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1 **Characterizing neonatal vitamin D deficiency in the modern era: a maternal-**
2 **neonatal birth cohort from Southern Europe**

3

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31 **Abstract**

32

33 Absence of adequate maternal vitamin D supplementation and decreased maternal
34 ultraviolet exposure during pregnancy are key determinants for the manifestation of
35 neonatal hypovitaminosis D at birth. These parameters may vary, according to country-
36 specific dietary patterns, health policies and sunshine exposure. We aimed to
37 investigate differences in calcium metabolism and anthropometric profiles according
38 to neonatal vitamin D status at birth, in a sunny region of Northern Greece. A secondary
39 aim was to identify maternal parameters as risk factors for developing neonatal vitamin
40 D deficiency at birth. A total of 129 mother-neonate pairs were included in the study
41 and classified into three groups, according to neonatal 25-hydroxy-D [25(OH)D]
42 concentrations at birth [deficiency (<30 nmol/l), insufficiency (30-50 nmol/l) and
43 sufficiency (>50 nmol/l)]. Neonatal biochemical and anthropometric profiles and
44 maternal demographic, social, dietary and biochemical profiles were comparatively
45 evaluated between the three groups. Univariate and multivariate logistic regression was
46 performed to identify independent associations of maternal factors with neonatal
47 vitamin D status. Vitamin D deficient-neonates manifested higher parathyroid hormone
48 (7.20 ± 2.60 vs 5.50 ± 1.50 pg/ml, $p=0.01$) and lower corrected calcium (10.70 ± 0.70 vs
49 11.30 ± 1.30 mg/dl, $p=0.02$) concentrations compared with vitamin D-insufficient
50 neonates. Mothers of vitamin D deficient and insufficient neonates had a lower total of
51 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)D₃
52 (27.4 ± 17.5 and 33.3 ± 19.9 vs 47.3 ± 36.7 nmol/l, $p<0.01$ and $p=0.04$, respectively)
53 concentrations respectively, compared with those of vitamin D-sufficient neonates.
54 Maternal use of alcohol during pregnancy was associated with a 5.57-fold higher risk
55 for neonatal vitamin D deficiency at birth (OR 5.57, 95% CI 1.17-26.56, $p=0.03$).
56 Newborns with vitamin D deficiency presented a 6.89-fold higher risk of having been

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

61

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

64 **1. Introduction**

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

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

85

86 **2. Methods**

87 ***2.1 Study criteria***

88 Pregnant women on regular follow-up, were recruited from the Maternity Unit of
89 the 1st Department of Obstetrics and Gynecology, Aristotle University, Thessaloniki,
90 Greece.

91 The inclusion criterion was full-term pregnancy (gestational week 37-42). Maternal
92 exclusion criteria were primary hyperparathyroidism, secondary osteoporosis, heavy
93 alcohol use (≥ 7 alcohol units per week or ≥ 6 units at any time during pregnancy),
94 hyperthyroidism, nephritic syndrome, inflammatory bowel disease, rheumatoid
95 arthritis, osteomalacia, obesity [body mass index (BMI) >30 kg/m²], gestational
96 diabetes and use of medications affecting Ca or vitamin D status (e.g. corticosteroids),
97 except for Ca and vitamin D supplements. Neonatal exclusion criteria were being
98 small-for-gestational age (SGA) and presence of severe congenital anomalies.
99 Informed consent was obtained from all mothers. The protocol received approval from
100 the Bioethics Committee of the Aristotle University of Thessaloniki, Greece (approval
101 number 1/19-12-2011).

102

103 ***2.2 Demographics and dietary assessment***

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

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

118

119 ***2.3 Biochemical and hormonal assays***

120 Blood samples were obtained from mothers by antecubital venipuncture 30-60 min
121 before delivery. Umbilical cord blood was collected immediately after clamping, from
122 the umbilical vein. Serum and umbilical cord specimens were stored at -20°C prior to
123 analysis for the following parameters: Ca, phosphorus (P), parathyroid hormone (PTH),
124 25-hydroxyvitamin D₂ [25(OH)D₂] and D₃ [25(OH)D₃]. Serum Ca and P
125 determinations were performed using the Cobas INTEGRA clinical chemistry system
126 (D-68298; Roche Diagnostics, Mannheim, Germany).

127 The inter- and intra-assay coefficients of variation were 0.99% and 3.5% for Ca, and
128 1.3% and 2.5% for P, respectively. PTH determinations were performed using the
129 electrochemiluminescence immunoassay ECLIA (Roche Diagnostics GmbH,
130 Mannheim, Germany). Reference range for PTH was 15-65 pg/ml, functional
131 sensitivity 6.0 pg/ml, within-run precision 0.6-2.8% and total precision 1.6-3.4%.
132 Concentrations of 25(OH)D₂ and 25(OH)D₃ were determined using novel assay, liquid
133 chromatography-tandem mass spectrometry (LC-MS/MS), with lower limits of
134 quantification (LLOQ): 25(OH)D₂ (0.5 ng/ml), 25(OH)D₃ (0.5 ng/ml). Briefly, the
135 assay involves analyte purification using liquid-liquid extraction followed by
136 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, $30 \leq 25(\text{OH})\text{D} \leq 50$ 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/m²) or overweight (25-30 kg/m²). 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, Brøndby, 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

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

166

167 ***2.6 UVB measurements***

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

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

178

179 ***2.7 Statistical analysis***

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

187 absolute numbers and frequencies (percentages). Univariate and multivariate logistic
188 regression was performed to identify independent associations of maternal factors with
189 neonatal vitamin D status. Maternal factors significantly associated to neonatal vitamin
190 D status in the univariate analysis were selected for the multivariate logistic regression,
191 indicating each factor's effect on neonates' vitamin D deficiency after adjusting for the
192 other factors. Statistical analysis was performed using IBM Statistics software SPSS
193 23.0 for Windows. A p-value <0.05 was considered as significant.

