High prevalence of neonatal vitamin D deficiency – rationale for reevaluation of vitamin D supplementation during pregnancy

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Abstract
It could be expected that Polish pregnant women and their offspring are vitamin D deficient due to latitude (49-55°N) and low vitamin D intake. As parathormone (PTH) is a marker of vitamin D deficiency in adults it is important to evaluate this correlation also among newborn infants. Material and method: Serum level of 25-hydroxyvitamin D (25-OHD), calcium, phosphates, alkaline phosphatase (ALP), intact parathormone (iPTH), calciluria and phosphaturia were measured in 41 term, appropriate for gestational age newborns before introduction of vitamin D supplementation. Comparison of measured parameter were done according to birth season. Results: Multivitamins were used during pregnancy regularly (> 3 months) by 80% mothers. The average daily vitamin D intake was 420 IU ± 80 IU/d. Mean neonatal 25-OHD concentration was 10.5 ± 4 ng/ml. Vitamin D deficiency defined as < 20 ng/ml of 25-OHD was found in all infants. No differences were observed in biochemical parameters despite of gender, birth season and maternal multivitamins intake. Conclusions: Vitamin D deficiency is common in Polish newborns irrespective of the birth season. Vitamin D supplementation in neonates should be started in the first few days of life. Parathormone concentration was 10.5 ± 4 ng/ml. Vitamin D deficiency defined as < 20 ng/ml of 25-OHD was found in all infants. No differences were observed in biochemical parameters despite of gender, birth season and maternal multivitamins intake. Vitamin D supplementation is recommended for all infants in Poland in dose 400 IU/d [15]. The time of introduction of vitamin D depend on maternal vitamin D status in pregnancy. In case of lack of prenatal multivitamins infants should receive vitamin D after birth but in everyday practice vitamin D supplementation starts around 3rd week of life. Because of insufficient vitamin D synthesis during winter months and low vitamin D consumption from diet it is likely that Polish mothers and their infants after birth are vitamin D deficient. To check this hypothesis vitamin D status in neonates was assessed taking into account season of the year and maternal vitamin D supplementation. Selected biochemical parameters of calcium-phosphate homeostasis were also measured as a potential markers of neonatal vitamin D status.

Introduction
Vitamin D is essential for normal intestinal calcium absorption, optimal growth of skeletal system and its mineralization. Deficiency of vitamin D is associated not only with rickets in growing children and osteomalacia in adults but also with diabetes mellitus, cardiovascular diseases (hypertension, metabolic syndrome), some carcinomas (breast, colon, prostate) and autoimmune diseases [1-3]. It is a result of vitamin D activity through its receptors located in many tissues [4]. As vitamin D has an impact on overall health it’s a crucial issue to establish optimal vitamin D status throughout whole life. Recent studies in adults showed that for many health aspects serum 25-hydroxyvitamin D levels should exceed 80 nmol/l (32 ng/ml) [5, 6]. The threshold for infants and children was set on 50 nmol/l (20 ng/ml) by American Academy of Pediatrics [7]. Currently proposed thresholds for vitamin D levels are much higher than it was before (around 10 ng/ml = 25 nmol/l).

The major source of vitamin D is skin synthesis after exposures to UV-B light. Full-body exposure during summer months for 10 to 15 minutes in adult with lighter pigmentation will generate between 10 000 and 20 000 IU of vitamin D per day. In Poland, because of latitude (49-55°N) endogenous skin synthesis of vitamin D is insufficient from October till March so it potentially could be vulnerable time to vitamin D deficiency. The other source of vitamin D is diet but dairy product are not fortified with vitamin D in Poland. Standard consumption of products containing substantial amounts of vitamin D like oily fish, eggs, butter or margarine is not sufficient to build adequate stores of vitamin D [8].

