Median (IQR)	Range
Maternal serum			
Total 25(OH)D (nmol/L)	1502	54.8 (42.6-69.0)	13.4-184.8
25(OH)D2 (nmol/L)	641	1.3 (0.8-2.7)	0.4-42.7
25(OH)D3 (nmol/L)	1502	53.0 (41.1-67.5)	12.4-177.2
3-epi-25(OH)D3 (nmol/L)	1419	1.9 (1.3-2.8)	0.4-14.2
3-epi-25(OH)D3 to 25(OH)D3 ratio (%)	1419	3.6 (2.8-4.5)	0.5-19.9
Umbilical cord serum			
Total 25(OH)D (nmol/L)	1321	40.7 (31.7-51.7)	11.8-219.5
25(OH)D2 (nmol/L)	140	1.4 (0.7-3.0)	0.4-27.6
25(OH)D3 (nmol/L)	1321	39.6 (30.5-50.4)	10.8-218.5
3-epi-25(OH)D3 (nmol/L)	1217	1.7 (1.1-2.4)	0.4-10.9
3-epi-25(OH)D3 to 25(OH)D3 ratio (%)	1217	4.2 (2.9-5.6)	0.7-15.3

Abbreviations: 3-epi-25(OH)D3, isomeric form of 25(OH)D3; IQR, interquartile range.
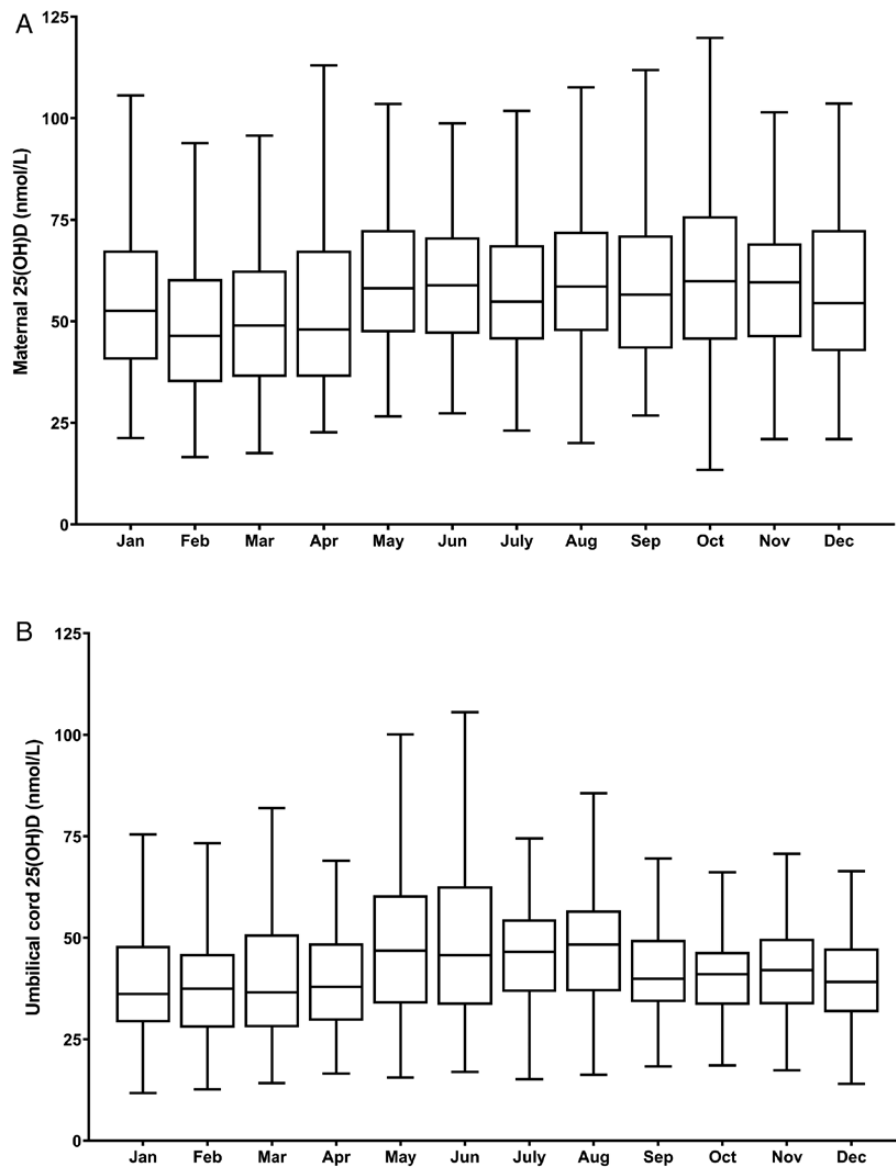
Figure 1. Box-and-whisker plot of (A) maternal serum total 25(OH)D levels at mid-gestation and (B) umbilical cord serum total 25(OH)D levels at delivery by month. Maternal serum total 25(OH)D levels in February were significantly lower than those from May to December (*P* values: <0.001-0.01), whereas its level in March was significantly lower than that in May, June, August, October, and November (*P* values: 0.001-0.03). Umbilical cord serum total 25(OH)D levels were significantly lower among newborns born between January and April than those born between May and August (*P* values: 0.001-0.04).

