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Cédric Annweiler, Spyridon Karras, Theocharis Koufakis, Vasiliki Antonopoulou, Dimitrios Goulis, Stefan Pilz, Helen Bili, Declan Naughton, Iltaf Shah, Vikentia Harizopoulou, et al.

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Spyridon N. Karras[1], Theocharis Koufakis[1], Vasiliki Antonopoulou[1], Dimitrios G. Goulis[2], Cedric Annweiler[3,4], Stefan Pilz[5], Helen Bili[2], Declan P. Naughton[6], Iltaf Shah[7], Vikentia Harizopoulou[2], Pantelis Zebekakis[1], Alkiviadis Bais[8], Kalliopi Kotsa[1]

[1] Division of Endocrinology and Metabolism, First Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA University Hospital, Thessaloniki, Greece

[2] Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3] Department of Neuroscience, Division of Geriatric Medicine, Angers University Hospital, Angers, France; University Memory Clinic, UPRES EA 4638, University of Angers, UNAM, Angers, France

[4] Robarts Research Institute, Department of Medical Biophysics, Schulich School of Medicine and Dentistry, the University of Western Ontario, London, Ontario, Canada

[5]Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University Graz, Graz, Austria

[6]School of Life Sciences, Kingston University London, UK

[7] Department of Chemistry, College of Science, UAE University, Al Ain, UAE

[8]Aristotle University of Thessaloniki, Laboratory of Atmospheric Physics, Thessaloniki, Greece

Correspondence: Dr. Spyridon N. Karras, Division of Endocrinology and Metabolism, First Department of Internal Medicine, AHEPA Hospital, S. Kiriakidi 1 str., Thessaloniki, Greece, karraspiros@yahoo.gr; Tel.: +30 2310324863.

Abstract

Absence of adequate maternal vitamin D supplementation and decreased maternal ultraviolet exposure during pregnancy are key determinants for the manifestation of neonatal hypovitaminosis D at birth. These parameters may vary, according to country-specific dietary patterns, health policies and sunshine exposure. We aimed to investigate differences in calcium metabolism and anthropometric profiles according to neonatal vitamin D status at birth, in a sunny region of Northern Greece. A secondary aim was to identify maternal parameters as risk factors for developing neonatal vitamin D deficiency at birth. A total of 129 mother-neonate pairs were included in the study and classified into three groups, according to neonatal 25-hydroxy-D [25(OH)D)] concentrations at birth [deficiency (<30 nmol/l), insufficiency (30-50 nmol/l) and sufficiency (>50 nmol/l)]. Neonatal biochemical and anthropometric profiles and maternal demographic, social, dietary and biochemical profiles were comparatively evaluated between the three groups. Univariate and multivariate logistic regression was performed to identify independent associations of maternal factors with neonatal vitamin D status. Vitamin D deficient-neonates manifested higher parathyroid hormone (7.20±2.60 vs 5.50±1.50 pg/ml, p=0.01) and lower corrected calcium (10.70±0.70 vs 11.30±1.30 mg/dl, p=0.02) concentrations compared with vitamin D-insufficient neonates. Mothers of vitamin D deficient and insufficient neonates had a lower total of 25(OH)D (31.7±19.2 and 36.5±22.3 vs 53.3±39.0 nmol/l, p<0.01) and 25(OH)D_3 (27.4±17.5 and 33.3±19.9 vs 47.3±36.7 nmol/l, p<0.01 and p=0.04, respectively) concentrations respectively, compared with those of vitamin D-sufficient neonates. Maternal use of alcohol during pregnancy was associated with a 5.57-fold higher risk for neonatal vitamin D deficiency at birth (OR 5.57, 95% CI1.17-26.56, p=0.03). Newborns with vitamin D deficiency presented a 6.89-fold higher risk of having been

given birth by vitamin D deficient mothers (OR 6.89, 95% CI 3.09-15.38, p<0.01). In conclusion, neonatal vitamin D deficiency is associated with maternal 25(OH)D concentrations at birth and maternal alcohol use. Further studies are required to replicate these findings in other regions and populations.

Keywords: Vitamin D; pregnancy; neonatal health; calcium; rickets.

1. Introduction

Despite the availability of supplementation and numerous published guidelines for its prevention [1,2], the resurgence of nutritional rickets (NR) secondary to vitamin D deficiency and/or dietary calcium (Ca) deficiency is becoming increasingly prevalent worldwide, highlighting the potential risks of not gaining sufficient vitamin D through diet, supplementation or exposure to ultraviolet (UVB) radiation. NR remains an important global, public health problem, with an established adverse impact on the skeletal and mental development of neonates and infants [3-5]. Recent reports detail the importance of maternal serum vitamin D concentrations in determining neonatal vitamin D status [6-10].