Neonatal vitamin D status at birth is highly correlated with maternal vitamin storage. Transplacental transport of 25-hydroxyvitamin D is a major source of vitamin D for fetus. Cord 25-OHD level is about 50-70% of maternal one [9-12]. Mothers with borderline storage, will probably deliver vitamin D deficient newborns. Appropriate vitamin D status in pregnancy is important in respect to women and their offspring health [13, 14]. Vitamin D supplementation is recommended for all infants in Poland in dose 400 IU/d [15]. The time of introduction of vitamin D depend on maternal vitamin D multivitamin intake during pregnancy. In case of lack of prenatal multivitamins infants should receive vitamin D after birth but in everyday practice vitamin D supplementation starts around 3rd week of life.

Material and methods
The study was approved by local medical ethics committee. Written parental consent was obtained before the beginning of the study. Only single, term, appropriate for gestational age infants without major medical problems were included. They were breast fed or formula fed. Children with congenital malformations, inborn errors of metabolism, endocrinological problems were excluded. Children with congenital malformations, inborn errors of metabolism, endocrinological problems were excluded.
disorders, liver or renal dysfunction and treated with anticonvulsants (phenobarbital) were excluded. All infants were recruited in Department of Neonatology and Intensive Care of Children’s Memorial Health Institute in Warsaw, Poland before introduction of routine vitamin D supplementation (24th week of life). According to the season of the year infants were allocated to summer-born group (May-October) or winter-born group (November-April) for further analysis. Maternal vitamin D intake from diet and prenatal multivitamins was calculated. Information on specific preparation, duration of supply and the dose received was obtained at the visit with the use of a simple questionnaire. Sun exposure was not calculated but sunbathing is not recommended for pregnant women.

Serum circulating 25-OHD level, alkaline phosphatase (ALP), intact parathormon (iPTH), calcium (Ca), phosphorus (P) and creatinine (crea.) were determined between 2nd and 12:00 am and stored at –80°C until assayed. 25-OHD were measured with electrochemiluminescence immunoassay (ECLIA), Roche Diagnostic, Mannheim, Germany, in the automated analyzer Elecsys 2010.

Serum 1,25(OH)2D were measured manually using Bio-source 1,25(OH)2D RIA CT Kit. Both inter- and intra-assay variations were < 15%. 25(OH)D and 1,25(OH)2D determinations are under international control of Vitamin D External Quality Assessment Scheme (DEQAS) with Certificates of Proficiency.

Vitamin D deficiency in infants was considered when 25-OHD levels were less than 20 ng/ml [7].

Serum intact PTH (84 amino acids) was quantitatively assayed using ELSA-PTH immunoradiometric (IRMA) kit from CIS bio international GifSur-Yvette Cedex, France. Intra- and inter-assay coefficient of variation was 7.5% and 6.8% respectively. Euparathyroid values were considered under 60 pg/ml [16].

Calcium, phosphorus, creatinine and ALP were measured in Dimension clinical chemistry system Dade Behring. Calcium and phosphaturia were assessed using respectively calcium/creatinine and phosphates/creatinine ratio in spot urine.

The normality of analyzed data distribution was controlled by Kolmogorow-Smirnow and Shapiro-Wilk tests. T-student or Mann-Whitney U tests were used for the evaluation of differences in all parameters measured between groups (stratified according to season of the year, maternal vitamin D status, gender). P value < 0.05 was considered as statistically significant. Results are presented as means ± SD otherwise stated.

### Results

There were 41 mother-infant (22 males and 19 females) pairs studied. Mean birth weight was 3577 ± 447 g, length 54.7 ± 3 cm and gestational age 39.1 ± 2 weeks. First minute Apgar score was 8-10 points in 87% of infants and 5-7 points in 13% of infants. None of infants had craniotabes.

