



**HAL**  
open science

## Vitamin D status during pregnancy and in cord blood in a large prospective French cohort.

Marie Courbebaisse, Jean-Claude Souberbielle, Amandine Baptiste, Joëlle Taieb, Vassilis Tsatsaris, Jean Guibourdenche, Marie-Victoire Senat, Hazar Haidar, Jacques Jani, Meriem Guizani, et al.

### ► To cite this version:

Marie Courbebaisse, Jean-Claude Souberbielle, Amandine Baptiste, Joëlle Taieb, Vassilis Tsatsaris, et al.. Vitamin D status during pregnancy and in cord blood in a large prospective French cohort.. Clinical Nutrition, 2018, 10.1016/j.clnu.2018.08.035 . hal-02618987

**HAL Id: hal-02618987**

**<https://hal.inrae.fr/hal-02618987v1>**

Submitted on 20 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Vitamin D status during pregnancy and in cord blood in a large prospective French**  
2 **cohort.**

3

4 **Authors:** Marie Courbebaisse<sup>a,\*</sup>, Jean-Claude Souberbielle<sup>b</sup>, Amandine Baptiste<sup>c</sup>, Joëlle  
5 Taieb<sup>d</sup>, Vassilis Tsatsaris<sup>e,f</sup>, Jean Guibourdenche<sup>g</sup>, Marie-Victoire Senat<sup>h</sup>, Hazar Haidar<sup>i</sup>,  
6 Jacques Jani<sup>j</sup>, Meriem Guizani<sup>j</sup>, Jean-Marie Jouannic<sup>k</sup>, Marie-Clotilde Haguët<sup>l</sup>,  
7 Norbert Winer<sup>m</sup>, Damien Masson<sup>n</sup>, Caroline Elie<sup>c</sup>, Alexandra Benachi<sup>e,o</sup>

8

9 **Affiliations:**

10 <sup>a</sup>. Department of Physiology, Georges Pompidou European Hospital, AP-HP, Université Paris  
11 Descartes, INSERM U1151-CNRS UMR8253, Paris, France

12 <sup>b</sup>. Department of Physiology, Necker-Enfants Malades Hospital, AP-HP, Paris, France

13 <sup>c</sup>. URC/CIC Paris Descartes Necker Cochin, Necker-Enfants Malades Hospital, AP-HP, Paris,  
14 France

15 <sup>d</sup>. Department of Biochemistry, Antoine-Béclère Hospital, AP-HP, Université Paris-Sud,  
16 Clamart, France

17 <sup>e</sup>. Fondation PremUp, Paris, France

18 <sup>f</sup>. Department of Obstetrics, Cochin Hospital, AP-HP, Université René Descartes, Paris,  
19 France

20 <sup>g</sup>. Department of Hormonal Biochemistry, Cochin Hospital, AP-HP, Université René  
21 Descartes, Paris, France

22 <sup>h</sup>. Department of Obstetrics and Gynecology, Bicêtre Hospital, AP-HP, Université Paris-Sud,  
23 Kremlin Bicêtre, France

24 <sup>i</sup>. Department of Molecular Genetics, Pharmacogenetics and Hormonology, Bicêtre Hospital,  
25 AP-HP, Université Paris-Sud, Kremlin Bicêtre, France

26 <sup>j</sup>. Department of Obstetrics and Gynecology, University Hospital Brugmann, Université Libre  
27 de Bruxelles, Brussels, Belgium

28 <sup>k</sup>. Fetal Medecine Department, Armand Trousseau Hospital, UPMC-Sorbonne Université,  
29 Paris, France

30 <sup>l</sup>. Department of Biochemistry, Armand Trousseau Hospital, UPMC-Sorbonne Université,  
31 Paris, France

32 <sup>m</sup>. Department of Obstetrics and Gynecology, Nantes University Hospital, 44000, France

33 <sup>n</sup>. Department of Biochemistry, Nantes University Hospital Hôtel-Dieu, Nantes 44000, France

34 <sup>o</sup>. Department of Obstetrics and Gynecology, Antoine-Béclère Hospital, Assistance Publique-  
35 Hôpitaux de Paris (AP-HP), Université Paris-Sud, Clamart, France

36

37 **\*Corresponding author:**

38 Marie Courbebaisse, MD, PhD

39 Department of Physiology, Unit of renal functional explorations

40 Georges Pompidou European Hospital

41 20 rue Leblanc, 75015, Paris, France

42 Tel: +33 1 56 09 39 73

43 Fax: +33 1 56 09 26 75

44 Email: [marie.courbebaisse@aphp.fr](mailto:marie.courbebaisse@aphp.fr)

45

46

47

48

49

50

51

52

53

54

55

56

57 **Abstract:**

58 **Background & Aims:** Vitamin D status during pregnancy and in newborns has never been  
59 studied in France. This study aims at determining the vitamin D status during the first and  
60 third trimesters of pregnancy (T1, T3) and in cord blood (CB) in the middle-north of France.

61 **Methods:** We conducted a prospective cohort study in five French centers (latitude 47.22 to  
62 48.86°N). Serum 25(OH)-vitamin D (25(OH)D) concentrations were measured using a  
63 radioimmunoassay during T1, T3 and in CB. According to the French guidelines, pregnant  
64 women received cholecalciferol, 100,000 IU, in the seventh month.

65 **Results:** Between April 2012 and July 2014, 2832 women were included, of whom 2803 were  
66 analyzed (mean±SD age: 31.5±5.0 years; phototypes 5-6: 21.8%). Three and 88.6% of  
67 participants received supplementation during the month before inclusion and in the seventh  
68 month, respectively. At T1, T3, and CB, mean 25(OH)D concentrations were 21.9±10.4,  
69 31.8±11.5, and 17.0±7.2 ng/mL, respectively, and 25(OH)D was <20 ng/mL in 46.5%, 14.0%,  
70 and 68.5%, respectively. At T1, body mass index  $\geq 25$  kg/m<sup>2</sup>, dark phototypes, sampling  
71 outside summer, and no supplementation before inclusion were independently associated with  
72 vitamin D insufficiency (25(OH)D<20ng/mL). Women who received cholecalciferol  
73 supplementation in month 7 had higher 25(OH)D at T3 than non-supplemented women  
74 (32.5±11.4 *versus* 25.8±11.4 ng/mL, p=<0.001) and marginally higher 25(OH)D in CB  
75 (17.2±7.2 *versus* 15.5±7.1 ng/mL, p=0.004).

76 **Conclusions:** Despite the recommended supplementation, vitamin D insufficiency is frequent  
77 during pregnancy and in newborns in France.

78

79 **Keywords:** Vitamin D; Pregnancy; Newborns; Epidemiology; Supplementation

80

81

82 **INTRODUCTION**

83 Vitamin D is a prohormone with effects beyond the prevention of rickets/osteomalacia. In  
84 addition to its protective effect against bone demineralization, vitamin D sufficiency is  
85 associated with a reduced risk of many chronic diseases including type 2 diabetes mellitus,  
86 cardiovascular diseases, cancers, and auto-immune and infectious diseases (1). During  
87 pregnancy, poor vitamin D status is associated with pregnancy complications such as pre-  
88 eclampsia (2,3), gestational diabetes mellitus (4), and increased risk of caesarean section (5)  
89 and of preterm birth (6,7). It is also associated with an increased risk of wheezing and asthma  
90 (8,9), of respiratory tract infections (10) and of low bone mass (11,12) in newborns and  
91 children.

92 The assessment of vitamin D status is based on the measurement of the serum concentration  
93 of 25(OH)-vitamin D (25(OH)D). During pregnancy, free 25(OH)D might reflect better the  
94 vitamin D status than total 25(OH)D due to the rise of vitamin D protein levels (13). Although  
95 there is a consensus to define vitamin D deficiency as serum 25(OH)D below 10 ng/mL (25  
96 nmol/L), the definition of vitamin D insufficiency is less consensual. Whereas the US Institute  
97 of Medicine (IOM) defines vitamin D insufficiency as serum 25(OH)D concentrations below  
98 20 ng/mL (50 nmol/L) in the general population (14), the Endocrine Society considers  
99 25(OH)D levels below 30 ng/mL (75 nmol/L) to be inadequate in chronically ill patients (15).

100 With low contemporary sun exposure and/or use of sunscreens, the relative contribution of the  
101 solar source to total basal input of vitamin D seems to be at best of 25%, the remaining  
102 coming from food sources (16). Regardless of the threshold for vitamin D insufficiency or  
103 inadequacy, prevalence of low serum 25(OH)D concentrations is high in most countries,  
104 including France, in all age groups (17).