Although guidelines resulted in an improvement of the management of maternal hypovitaminosis D in the daily clinical setting [11], many aspects of such an approach are largely affected by country-specific dietary patterns, public health policies and variation of UVB exposure, due to cultural and life-style reasons [8,12]. In this context, results from regional studies are essential for establishing appropriate preventive strategies during pregnancy, to optimize vitamin D status in the mother and the neonate, in a country-specific approach. The aim of this maternal-neonatal birth cohort study was to investigate differences in Ca metabolism and anthropometric profiles according to neonatal vitamin D status at birth, in a sunny region of Northern Greece. A secondary aim was to identify maternal parameters as risk factors for developing neonatal vitamin D deficiency at birth.

2. Methods

2.1 Study criteria

Pregnant women on regular follow-up, were recruited from the Maternity Unit of the1st Department of Obstetrics and Gynecology, Aristotle University, Thessaloniki, Greece.

The inclusion criterion was full-term pregnancy (gestational week 37-42). Maternal exclusion criteria were primary hyperparathyroidism, secondary osteoporosis, heavy alcohol use (≥7 alcohol units per week or ≥6 units at any time during pregnancy), hyperthyroidism, nephritic syndrome, inflammatory bowel disease, rheumatoid arthritis, osteomalacia, obesity [body mass index (BMI)>30 kg/m^2], gestational diabetes and use of medications affecting Ca or vitamin D status (e.g. corticosteroids), except for Ca and vitamin D supplements. Neonatal exclusion criteria were being small-for-gestational age (SGA) and presence of severe congenital anomalies. Informed consent was obtained from all mothers. The protocol received approval from the Bioethics Committee of the Aristotle University of Thessaloniki, Greece (approval number 1/19-12-2011).

2.2 Demographics and dietary assessment

At enrolment, maternal demographic and social characteristics, as well as dietary habits, were recorded. Ca and vitamin D dietary intake during the last month of pregnancy were assessed through a validated, semi-quantitative, food frequency questionnaire that includes 150 foods and beverages [13]. For each dietary item, participants were asked to report their frequency of dairy products consumption and portion size. From these data, calculations were made for estimations of consumed quantities (in gr per day) based on a food composition database, modified to accommodate the particularities of the Greek diet [14] for estimating daily dietary Ca and vitamin D intake. Maternal education was classified as elementary (primary),

standard (secondary) and higher (tertiary and holding of academic degrees). Maternal alcohol use during pregnancy was treated as a dichotomous variable, defined either as none (subdivided in never drinking alcohol or drinking alcohol but not during pregnancy) or light (1-2 units per week or at any one time during pregnancy) / moderate (3-6 units per week or at any one time during pregnancy) [15].

2.3 Biochemical and hormonal assays

Blood samples were obtained from mothers by antecubital venipuncture 30-60 min before delivery. Umbilical cord blood was collected immediately after clamping, from the umbilical vein. Serum and umbilical cord specimens were stored at -20°C prior to analysis for the following parameters: Ca, phosphorus (P), parathyroid hormone (PTH), 25-hydroxyvitamin D_2 [$25(OH)D_2$] and D_3 [$25(OH)D_3$]. Serum Ca and P determinations were performed using the Cobas INTEGRA clinical chemistry system (D-68298; Roche Diagnostics, Mannheim, Germany).

The inter- and intra-assay coefficients of variation were 0.99% and 3.5% for Ca, and 1.3% and 2.5% for P, respectively. PTH determinations were performed using the electrochemiluminescence immunoassay ECLIA (Roche Diagnostics GmbA, Mannheim, Germany). Reference range for PTH was 15-65 pg/ml, functional sensitivity 6.0 pg/ml, within-run precision 0.6-2.8% and total precision 1.6-3.4%. Concentrations of $25(OH)D_2$ and $25(OH)D_3$ were determined using novel assay, liquid chromatography-tandem mass spectrometry (LC-MS/MS), with lower limits of quantification (LLOQ): $25(OH)D_2$ (0.5 ng/ml), $25(OH)D_3$ (0.5 ng/ml). Briefly, the assay involves analyte purification using liquid-liquid extraction followed by chromatographical separation using a chiral column in tandem with a rapid resolution