<table>
<thead>
<tr>
<th></th>
<th>Summer (N = 20)</th>
<th>Winter (N = 21)</th>
<th>Total (N = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3551 ± 491</td>
<td>3602 ± 410</td>
<td>3577 ± 447</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>54 ± 2.7</td>
<td>53 ± 3.1</td>
<td>54.7 ± 3</td>
</tr>
<tr>
<td>Maternal vit. D (IU/d)</td>
<td>433 ± 97</td>
<td>408 ± 55</td>
<td>420 ± 80</td>
</tr>
<tr>
<td>Maternal vit. D (moths)</td>
<td>6.5 ± 2.6</td>
<td>4.6 ± 2.9</td>
<td>5.5 ± 2.9</td>
</tr>
<tr>
<td>Ca/crea. (mol/l)</td>
<td>2.53 ± 0.1</td>
<td>2.56 ± 0.1</td>
<td>2.54 ± 0.13</td>
</tr>
<tr>
<td>P/crea. (mol/l)</td>
<td>2.21 ± 0.2</td>
<td>2.14 ± 0.2</td>
<td>2.17 ± 0.2</td>
</tr>
<tr>
<td>Ca/creat. (mg/mg)</td>
<td>0.6 ± 0.4</td>
<td>0.64 ± 0.7</td>
<td>0.62 ± 0.6</td>
</tr>
<tr>
<td>P/creat. (mg/mg)</td>
<td>0.84 ± 0.8</td>
<td>0.78 ± 0.9</td>
<td>0.81 ± 0.9</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>273 ± 89</td>
<td>253 ± 76</td>
<td>263 ± 22</td>
</tr>
<tr>
<td>25-OHD (ng/ml)</td>
<td>10.2 ± 3.5</td>
<td>10.8 ± 4.6</td>
<td>10.5 ± 4</td>
</tr>
<tr>
<td>1,25(OH)2D (pg/ml)</td>
<td>55 ± 20</td>
<td>58 ± 25</td>
<td>56.46 ± 22.56</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>18.4 ± 10</td>
<td>22 ± 13</td>
<td>20.32 ± 11.8</td>
</tr>
</tbody>
</table>

*p < 0.05*

Maternal vitamin D intake during pregnancy was based in 100% on multivitamin preparations. All contain cholecalciferol (vitamin D3). Pure vitamin D preparations were not used in any case. Multivitamins containing vitamin D were used regularly (> 3 months) during pregnancy by 80% of mothers whereas for longer than 6 months by 58.5% mothers. The average daily vitamin D intake from supplements was 420 IU ± 80 IU/d.

Mean infants age of the blood sample collection was 15 ± 6 days and body weight 3869 ± 484 g. The mean serum 25-OHD level was 10.5 ± 4 ng/ml. All infants had 25-OHD level < 20 ng/ml considered as vitamin D deficient, whereas 19 newborns (46.3%) had mild vitamin D deficiency (25-OHD level between 10 and 20 ng/ml) and 22 newborns (53.7%) had severe deficiency (25-OHD level < 10 ng/ml).

No sex differences were found in serum 25-OHD level (male 9.95 ± 3.7 ng/ml vs female 11.2 ± 4.5 ng; p > 0.05). There were also no differences in 25-OHD level despite of maternal multivitamin intake during pregnancy (10.28 vs 10.35 ng/ml; p > 0.05). There was very high prevalence of exclusive breastfeeding – 90.2%. Only 3 infants were formula fed. Because of small number of formula fed infants the influence of infant’s diet wasn’t assessed.

All infants had serum calcium, phosphorus ALP, iPTH within normal values (results presented in table 1). There was significant inverse correlation observed between serum 25-OHD and ALP (R = –0.37, p < 0.05) but with no other biochemical parameters analyzed.

There were 20 summer- and 21 winter-born infants. There were no differences in anthropometric parameters or daily maternal vitamin D intake between compared summer- and winter-born infants although duration of maternal vitamin supplementation was significantly shorter in winter born infants.

### Table 1. Comparison of biochemical and anthropometric measurements in infants and maternal vitamin D (vit. D) supply according to the season of the year (mean ± SD)
Serum 25-OHD levels and other biochemical parameters measured did not differ according to the season of birth (table 1).