105 Vitamin D status during pregnancy or in cord blood has been evaluated in studies conducted  
106 in North America (18,19) and in many European countries, mainly northern Europe, although

107 few studies have measured 25(OH)D throughout the pregnancy or in cord blood in large  
108 cohorts (20–22). Considering i) the high prevalence of vitamin D deficiency or insufficiency  
109 during pregnancy reported in most of these studies, ii) the potential deleterious consequences  
110 of low 25(OH)D circulating levels on health of both mother and child, and iii) the absence of  
111 uniform guidelines for vitamin D supplementation in pregnant women, there is an urgent need  
112 to assess vitamin D status in large populations of pregnant women and newborns and, for each  
113 country, to systematically evaluate recommendations for vitamin D supplementation during  
114 pregnancy. To our best knowledge, such a study has never been conducted in France. That's  
115 why we felt important to gather national data given the particularity of the French  
116 recommendation for vitamin D supplementation during pregnancy (23) and the lack of food  
117 fortification in France.

118 The aims of the present study were to determine the vitamin D status and its evolution in a  
119 large cohort of pregnant women living in France by analysis in the first and third trimesters  
120 and in cord blood. We sought to assess the determinants of vitamin D status at each time point  
121 and to study the impact of the French recommendations regarding vitamin D supplementation  
122 during pregnancy. To answer these questions, we used the prospective observational FEPED  
123 cohort study including pregnant women first seen during the first trimester in five centers of  
124 the middle-north of France.

125

## 126 **PATIENTS AND METHODS**

### 127 *Study protocol*

128 The FEPED study was initially designed to investigate the association of vitamin D status  
129 during pregnancy with pre-eclampsia in six centers (five French and one Belgian). Written  
130 informed consent was obtained from each patient before inclusion in the study. The protocol  
131 was conducted in accordance with the Declaration of Helsinki and was approved by a local

132 independent Ethics Committee (2011/13NICB). It is registered with the ClinicalTrials.gov  
133 (identifier NCT01648842). Samples were stored in the Perinat Collection (ANR-10-EQPX-  
134 0010).

135

136 For the purpose of the present epidemiological study, which aimed to determine the 25(OH)D  
137 status of pregnant women living in France and in their newborns (in cord blood), we did not  
138 include women recruited in Bruxelles (Belgium). The patients included in this study were  
139 recruited between April 2012 and July 2014 in five French maternity departments ensuring  
140 the obstetrical follow-up from the first trimester of pregnancy until delivery. Four of these  
141 departments are located in Paris area (Béclère, Bicêtre, Cochin, and Trousseau University  
142 hospitals, latitude 48.86°N) and one is located in Nantes in the mid-western part of France  
143 (latitude 47.22°N). Patients were told about the study by the obstetrician or the midwife  
144 during the first consultation for pregnancy follow-up if the following inclusion and exclusion  
145 criteria were fulfilled. Inclusion criteria were: age  $\geq 18$  years, single pregnancy, gestational  
146 age from 10 to  $< 15$  weeks of amenorrhea (WA), corresponding to 8 to  $< 13$  gestational weeks  
147 (GW), at inclusion, and healthcare coverage. Exclusion criteria were conditions for which  
148 vitamin D level could have been modified or vitamin D supplementation during the third  
149 trimester could have been contra-indicated or inefficient, including serum calcium levels  
150  $> 2.65$  mmol/L or other known pathologies of mineral metabolism, constitutive bone disease,  
151 history of urinary stones, lithium treatment, or intestinal malabsorption, and conditions  
152 susceptible to interfere with the diagnosis of pre-eclampsia, including uncontrolled  
153 hypertension ( $> 140/90$  mm Hg from the beginning of pregnancy) and renal insufficiency  
154 (serum creatinine  $> 120$   $\mu\text{mol/L}$ ). For all included patients, a blood sample was collected for  
155 25(OH)D measurement between 11 and 14 WA, or between 9 and 12 GW, (the first  
156 trimester, T1, sample), during the third trimester (between 28 WA, or 26 GW, and delivery,

157 the T3 sample) and from cord blood (CB) within the framework of the research protocol. The  
158 patients were not required to fast before blood sample collection. Vitamin D (100,000 IU of  
159 cholecalciferol) was prescribed to all women in the seventh month of pregnancy as a routine  
160 procedure in agreement with the French guidelines (23). At obstetrics clinic from 28 GW the  
161 patient was asked whether and when she took the vitamin D supplementation and the date was  
162 recorded in the patient file. Follow-up outcomes were recorded such as outcome at birth (live  
163 birth, per partum demise, termination of pregnancy), gestational age at delivery, birth weight,  
164 vitamin D status in the first, third trimester and cord blood.

165 We defined vitamin D deficiency, insufficiency, inadequacy, and sufficiency as serum  
166 25(OH)D concentrations of <10 ng/mL, <20 ng/mL, <30 ng/mL, and  $\geq 30$  ng/mL,  
167 respectively. The phototype of each subject was determined according to the Fitzpatrick skin  
168 type classification (24). Pre-pregnancy body mass index (BMI) calculated from height and  
169 pre-pregnancy weight was classified using the WHO cut-off for overweight (<25 or  $\geq 25$   
170 kg/m<sup>2</sup>) (25).

### 171 ***Biological analysis***

172 All blood samples were centrifuged and stored locally at -20°C and were subsequently  
173 transferred monthly to the Department of Physiology of Necker University Hospital (Paris,  
174 France) for centralized 25(OH)D measurement from serum. 25(OH)D was measured with the  
175 DiaSorin radioimmunoassay (RIA). The Necker Hospital Physiology Laboratory participates  
176 in the DEQAS proficiency control with excellent results. A value of 4 ng/mL, corresponding  
177 to the limit of quantification that we determined in our laboratory, was assigned to any  
178 undetectable concentration.

### 179 ***Statistical analyses***

180 All statistical analyses were undertaken using R 2.11.1 software. Statistical tests were two-  
181 sided and p values less than 0.05 were considered statistically significant. Baseline



182 characteristics of women were described as means  $\pm$  standard deviations for quantitative  
183 variables and frequencies (%) for qualitative variables.

184 Prevalence of vitamin D insufficiency and inadequacy (serum 25(OH)D concentrations  $<20$   
185 ng/ml and  $<30$  ng/ml respectively) were estimated on the available samples at each time (T1,  
186 T3, and CB) along with their corresponding 95% Wald CI.

187 Associations between characteristics of women and 25(OH)D insufficiency were investigated  
188 using chi-squared test (or Fisher's test when it was appropriate) for qualitative factors and  
189 Student's t test for quantitative parameters. Pearson's r and p value of its test were computed  
190 to examine correlations between continuous variables. Multiple logistic regression models  
191 were performed to assess determinants of 25(OH)D insufficiency at T1 and T3 and in CB.  
192 Initial models included all significant factors identified by univariate analysis ( $p < 0.05$ ). A  
193 backward selection procedure based on likelihood ratio tests was used. Odds ratios, 95% CI,  
194 and p values of determinants in the final models were computed.

