Journal of Steroid Biochemistry & Molecular Biology 167 (2017) 162–168

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Vitamin D metabolite concentrations in umbilical cord blood serum and associations with clinical characteristics in a large prospective mother-infant cohort in Ireland

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ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form 14 December 2016
Accepted 16 December 2016
Available online 19 December 2016

Keywords:
Vitamin D
25-Hydroxyvitamin D
3-epi-25-hydroxyvitamin D
Vitamin D deficiency
umbilical cord blood
birth cohort

ABSTRACT

Vitamin D deficiency is widespread among mothers and neonates and quality clinical and analytical data are lacking. We used a CDC-accredited LC–MS/MS method to analyze vitamin D metabolites in cord sera from 1050 maternal-infant dyads in the prospective SCOPE Ireland Pregnancy and BASELINE Birth cohort studies, based in Cork, Ireland. The mean ± SD total 25(OH)D was 34.9 ± 18.1 nmol/L; 35% of cords (50% during winter) had 25(OH)D <25 nmol/L, 46% were <30 nmol/L and 80% were <50 nmol/L. In this predominantly white cohort, the main predictor of cord 25(OH)D [adj. mean difference in nmol/L (95% CI)] was summer delivery [19.2 (17.4, 20.9), $P < 0.0001$]. Maternal smoking during pregnancy (9% prevalence) was negatively associated ($P < 0.002$) with cord 25(OH)D [−4.83 (−7.9, −1.5) nmol/L]. There were no associations between cord 25(OH)D and birth weight or any anthropometric measures at birth. Despite the high prevalence of vitamin D deficiency at birth, there were no documented musculoskeletal complications during infancy, which was likely due to widespread supplementation with vitamin D. The mean ± SD concentration of 3-epi-25(OH)D_3, detectable in 99.4% of cord samples, was 3.3 ± 1.9 nmol/L. The proportion of 25(OH)D as 3-epi-25(OH)D_3 was 11.2%. Cord 3-epi-25(OH)D_3 concentrations were positively predicted by cord 25(OH)D_3 [0.101 (0.099, 0.103) nmol/L, $P < 0.0001$] and negatively by gestational age [−0.104 (−0.131, −0.076) nmol/L, $P < 0.0001$] and maternal age [−0.010 (−0.019, −0.001) nmol/L, $P < 0.05$]. 25(OH)D_2 was detected in 98% of cord sera (mean ± SD; 2.2 ± 1.9 nmol/L) despite low antenatal consumption of vitamin D_2 supplements. In conclusion, these first CDC-accredited data of vitamin D metabolites in umbilical cord blood emphasise the high risk of very low vitamin D status in infants born to un-supplemented mothers. Experimental data to define maternal vitamin D requirements for prevention of neonatal deficiency at high latitude are required.

1. Introduction

As fetal and neonatal circulating 25-hydroxyvitamin D (25(OH)D) concentrations are dependent on maternal status, it is important to prevent vitamin D deficiency during pregnancy [1]. Infants born to vitamin D-deficient mothers have the highest risk of neonatal deficiency and consequent complications in the neonatal period [2]. However, maternal vitamin D deficiency and a high prevalence of low cord serum 25-hydroxyvitamin D (25(OH)D) concentrations has been reported extensively around the

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 3-epi-25(OH)D_3, 3-epimer of 25-hydroxyvitamin D_3; 1,25$(OH)_2D_3$, 1-25 dihydroxyvitamin D_3; 3-epi-1,25$(OH)_2D_3$, 1-25 dihydroxy-3-epi-vitamin D_3; CDC, Centres for Disease Control; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LOD/LOQ, limit of detection/limit of quantitation; NIST, National Institute of Standards and Technology; SRM, standard reference material.

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http://dx.doi.org/10.1016/j.jsbmb.2016.12.006
0960-0760/

world [3]. In the absence of reference intervals for umbilical cord 25(OH)D concentrations, thresholds of vitamin D deficiency, insufficiency and sufficiency vary across studies. Currently, the Institute of Medicine [4] defines cord serum 25(OH)D <30 nmol/L as the level at which there is an increased risk of vitamin D deficiency, 40 nmol/L as the adequacy threshold and vitamin D sufficiency at concentrations ≥50 nmol/L. International comparisons are difficult due to variable analytical methods and a lack of transparency in data reporting [3,5]. Concentrations of 25(OH)D in cord sera <25–30 nmol/L are consistent with an increased risk of nutritional rickets, particularly in the absence of neonatal and infant vitamin D supplementation exacerbated by inadequate calcium intakes during complementary feeding (2). Association studies have suggested adverse impacts of low cord 25(OH)D concentrations on non-skeletal health and developmental outcomes [6,7]. There is a need to understand the impact of vitamin D status at birth and during the early years on healthy growth and development, including bone and soft tissue, neurological development and immune function.

