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

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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

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.

1. Introduction

64

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

2. Methods

86

87 *2.1 Study criteria*

88 Pregnant women on regular follow-up, were recruited from the Maternity Unit of

89 the1st 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 ($\geq$7 alcohol units per week or $\geq$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/m^2], 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 D_2 [25(OH)D_2] and D_3 [25(OH)D_3]. 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 GmbA,

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)D_2 and 25(OH)D_3 were determined using novel assay, liquid

133   chromatography-tandem mass spectrometry (LC-MS/MS), with lower limits of

134   quantification (LLOQ): 25(OH)D_2 (0.5 ng/ml), 25(OH)D_3 (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≤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

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 ± 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)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**

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.

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

synthesis, resulting in low levels of vitamin D binding protein and – subsequently - of the active form of vitamin D [31]. Shankar et al. [32] have demonstrated that alcohol reduces circulating 1,25 (OH)2 D_3 levels by inducing CYP24A1, resulting from renal oxidative stress due to local ethanol metabolism. On the other hand, a systematic review on the relationship between alcohol use and vitamin D [33] concluded that studies reporting positive associations between alcohol units per day and vitamin D serum concentrations had better study designs and larger sample sizes compared with those that established the opposite findings. Greater exposure to sunlight due to the homeless status of many alcoholics was suggested by the authors as a plausible explanation for this observation. It is evident that the relationship between alcohol and vitamin D is mediated by complex factors, equally related to the social and health consequences of alcohol abuse, thus warranting further evaluation by future studies.

Two characteristics of this cohort deserve special attention. First, the study did not observe differences between maternal groups in dietary Ca and vitamin D intake during pregnancy, nor an impact of these parameters on the risk of neonatal hypovitaminosis D. A systematic review and meta-analysis on micronutrient intake during pregnancy in developed countries [34], reported that dietary vitamin D intake among pregnant women is insufficient; still, it has a limitted impact on neonatal vitamin D status. In addition, recent findings from a birth-cohort of 567 women from northern Sweden [35], indicated that more than half of the women participated, had intake levels of vitamin D lower than those recommended (median level of 0.85 μg/MJ/day vs. recommended level of 0.98 μg/MJ/day). These results are in accordance with our findings, derived from a sunny region of Southern Europe. Moreover, the vast majority of the women 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 phenotypes [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.

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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
$3\text{-epi-}25(OH)D_2$ (nmol/l)	3.6 ± 5.4	4.8 ± 4.0	3.4 ± 3.9	p=0.91
$3\text{-epi-}25(OH)D_3$ (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 3rd 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.