195

196

## 197 **RESULTS**

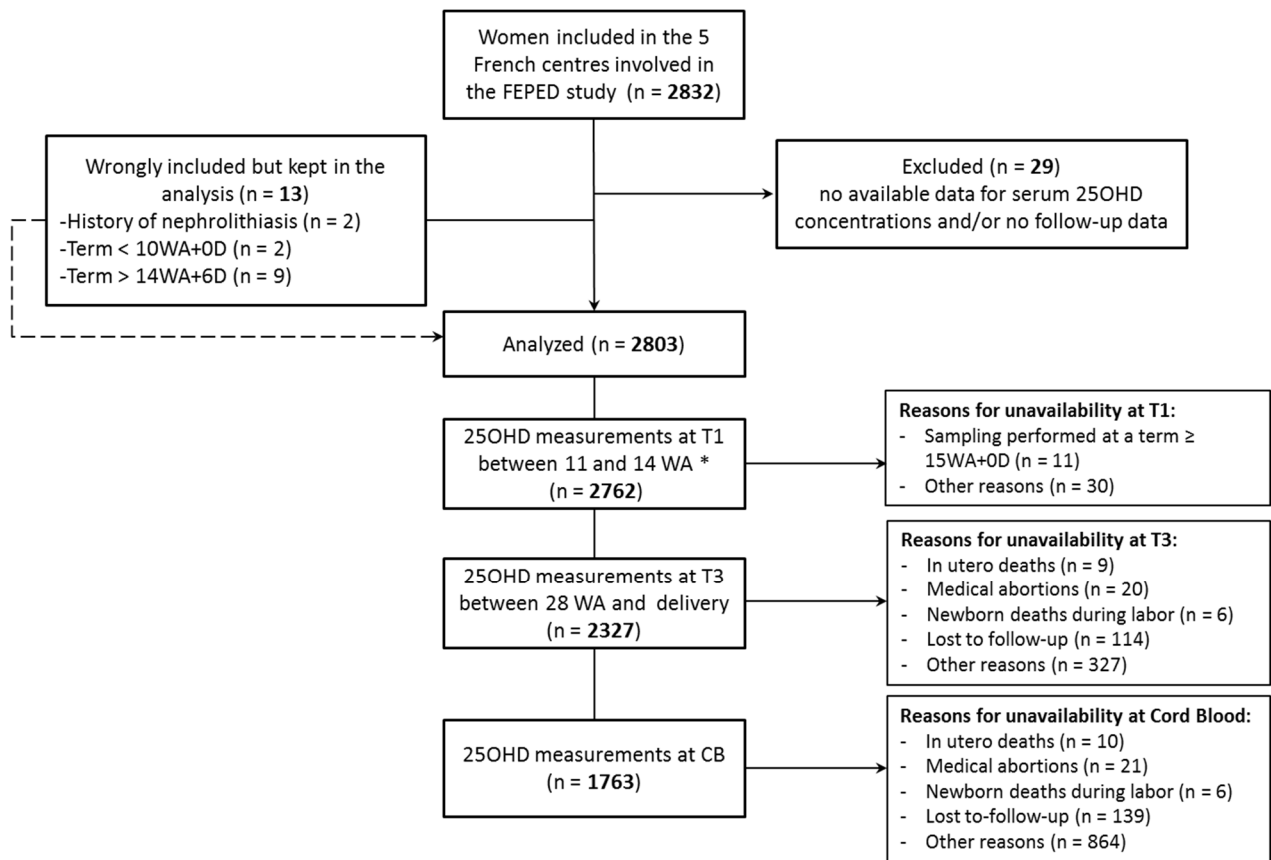
### 198 *Description of the study and of the study population*

199 The flow chart of the study protocol is shown in Figure 1. None of the patients were included  
200 twice during the study. Among the 2658 women with available data regarding pregnancy  
201 outcomes, pregnancies were terminated as follows: 2621 (98.6%) live births, six (0.2%)  
202 newborn deaths during labor, 10 (0.4%) in utero deaths, and 21 (0.8%) medical abortions. The  
203 mean delivery term was  $37.5 \pm 1.8$  GW.

204

205 **Figure 1:** Flow chart of the study protocol. 25(OH)D: 25(OH)-vitamin D, D: day, T1: first  
206 trimester, T3: third trimester, CB: cord blood, GW: gestational weeks. \*25(OH)D

207 measurements performed for women at a term  $\geq 13$  GW+0D at the time of T1 sampling were  
 208 excluded from the analysis.



209  
 210  
 211 Characteristics of the cohort are shown in Table 1. Among the 2779 women with available  
 212 data regarding ethnical origin, 1550 (55.8%) patients originated from Continental France, 63  
 213 (2.3%) from northern Europe, 122 (4.4%) from southern Europe, 420 (15.1%) from northern  
 214 Africa, 292 (10.5%) from Sub-Saharan Africa, 116 (4.2%) from French West Indies, 105  
 215 (3.8%) from Asia, and 111 (4%) from other countries. Of note, most women received vitamin  
 216 D after inclusion in agreement with the French national guidelines.

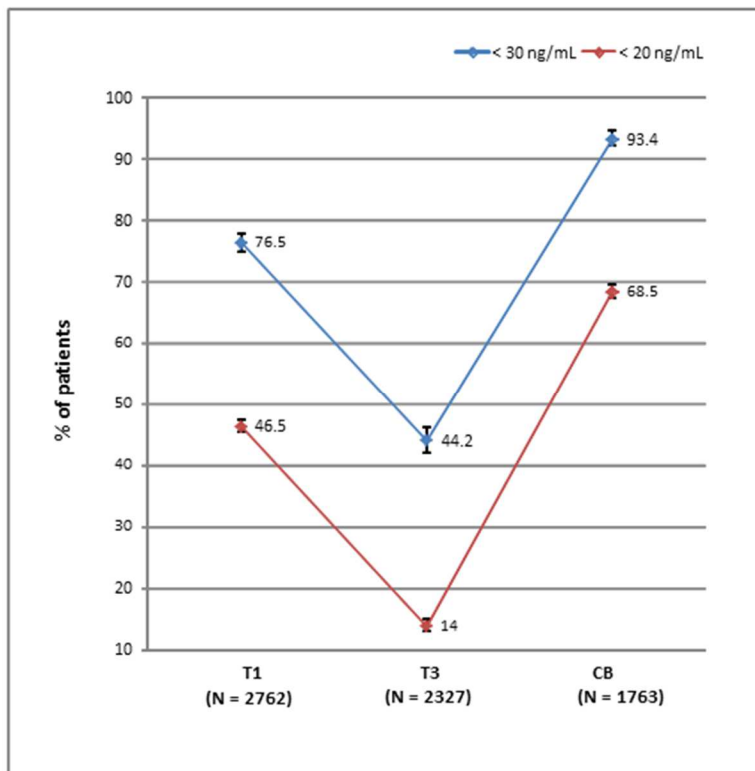
217  
 218 ***Evolution of vitamin D status during pregnancy and in cord blood***

219 Mean serum 25(OH)D concentrations for all women with available samples at each time point  
 220 are presented in Table 2. Vitamin D deficiency (25(OH)D <10 ng/mL) was present in more

221 than 10% of cases during the first trimester and a cord blood but was nearly absent during the  
222 third trimester. As shown in Figure 2, around half of the women had vitamin D insufficiency  
223 (25(OH)D <20 ng/mL) during the first trimester but only 14% had vitamin D insufficiency  
224 during the third trimester. Vitamin D inadequacy (25(OH)D <30 ng/mL) was found in three-  
225 quarters of women during the first trimester and was present in nearly half of women during  
226 the third trimester. Of note, vitamin D insufficiency or inadequacy was highly prevalent in  
227 newborns based on our cord blood analyses.

228

229 **Figure 2.** Prevalence of 25(OH)-vitamin D (25(OH)D) insufficiency (serum 25(OH) D <20  
230 ng/mL, red line) and inadequacy (serum 25(OH)D <30 ng/mL, blue line) at the first and third  
231 trimesters (T1 and T3) and in cord blood (CB) (with 95% confidence interval). N= number of  
232 patients for whom serum 25(OH)D measurements were performed at T1, T2 and T3.