137 microbore column. Full method validation parameters have been previously reported
138 [16,17].
139
140 ***2.4 Neonatal and maternal vitamin D status***
141 Neonates were classified into three groups according to 25(OH)D status: Group A
142 (deficiency, 25(OH)D <30nmol/l), Group B (insufficiency, 30≤25(OH)D ≤50 nmol/l)
143 and Group C (sufficiency 25(OH)D>50 nmol/l) [2]. Respectively, mothers were
144 classified according to neonatal 25(OH)D concentrations at birth, into the following
145 groups: those who gave birth to deficient (group A), insufficient (group B) or sufficient
146 neonates (group C). Maternal vitamin D status was also included in subsequent uni-
147 and multivariate analysis as follows: vitamin D sufficiency ≥50 nmol/l and Vitamin D
148 insufficiency <50 nmol/l [18].
149
150 ***2.5 Maternal and neonatal anthropometry***
151 At enrolment, maternal and neonatal anthropometry was recorded. Maternal pre-
152 pregnancy BMI was either normal (18-25 kg/m^2) or overweight (25-30 kg/m^2). All
153 neonatal anthropometric measurements were performed by the same trained nurse,
154 between 12 and 72 hours of age. The following measurements were recorded: birth
155 weight, height, neck-rump, upper arm, femur and knee heel lengths; head, chest,
156 abdominal, upper arm and middle thigh circumferences; and abdominal skin fold
157 thickness. The birth weight of the neonates was measured on regularly calibrated scales.
158 The knee-heel length was measured with a hand-held BK5 infant knemometer (Force
159 Technology, Brondby, Denmark). Instrument software calculated the mean of 10
160 sequential readings and generated a printed report of all readings and the calculated
161 mean. Neonatal height was measured to the nearest mm using an Ellard newborn length

board (Ellard Instrumentation Ltd., Seattle, WA). Abdominal, upper arm and middle thigh head, mid-upper arm, and maximal head circumferences were measured using a plastic encircling tape (Child Growth Foundation, London, UK). Abdominal skinfold was measured using Holtain calipers (Holtain, Crymych, UK).

2.6 UVB measurements

UVB radiation includes wavelengths from 280 to 320 nm. UVB data for the broad geographical region of Thessaloniki, Greece were collected at the Laboratory of Atmospheric Physics, School of Physics, Aristotle University of Thessaloniki.

The daily integral of vitamin D effective UVB radiation (09:00 to 16:00 local time) was used as the most representative parameter for UVB exposure. These hours were selected as indicatives, since they are related to the beginning and the end of the working period for the majority of the population. Individual sunlight exposure was recorded for each participant during that period. Finally, mean UVB exposure during the previous 45 days (daily integral) before blood sample collection (estimated mean half-life of vitamin D) was calculated for each participant.

2.7 Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check the normality of distribution of the continuous variables in the whole study sample and in the neonates' vitamin D status groups, respectively. Normally distributed continuous data were presented as mean ± standard deviation (SD) and non-normally distributed as median (interquartile range) (IQR). One-way analysis of variance (ANOVA) was used to compare means among groups. Chi-square test was used for between-group comparisons, in case of categorical variables. Categorical data were presented as

absolute numbers and frequencies (percentages). Univariate and multivariate logistic regression was performed to identify independent associations of maternal factors with neonatal vitamin D status. Maternal factors significantly associated to neonatal vitamin D status in the univariate analysis were selected for the multivariate logistic regression, indicating each factor's effect on neonates' vitamin D deficiency after adjusting for the other factors. Statistical analysis was performed using IBM Statistics software SPSS 23.0 for Windows. A p-value <0.05 was considered as significant.

3. Results

A total of consecutive 129 mother-neonate pairs were included in the analysis. Recruitment period was from February to November. Mean gestational age was 38.6±1.5 weeks, and 53.6% of births occurred between September and March. Mean birth weight of neonates was 3269 ± 408 gr and mean neonatal 25(OH)D concentrations were 37.5±27.4 nmol/l. Neonates at the range of severe vitamin D deficiency manifested mean 25(OH)D concentrations of 15.9±7.5 nmol/L compared with 38.4±5.0 nmol/l and 71.0±30.5 nmol/l of insufficient and sufficient ones, respectively. Vitamin D deficient neonates manifested higher PTH (7.20±2.60 vs 5.50±1.50 pg/ml, p=0.01) and lower corrected Ca (10.70±0.70 vs 11.30±1.30 mg/dl, p=0.02) concentrations compared with vitamin D insufficient neonates, being not different from neonates with sufficiency [(PTH: 7.2 ± 2.6 vs 6.3 ± 2.0 pg/ml, p=0.22) and (corrected Ca: 10.7 ± 0.7 vs 10.8 ± 0.7, p=1.00)]. Epimers did not differ among various groups **(Table 1)**.