P < 0.001) between maternal and umbilical cord sera 3-epi-25(OH)D3 levels, but a weak association (Pearson correlation coefficient *R* = 0.31; *P* < 0.001) between maternal and umbilical cord sera 25(OH)D3 levels (supplementary Fig. 3) [26].

Discussion

In this cohort of 1502 mothers and 1321 infants, we reported that 3-epi-25(OH)D3 could be identified in 94.5% of maternal and 92.1% of neonatal umbilical cord sera. The highest absolute 3-epi-25(OH)D3 levels reached

14.2 nmol/L and 10.9 nmol/L in maternal and umbilical cord serum, accounting for 19.9% and 15.3% of the maternal and umbilical cord serum 25(OH)D3 levels, respectively. Hence, an immunoassay that does not distinguish total 25(OH)D from other biologically inactive metabolites and LC-MS/MS methods that do not preclude coelution of 3-epi-25(OH)D3 with 25(OH)D3 will result in underestimation of hypovitaminosis D in both mothers and newborns.

The present study examined the factors associated with 3-epi-25(OH)D3 levels in the maternal and neonatal circulation of a Chinese population. Maternal serum

Table 2. Maternal characteristics according to different maternal serum 3-epi-25(OH)D3 level at the mid-gestation of pregnancy

	Maternal serum 3-epi-25(OH)D3 (nmol/L)					
	≤1.00	1.01-1.50	1.51-2.00	2.01-3.00	> 3.00	*P*
	(n = 275)	(n = 281)	(n = 261)	(n = 409)	(n = 276)	
Maternal age, y	30.0 ± 4.8	30.7 ± 4.8	30.9 ± 5.0	30.9 ± 4.7	31.5 ± 4.8	0.01
Primigravida	188 (68.4)	175 (62.3)	166 (63.6)	249 (60.9)	155 (56.2)	0.06
Prepregnancy BMI, kg/m^2	20.9 ± 3.3	20.5 ± 2.5	20.6 ± 2.7	20.6 ± 2.8	20.6 ± 2.7	0.68
BMI at blood sampling, kg/m^2	24.9 ± 3.5	24.3 ± 2.9	24.2 ± 2.8	24.3 ± 2.8	24.3 ± 2.8	0.08
Maternal pre-pregnant weight, kg	52.4 ± 9.0	51.9 ± 7.0	52.0 ± 7.9	51.6 ± 7.3	51.3 ± 7.3	0.50
Maternal weight at blood sampling, kg	62.5 ± 9.7	61.5 ± 8.1	61.1 ± 8.5	60.8 ± 7.7	60.6 ± 7.5	0.054
Smoker	7 (2.5)	7 (2.5)	5 (1.9)	5 (1.2)	10 (3.6)	0.33
Alcohol drinker	2 (0.7)	0	0	0	1 (0.4)	0.19
Maternal glycemic status, AUC$_{glu}$	752.6 ± 121.5	769.8 ± 121.9	789.3 ± 140.5	794.2 ± 132.5	804.8 ± 138.0	<0.001
Gestation age at blood sampling, wk	27.8 ± 1.3	27.7 ± 1.2	27.7 ± 1.3	27.7 ± 1.4	27.7 ± 1.3	0.90
Baby's gender (male)	121 (44.0)	147 (52.3)	130 (49.8)	210 (51.3)	164 (59.4)	0.01
Ambient solar radiation, MJ/m^2	352.0 ± 73.0	371.7 ± 80.5	389.0 ± 69.2	407.2 ± 73.1	423.4 ± 74.5	<0.001
Maternal 25(OH)D3 level, nmol/L	37.1 ± 14.0	45.7 ± 12.4	52.4 ± 11.8	62.8 ± 14.3	75.4 ± 17.7	<0.001