233

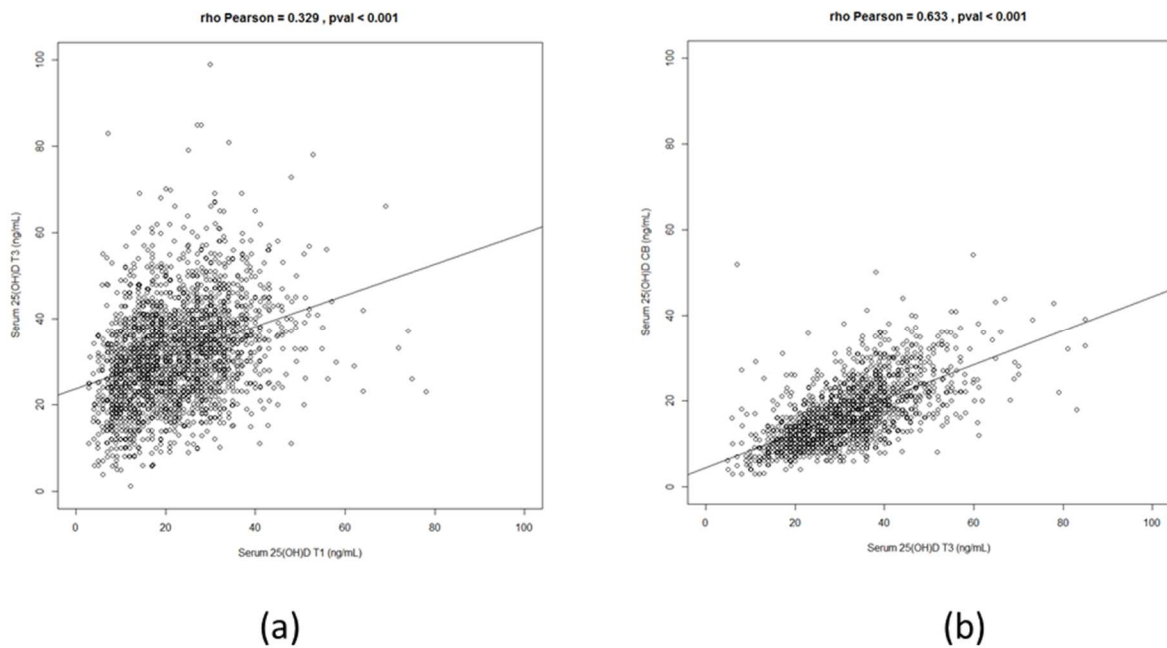
234

235 Serum 25(OH)D significantly increased between the first and the third trimesters among the  
236 2289 women with serum 25(OH)D measurements available at these two visits ( $22.2 \pm 10.5$   
237  $\text{ng/mL}$  versus  $31.8 \pm 11.5 \text{ ng/mL}$ , respectively, with a mean difference of  $9.5 \pm 12.8 \text{ ng/mL}$ ,  
238  $p < 0.001$ ). Serum 25(OH)D significantly decreased between the third trimester and cord blood  
239 among the 1606 women with serum 25(OH)D measurements available at these two visits  
240 ( $31.7 \pm 11.5 \text{ ng/mL}$  versus  $17.1 \pm 7.3 \text{ ng/mL}$  respectively, with a mean difference of  $-14.7 \pm 8.9$   
241  $\text{ng/mL}$ ,  $p < 0.001$ ). Figure 3a shows that serum 25(OH)D during the third trimester positively  
242 correlates with serum 25(OH)D during the first trimester. Figure 3b shows that the positive  
243 correlation between serum 25(OH)D during the third trimester and in cord blood is even  
244 stronger.

245

246 **Figure 3. a:** Correlation between serum 25(OH)-vitamin D (25(OH)D) at the first trimester  
247 (T1) and at the third trimesters (T3) for the 2289 women with serum 25(OH)D measurements  
248 at T1 and at T3. **b:** Correlation between serum 25(OH)D at T3 and in cord blood (CB) for the  
249 1606 women with serum 25(OH)D measurements at T3 and at CB.

250



251  
252

253  
254

255 ***Determinants of vitamin D status during pregnancy and in newborns***

256 Univariate analysis of determinants of vitamin D insufficiency (25(OH)D <20 ng/mL) during  
257 pregnancy and in cord blood is summarized in Table 3. Table 4 shows 25(OH)D  
258 concentrations and categories among women who received or did not receive the  
259 recommended supplementation during the seventh month of pregnancy. Vitamin D  
260 supplementation during the seventh month did not significantly influence the presence of  
261 vitamin D insufficiency in cord blood (Table 3). However, serum 25(OH)D was slightly but  
262 significantly higher in cord blood in case of vitamin D supplementation during the seventh  
263 month of pregnancy (Table 4). Moreover, there was less vitamin D deficiency (25(OH)D <10  
264 ng/mL) in cord blood in supplemented women (Table 4)).

265 Supplementation had a significant impact on serum 25(OH)D concentration during the third  
266 trimester since there was virtually no vitamin D deficiency during the third trimester in  
267 supplemented women (Table 4). Of note, 11.6% and 41.8% of the supplemented women had  
268 vitamin D below 20 or 30 ng/mL, respectively, during the third trimester.

269 Weight gain during pregnancy was not associated with vitamin D insufficiency during the  
270 third trimester (+8.7±5.0 kg in the 308 women with serum 25(OH)D <20 ng/mL *versus*  
271 +8.5±4.3 kg in the 1908 women with serum 25(OH)D ≥ 20 ng/mL, p= 0.54).

272 Table 5 shows variables independently associated with serum 25(OH)D concentration <20  
273 ng/mL during pregnancy and in cord blood. In multivariate analysis, overweight before  
274 pregnancy, dark phototype, sampling during fall, winter, or spring, and absence of vitamin D  
275 supplementation at the very beginning of pregnancy were independently associated with  
276 vitamin D insufficiency in the first trimester. In the third trimester, age below 35 years, parity  
277 ≥1, dark phototype, sampling during fall, winter, or spring, absence of vitamin D  
278 supplementation during pregnancy, and presence of vitamin D insufficiency or deficiency in

279 the first trimester were independently associated with vitamin D insufficiency. Vitamin D  
280 insufficiency in newborns was independently associated with overweight before pregnancy,  
281 sampling during fall, winter, or spring, and presence of vitamin D insufficiency or deficiency  
282 in the third trimester.

283

## 284 **DISCUSSION**

285 We report the first large scale population cohort study describing serum 25(OH)D status  
286 during pregnancy and in cord blood in French women. Given the disparities in results from  
287 vitamin D studies conducted during pregnancy world-wide, we will compare our results to  
288 those from other European studies.

289 As highlighted in table 6, during early or mid-pregnancy in countries of northern Europe,  
290 serum 25(OH)D levels are relatively low with a mean or median value around 20 to 22  
291 ng/mL, as in our study (20,21,26–28). In the two studies with serum 25(OH)D values above  
292 30 ng/mL despite the high latitudes (Finland and northwest of England) (29,30), a large  
293 proportion of women received vitamin D supplementation (95% in the Finland study and 73%  
294 in England study). Moreover, there are vitamin D food fortification policies in Finland (31);  
295 thus, in the Finnish study the mean vitamin D intake was 15.7 µg/day (29). Mean vitamin D  
296 intake for the French general population is 2.3 µg/day (32), a value that can be extrapolated to  
297 French pregnant women before the third trimester in the absence of vitamin D  
298 supplementation and of food fortification policies in France. Low vitamin D levels are also  
299 frequently found in pregnant women in countries from southern Europe despite theoretically  
300 higher and more efficient UVB radiation (33). The close values between northern and  
301 southern European countries may be explained by high consumption of fatty fish in northern  
302 European countries and by vitamin D food fortification policies in some countries (31,33), but  
303 also by less voluntary sun exposure and by darker phototype in southern Europe.

304 We observed a clear increase in serum 25(OH)D concentrations between the first and third  
305 trimester. Our results must be interpreted taking into account the vitamin D supplementation  
306 currently recommended in France (100,000 IU of cholecalciferol during the seventh month)  
307 (23). Consequently, 25(OH)D levels were overall higher in our study during the third  
308 trimester than levels reported in most European studies (table 6) (26,27,33,34).

309 We observed a dramatic decrease in 25(OH)D levels between the third trimester and sampling  
310 of cord blood. As observed in previous studies, 25(OH)D levels were approximately two-fold  
311 lower in cord blood than in mother's serum during pregnancy (20–22,30,35). In our study, as  
312 in these previous studies, sampling in the mother was performed several weeks before  
313 delivery. By contrast, in two studies with maternal sampling performed at delivery, exactly at  
314 the same time as cord blood sampling, 25(OH)D concentrations in mothers were similar to the  
315 ones found in newborns (34,36). This finding suggests that the decrease in serum 25(OH)D  
316 observed between the second or third trimesters and cord blood may be due to a rapid  
317 decrease in serum 25(OH)D concentrations between sampling and delivery. Possible  
318 explanations for this rapid decrease in 25(OH)D levels during the last 4 weeks of pregnancy  
319 (mean delay between the third trimester and cord blood samplings in our study) in the absence  
320 of further supplementation could be reduced outdoor activity and sun exposure combined with  
321 an increase in fat mass at the end of pregnancy. Another explanation could be the rapid  
322 decrease in serum 25(OH)D concentrations after the single administration of 100,000 IU of  
323 vitamin D<sub>3</sub>, as recently described (37). Another hypothesis to explain the discrepancy between  
324 25(OH)D levels in mothers during the second or third trimesters and in newborns could be  
325 that 3-epi-25(OH)D<sub>3</sub>, an isoform not detected by the current immunoassays, may be present at  
326 high concentrations in cord blood. However, this theory was ruled out by a study showing that  
327 the proportion of 25(OH)D as 3-epi-25(OH)D<sub>3</sub> was only 11.2% in cord blood (22) and by  
328 another study reporting similar levels of 3-epi-25(OH)D<sub>3</sub> in mothers at delivery and in

329 newborns (36). We would like to emphasize that our study demonstrates that this high dose  
330 of cholecalciferol given once, 100 000 IU theoretically corresponding to 1100 IU daily during  
331 3 months (23), is clearly insufficient to obtain serum 25(OH)D levels above 20 ng/mL in most  
332 newborns and is also insufficient to completely prevent vitamin D deficiency.

