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1           **Relationship between vitamin D status in the first trimester of pregnancy and**  
2                           **gestational diabetes mellitus - A nested case-control study**

3  
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48 **Abbreviations**

49 GDM: Gestational diabetes mellitus

- 50 PGDM: Diabetes prior to pregnancy
- 51 SGA: Small for gestational age
- 52 BMI: Body mass index
- 53 WA: Weeks of amenorrhea
- 54 25OHD: 25-hydroxyvitamin D
- 55 1,25(OH)2D: 1,25-dihydroxyvitamin D
- 56 OGTT: Oral glucose tolerance test

57 **ABSTRACT**

58 **Background & Aims:**

59 Gestational diabetes mellitus (GDM) is one of the most frequent medical complications  
60 during pregnancy. It has been associated with many adverse pregnancy, fetal and neonatal  
61 outcomes, as well as with an increased risk for mothers and children in the long term. There is  
62 a growing interest in vitamin D and its potential role in the development of metabolic  
63 disorders. However, the medical literature is not consensual. The aim of this study was to  
64 assess the risk of GDM according to vitamin D status during the first trimester.

65

66 **Methods:**

67 This study is a nested case-control study performed from a multicenter prospective  
68 observational cohort of pregnant women assessed for 25-hydroxyvitamin D levels (25OHD).  
69 Three hundred ninety-three patients were included in the initial cohort. After applying  
70 exclusion criteria, a total of 1191 pregnant women were included. Two hundred fifty women  
71 with GDM (cases) were matched to 941 women without GDM (controls) for parity, age, body  
72 mass index before pregnancy, the season of conception, and phototype. This study was funded  
73 by a grant from the “Programme Hospitalier de Recherche Publique 2010”.

74

75 **Results:**

76 The GDM risk was significantly greater for patients with 25OHD levels < 20 ng/mL  
77 (OR=1.42, 95% CI 1.06-1.91; p = 0.021). However, there was no significant relationship with  
78 other thresholds. The study of 25OHD levels with the more precise cutting of 5 units intervals  
79 showed a variable relationship with GDM risk, as the risk was low for very low 25OHD  
80 levels, increased for moderated levels, decreased for normal levels, and finally increased for  
81 higher levels.

82 **Conclusion:**

83 According to our study, there seems to be no linear relationship between GDM and 25OHD  
84 levels in the first trimester of pregnancy since GDM risk does not continuously decrease as  
85 25OHD concentrations increase. Our results most probably highlight the absence of an  
86 association between 25OHD levels and GDM risk.

87

88 **KEYWORDS**

89 Vitamin D status; gestational diabetes mellitus; nested case-control study.

## 90 INTRODUCTION

91 Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of variable severity  
92 with onset or first recognition during pregnancy (1). The most common risk factors for GDM  
93 are maternal age, a history of GDM or a family history of type 2 diabetes, obesity or  
94 overweight, previous fetal death or delivery of a macrosomic infant, and ethnicity (1,2).  
95 GDM is the most common complication during pregnancy in Europe (3), affecting 1 to 14%  
96 of pregnancies (4), and its incidence is rising. GDM has been associated with adverse  
97 pregnancy outcomes, fetal and neonatal complications, as well as with an increased risk of  
98 obesity, metabolic syndrome, type 2 diabetes and cardiovascular disease in the long term for  
99 both mother and child (5,6). Hence, the challenge is to identify the mechanisms leading to  
100 GDM in order to improve its prevention and treatment. The causes of GDM are actively  
101 investigated, with a growing interest in poor vitamin D status as a potential cause.

102 Vitamin D is a steroid with a hormone-like activity that regulates the expression of a wide  
103 range of genes. Vitamin D status is evaluated by measuring serum concentration of 25-  
104 hydroxyvitamin D (25OHD). Vitamin D deficiency, i.e., 25OHD levels  $\leq 50$  nmol/L or 20  
105 ng/mL (7), is widely prevalent worldwide (8). Pregnant women present a greater risk of  
106 vitamin D deficiency; it has been estimated that 18% of pregnant women in England, 25% in  
107 the United Arab Emirates, 80% in Iran, 42% in Northern India, and 60-84% of non-western  
108 women in the Netherlands, have 25OHD concentrations below 25 nmol/L (9–13). A recent  
109 large cohort study of pregnant women living in France showed that vitamin D insufficiency  
110 was highly prevalent at the beginning of pregnancy, with 50% of women with 25OHD below  
111 20 ng/mL (14). One possible explanation for high rates of vitamin D deficiency during  
112 pregnancy may be the increase of vitamin D needs during pregnancy without increasing the  
113 intakes. Furthermore, the reduced outdoor activity and sun exposure combined with an  
114 increase in fat mass at the end of pregnancy could also explain this association. Vitamin D's

115 function in maintaining phosphocalcic homeostasis and promoting bone mineralization is now  
116 well established. There is an increasing interest in a wide range of mechanisms involving  
117 vitamin D, particularly during pregnancy, and in its effects on placental function, glucose  
118 homeostasis, and inflammatory response.