Continuous and categorical variables were expressed as mean ± SD and n (proportion in %), and were compared by ANOVA and χ^2 tests, respectively.
Abbreviations: 3-epi-25(OH)D3, isomeric form of 25(OH)D3; AUC$_{glu}$, area under the curve of maternal glucose levels at the oral glucose tolerance test during pregnancy; BMI, body mass index.

Table 3. Maternal characteristics and birth outcomes according to different umbilical cord serum 3-epi-25(OH)D3 level at delivery

	Umbilical cord serum 3-epi-25(OH)D3 (nmol/L)					
	≤ 1.00	1.01-1.50	1.51-2.00	2.01-3.00	> 3.00	*P*
	(n = 358)	(n = 267)	(n = 258)	(n = 282)	(n = 156)	
Maternal characteristics						
Maternal age, y	29.8 ± 4.9	30.3 ± 4.7	30.8 ± 4.9	31.7 ± 4.7	32.3 ± 4.5	<0.001
Primigravida	242 (67.6)	175 (65.5)	155 (60.1)	146 (51.8)	72 (46.2)	<0.001
Prepregnancy BMI, kg/m^2	20.6 ± 2.9	20.7 ± 2.9	20.5 ± 2.7	20.5 ± 2.5	20.8 ± 2.8	0.77
Maternal prepregnant weight, kg	51.9 ± 7.6	52.2 ± 7.8	51.6 ± 7.6	51.5 ± 6.8	51.8 ± 7.7	0.85
Maternal weight at delivery, kg	68.0 ± 9.2	68.1 ± 8.7	66.7 ± 8.8	66.5 ± 8.0	66.1 ± 8.7	0.02
Maternal gestational weight gain, kg	16.1 ± 4.6	16.0 ± 4.6	14.8 ± 4.2	15.0 ± 4.4	14.3 ± 4.1	<0.001
BMI at delivery, kg/m^2	27.1 ± 3.5	27.2 ± 3.0	26.6 ± 3.1	26.5 ± 3.1	26.4 ± 2.8	0.02
Smoker	11 (3.1)	5 (1.9)	3 (1.2)	7 (2.5)	2 (1.3)	0.48
Alcohol drinker	0	1 (0.4)	0	0	1 (0.6)	0.32
Maternal glycemic status, AUC$_{glu}$	768 ± 126	770 ± 133	778 ± 132	795 ± 140	815 ± 122	0.001
Ambient solar radiation at delivery, MJ/m^2	357.1 ± 67.0	375.0 ± 67.4	387.2 ± 77.7	402.7 ± 77.0	413.6 ± 80.3	<0.001
Maternal 25(OH)D3 level, nmol/L	45.6 ± 16.1	49.8 ± 15.8	56.7 ± 15.5	63.1 ± 18.0	73.1 ± 22.2	<0.001
Birth outcomes						
Gestation age at delivery, wk	39.5 ± 1.4	39.5 ± 1.4	39.4 ± 1.3	39.3 ± 1.4	39.1 ± 1.5	0.01
Preterm (≤37 wk)	16 (4.5)	8 (3.0)	9 (3.5)	14 (5.0)	10 (6.4)	0.48
Cesarean delivery	86 (24.0)	58 (21.7)	60 (23.3)	64 (22.7)	39 (25.0)	0.94
Baby's sex (male)	185 (51.7)	138 (51.7)	140 (54.3)	147 (52.1)	82 (52.6)	0.97
LGA	21 (6.1)	17 (6.6)	17 (6.8)	17 (6.3)	11 (7.5)	0.99
SGA	38 (11.1)	15 (5.8)	26 (10.4)	20 (7.5)	14 (9.6)	0.16
Umbilical cord serum 25(OH)D3 level (nmol/L)	33.4 ± 17.1	39.3 ± 14.4	42.3 ± 13.1	49.2 ± 16.9	58.0 ± 19.4	<0.001