333 Finally, we analyzed the determinants of vitamin D insufficiency (25(OH)D <20 ng/mL) at  
334 each time point. Most studies found, as we did, that season of sampling was strongly  
335 associated with 25(OH)D status during pregnancy, with maximal concentrations reached  
336 during summer (20,26–28,30,33,38). As in our study, dark phototype was also reported to be  
337 independently associated with maternal vitamin D status (26,27,30,33,39). As in other  
338 European studies (20,26–28,30,33), we found that vitamin D supplementation during  
339 pregnancy was a strong independent determinant of vitamin D status. Whereas some studies  
340 found no association between BMI and vitamin D status in pregnant women (27,34), others  
341 found, like us, a negative association (40,41) .

342 Few European studies have described the determinants of vitamin status in cord blood. A  
343 study from Scotland (20) and a study from Ireland (22), found, like us, that seasonal variation  
344 and maternal 25(OH)D status were independently associated with 25(OH)D levels in cord  
345 blood. Whereas the study from Scotland (20) found, as we did, that vitamin D  
346 supplementation during pregnancy did not influence vitamin D insufficiency in cord blood,  
347 two studies (22,29), reported a positive association between antenatal vitamin D supplements  
348 and vitamin D concentrations in cord blood. Finally, unlike us, others did not find that  
349 maternal BMI was an independent determinant of 25(OH)D levels in cord blood (20,22,29).

350 We must acknowledge that our study has some limitations. First, although the DiaSorin RIA  
351 used to measure 25(OH)D concentration in our study is a “historic” 25(OH)D assay that has  
352 been used in many studies, it does not allow a strict comparison with the previously published  
353 data on vitamin D status in pregnant women due to a certain degree of inter-method



354 variability. Such a comparison is, however, important to develop evidence-based international  
355 guidelines for vitamin D supplementation during pregnancy. A way to achieve this goal  
356 would have been to collaborate with a laboratory that uses a CDC-certified chromatography  
357 tandem-mass spectrometry (LC-MS/MS) method in order to apply the VDSP protocols for  
358 standardizing existing 25(OH)D data from national surveys around the world (42). However,  
359 when the present study was designed (in 2009), the new international and the standard LC-  
360 MS/MS reference method for measuring 25(OH)D were not published so such a collaboration  
361 was not possible. Moreover, we did not assess the concentration of the biologically active free  
362 25(OH)D. Of note, Patients were recruited only in centers from the middle-north of France, so  
363 we cannot extrapolate our results to the whole French territory. Finally, some data susceptible  
364 to modify serum 25(OH)D concentration including dietary habits, sun exposure, use of  
365 sunscreen and outdoor activity were not recorded in the present study.

366 Our study also has several strengths. To our best knowledge, it is the first study to evaluate  
367 the vitamin D status of a French cohort during pregnancy and in cord blood and this study is  
368 the largest European study regarding this issue. We also evaluated the effects of the  
369 supplementation recommended by current French guidelines (23), which is of high  
370 importance to improve the care of pregnant women and newborns.

371 In conclusion, vitamin D insufficiency is highly prevalent at the beginning of pregnancy and  
372 in cord blood in the middle-north of France. The supplementation with cholecalciferol  
373 100,000 IU during the seventh month of pregnancy is insufficient to prevent vitamin D  
374 insufficiency and deficiency in newborns and should therefore be reevaluated.

375 **Acknowledgments:**

376 We would like to thank S. Albert, V. Buth, V. da Costa, S. Larrède, E. Etienne, L. Peaudecerf,  
377 and I. Rieger for monitoring the data, and A. Bellino and M. Delattre for coordinating the  
378 study. We are grateful to the participating doctors, midwives, and nurses for patient

379 management. We would also like to thank the “Centre de Ressources Biologiques”, Centre  
380 Hospitalier Intercommunal de Créteil, for the management and storage of biospecimens. We  
381 also thank the patients for their help in making this trial a success.

382

383 **Statement of Authorship:**

384 MC interpreted the data and wrote the manuscript.

385 ABa and CE analyzed and interpreted the data.

386 ABe, CE and JCS conceived and designed the study.

387 JCS performed 25(OH)D measurements.

388 JT, VT, JG, MVS, HH, JJ, MG, JMJ, MCH, NW, and DM included patients.

389 All the authors contributed substantially to the acquisition of data and to drafting the article or  
390 revising it critically for important intellectual content and to final approval of the version to be  
391 published and agree to be accountable for all aspects of the work in ensuring that questions  
392 related to the accuracy or integrity of any part of the work are appropriately investigated and  
393 resolved.

394

395 **Conflict of Interest Statement:**

396 JC. Souberbielle reports lecture fees and/or travel/hotel expenses from DiaSorin, Roche  
397 Diagnostics, Abbott, Amgen, Shire, MSD, Lilly, and Rottapharm/Meda. The other authors  
398 declare no conflicts of interest.

399

400 **Funding sources:**

401 The study was sponsored by the Assistance Publique-Hôpitaux de Paris (AP-HP) and was  
402 funded by a grant from the Programme Hospitalier de Recherche Publique – PHRC national  
403 2010 (Ministry of Health – AOM10113).

404

405

406

407

408

409 **References**

410

411 1. Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskeletal health and the  
412 need for supplementation. *Nutrients*. 10 janv 2013;5(1):111-48.

413 2. Bodnar LM, Simhan HN, Catov JM, Roberts JM, Platt RW, Diesel JC, et al. Maternal  
414 Vitamin D Status and the Risk of Mild and Severe Preeclampsia: *Epidemiology*. mars  
415 2014;25(2):207-14.

416 3. Mirzakhani H, Litonjua AA, McElrath TF, O'Connor G, Lee-Parritz A, Iverson R, et  
417 al. Early pregnancy vitamin D status and risk of preeclampsia. *J Clin Invest*. 14 nov  
418 2016;126(12):4702-15.

419 4. Lu M, Xu Y, Lv L, Zhang M. Association between vitamin D status and the risk of  
420 gestational diabetes mellitus: a meta-analysis. *Arch Gynecol Obstet*. mai 2016;293(5):959-66.

421 5. Scholl TO, Chen X, Stein P. Maternal vitamin D status and delivery by cesarean.  
422 *Nutrients*. 2012;4(4):319-30.

423 6. Miliku K, Vinkhuyzen A, Blanken LM, McGrath JJ, Eyles DW, Burne TH, et al.  
424 Maternal vitamin D concentrations during pregnancy, fetal growth patterns, and risks of  
425 adverse birth outcomes. *Am J Clin Nutr*. 1 juin 2016;103(6):1514-22.

- 426 7. Amegah AK, Klevor MK, Wagner CL. Maternal vitamin D insufficiency and risk of  
427 adverse pregnancy and birth outcomes: A systematic review and meta-analysis of longitudinal  
428 studies. Nguyen TV, éditeur. PLOS ONE. 17 mars 2017;12(3):e0173605.
- 429 8. Christesen HT, Elvander C, Lamont RF, JøRrgensen JS. The impact of vitamin D in  
430 pregnancy on extraskeletal health in children: a systematic review: Impact of vitamin D in  
431 pregnancy on children. Acta Obstet Gynecol Scand. déc 2012;91(12):1368-80.
- 432 9. Camargo CA, Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, et al. Cord-  
433 blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma.  
434 Pediatrics. janv 2011;127(1):e180-187.
- 435 10. Fried DA, Rhyu J, Odato K, Blunt H, Karagas MR, Gilbert-Diamond D. Maternal and  
436 cord blood vitamin D status and childhood infection and allergic disease: a systematic review.  
437 Nutr Rev. 2016;74(6):387-410.
- 438 11. Dror DK, King JC, Fung EB, Van Loan MD, Gertz ER, Allen LH. Evidence of  
439 associations between feto-maternal vitamin D status, cord parathyroid hormone and bone-  
440 specific alkaline phosphatase, and newborn whole body bone mineral content. Nutrients.  
441 2012;4(2):68-77.
- 442 12. Zhu K, Whitehouse AJ, Hart PH, Kusel M, Mountain J, Lye S, et al. Maternal Vitamin  
443 D Status During Pregnancy and Bone Mass in Offspring at 20 Years of Age: A Prospective  
444 Cohort Study: MATERNAL 25OHD IN PREGNANCY AND OFFSPRING BONE MASS  
445 AT 20 YEARS OLD. J Bone Miner Res. mai 2014;29(5):1088-95.
- 446 13. Tsuprykov O, Buse C, Skoblo R, Haq A, Hocher B. Reference intervals for measured  
447 and calculated free 25-hydroxyvitamin D in normal pregnancy. J Steroid Biochem Mol Biol.  
448 juill 2018;181:80-7.
- 449 14. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for  
450 Vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin D [Internet].  
451 Ross AC, Taylor CL, Yaktine AL, Del Valle HB, éditeurs. Washington (DC): National  
452 Academies Press (US); 2011 [cité 12 févr 2018]. (The National Academies Collection:  
453 Reports funded by National Institutes of Health). Disponible sur:  
454 <http://www.ncbi.nlm.nih.gov/books/NBK56070/>