194

195 **3. Results**

196 A total of consecutive 129 mother-neonate pairs were included in the analysis.
197 Recruitment period was from February to November. Mean gestational age was
198 38.6 ± 1.5 weeks, and 53.6% of births occurred between September and March. Mean
199 birth weight of neonates was 3269 ± 408 gr and mean neonatal 25(OH)D concentrations
200 were 37.5 ± 27.4 nmol/l. Neonates at the range of severe vitamin D deficiency
201 manifested mean 25(OH)D concentrations of 15.9 ± 7.5 nmol/L compared with 38.4 ± 5.0
202 nmol/l and 71.0 ± 30.5 nmol/l of insufficient and sufficient ones, respectively. Vitamin
203 D deficient neonates manifested higher PTH (7.20 ± 2.60 vs 5.50 ± 1.50 pg/ml, $p=0.01$)
204 and lower corrected Ca (10.70 ± 0.70 vs 11.30 ± 1.30 mg/dl, $p=0.02$) concentrations
205 compared with vitamin D insufficient neonates, being not different from neonates with
206 sufficiency [(PTH: 7.2 ± 2.6 vs 6.3 ± 2.0 pg/ml, $p=0.22$) and (corrected Ca: 10.7 ± 0.7
207 vs 10.8 ± 0.7 , $p=1.00$)]. Epimers did not differ among various groups (**Table 1**).
208 Vitamin D insufficient neonates had higher lower leg length compared with sufficient
209 ones (15.6 ± 1.5 vs 13.0 ± 1.9 cm, $p<0.01$), whereas manifested lower knee-heel length
210 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)D₃ [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% CI 1.87-11.42, p=0.03); this association did not
232 retain its significance in the multivariate analysis.

233

234 **4. Discussion**

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

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

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

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

272 Neonatal anthropometry at birth was not remarkably affected by neonatal vitamin D
273 deficiency in our cohort. Although lower mean neonatal knee-heel length at birth has
274 been associated with lower maternal 25(OH)D levels between 28 and 32 weeks of
275 gestation [25], this observation was not replicated in vitamin D deficient neonates at
276 birth. Our results are in agreement with the findings reported by Czech-Kowalska et al
277 [26], who failed to establish an association between neonatal vitamin D status and
278 neonatal anthropometry and adiposity in a similar to our study population, including
279 appropriate for gestational age neonates. Contrariwise, Sauder et al. [27] proved a
280 relationship between higher neonatal 25(OH)D concentrations and lower birthweight
281 and neonatal adiposity, after adjustment for gestational age at birth. Therefore, the fact
282 that SGA infants were not included in our analysis, might have had an impact on the
283 pattern of our findings.

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

295 The present study revealed an association between maternal alcohol use, defined as ≥ 1
296 unit per week, and hypovitaminosis D during pregnancy and the development of
297 neonatal vitamin D deficiency. There is a paucity of studies concerning the effects of
298 alcohol on vitamin D status during pregnancy. In a cohort of Ukrainian pregnant women
299 [28], alcohol-exposed women had lower 25(OH)D concentrations than low/unexposed
300 women during spring and winter. Of major interest, vitamin D concentrations were
301 lower in patients with alcohol use disorders, whose last alcohol intake was within the
302 last 30 days compared with those who had abstained >30 days from alcohol [29]. In
303 non-pregnant rats, chronic alcohol consumption can lead to depletion of vitamin D
304 stores [30]. Multiple factors have been proposed as potential explanations for the
305 inverse association between alcohol use and vitamin D status, including poor diet,
306 malabsorption and restricted exposure to natural sunlight, which are all commonly seen
307 in heavy drinkers [28]. A direct effect of alcohol on vitamin D biodynamics has been
308 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 D₃ 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 limited 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

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

338 A second finding was the inverse association (although not confirmed in the
339 multivariate analysis) between UVB exposure and vitamin D equilibrium. Whole-body
340 sun exposure at the right time of the year is required, in order for circulating vitamin D
341 levels to be affected [28,29]. In contrast, partial exposure (5-10%) of total body surface
342 to intense sunlight produces only a limited amount of vitamin D. It could be
343 hypothesized that, under high-temperature climatic conditions that are present during
344 spring and summer months in Greece, most pregnant women limit their outdoor
345 activities during the morning and afternoon hours. In this context, high UVB radiation,
346 especially during hot summer months, might comprise a risk factor for reduced
347 maternal sunshine exposure which could in turn adversely affect maternal vitamin D
348 status during pregnancy, leading to a high prevalence of neonatal vitamin D deficiency
349 [36,37]. Additional studies are required in this direction.

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

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

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

369 **Declaration of interest:** Authors report no conflict of interest.

370

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372

373 **Informed consent:** Informed consent was obtained from all participants.

374

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

377

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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/l/Values 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)	Group B (n=47)	Group C (n=32)	Comparisons
	Deficiency	Insufficiency	Sufficiency	
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 3 rd trimester (mcg)	2.7 ± 1.4	2.5 ± 1.5	3.0 ± 1.4	p=1.00
UVB exposure (Wh/m ²)	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.