Discussion

Adequate vitamin D status is very important for both pregnant women and their offspring. Vitamin D deficiency during pregnancy could cause fetal intrauterine growth retardation, inborn rickets and osteomalacia even with bone fractures, enamel and dental hypoplasia, neonatal hypocalcaemia and delay in growth velocity in the first year of life [14, 17-19]. Increased risk of diabetes mellitus type 1 as the long term effect was also documented in epidemiological study [2]. Unfortunately there is shortage of well designed studies evaluating influence of vitamin D status of pregnant women on their offspring’s health. Cochrane Library review on vitamin D supplementation during pregnancy is also not very informative because the analysis contains only two old papers from eighties. Nevertheless they gave evidence for lower number of craniotabes and hypocalcaemia in infants born by mother supplemented with vitamin D but results related to birth weight were inconsistent [20].

Our results indicate very high prevalence of vitamin D deficiency among Polish newborns. All studied newborns were vitamin D deficient while 53.7% of them had sever deficiencies. Previous reports showed lower percentage of vitamin D deficiency (about 40%) in Polish newborns but that time vitamin deficiency was defined as 25-OHD level < 10 ng/ml (25 mmol/l) [21, 22]. If we recalculated that data using a new threshold (20 ng/ml) vitamin D deficiency would be found in 60-70% of neonates. Researchers from other countries also stress the scale of the problem [23-27]. In the sunny country like Greece the severe vitamin D deficiency was found in 56% of the summer-born neonates and for all in (91.3%) of the winter-born ones [23]. High prevalence of vitamin D deficiency in Polish newborns gives evidence to introduce vitamin D supplements in infants in the first few days of life which is consistent with recent American Academy of Pediatrics recommendations [7].

Despite of high percentage of severe vitamin D deficiency we did not find any clinical symptoms of vitamin D deficiency in newborn infants, such as: craniotabes, hypocalcemia, secondary hyperparathyroidism (with elevated iPTH), elevated ALP, increased phosphaturia nor hypophosphatemia, while ALP correlated with 25-OHD level. Vitamin D deficiency triggers the release of PTH in adults, children and older infants but is not necessarily seen in neonates and young infants [7, 16, 27, 28]. According to this results serum iPTH level seems not to be a reliable functional marker of vitamin D sufficiency or deficiency states in newborn infants.

It was already established that newborn’s vitamin D status is strictly related to fetal storages which are built through transplacental transfer from the mother during pregnancy [9-12]. Mother’s vitamin D status is a key issue here. Vitamin D deficiency in newborns indicate inadequate vitamin D status in their mothers during pregnancy. Poor maternal vitamin D status could be explained by insufficient vitamin D endogenous skin synthesis and intake from diet and/or supplements. Taking into account lack of skin vitamin D synthesis in Poland during winter time, seasonal differences in neonatal vitamin D status could be expected. However we did not find such differences in infant’s serum levels of vitamin D metabolites, calcium-phosphates homeostasis parameters and markers of vitamin D deficiency as ALP and iPTH. Individual, mother sun exposure during pregnancy was not assessed but sunbathing during pregnancy is not recommended. As we found low 25-OHD levels even in summer born infants, substantial vitamin D skin synthesis is not expected in pregnant woman.

We also did not report any seasonal variation of serum 25-OHD level in our previous study [22]. Another elder Polish data had shown better vitamin D status in summer-born infants and severe vitamin deficiency during winter in both mothers and their newborn babies [11, 12]. Also Greek pregnant women who delivered in summer as well as their neonates had higher levels of 25-OHD than those who delivered in winter but both have the average 25-OHD concentration in the deficient range [23, 24]. Lack of seasonal differences in vitamin D status in our study could be explained by the growing avoidance of sun exposure or usage of sun blockers as a protection against skin cancer. On the other hand sunbathing is not recommended for pregnant women and against Polish tradition in this issue. The environmental pollution could be additional important factor [29]. Namgung et al. also did not observed any seasonal differences in cord serum 25-OHD concentrations of infants born in Cincinnati (USA). Authors speculated that this finding may be due to a large percentage (> 80%) of women taking prenatal vitamins towards the end of pregnancy, which would obscure any sunshine-related effects on vitamin D metabolism [30]. This could also be expected in our population, where prenatal vitamin D supplementation was reported in as high as 80% of studied women. Serum 25-OHD level in winter-born infants was not lower compared to summer-born infants although mothers of winter-born infants had also significantly shorter duration of vitamin D supplementation during pregnancy.