Vitamin D insufficient neonates had higher lower leg length compared with sufficient ones (15.6±1.5 vs 13.0±1.9 cm, p<0.01), whereas manifested lower knee-heel length compared with vitamin D deficient ones (8.0±1.3 vs 9.0±0.8 cm, p<0.01) (**Table 2**).

211 Mothers of vitamin D deficient and insufficient neonates had lower total 25(OH)D
212 [31.7±19.2 and 36.5±22.3 vs 53.3±39.0 nmol/l, $p<0.01$] and 25(OH)D_3 [27.4±17.5 and
213 33.3±19.9 vs 47.3±36.7 nmol/l, $p<0.01$ and $p=0.04$, respectively] concentrations,
214 compared with sufficient ones **(Table 3)**. The 37.5% of mothers of sufficient neonates
215 at birth had higher education, compared with 10.6% of mothers of insufficient ones
216 ($p<0.01$) (**Table 4)**.
217 In a univariate analysis, various maternal factors (age, smoking, alcohol consumption,
218 vitamin D status, Ca supplementation, dietary daily Ca and vitamin D intake during
219 the third trimester, pre-pregnancy BMI, delivery BMI, education level, UVB exposure)
220 were included, to assess their impact on neonatal vitamin D status. Maternal use of
221 alcohol during pregnancy (≥1 unit per week) was associated with a 5.57-fold higher
222 probability for neonatal vitamin D deficiency at birth (OR 5.57, 95% CI 1.17-26.56,
223 $p=0.03$). These results remained significant in a multivariate analysis [OR 6.18
224 (adjusted for maternal vitamin D status and UVB exposure)], 95% CI 1.18-32.51,
225 $p=0.03$] (**Table 5)**. Risk of neonatal vitamin D deficiency was 6.89-fold increased for
226 infants born by vitamin D deficient (<50 nmol/l), when compared with vitamin D
227 sufficient mothers (>50 nmol/l) (OR 6.89, 95% CI 3.09-15.38, $p<0.01$). The results
228 remained significant in the multivariate analysis [OR (adjusted for alcohol consumption
229 and UVB exposure)] 7.62, 95% CI 3.27-17.78] (**Table 5**). UVB exposure during
230 summer months, was associated with 3.51-fold higher probability for neonatal vitamin
231 D deficiency at birth (OR 3.51, 95% CI1.87-11.42, $p=0.03$); this association did not
232 retain its significance in the multivariate analysis.
233
234 **4. Discussion**

The present study captures the current status of neonatal vitamin D deficiency at birth in a sunny Mediterranean region, characterized by both PTH and corrected Ca concentrations within the normal range, however, significantly decreased compared with insufficient neonates. Neonatal birth anthropometry was not affected by neonatal vitamin D deficiency, with the exclusion of a significant increase in knee-heel length. It was also demonstrated that maternal alcohol use and maternal vitamin D insufficiency (<50 nmol/l) during pregnancy are risk factors for the development of neonatal vitamin D deficiency at birth.

Neonatal vitamin D deficiency is a major risk factor for the development of acute and chronic metabolic complications including NR, hypocalcemia and impairment of optimal skeletal development of the offspring [1-3]. Recent reports underline the importance of maternal circulating vitamin D concentrations in determining neonatal vitamin D status [6-8]. Preventive strategies are primarily focused on the sufficiency of maternal vitamin D status during pregnancy, either through appropriate vitamin D supplementation or sunshine exposure. In accordance with our previous findings [19] regarding a high prevalence of maternal hypovitaminosis D during pregnancy in the Mediterranean countries, results from this cohort describe a high prevalence of neonatal vitamin D sufficiency at birth, in this region. The main reasons behind this phenomenon seem to be racial, social and cultural particularities, as well as the absence of preventive strategies, including food fortification policies in our region, that mitigate the benefits of sun exposure. In detail, dark skin, dressing habits, and sunshine avoidance, especially during the hot summer months, have been associated with an increased prevalence of maternal hypovitaminosis D during pregnancy [12,19].

PTH becomes a major regulator of mineral and bone homeostasis within the first hours after birth [20]. Parathyroid glands increase the synthesis and secretion of PTH, which

acts to raise serum Ca, lower P, stimulate calcitriol synthesis, inhibit calcitriol catabolism, reabsorb Ca in the kidney tubules, and up regulate bone formation [21]. The significant increase of PTH concentrations - albeit within the normal range - in vitamin D deficient neonates observed in this study, might result from an *in utero* adaptive up-regulation of fetal PTH, as a result of maternal-fetal hypovitaminosis D, to maintain adequate Ca supply for the developing infant [9]. On the other hand, absence of correlation between 25(OH)D and intact PTH in cord blood has been previously reported [22-24], indicating that the well documented rise of PTH in adults with secondary hyperparathyroidism, is not evident in neonates after birth, as a result of a temporary PTH suppression. Further studies are necessary in order to evaluate the magnitude of neonatal PTH variation in the context of neonatal vitamin D status at birth.