Continuous and categorical variables were expressed as mean ± SD and n (proportion in %), and were compared by ANOVA and χ^2 tests, respectively.
Abbreviations: 3-epi-25(OH)D3, isomeric form of 25(OH)D3; AUC$_{glu}$, area under the curve of maternal glucose levels at the oral glucose tolerance test during pregnancy; BMI, body mass index; LGA, large for gestational age; SGA, small for gestational age.

3-epi-25(OH)D3 was associated with carrying a male fetus, maternal glycemic levels, and maternal 25 (OH) D3 levels. In contrast to a previous finding from Ireland that maternal age was a negative predictor of maternal 3-epi-25(OH)D3 concentrations, the apparent positive association between maternal age and maternal 3-epi-25(OH)D3 level was not statistically significant after adjusting for maternal 25(OH)D3 levels and prepregnancy body mass index [27]. Umbilical cord 3-epi-25(OH)D3 level was associated with advanced maternal age, multiparity, maternal gestational weight gain below the Institute of Medicine recommendation, maternal glycemic status, earlier gestational age at delivery, and both maternal and umbilical sera 25(OH)D3 levels. Both maternal and umbilical cord 3-epi-25(OH)D3 levels were associated with ambient solar radiation at the time of collection of the blood sample. Our finding is consistent with the previous finding that more adequate total 25(OH)D was associated with detectable 3-epi-25(OH)D3 [28].

Although we observed a slightly greater correlation between maternal and umbilical cord serum 3-epi-25(OH)D3 levels compared with 25(OH)D3 levels, previous studies suggested that umbilical cord 3-epi-25(OH)D3 is derived from endogenous fetal production from maternal 25(OH)D3. It has been previously suggested that 3-epi-25(OH)D does not transfer efficiently across the placenta and that high levels of 3-epimer concentrations in infancy are due to postnatal epimerization of 25(OH)D [29]. In a recent study in which all children were provided with daily vitamin D supplementation, 3-epi-25(OH)D3 was detectable only at low concentrations in the cord blood and first week of life in nearly all infants, but rose remarkably to 55% and 36% of total 25(OH)D3 in both preterm and term infants by day 10 of life [30]. The increase in 3-epi-25(OH)D3 after the first week of life coincided with the commencement of vitamin D supplementation and was more pronounced among preterm infants. It was speculated that high C3 epimer concentrations in infancy are due to postnatal epimerization from 25(OH)D3 instead of through placental passage. Furthermore, hepatic immaturity accounts for the increased 3-epi-25(OH)D3 levels during the first 3 months of age, coinciding with the commencement of vitamin D supplementation, particularly among the preterm infants [30]. Meanwhile, we also demonstrated an association of maternal 3-epi-25(OH)D3 levels at an earlier gestational age with higher levels of umbilical cord 3-epi-25(OH)D3 at birth, even when analyses were confined to term infants.