119 Low vitamin D status has been associated with adverse pregnancy outcomes such as  
120 preeclampsia, preterm labor, small for gestational age (SGA), and neonatal hypocalcemia  
121 (15–17). Furthermore, several epidemiological studies have suggested an association between  
122 low serum concentration of 25OHD and impaired glucose metabolism or metabolic syndrome  
123 (18–21) as well as GDM. However, the evidence is inconsistent (22,23). While some studies  
124 indicate an increased risk of GDM when maternal blood 25OHD is low during the first  
125 trimester (15,24–32), others fail to show such an association (33–35). Most studies on the  
126 topic are cross-sectional, with few participants, different cut-offs and methods of quantifying  
127 25OHD, and lack of adjustments for possible confounding factors, which makes it difficult to  
128 draw reliable conclusions.

129 Regarding interventional studies, a recent randomized controlled trial conducted in Iran, in  
130 women at risk for developing GDM, suggested that vitamin D supplementation during  
131 pregnancy reduces the incidence of GDM (36). Another meta-analysis of 87 observational  
132 studies and 25 randomized controlled trials showed that vitamin D supplementation during  
133 pregnancy could influence not only 25OHD levels but also other biomarkers related to GDM  
134 (25). However, the literature is conflicting (37,38). The aim of the present study was to assess  
135 the risk of GDM according to vitamin D status during the first trimester of women with a  
136 singleton pregnancy in five centers of the middle-north of France and one Belgian center.



## 137 MATERIAL AND METHODS

### 138 Study design

139 This nested case-control study was a secondary analysis of a prospective observational cohort  
140 (FEPED cohort) study, conducted from April 2012 through July 2014, which included  
141 pregnant women from six centers: one in Belgium (latitude 50·83°N) and five in France. Four  
142 of the French centers were in Paris, or its suburbs (Béclère, Bicêtre, Cochin and Trousseau  
143 university hospitals, latitude 48·86°N) and the fifth was in Nantes (latitude 47·22°N). The first  
144 aim of this study was to assess the association between maternal 25OHD levels during  
145 pregnancy and the risk of preeclampsia (39).

146 Eligible women were included in the cohort in the first trimester (i.e., from 10 to <15 weeks  
147 of amenorrhea, (WA)) of a singleton pregnancy. The enrolment took place at the first prenatal  
148 visit in one of the maternity units participating in the study. Exclusion criteria were: 1) any  
149 condition interfering with 25OHD levels such as a history of hypercalcemia (> 2·65 mmol/L)  
150 or any other phosphocalcic disorder, bone disease, lithium therapy, bowel malabsorption or  
151 kidney stone disease; 2) any condition susceptible to interfere with the diagnosis of pre-  
152 eclampsia including uncontrolled hypertension (>140/90 mmHg since first trimester) and  
153 renal insufficiency (creatinine >120 µmol/L). Furthermore, patients were excluded from case  
154 selection if pregestational diabetes mellitus (PGDM) had been diagnosed, if we could not  
155 insure whether GDM or PGDM had previously occurred, if the pregnancy was interrupted  
156 (abortion, intrauterine fetal death), if missing data on delivery and/or pre-eclampsia. Eligible  
157 controls were pregnant women without GDM who had delivered after at least 37 WA, whose  
158 newborn was alive in the delivery room and presented no intrauterine growth restriction (<5<sup>th</sup>  
159 percentile) at birth, with first trimester 25OHD measurement available and with no missing  
160 data on any matching factor.

161 Written informed consent was obtained from each patient prior to inclusion in the study. The  
162 protocol was conducted in accordance with the Declaration of Helsinki and was approved by  
163 the National Data Protection Authority (CNIL no. 911432), and the committee for the  
164 protection of people participating in biomedical research (2011/13NICB).

165 According to current French recommendations, patients at the 7<sup>th</sup> month of pregnancy were  
166 prescribed a bolus vitamin D dose (100 000 IU of cholecalciferol) (40). Patients were  
167 specifically asked, during follow up, whether recommended vitamin D supplementation was  
168 correctly administrated as well as when it took place.