Vitamin D metabolites, such as 3-epi-25-hydroxyvitamin D_3 (3-epi-25(OH)D_3) and 25-hydroxyvitamin D_2 (25(OH)D_2), are of interest. C-3 epimerisation may be a common metabolic pathway for vitamin D_3 metabolites in selected cells, independent of cytochrome P-450 enzymes [8–10]. Despite demonstrated *in vitro* biological activity, the clinical significance of 3-epi-25(OH)D_3 is still unknown and it is not included in the calculation of total 25(OH)D [11,12]. However, 3-epi-25(OH)D_3 is present in most individuals in low circulating concentrations and in a relatively higher proportion of total 25(OH)D in infants [13–15]. There have been calls for more clinical studies describing the relationship between serum 25(OH)D_3 and 3-epi-25(OH)D_3 [11]. There are currently limited data on 3-epi-25(OH)D_3 in small samples of umbilical cord blood [15–17]. Similarly, data on circulating 25(OH)D_2 in cord sera are scarce. While the metabolism of vitamin D_2 and D_3 in the human body are similar [18], two systematic reviews [19,20] reported that vitamin D_3 is more effective in raising serum 25(OH)D concentrations than vitamin D_2. This is potentially important in populations that may be vulnerable to vitamin D deficiency, such as infants.

We recently reported a relatively high prevalence of vitamin D deficiency among a large cohort of pregnant women in Ireland compared with most international estimates in similarly sized cohorts [21]. This study describes the distribution and determinants of cord serum 25(OH)D_3, 3-epi-25(OH)D_3 and 25(OH)D_2 and from this maternal-infant cohort resident at 52 °N.

2. Methods

2.1. Study design

The Cork BASELINE (Babies After SCOPE: Evaluating the Longitudinal Impact Using Neurological and Nutritional Endpoints) Birth Cohort Study was established as a follow-up to the SCOPE (Screening for Pregnancy Endpoints) pregnancy cohort study in Ireland. SCOPE is a worldwide multicenter study in primiparous low-risk women aimed at establishing biomarkers to assist with prediction and prevention of the major diseases of late pregnancy [22]. In March 2008, SCOPE Ireland commenced recruitment in Cork and enrolled a total of 1768 women, of whom 87% consented to participate in the Cork BASELINE Birth Cohort study. Complete details of the SCOPE study and data collection have been provided elsewhere [23]. The main inclusion criteria were low risk, singleton pregnancy and no previous pregnancy beyond 20 weeks' gestation. All women provided written informed consent and had their first SCOPE visit at 15 weeks of gestation (range, 14–16 weeks). Research midwives interviewed participants and collected information on socio-economic status, occupation, educational attainment, marital/relationship status and a complete medical history. Data on nutritional supplement use including brand level data, alcohol consumption, smoking history and recreational drugs were recorded for the period before conception, during the first trimester, and at the time of the 15-week visit. Maternal anthropometric measurements (height and weight for calculation of BMI) were carried out. Blood was processed within three hours to serum and stored at −80 °C for future analysis.

A detailed overview of the BASELINE birth cohort methodology has been described (24). Umbilical cord blood was collected from 1050 infants, processed to serum within three hours, and stored at −80 °C for later analysis. Clinical assessments were carried out at 2 days of life and at 2, 4, 6, 12 and 24 months of age. Infant anthropometric measurements were completed at each time point and body composition was assessed using air displacement plethysmography at 2 days and 2 months. Detailed interviewer-led assessments were completed and captured comprehensive information on infant feeding, supplementation, medication, illness, medical care and overall health.

2.2. Ethics

The SCOPE Ireland study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals [ECM5(10)05/02/08) and is registered at the Australian New Zealand Clinical Trials Registry (ACTRN12607000551493). The Cork BASELINE Birth Cohort was conducted according to the guidelines laid down in the Declaration of Helsinki as revised in 1983 and all procedures were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, ref ECM 5 (9) 01/07/2008. The Cork BASELINE Birth Cohort is registered at the United States National Institutes of Health Clinical Trials Registry (http://www.clinicaltrials.gov), ID: NCT01498965. Written informed consent was obtained from mothers in SCOPE and on behalf of their infants in BASELINE.

2.3. Laboratory analysis

Circulating 25(OH)D_3, 3-epi-25(OH)D_3 and 25(OH)D_2 concentrations were measured at the Cork Centre for Vitamin D and Nutrition Research laboratory using an LC–MS/MS method, which has been fully described earlier [25,26]. The instrument used was a Waters Acquity UPLC system coupled to an Acquity Triple Quadrupole TQD mass spectrometer detector (Waters, Milford, USA). Concentrations of 25(OH)D_3 and 25(OH)D_2 were quantified individually and their values summed to generate total 25(OH)D. Chromatographic separation and quantitation of 3-epi-25(OH)D_3 was also achieved. Four levels of serum-based NIST (National Institute of Standards and Technology) certified quality assurance material (SRM 972) were used for method validation while quality control materials assayed in parallel to all samples were purchased from Chromsystems (Germany). NIST Calibrators were used throughout the analysis (SRM 2972). The inter-assay CV was <5% for all metabolites and the intra-assay CV was <6%. The limit of detection (LOD) for 25(OH)D_3, 3-epi-25(OH)D_3 and 25(OH)D_2 was 0.31, 0.20 and 0.44 nmol/L, respectively. The limit of quantitation (LOQ) was 1.03, 0.66 and 1.43 nmol/L, respectively. Our method does not measure C-3 epimer of 25(OH)D_2, but given the low concentrations of 25(OH)D_2 found in human sera [current median (IQR) 1.9 (1.3–2.7) nmol/L], we would expect the concentrations of 3-epi 25(OH)D_2 to be extremely low. The quality and accuracy of vitamin D metabolite analysis in our laboratory using this LC–MS/MS method is assessed on an on-going basis by participation in the Vitamin D External Quality Assessment