Neonatal anthropometry at birth was not remarkably affected by neonatal vitamin D deficiency in our cohort. Although lower mean neonatal knee-heel length at birth has been associated with lower maternal 25(OH)D levels between 28 and 32 weeks of gestation [25], this observation was not replicated in vitamin D deficient neonates at birth. Our results are in agreement with the findings reported by Czech-Kowalska et al [26], who failed to establish an association between neonatal vitamin D status and neonatal anthropometry and adiposity in a similar to our study population, including appropriate for gestational age neonates. Contrariwise, Sauder et al. [27] proved a relationship between higher neonatal 25(OH)D concentrations and lower birthweight and neonatal adiposity, after adjustment for gestational age at birth. Therefore, the fact that SGA infants were not included in our analysis, might have had an impact on the pattern of our findings.

Neonatal 25(OH)D concentrations at birth roughly follow the maternal pattern in the deficient and insufficient mother groups, while resembling uniform distribution in the group of mothers with sufficient vitamin D status [6]. The results of this study suggest that both mothers of deficient and insufficient neonates manifested significant differences in their vitamin D status compared to those of sufficient ones, indicating that both profiles fall within the same pathophysiological high-risk zone, for development of neonatal vitamin D deficiency at birth. On the other hand, mothers who gave birth to vitamin D sufficient neonates demonstrated mean total 25(OH)D concentrations >50 nmol/l, identifying a potential, maternal, low-threshold to be targeted during pregnancy. Of course, these values are country-specific and might not be applied to other regions and countries.

The present study revealed an association between maternal alcohol use, defined as ≥1 unit per week, and hypovitaminosis D during pregnancy and the development of neonatal vitamin D deficiency. There is a paucity of studies concerning the effects of alcohol on vitamin D status during pregnancy. In a cohort of Ukrainian pregnant women [28], alcohol-exposed women had lower 25(OH)D concentrations than low/unexposed women during spring and winter. Of major interest, vitamin D concentrations were lower in patients with alcohol use disorders, whose last alcohol intake was within the last 30 days compared with those who had abstained >30 days from alcohol [29]. In non-pregnant rats, chronic alcohol consumption can lead to depletion of vitamin D stores [30]. Multiple factors have been proposed as potential explanations for the inverse association between alcohol use and vitamin D status, including poor diet, malabsorption and restricted exposure to natural sunlight, which are all commonly seen in heavy drinkers [28]. A direct effect of alcohol on vitamin D biodynamics has been also postulated, possibly related to alcoholic liver disease that disrupts protein

309 synthesis, resulting in low levels of vitamin D binding protein and – subsequently - of
310 the active form of vitamin D [31]. Shankar et al. [32] have demonstrated that alcohol
311 reduces circulating 1,25 (OH)2 D_3 levels by inducing CYP24A1, resulting
312 from renal oxidative stress due to local ethanol metabolism. On the other hand, a
313 systematic review on the relationship between alcohol use and vitamin D [33]
314 concluded that studies reporting positive associations between alcohol units per day
315 and vitamin D serum concentrations had better study designs and larger sample sizes
316 compared with those that established the opposite findings. Greater exposure to sunlight
317 due to the homeless status of many alcoholics was suggested by the authors as a
318 plausible explanation for this observation. It is evident that the relationship between
319 alcohol and vitamin D is mediated by complex factors, equally related to the social and
320 health consequences of alcohol abuse, thus warranting further evaluation by future
321 studies.
322 Two characteristics of this cohort deserve special attention. First, the study did not
323 observe differences between maternal groups in dietary Ca and vitamin D intake during
324 pregnancy, nor an impact of these parameters on the risk of neonatal hypovitaminosis
325 D. A systematic review and meta-analysis on micronutrient intake during pregnancy in
326 developed countries [34], reported that dietary vitamin D intake among pregnant
327 women is insufficient; still, it has a limitted impact on neonatal vitamin D status. In
328 addition, recent findings from a birth-cohort of 567 women from northern Sweden [35],
329 indicated that more than half of the women participated, had intake levels of vitamin D
330 lower than those recommended (median level of 0.85 μg/MJ/day vs. recommended
331 level of 0.98 μg/MJ/day). These results are in accordance with our findings, derived
332 from a sunny region of Southern Europe. Moreover, the vast majority of the women
333 included in this analysis regularly followed the typical Mediterranean diet during

pregnancy. Given that the analysis lacks a detailed evaluation of dietary protein and fat intake as potential food sources of vitamin D, future studies are needed to elucidate the hypothesis about a potential neutral effect of this dietary pattern on maternal vitamin D status.