Our study also reflected a high prevalence of vitamin D deficiency in Chinese mothers and newborns in this subtropical area at the latitude of 22.3°N with year-round abundant sunlight. It is possible that local young women may fear skin pigmentation from direct sunlight and may use different strategies to reduce sunlight exposure. Compared with studies in pregnant women residing in regions of similar latitude, the median serum 25(OH)D level in our population was slightly below that of Guangzhou (67.6 nmol/L) at 23.1°N, and much below that of Brisbane (132.5 nmol/L) at 27.5°S [31, 32]. These previous studies also showed a seasonal variation in maternal vitamin D status similar to that seen in the present study. Most importantly, nearly three-quarters of the newborns were vitamin D deficient in the present study. The median umbilical cord 25(OH)D concentration in the present study is similar to that previously reported from the Anhui (31.9°N) and Sichuan (30.7°N) provinces of China [33]. However, a report from Shanghai (31.2°N) situated at the same latitude showed a median umbilical 25(OH)D level of 56.0 nmol/L [34]. The higher level could be related to vitamin D supplementation in mothers. In studies from Ireland, median umbilical cord 25(OH)D levels were 32 to 39 nmol/L but increased to 44 and 50 nmol/L when mothers were put on daily vitamin D supplementation of 400 IU and 800 IU, respectively [14, 35]. In a recent local pilot study of 155 infants at an average age of 3 months, vitamin D deficiency was present in 33.5% and severe deficiency in 21.9% of infants; the rate of vitamin D deficiency was up to 97.4% at 3 months of age among infants on exclusive breast feeding, and their mean 25(OH)D concentration was 19.7 nmol/L [36]. This highlights the importance of providing vitamin D supplementation to pregnant women and infants while promoting exclusive breast feeding. At the same time, we should emphasize the importance of having adequate sunshine to minimize maternal and neonatal vitamin D deficiency.

The present study is the largest population reporting 3-epi-25(OH)D3 concentrations in the maternal and fetal circulation as well as its percent contribution to total 25(OH)D. It is also the first study in a Chinese population, with similar findings to those previously reported in Europe and North America [11-15]. However, the present study has several limitations. A recent study suggested that oral vitamin D supplementation might be associated with the presence and a higher level of C3-epimer in the circulation in animal models but not in humans [37]. Because of the limitation from a lack of data on dietary intake, including calcium and vitamin D, and vitamin D and calcium supplementation in the original HAPO Study, we cannot examine further the association between vitamin D supplementation and 3-epi-25(OH)D3 levels in newborns. Furthermore, the apparent association between higher maternal glucose levels and increased 3-epi-25(OH)D3 levels could be confounded by dietary vitamin D intake and supplementation as well as exposure to sunlight that was not measured. Nonetheless, mothers who had significant medical diseases, such as

autoimmune or chronic kidney disease, that required empirical vitamin D supplementation were not eligible for the HAPO Study. During the HAPO Study (ie, between 2000 and 2006), routine vitamin D or nutritional supplementation was uncommon. This could possibly explain the lower levels and rate of detection of 25(OH)D2 in the mothers and neonates of our cohort. However, it remains uncertain why several neonates had relatively high 3-epi-25(OH)D3 levels, with the highest level reaching 15% of 25(OH)D3.

Another limitation of the study was the lack of assessment of an individual participant's sunlight exposure and use of sunscreen. Nevertheless, the close linkage of the monthly variation of maternal and umbilical cord vitamin D levels to the ambient solar radiation suggests that the main source of vitamin D in the study population was predominantly from 25(OH)D3 synthesis in the skin because of UV light exposure. Because of the original study design, we had only a single measurement of vitamin D metabolites in mid-gestation. Studies have suggested that maternal 25(OH)D levels and hence vitamin D status increase as gestation advances, regardless of whether the mothers received vitamin D supplementation [38-40]. Moreover, both maternal 3-epi-25(OH)D3 and the 3-epi-25(OH)D3: 25(OH)D3 ratio have been reported to increase with gestational age [40, 41]. The association between maternal and umbilical cord serum 25(OH)D levels, as well as 3-epi-25(OH)D3 levels, would be better reflected if maternal vitamin D status was assayed at delivery.

Conclusion

In this first Chinese cohort reporting the level of serum 3-epi-25(OH)D3 and its association with serum 25(OH)D3, ambient solar radiation and other maternal and infant characteristics, we observed that 3-epi-25(OH)D3 is present and represents up to 20% and 15% of 25(OH)D3 in mothers during pregnancy and newborns at birth, respectively. In addition to maternal 25(OH)D3 level and ambient solar radiation, higher maternal glycemia and carrying a boy were also significantly associated with higher 3-epi-25(OH)D3 levels in the maternal circulation. Likewise, maternal glycemic status, earlier gestational age at delivery, ambient solar radiation, and both maternal and umbilical cord sera 25(OH)D3 levels were independent factors associated with higher umbilical cord 3-epi-25(OH)D3 levels at birth. Further study will be needed to determine the mechanisms underlying these findings.

Acknowledgments

Additional Information

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