- 455 15. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP,  
456 et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society  
457 clinical practice guideline. *J Clin Endocrinol Metab.* juill 2011;96(7):1911-30.
- 458 16. Heaney RP, Armas LAG, French C. All-source basal vitamin D inputs are greater than  
459 previously thought and cutaneous inputs are smaller. *J Nutr.* mai 2013;143(5):571-5.
- 460 17. Souberbielle J-C, Massart C, Brailly-Tabard S, Cavalier E, Chanson P. Prevalence and  
461 determinants of vitamin D deficiency in healthy French adults: the VARIETE study.  
462 *Endocrine.* août 2016;53(2):543-50.
- 463 18. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High  
464 prevalence of vitamin D insufficiency in black and white pregnant women residing in the  
465 northern United States and their neonates. *J Nutr.* févr 2007;137(2):447-52.
- 466 19. Woolcott CG, Giguère Y, Weiler HA, Spencer A, Forest J-C, Armson BA, et al.  
467 Determinants of vitamin D status in pregnant women and neonates. *Can J Public Health Rev*  
468 *Can Sante Publique.* 27 déc 2016;107(4-5):e410-6.
- 469 20. Haggarty P, Campbell DM, Knox S, Horgan GW, Hoad G, Boulton E, et al. Vitamin  
470 D in pregnancy at high latitude in Scotland. *Br J Nutr.* 14 mars 2013;109(5):898-905.
- 471 21. Kiely ME, Zhang JY, Kinsella M, Khashan AS, Kenny LC. Vitamin D status is  
472 associated with uteroplacental dysfunction indicated by pre-eclampsia and small-for-  
473 gestational-age birth in a large prospective pregnancy cohort in Ireland with low vitamin D  
474 status. *Am J Clin Nutr.* août 2016;104(2):354-61.
- 475 22. Kiely M, O'Donovan SM, Kenny LC, Hourihane JO, Irvine AD, Murray DM. Vitamin  
476 D metabolite concentrations in umbilical cord blood serum and associations with clinical  
477 characteristics in a large prospective mother-infant cohort in Ireland. *J Steroid Biochem Mol*  
478 *Biol.* 2017;167:162-8.
- 479 23. RPC [Internet]. [cité 27 févr 2018]. Disponible sur: [http://www.cngof.fr/pratiques-](http://www.cngof.fr/pratiques-cliniques/recommandations-pour-la-pratique-clinique?folder=RPC%2BCOLLEGE)  
480 [cliniques/recommandations-pour-la-pratique-clinique?folder=RPC%2BCOLLEGE](http://www.cngof.fr/pratiques-cliniques/recommandations-pour-la-pratique-clinique?folder=RPC%2BCOLLEGE)
- 481 24. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI.  
482 *Arch Dermatol.* juin 1988;124(6):869-71.

- 483 25. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert  
484 Committee. World Health Organ Tech Rep Ser. 1995;854:1-452.
- 485 26. Vandevijvere S, Amsalkhir S, Van Oyen H, Moreno-Reyes R. High prevalence of  
486 vitamin D deficiency in pregnant women: a national cross-sectional survey. PloS One.  
487 2012;7(8):e43868.
- 488 27. Bärebring L, Schoenmakers I, Glantz A, Hulthén L, Jagner Å, Ellis J, et al. Vitamin D  
489 Status during Pregnancy in a Multi-Ethnic Population-Representative Swedish Cohort.  
490 Nutrients. 22 oct 2016;8(10).
- 491 28. Bjørn Jensen C, Thorne-Lyman AL, Vadgård Hansen L, Strøm M, Odgaard Nielsen  
492 N, Cohen A, et al. Development and validation of a vitamin D status prediction model in  
493 Danish pregnant women: a study of the Danish National Birth Cohort. PloS One.  
494 2013;8(1):e53059.
- 495 29. Hauta-Alus HH, Holmlund-Suila EM, Rita HJ, Enlund-Cerullo M, Rosendahl J,  
496 Valkama SM, et al. Season, dietary factors, and physical activity modify 25-hydroxyvitamin  
497 D concentration during pregnancy. Eur J Nutr. 2 mars 2017;
- 498 30. Emmerson AJB, Dockery KE, Mughal MZ, Roberts SA, Tower CL, Berry JL. Vitamin  
499 D status of White pregnant women and infants at birth and 4 months in North West England:  
500 A cohort study. Matern Child Nutr. janv 2018;14(1).
- 501 31. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in  
502 Europe. Nutr Bull. déc 2014;39(4):322-50.
- 503 32. ENNS : étude nationale nutrition santé / Enquêtes et études / Nutrition et santé /  
504 Maladies chroniques et traumatismes / Dossiers thématiques / Accueil [Internet]. [cité 13 févr  
505 2018]. Disponible sur: [http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-  
506 chroniques-et-traumatismes/Nutrition-et-sante/Enquetes-et-etudes/ENNS-etude-nationale-  
507 nutrition-sante](http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-chroniques-et-traumatismes/Nutrition-et-sante/Enquetes-et-etudes/ENNS-etude-nationale-nutrition-sante)
- 508 33. Karras S, Paschou SA, Kandaraki E, Anagnostis P, Annweiler C, Tarlatzis BC, et al.  
509 Hypovitaminosis D in pregnancy in the Mediterranean region: a systematic review. Eur J Clin  
510 Nutr. sept 2016;70(9):979-86.
- 511 34. Wuertz C, Gilbert P, Baier W, Kunz C. Cross-sectional study of factors that influence  
512 the 25-hydroxyvitamin D status in pregnant women and in cord blood in Germany. Br J Nutr.  
513 nov 2013;110(10):1895-902.

- 514 35. Saraf R, Morton SMB, Camargo CA, Grant CC. Global summary of maternal and  
515 newborn vitamin D status - a systematic review. *Matern Child Nutr.* 2016;12(4):647-68.
- 516 36. Karras SN, Shah I, Petroczi A, Goulis DG, Bili H, Papadopoulou F, et al. An  
517 observational study reveals that neonatal vitamin D is primarily determined by maternal  
518 contributions: implications of a new assay on the roles of vitamin D forms. *Nutr J.* 7 juin  
519 2013;12:77.
- 520 37. Välimäki V-V, Löyttyniemi E, Pekkarinen T, Välimäki MJ. How well are the optimal  
521 serum 25OHD concentrations reached in high-dose intermittent vitamin D therapy? a placebo-  
522 controlled study on comparison between 100 000 IU and 200 000 IU of oral D3 every 3  
523 months in elderly women. *Clin Endocrinol (Oxf).* juin 2016;84(6):837-44.
- 524 38. Gellert S, Ströhle A, Bitterlich N, Hahn A. Higher prevalence of vitamin D deficiency  
525 in German pregnant women compared to non-pregnant women. *Arch Gynecol Obstet.* juill  
526 2017;296(1):43-51.
- 527 39. Richard A, Rohrmann S, Quack Lötscher KC. Prevalence of Vitamin D Deficiency  
528 and Its Associations with Skin Color in Pregnant Women in the First Trimester in a Sample  
529 from Switzerland. *Nutrients.* 10 mars 2017;9(3).
- 530 40. Perez-Ferre N, Torrejon MJ, Fuentes M, Fernandez MD, Ramos A, Bordiu E, et al.  
531 Association of low serum 25-hydroxyvitamin D levels in pregnancy with glucose homeostasis  
532 and obstetric and newborn outcomes. *Endocr Pract Off J Am Coll Endocrinol Am Assoc Clin*  
533 *Endocrinol.* oct 2012;18(5):676-84.
- 534 41. Fernández-Alonso AM, Dionis-Sánchez EC, Chedraui P, González-Salmerón MD,  
535 Pérez-López FR, Spanish Vitamin D and Women's Health Research Group. First-trimester  
536 maternal serum 25-hydroxyvitamin D<sub>3</sub> status and pregnancy outcome. *Int J Gynaecol Obstet*  
537 *Off Organ Int Fed Gynaecol Obstet.* janv 2012;116(1):6-9.
- 538 42. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM, Vitamin D  
539 Standardization Program (VDSP). Vitamin D status as an international issue: national surveys  
540 and the problem of standardization. *Scand J Clin Lab Investig Suppl.* 2012;243:32-40.