A second finding was the inverse association (although not confirmed in the multivariate analysis) between UVB exposure and vitamin D equilibrium. Whole-body sun exposure at the right time of the year is required, in order for circulating vitamin D levels to be affected [28,29]. In contrast, partial exposure (5-10%) of total body surface to intense sunlight produces only a limited amount of vitamin D. It could be hypothesized that, under high-temperature climatic conditions that are present during spring and summer months in Greece, most pregnant women limit their outdoor activities during the morning and afternoon hours. In this context, high UVB radiation, especially during hot summer months, might comprise a risk factor for reduced maternal sunshine exposure which could in turn adversely affect maternal vitamin D status during pregnancy, leading to a high prevalence of neonatal vitamin D deficiency [36,37]. Additional studies are required in this direction.

This study has several limitations, including the relatively small sample size, which may explain why additional parameters known to affect maternal vitamin D status during pregnancy and result in neonatal vitamin D deficiency, were not found to be regulators of 25(OH)D concentrations. In this regard, skin color has been reported to be a determinant of vitamin D status [38]. However, in this study we chose not to include a rough estimate of skin pigmentation as, for example, the Fitzpatrick's scale, given that most women of Greek origin belong to a relatively homogenous group of mild dark or white skin phototypes [36]. Finally, the exclusion of obese pregnant

women from our cohort could be considered as an additional limitation, since maternal obesity is a well-known risk factor for maternal vitamin D deficiency [39].

In conclusion, this study reported results from a maternal-neonatal Greek cohort, primarily focusing on neonatal vitamin D deficiency. It demonstrated a high prevalence of vitamin D deficient neonates at birth. This phenomenon was primarily mediated by maternal 25(OH)D concentrations at birth and maternal alcohol use. Adequate and appropriate sun exposure may not be indispensable for avoiding maternal hypovitaminosis D, even in the sunny Mediterranean region. These data could provide a targeted approach based on specific-population characteristics for future vitamin D supplementation studies and help to recognize parameters necessary for developing health policies to prevent neonatal vitamin D deficiency in this region.

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Ethical approval: The study protocol was approved by the Bioethics Committee of the Aristotle University of Thessaloniki.

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Table 1. Biochemical parameters of neonates born with Vitamin D deficiency, insufficiency or sufficiency.

Parameter	**Group A (n=50) Deficiency**	**Group B (n=47) Insufficiency**	**Group C (n=32) Sufficiency**	**Comparisons**
Total 25(OH)D (nmol/l)	15.9 ± 7.5	38.4 ± 5.0	71.0 ± 30.5	**A vs B p<0.01** **A vs C p<0.01** **B vs C p<0.01**
25(OH)D_2 (nmol/l)	2.6 ± 3.2	3.4 ± 4.4	6.9 ± 7.8	**A vs C p=0.01** **B vs C p=0.02**
25(OH)D_3 (nmol/l)	13.4 ± 7.6	35.8 ± 6.1	64.1 ± 29.8	**A vs B p<0.01** **A vs C p<0.01** **B vs C p<0.01**
3-epi-25(OH)D_2 (nmol/l)	3.6 ± 5.4	4.8 ± 4.0	3.4 ± 3.9	p=0.91
3-epi-25(OH)D_3 (nmol/l)	4.3 ± 7.6	3.3 ± 3.9	7.2 ± 8.5	**B vs C p=0.03**
PTH (pg/ml)	7.2 ± 2.6	5.5 ± 1.5	6.3 ± 2.0	**A vs B p=0.01**
Corrected calcium (mg/dl)	10.7 ± 0.7	11.3 ± 1.3	10.8 ± 0.7	**A vs B p=0.02**
Phosphorus (mg/dl)	5.7 ± 0.5	5.5 ± 0.5	5.8 ± 0.6	p=0.65
Total bilirubin (mg/dl)	1.6 ± 0.4	1.9 ± 0.5	1.7 ± 0.4	p=0.11

Vitamin D deficiency <30 nmol/l, insufficiency 30-50 nmol/l, sufficiency >50 nmol/l. Values are presented as mean ± standard deviations. Significant differences are presented in bold. **Abbreviations:** PTH: parathyroid hormone; 25(OH)D: 25-hydroxy-Vitamin D

Table 2. Demographic and anthropometric parameters of neonates born with Vitamin D deficiency, insufficiency or sufficiency.