169 After delivery, sociodemographic and clinical data were abstracted from medical records by  
170 personnel trained for the study. Sociodemographic maternal characteristics included parity,  
171 maternal age, height, pre-pregnancy weight, ethnicity and smoking status before, and during  
172 pregnancy. Information was also collected on medical and obstetrical history (diabetes,  
173 chronic hypertension, auto-immune diseases, obstetrical history of SGA infant, stillbirth and  
174 other pregnancy complications), ultrasound, complications of the current pregnancy (pre-  
175 eclampsia, HELLP syndrome, gestational diabetes), the delivery, and the infant's health status  
176 at birth. The phototype of each subject was determined according to the Fitzpatrick skin type  
177 classification (41). Pre-pregnancy body mass index (BMI), calculated from height and pre-  
178 pregnancy weight, was classified using the WHO cut-off for overweight ( $<25$  or  $\geq 25$  kg/m<sup>2</sup>)  
179 (42).

180 The primary objective of our nested case-control study was to assess the risk of GDM  
181 according to vitamin D status during the first trimester, before 15 WA.

182

### 183 **Selection of cases and controls**

184 For each case, four controls were matched for parity (primipara or more), age ( $<35$  or  $\geq 35$   
185 years), body mass index (BMI) before pregnancy ( $<25$  kg/m<sup>2</sup> or  $\geq 25$  kg/m<sup>2</sup>), season of

186 conception (autumn/winter or spring/summer) and phototype ( $<V$  or  $\geq V$  Fitzpatrick scale). In  
187 addition, cases were planned to be matched to controls with the closest age whenever  
188 possible. Phototype was preferred over ethnicity, because its use seemed more reliable. The  
189 matching criteria were chosen according to the main potential confounding factors influencing  
190 both 25OHD levels and GDM risk.

191

### 192 **Assessment of vitamin D status**

193 The measurement of 25OHD level was performed on maternal blood samples collected during  
194 the first trimester of pregnancy (11 to  $<15$  WA). All blood samples were centrifuged and  
195 stored locally at  $-20^{\circ}\text{C}$  and then transferred for centralized 25OHD serum measurement to the  
196 Department of Physiology of Necker University Hospital (Paris, France), which has excellent  
197 results in the DEQAS proficiency program. 25OHD was measured with the DiaSorin RIA.  
198 The limit of detection was arbitrarily set to a value of 4 ng/mL, and any undetectable quantity  
199 was assigned a concentration of 4 ng/mL. Vitamin D deficiency, insufficiency, inadequacy  
200 and sufficiency were defined as a serum 25OHD level below 10 ng/mL, 20 ng/mL, 30 ng/mL  
201 and  $\geq 30$  ng/mL, respectively.

202

### 203 **Assessment of gestational diabetes mellitus**

204 GDM was diagnosed according to World Health Organization guidelines (43). A fasting oral  
205 glucose tolerance test (OGTT) was performed after ingestion of 75 g glucose between 24 and  
206 28 WA. GDM was diagnosed if one or more of the following criteria were met: fasting  
207 plasma glucose 5.1 - 6.9 mmol/L (92 - 125 mg/dL), 1-hour plasma glucose  $\geq 10.0$  mmol/L  
208 (180 mg/dL) and/or 2-hour plasma glucose 8.5 - 11.0 mmol/L (153 - 199 mg/dL) following a  
209 75 g oral glucose load. Patients with fasting plasma glucose  $\geq 7$  mmol/L (126 mg/mL) or 2-  
210 hour plasma glucose  $\geq 11.1$  mmol/L (200 mg/dL) were considered to have a type 2 diabetes

211 prior to pregnancy (PGDM). Seventy-eight-point four percent of patients were screened for  
212 GDM. Among them, 90,3% had an OGTT and 9,7% had another type of screening. Were  
213 qualified as PGDM, both patients with type 2 diabetes prior to pregnancy as well patients who  
214 were diagnosed with GDM with early onset (before 22 WA).

215

### 216 **Statistical analysis**

217 Statistical analysis was performed with R 3.3.1 software. Statistical tests were two-sided, and  
218 p values less than 0.05 were considered statistically significant. The baseline characteristics of  
219 each group were described as mean  $\pm$  standard deviation for quantitative variables and  
220 frequencies (%) for qualitative variables. Conditional logistic regression models were used to  
221 compare cases and controls and to test the association between 25OHD levels and GDM  
222 (through likelihood ratio tests). Analyses were not adjusted for multiple comparisons. Post-  
223 hoc power analysis was not performed because no a priori hypothesis were made on the true  
224 OR.