541

542

	<b>n</b>	<b>Mean ± SD or n (% of patients)</b>
<b>Age at inclusion (years)</b>	2803	31.5±5.0
Age ≥35 (years)		734 (26.2%)
<b>Gestational age at inclusion (GW)</b>	2803	10.8±0.8
<b>BMI before the beginning of pregnancy (kg/m<sup>2</sup>)</b>	2773	23.5±4.6
BMI ≥25 (kg/m <sup>2</sup> )		861 (31.1%)
<b>Pre gestational diabetes mellitus</b>	2778	103 (3.7%)
<b>Season of conception</b>	2803	
Summer		712 (25.4%)
Fall		692 (24.7%)
Winter		704 (25.1%)
Spring		695 (24.8%)
<b>Phototype*</b>	2803	
Types 1 to 4		2191 (78.2%)
Types 5 and 6		612 (21.8%)
<b>Parity**</b>	2781	
0		1349 (48.5%)
1		966 (34.7%)
>1		466 (16.8%)
<b>Smoking</b>		
Before the ongoing pregnancy	2760	569 (20.6%)
Active at the beginning of pregnancy	2743	317 (11.6%)
Active at inclusion	2727	225 (8.2%)
<b>Vitamin D supplementation during the month before inclusion</b>	2452	74 (3.0%)
<b>Vitamin D supplementation in 7<sup>th</sup> month***</b>	2592	2296 (88.6%)

546

547

**Table 1.** Characteristics of the population.



548 BMI: body mass index, GW: gestational weeks, n: number of patients with available data, SD:  
 549 standard deviation. \*According to the Fitzpatrick phototyping scale, \*\*excluding the ongoing  
 550 pregnancy, \*\*\* vitamin D supplementation in 7<sup>th</sup> month of pregnancy consists of 100,000 IU  
 551 cholecalciferol, according to the French guidelines.

552  
 553

		<b>1<sup>st</sup> trimester</b>	<b>3<sup>rd</sup> trimester</b>	<b>Cord blood</b>
	<b>n</b>	<b>2762</b>	<b>2327</b>	<b>1763</b>
<b>Gestational age (GW)</b>	<b>Mean ± SD [Min-Max]</b>	10.8±0.8 [6.6-12.9]	33.4±2.4 [23.9-39.9]	37.6±1.5 [25.4-40.7]
<b>Serum 25(OH)D (ng/mL)</b>	<b>Mean ± SD [Min-Max]</b>	21.9±10.4 [3.0-78.0]	31.8±11.5 [1.0-99.0]	17.0±7.2 [3.0-54.0]
<b>25(OH)D categories n (%)</b>	<b>&lt;10 ng/mL</b>	286 (10.4%)	29 (1.2%)	231 (13.1%)
	<b>10-20 ng/mL</b>	998 (36.1%)	297 (12.8%)	977 (55.4%)
	<b>20-30 ng/mL</b>	829 (30.0%)	703 (30.2%)	439 (24.9%)
	<b>≥30 ng/mL</b>	649 (23.5%)	1298 (55.8%)	116 (6.6%)

554  
 555 **Table 2.** Serum 25(OH)-vitamin D during pregnancy and at cord blood.

556 n: number of patients, SD: standard deviation, Min: minimum, Max: maximum, 25(OH)D:  
 557 25(OH)-vitamin D, GW: gestational weeks.

558

	1 <sup>st</sup> trimester			3 <sup>rd</sup> trimester			Cord blood		
	25(OH)D (ng/mL)		P value	25(OH)D (ng/mL)		P value	25(OH)D (ng/mL)		P value
	<20 n=1284	≥20 n=1478		<20 n=326	≥20 n=2001		<20 n=1208	≥20 n=555	
<b>Age (years)</b>			0.029			0.017			0.18
<35	973 (75.8)	1066 (72.1)		256 (78.5)	1445 (72.2)		913 (75.6)	403 (72.6)	
≥35	311 (24.2)	412 (27.9)		70 (21.5)	556 (27.8)		295 (24.4)	152 (27.4)	
<b>BMI (kg/m<sup>2</sup>)</b>			<0.001			0.012			<0.001
<25	827 (65.3)	1056 (72.0)		205 (63.3)	1401 (70.2)		797 (66.4)	420 (75.8)	
≥25	439 (34.7)	411 (28.0)		119 (36.7)	594 (29.8)		403 (33.6)	134 (24.2)	
<b>Phototype*</b>			<0.001			<0.001			<0.001
1 to 4	901 (70.2)	1259 (85.2)		209 (64.1)	1657 (82.8)		931 (77.1)	483 (87.0)	
5 and 6	383 (29.8)	219 (14.8)		117 (35.9)	344 (17.2)		277 (22.9)	72 (13.0)	
<b>Parity**</b>			0.026			0.002			0.062
0	587 (46.2)	741 (50.4)		135 (41.5)	1015 (50.7)		573 (47.5)	290 (52.2)	
≥1	684 (53.8)	728 (49.6)		190 (58.5)	985 (9.3)		634 (52.5)	265 (47.8)	
<b>Smoking***</b>			0.41			0.64			1
no	1112 (89.0)	1277 (87.9)		287 (89.4)	1750 (88.5)		1050 (88.2)	484 (88.2)	
yes	138 (11.0)	175 (12.1)		34 (10.6)	227 (11.5)		141 (11.8)	65 (11.8)	
<b>Season****</b>			<0.001			0.014			<0.001
Summer	160 (12.5)	455 (30.8)		59 (18.2)	529 (26.5)		245 (20.3)	207 (37.3)	
Fall	305 (23.7)	416 (28.2)		103 (31.7)	595 (29.7)		326 (27.0)	154 (27.7)	
Winter	394 (30.7)	274 (18.5)		70 (21.5)	366 (18.3)		284 (23.5)	82 (14.8)	
Spring	425 (31.1)	333 (22.5)		93 (28.6)	510 (25.5)		353 (29.2)	112 (20.2)	
<b>Vitamin D before inclusion<sup>‡</sup></b>			<0.001						
no	1101 (99.1)	1245 (95.2)		NA	NA		NA	NA	

yes	10 (0.9)	63 (4.8)		NA	NA		NA	NA	
<b>Vitamin D in 7<sup>th</sup> month<sup>W</sup></b>						<0.001			0.20
no				74 (23.8)	148 (7.6)		119 (10.2)	44 (8.2)	
yes				237 (76.2)	1802 (92.4)		1052 (89.8)	493 (91.8)	
<b>Previous 25(OH)D<sup>WY</sup></b>						<0.001			<0.001
≥30 ng/mL				28 (8.7)	530 (26.9)		436 (39.7)	450 (88.7)	
20-30 ng/mL				77 (24.1)	624 (31.7)		456 (41.5)	43 (8.5)	
10-20 ng/mL				136 (42.5)	664 (33.7)		190 (17.3)	12 (2.4)	
<10 ng/mL				79 (24.7)	151 (7.7)		17 (1.5)	2 (0.4)	

560

561 **Table 3.** Univariate analysis of the determinants of severe vitamin D insufficiency (defined as  
562 serum 25(OH)-vitamin D <20 ng/mL) during pregnancy and in cord blood.

563 BMI: body mass index, 25(OH)D: serum 25(OH)-vitamin D concentration, NA: non-  
564 applicable. \*According to the Fitzpatrick phototyping scale,\*\*excluding the ongoing  
565 pregnancy,\*\*\*active at the beginning of pregnancy, \*\*\*\*season of sampling, ¥vitamin D  
566 supplementation during the month before inclusion, ¥¥vitamin D supplementation in 7<sup>th</sup> month  
567 of pregnancy (cholecalciferol, 100,000 IU), ¥¥¥ serum 25(OH)D concentrations at the previous  
568 visit (for the third trimester, the previous visit took place in the first trimester; for cord blood,  
569 the previous visit took place in the third trimester. Results are shown as n (%).