Parameter	**Group A (n=50) Deficiency**	**Group B (n=47) Insufficiency**	**Group C (n=32) Sufficiency**	**Comparisons**
Male (%)	23 (46)	29 (61.7)	13 (40.6)	p=0.12
Season of birth (Sep-Mar / Apr-Aug)	42 / 8	36 / 11	27 / 5	p=0.35
Height (cm)	50.3 ± 1.9	50.8 ± 1.7	50.2 ± 2.3	p=0.73
Birth weight (kg)	3290.9 ± 331.2	3334.4 ± 355.8	3145.7 ± 428.2	p=1.00
Apgar score at 1 min	7.8 ± 0.5	8.0 ± 0.2	8.0 ± 0.0	p=0.20
Apgar score at 5 min	8.7 ± 0.8	9.0 ± 0.2	8.9 ± 0.5	p=0.10
Head circumference (cm)	34.7 ± 4.0	34.8 ± 0.8	33.6 ± 1.9	p=1.00
Neck-rump length (cm)	17.6 ± 2.0	17.9 ± 1.2	17.9 ± 2.5	p=1.00
Chest circumference (cm)	30.8 ± 2.0	31.0 ± 1.5	31.3 ± 2.1	p=1.00
Abdominal circumference (cm)	28.2 ± 2.0	28.3 ± 1.6	28.8 ± 2.5	p=1.00
Abdominal circumference (iliac)	25.8 ± 1.4	26.0 ± 1.3	26.3 ± 2.0	p=1.00
Upper arm length (cm)	13.2 ± 0.7	13.7 ± 0.6	13.9 ± 1.5	p=0.17
Upper arm circumference (cm)	9.8 ± 0.7	9.8 ± 0.7	9.8 ± 0.7	p=1.00

Lower arm-radial circumference (cm)	9.0 ± 0.6	9.0 ± 0.5	9.0 ± 0.7	p=1.00
Thigh circumference (high) (cm)	15.0 ± 1.0	15.4 ± 1.4	15.5 ± 1.7	p=0.79
Thigh circumference (middle) (cm)	13.3 ± 1.0	13.3 ± 1.0	13.3 ± 1.4	p=1.00
Lower leg-calf circumference (maximum) (cm)	10.4 ± 0.7	10.3 ± 0.7	10.3 ± 0.9	p=1.00
Lower leg length (cm)	13.9 ± 0.9	15.6 ± 1.5	13.0 ± 1.9	**B vs C p<0.01**
Knee-heel length (cm)	9.0 ± 0.8	8.0 ± 1.3	8.8 ± 0.9	**A vs B p<0.01**
Femur length (cm)	9.6 ± 0.9	10.0 ± 0.5	9.4 ± 1.1	p=0.19
Skin fold-subscapular (cm)	2.8 ± 0.4	2.6 ± 0.4	2.9 ± 0.7	p=0.14
Skin fold-abdominal (cm)	2.9 ± 0.7	2.8 ± 0.3	2.9 ± 0.5	p=0.82
Skin fold-anterior thigh (cm)	3.6 ± 0.6	3.7 ± 0.4	3.7 ± 0.5	p=1.00

Vitamin D deficiency <30 nmol/l, insufficiency 30-50 nmol/l, sufficiency >50 nmol/lValues are presented as mean ± standard deviation or as absolute values (percentage). Significant differences are presented in bold. **Abbreviations:** min: minute(s); cm: centimeter(s); kg: kilogram(s).

Table 3. Biochemical parameters of mothers who gave birth to neonates with Vitamin D deficiency, insufficiency or sufficiency.

Parameter	**Group A (n=50)** **Deficiency**	**Group B (n=47)** **Insufficiency**	**Group C (n=32)** **Sufficiency**	**Comparisons**
Total 25(OH)D (nmol/l)	31.7 ± 19.2	36.5 ± 22.3	53.3 ± 39.0	**A vs C p<0.01** **B vs C p=0.01**
$25(OH)D_2$ (nmol/l)	4.2 ± 5.4	3.9 ± 6.0	6.0 ± 8.0	p=1.00
$25(OH)D_3$ (nmol/l)	27.4 ± 17.5	33.3 ± 19.9	47.3 ± 36.7	**A vs C p<0.01** **B vs C p=0.04**
3-epi-$25(OH)D_2$ (nmol/L)	4.0 ± 5.1	3.1 ± 3.9	7.5 ± 13.9	p=1.00
epi-$25(OH)D_3$ (nmol/l)	4.5 ± 4.9	3.4 ± 3.6	7.7 ± 14.1	p=1.00
PTH (pg/ml)	32.2 ± 9.1	30.8 ± 15.5	31.9 ± 13.1	p=1.00
Calcium (mg/dl)	8.1 ± 1.2	8.7 ± 0.9	8.4 ± 1.0	p=0.076
Corrected calcium (mg/dl)	9.5 ± 0.5	9.9 ± 0.3	9.9 ± 0.4	p=0.06
Phosphorus (mg/dl)	3.6 ± 0.6	3.6 ± 0.6	3.5 ± 0.7	p=1.00