## 225 **RESULTS**

### 226 **Demographic and clinical characteristics of cases and controls**

227 The selection of cases and controls is summarized in **Figure 1**. In the original cohort, 3129  
228 women were included; however, 36 women were excluded because they had one exclusion  
229 criterion or no blood test to measure 25OHD levels. Thus, 3093 pregnant women were  
230 included from April 2012 to July 2014 with the last delivery in February 2015. Among the  
231 258 cases of GDM (8.3%), eight were excluded due to missing data on 25OHD assay in the  
232 first trimester or missing data on matching criteria (n=7) and neonatal death at delivery (n=1).  
233 Finally, 250 cases of GDM were matched among the 1852 eligible controls. For 43 cases with  
234 the least common profiles, we could not match the four controls required. Overall, the 250  
235 cases were matched to four controls (n=207), three controls (n=27) or two controls (n=16),  
236 leading to a total number of 941 controls.

237 The characteristics of the 250 cases of GDM and the 941 matched controls are summarized in  
238 **Table 1**. As expected, cases and controls were similar in matching characteristics (age, BMI  
239 before pregnancy, conception season, phototype, and parity).

240

### 241 **Routine vitamin D supplementation**

242 In agreement with French national guidelines, 83.2% of women received vitamin D after  
243 inclusion. The time at first vitamin D supplementation did not differ between cases and  
244 controls (p=0.76). In both groups, some women received a vitamin D supplementation in the  
245 month before inclusion (2.8% in the control group and 2.7% among cases, p=0.89) (**Table 2**).

246

### 247 **Levels of 25OHD during pregnancy and association with gestational diabetes**

248 In our cohort, mean 25OHD levels in the first trimester were 21.1 ng/mL among cases and  
249 22.7 ng/mL in the control group (p=0.028). The risk of GDM in inadequate vitamin D patients

250 (25OHD level <30 ng/mL) in the first trimester of pregnancy did not differ significantly from  
251 that of sufficient vitamin D patients (OR 1.34, 95% CI 0.94-1.90, p=0.1). The GDM risk was  
252 significantly greater for patients with vitamin D insufficiency (25OHD level < 20 ng/mL):  
253 OR= 1.42 (95% CI 1.06-1.91; p = 0.021). With other thresholds of 25OHD, such as 10  
254 ng/mL, the difference in risk was not significant anymore (**Table 2**). To explore this  
255 discrepancy, the GDM risk was further tested on 5 units intervals of 25OHD level (**Figure 2**).  
256 This revealed that GDM risk was not constant over increasing 25OHD levels, highlighting  
257 that relationship between the first trimester 25OHD level and the risk of GDM is not linear.  
258 The GDM risk was low for very low 25OHD levels (<10 ng/mL), increased for moderate  
259 levels (10 to 25 ng/mL), decreased for normal levels (25 to 40 ng/mL) and finally increased  
260 for higher levels (> 40 ng/mL).

261 **DISCUSSION**

262 Low vitamin D status in the first trimester may increase GDM risk, but the evidence in the  
263 literature is insufficient to draw reliable conclusions. This study was designed to assess the  
264 risk of GDM according to vitamin D status during the first trimester of pregnancy.

265 While the criteria set for the different subcategories of vitamin D are not consensual, it is  
266 commonly admitted that a blood level below 30 ng/mL is inadequate (44). The recommended  
267 daily intake for vitamin D is 200 IU for children over three years old and adults younger than  
268 65 years and 400 to 600 IU for those over 65 years old (45).

269 Vitamin D is well known for its role in maintaining phosphocalcic homeostasis and  
270 stimulating bone mineralization. However, interest in other potential roles has grown in recent  
271 years, notably a possible association between low vitamin D in early pregnancy and increased  
272 risk of adverse pregnancy outcomes such as preeclampsia, preterm births, SGA, and neonatal  
273 hypocalcemia (15–17). Regarding the association between vitamin D deficiency and the risk  
274 of GDM, controversy remains as the findings have been inconsistent: disparate populations  
275 and conflicting data have been reported. Most studies in the medical literature are  
276 inconclusive because of their cross-sectional design, a limited number of participants, or  
277 absence of adjustments for major confounding factors. Moreover, vitamin D deficiency is  
278 variably defined between studies and is sometimes only analyzed in late pregnancy, plus the  
279 diagnostic criteria for GDM are heterogeneous across studies, all of which complicate  
280 comparisons. A 2016 meta-analysis of 20 observational studies showed a 45% increase in the  
281 risk of GDM in the case of vitamin D deficiency during pregnancy (31). Three previous meta-  
282 analyses of 7, 12 and 22 observational studies, yielded similar results and concluded that there  
283 is a statistically significant association between maternal vitamin D deficiency during  
284 pregnancy and an increased risk of GDM (15,28,29). Additionally, a 2017 systematic review  
285 and meta-analysis of 38 studies, by Zhang et al., included more studies and parameters than

286 previous analyses, in an attempt to draw a definitive conclusion regarding the relationship  
287 between vitamin D and GDM. The results were consistent with the previous meta-analyses:  
288 patients with vitamin D deficiency or insufficiency have a higher risk of GDM (OR 1.85, 95%  
289 CI 1.471-2.328) and 25OHD levels are significantly lower in patients with GDM ( $p < 0.001$ )  
290 (25). Lastly, a 2018 meta-analysis by Hu et al. indicates a significant association between  
291 vitamin D insufficiency and increased risk of GDM (46).