570

571

572

573

574

575

576

		3 <sup>rd</sup> trimester			Cord blood		
	Supplementation*	No	Yes	P value	No	Yes	p value
	n	222	2039		163	1545	
<b>Serum 25(OH)D (ng/mL)</b>	<b>Mean ± SD</b>	25.8±11.1	32.5±11.4	<0.001	15.5±7.1	17.2±7.22	0.004
<b>25(OH)D categories n (%)</b>	<b>&lt;10 ng/mL</b>	12 (5.4%)	16 (0.8%)	<0.001	33 (20.2%)	191 (12.4%)	0.03
	<b>10-20 ng/mL</b>	62 (27.9%)	221 (10.8%)		86 (52.8%)	861 (55.7%)	
	<b>20-30 ng/mL</b>	68 (30.6%)	616 (30.2%)		37 (22.7%)	388 (25.1%)	
	<b>≥30 ng/mL</b>	80 (36.1%)	1186 (58.2%)		7 (4.3%)	105 (6.8%)	

577 **Table 4.** Serum 25(OH)-vitamin D at the third trimester and in cord blood for subjects who  
578 were and who were not supplemented as recommended by the French guidelines in the 7<sup>th</sup>  
579 month of pregnancy (cholecalciferol, 100,000 IU)\*.

580 n: number of subjects, SD: standard deviation, 25(OH)D: 25(OH)-vitamin D.

	1 <sup>st</sup> trimester		3 <sup>rd</sup> trimester		Cord blood	
	OR [95% CI]	p value	OR [95% CI]	p value	OR [95% CI]	p value
<b>Age (years)</b>						
<35			1.00			
≥35			0.66 [0.48-0.91]	0.01		
<b>BMI (kg/m<sup>2</sup>)</b>						
<25	1.00				1.00	
≥25	1.33 [1.10-1.61]	0.003			1.32 [1.00-1.73]	0.049
<b>Phototype*</b>						
1-4	1.00		1.00			
5-6	2.70 [2.17-3.36]	<0.001	1.80 [1.34-2.40]	<0.001		
<b>Parity**</b>						
0			1			
≥1			1.47 [1.12-1.92]	0.005		
<b>Season***</b>						
Summer	1.00		1.00		1.00	
Fall	2.03 [1.57-2.63]	<0.001	1.86 [1.27-2.72]	0.002	2.18 [1.59-2.99]	<0.001
Winter	4.43 [3.41-5.76]	<0.001	4.04 [2.62-6.23]	<0.001	3.15 [2.19-4.51]	<0.001
Spring	4.16 [3.22-5.38]	<0.001	2.24 [1.51-3.31]	<0.001	1.96 [1.39-2.75]	<0.001
<b>Vitamin D before inclusion<sup>¥</sup></b>			NA	NA	NA	NA
no	1.00		NA	NA	NA	NA
yes	0.16 [0.08-0.31]	<0.001	NA	NA	NA	NA

<b>Vitamin D in 7<sup>th</sup> month<sup>¥¥</sup></b>	NA	NA				
no			1.00			
yes			0.21 [0.15-0.29]	<0.001		
<b>Previous 25(OH)D<sup>¥¥¥</sup></b>	NA	NA				
≥30	NA	NA	1.00		1.00	
20-30	NA	NA	2.83 [1.77-4.53]	<0.001	10.59 [7.49-14.98]	<0.001
10-20	NA	NA	4.71 [2.98-7.47]	<0.001	16.26 [8.89-29.74]	<0.001
<10	NA	NA	13.84 [8.18-23.43]	<0.001	7.66 [1.73-33.90]§	0.007

581

582 **Table 5.** Multivariate analysis of the determinants of severe vitamin D insufficiency (defined  
583 as serum 25(OH)-vitamin D<20 ng/mL) during pregnancy and in cord blood.

584 BMI: body mass index, 25(OH)D: serum 25(OH)-vitamin D concentration, NA: non-  
585 applicable. \*According to the Fitzpatrick phototyping scale,\*\*excluding the ongoing  
586 pregnancy, \*\*\*season at the time of sampling, ¥vitamin D supplementation during the month  
587 before inclusion, ¥¥vitamin D supplementation in 7<sup>th</sup> month of pregnancy (cholecalciferol,  
588 100,000 IU), ¥¥¥ serum 25(OH)D concentrations at the previous visit (for the third trimester,  
589 the previous visit took place at the first trimester; for cord blood, the previous visit took place  
590 at the third trimester), §among women with 25(OH)D below 10 ng/mL at the third trimester,  
591 only two had 25(OH)D above 20 ng/mL in cord blood.

592

Country	Latitude	n	Pregnancy		Cord Blood	Method for 25(OH)D measurement
			Timing of sampling	25(OH)D concentrations (ng/mL), and by category when available (%)	25(OH)D concentrations (ng/mL), and by category when available (%)	
Finland (29)	60°N	584	T1 GW 6–13	Mean ±SD 35.5±7.6 -1% <20 ng/mL	Mean ±SD 35.3 ± 8.8 -1% <20 ng/mL	CLIA
south-western Sweden Gravid study (27)	57-58°N	1985	T1 before GW 17	Mean ±SD 25.8±9.8 - 25% <20 ng/L - 10% <12 ng/mL	NO	LC-MS/MS
		1836	T3 after GW 31	Mean ±SD 29.8±13.8		
Scotland (20)	57°N	1205 (T1 and cord blood)	T2 GW 19	Mean (95%CI) 16.0 (15.4-16.7) - 21.5% <10 ng/mL	Mean (95%CI) 8.7 (8.2-9.4) - 50 % <10 ng/mL	LC-MS/MS
Denmark (28)	54-57°N	1494	T2 GW 25	Mean ±SD 22.7±9.8 - 76.9% <.30 ng/ml - 42.3% < 20 ng/ml - 10.1% < 10 ng/ml	NO	LC-MS/MS
North West of England (30)	53°N	- Mother: 608 - CB: 345	T2/T3 26.9 GW (range 26.0-28.7)	Median (IQR) 30.6 (19.2–38.1) -27% <20 ng/mL -7% <10 ng/mL	Median (IQR) - 15.4 (9.8–22.4) - 65% <20 ng/mL - 26% <10 ng/mL	LC-MS/MS
Ireland SCOPE study (21)	52°N	1768	T2 GW 15 (range, 14-16)	Mean ±SD 22.7±10.4 - 75% <30 ng/mL - 44% <20 ng/mL - 11% <10 ng/mL	NO	LC-MS/MS
Ireland SCOPE study (22)	52°N	1050			Mean ±SD 14.0±7.2  - 80% <20 ng/mL - 35% (50% during winter) <10 ng/mL	LC-MS/MS

Belgium (26)	49- 51°N	640	T1	Median 20.4	NO	RIA
		666	T3	Median 22.7		
		1311	T1+ T3 (+5 patients at T2)	- 74.1% <30 ng/mL - 44.6% <20 ng/mL - 12.1% <10 ng/mL		
Germany (34)	47- 54°N	- Mother: 261 - CB: 328	delivery or within 72 h post-partum	Median (IQR) 10.0 (5.0–18.2) -77% <20 ng/mL	Median (IQR) 13.6 (7.1–23.4) - 69% <20 ng/mL	CLIA
Germany (38)	47- 54°N	429	between the 2 <sup>nd</sup> and 41 <sup>st</sup> GW (mean±SD: 23.8 ±11.5)	Mean ±SD 14.2± 8	NO	CLIA
Switzerland (39)	47°N	204	7 GW	63% <20 ng/mL	NO	CLIA
		n=75 Vit ≥20 ng/ml	7 GW	Mean (95%CI) 26.1 (24.8–27.4)		
		n=129 Vit <20 ng/ml	7 GW	Mean (95%CI) 10.5 (9.7–11.5)		

593

594 **Table 6:** Vitamin D status during pregnancy and in cord blood reported in studies from  
595 northern Europe (countries with latitudes equal or higher than the one reported in the present  
596 study: 47-49°N). Studies are presented according to the latitude of the country (higher to  
597 lower latitudes). Results from the countries of southern Europe are not mentioned in this table  
598 since it was the purpose of a review by Karras et al published in 2016 (33).

599 CLIA: chemiluminescence immunoassay, LC-MS/MS: chromatography tandem-mass  
600 spectrometry, RIA: Radioimmunoassay, GW: gestational weeks, T1: first trimester, T2:  
601 second trimester, T3: third trimester, CB: cord blood, SD: standard deviation, IQR:  
602 interquartile range, CI: confidence interval, 25(OH)D: serum 25(OH)-vitamin D  
603 concentration, n: number of patients.

604