Vitamin D deficiency < 30 nmol/l, insufficiency 30-50 nmol/l, sufficiency > 50 nmol/l. Values are presented as mean ± standard deviation. Significant differences are presented in bold. **Abbreviations:** PTH: parathyroid hormone; 25(OH)D: 25-hydroxy-Vitamin D

Table 4. Demographic and anthropometric parameters of mothers who gave birth to neonates with Vitamin D deficiency, insufficiency or sufficiency.

Parameter	**Group A (n=50) Deficiency**	**Group B (n=47) Insufficiency**	**Group C (n=32) Sufficiency**	**Comparisons**
Age (years)	33.0 ± 4.5	32.5 ± 5.1	32.5 ± 5.0	p=1.00
Height (cm)	169.4 ± 8.9	168.2 ± 7.2	166.8 ± 6.0	p=1.00
Father's height (cm)	183.3 ± 10.7	181.2 ± 10.3	181.6 ± 8.0	p=1.00
Weight before pregnancy (kg)	64.3 ± 9.1	66.8 ± 10.5	62.6 ± 8.7	p=0.63
Weight at term (kg)	78.5 ± 10.0	81.6 ± 12.1	75.7 ± 9.2	p=0.49
BMI before pregnancy (kg/m^2)	22.6 ± 3.7	23.9 ± 3.8	22.6 ± 3.2	p=0.36
BMI at term (kg/m^2)	27.4 ± 3.3	28.2 ± 6.1	27.2 ± 3.1	p=1.00
Gestation weeks	39.0 ± 1.7	38.9 ± 1.6	38.9 ± 1.0	p=1.00
Previous live births	0.9 ± 0.7	0.8 ± 0.8	1.2 ± 1.0	p=1.00
Daily calcium supplementation (mg)	416.6 ± 304.8	474.3 ± 292.7	403.4 ± 9280.6	p=1.00
Daily dietary calcium intake during 3^{rd} trimester	786.2 ± 401.8	796.5 ± 334.0	776.2 ± 338.7	p=1.00

(mg)				
Daily dietary vitamin D intake during 3rd trimester (mcg)	2.7 ± 1.4	2.5 ± 1.5	3.0 ± 1.4	p=1.00
UVB exposure (Wh/m²)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	p=0.55
Smoking (%)	5 (10)	4 (8.5)	0 (0)	p=0.80
Higher education (%)	11 (22)	5 (10.6)	12 (37.5)	**B vs C p<0.01**
Alcohol consumption (%)	None 32 (64) Light 11 (22) Moderate 7 (14)	None 40 (85.1) Light 5 (10.6) Moderate 2 (4.3)	None 29 (90.6) Light 2 (6.3) Moderate 1 (3.1)	p=0.09

Vitamin D deficiency <30 nmol/l, insufficiency 30-50 nmol/l, sufficiency >50 nmol/l. Values are presented as mean ± standard deviation. Frequencies are presented as absolute values (percentage). Significant differences are presented in bold. **Abbreviations:** BMI: Body Mass Index; UVB: Ultraviolet B radiation.

Table 5. Maternal factors associated to neonatal vitamin D status.

Maternal factors	**Univariate analysis**			**Multivariate analysis**		
	OR	95% CI	p-value	OR (adjusted)	95% CI	p-value
Alcohol consumption	5.57	1.17-26.56	**0.03**	6.18¶	1.18-32.51	**0.03**
Vitamin D status	6.89	3.09-15.38	**<0.01**	7.62§	3.27-17.78	**<0.01**

Vitamin D deficient: 25(OH)D<30 nmol/l, Vitamin D non-deficient: 25(OH)D≥30 nmol/l in univariate and multivariate analysis. Significant differences are presented in bold..The maternal factors that were significantly associated to neonates' vitamin D status in the univariate analysis (alcohol consumption, vitamin D status and UVB exposure) were selected for the multivariate analysis.¶adjusted for vitamin D status and UVB exposure, §adjusted for alcohol consumption and UVB exposure. **Abbreviations:** OR: odds ratio; CI: confidence interval.