Although standard prenatal vitamins provide around 400 IU of vitamin D per day, maternal vitamin D supplementation during pregnancy at such level was not enough to keep appropriate newborns vitamin D stores neither during winter nor summer. Whereas diet is a potential source of vitamin D – it was established within another study performed by Institute on Food and Nutrition – its mean intake during pregnancy in Polish women is 136 ± 164 IU/day [8]. According to the results of the study one can conclude that diet is not substantial source of vitamin D for pregnant women in Poland. Then, the lack of precise calculation of maternal vitamin D consumption from diet should not essentially influence our results.

Cockburn et al. comparing vitamin D supply during pregnancy in dose 400 IU/d with placebo showed significant difference in mother’s serum 25-OHD levels between the groups, although both remained in the range of severe deficiency [31]. It was recently documented that daily supplementation with
1000 IU of vitamin D throughout the third trimester of pregnancy resulted in increase of serum 25-OHD concentration from 25 nmol/l (10 ng/ml) to 65 ± 17.5 nmol/l (26 ± 7 ng/ml) [7]. These data suggest that doses exceeding 1000 IU of vitamin D per day are necessary to achieve 25-OHD concentration of > 80 nmol/l (32 ng/ml). Another study shows that supplementation within whole pregnancy in dose 800 IU/d up to 1600 IU/d was not enough to rebuilt vitamin D stores in deficient women [32].

Giving the growing evidence that adequate maternal vitamin D status during pregnancy is essential, not only for maternal well-being but also for fetal development, health care professionals who provide obstetric care should consider assessing maternal vitamin D status by measuring the 25-OHD concentration during pregnancy. American Academy of Pediatrics recommends vitamin D supplementation during pregnancy on individual basis to ensure 25-OHD levels in a sufficient range (> 80 nmol/l = 32 ng/ml) [7]. According to the Australian and New Zealand consensus statement pregnant women should have their serum 25-OHD concentrations evaluated during the first trimester of pregnancy. If they are moderately to severely vitamin D deficient, then should be treated with 3000-5000 IU daily until the serum 25-OHD concentration is over 50 nmol/l. After this serum concentration is achieved, they should receive 400 IU daily, same as women with mild deficiency. Routine vitamin D supplementation is not recommended but sunlight exposure is a major source of vitamin D (> 80%) in Australia and New Zealand [33]. Canadian Pediatrics Society recommends to consider vitamin D supplementation during pregnancy in dose 2000 IU daily (especially during winter). Efficiency and safety of such amount of vitamin D intake should be checked on regular basis by measuring serum calcium and 25-OHD concentrations [34].

Health care professionals involved in care of pregnant women should be better informed that prenatal 400 IU of vitamin D supplementation – as available in daily dose of standard multivitamin preparations – has little effect on maternal and newborn 25-OHD concentrations, especially during winter months [7, 14, 19]. Data from adults, taking into account all aspects of health, emphasize possible need for much higher vitamin D doses, exceeding 1000 IU per day (2000-10000 IU per day) [1, 5, 6, 14].

Conclusions
1) Vitamin D deficiency is common in Polish newborns irrespective of the season of birth.
2) It is justified to start vitamin D supplementation in neonates in the first few days of life.
3) Parathormon seems not to be a reliable functional marker of vitamin D deficiency in newborns.
4) Maternal vitamin D supplementation in dose 400 IU per day during pregnancy is not enough to build up appropriate offspring’s vitamin D stores. Farther researches with higher doses of vitamin D supplementation during pregnancy are necessary.

References