292 However, many authors in the medical literature did not demonstrate a significant correlation  
293 between vitamin D deficiency and GDM. Farrant et al. found no significant association  
294 between vitamin D insufficiency and GDM in a prospective observational cohort of 559  
295 Indian mothers (47). Likewise, in their nested case-control study of 1100 patients, Savvidou et  
296 al. found no significant difference in maternal 25OHD levels during the first trimester  
297 between pregnant women with GDM and control patients (48). Magkoba et al., in a nested  
298 case-control study of 90 patients with GDM and 158 healthy controls, also concluded that the  
299 increase in the risk of GDM in the case of vitamin D deficiency during first trimester of  
300 pregnancy was not significant (49). Baker et al. reported similar results in a case-control study  
301 of 60 cases of GDM and 120 controls in a North Carolina Hospital (50). In a large prospective  
302 cohort of 1953 patients in Guangzhou Hospital in south China, Zhou et al. observed a higher  
303 prevalence of GDM in the vitamin D sufficient group than in the low and medium vitamin D  
304 group. Thus they concluded that the risk of GDM decreased with vitamin D deficiency (33).

305 Two recent cross-sectional studies showed no significant difference in 25OHD levels between  
306 women with and women without GDM, even after adjustment for confounding factors  
307 (34,35). In an attempt to reach a reliable conclusion, two recent reports summarized several  
308 meta-analyses, but failed to give a clear answer regarding the relationship between vitamin D  
309 and the risk of GDM (22,23).



310 The question of 25OHD levels during early pregnancy and GDM risk therefore remains  
311 unresolved. Our study did not show that vitamin D insufficiency during the first trimester  
312 increases the risk of GDM. Indeed, this risk seems to oscillate with 25OHD level. These  
313 results question the consistency of the association between GDM and vitamin D deficiency  
314 previously suggested by many authors, as only usual cut-offs were tested, without studying  
315 more precisely various 25OHD levels.

316 The strength of our study relies on its prospective design, the large sample size, as well as the  
317 choice of potential confounding factors as matching profile criteria for cases and controls.  
318 However, our study does have some limitations. First, the stated amount of vitamin D taken  
319 during pregnancy might not be perfectly accurate as intake may have been increased by the  
320 use of additional nutritional supplements and/or because of dietary habits, in addition to the  
321 7<sup>th</sup> month's national recommended intake. Nevertheless, as GDM is diagnosed late in  
322 pregnancy, the latter might not be an issue. Furthermore, there is no information on the extent  
323 of solar exposure, which depends on clothing and outdoor activities. Finally, other potential  
324 confounding factors such as physical activity, smoking, alcohol intake, socioeconomic status  
325 and a family history of diabetes were not taken into consideration in the design of this study.

326 Several mechanisms can explain the potential physiopathological association between vitamin  
327 D insufficiency and the risk of developing GDM. Vitamin D enhances pancreatic insulin  
328 secretion, via a beta cell vitamin D receptor, thus lowering circulating glucose levels (19,51).  
329 Furthermore, vitamin D, via its receptor, stimulates the expression of insulin receptors, which  
330 stimulates insulin sensitivity and thus increases glucose transport inside the cells (52). Finally,  
331 it plays a major role in the balance of the extracellular and intracellular calcium pools which  
332 are essential to intracellular insulin-mediated mechanisms of insulin-sensitive tissues, also  
333 resulting in glucose transportation (53). Vitamin D deficiency may worsen pre-existing  
334 insulin resistance with impaired compensation due to pregnancy, leading to GDM.

335 Furthermore, it is possible that the association between vitamin D deficiency and GDM is  
336 indirectly regulated via other common risk factors, such as obesity. Our data do not enable us  
337 to evaluate the amount of vitamin D at the onset of GDM as only first-trimester and late third-  
338 trimester samples are available.

339 Recommended vitamin D supplementation varies from one country to another, and there is  
340 still no standard recommendation. The French National College of Gynecologists and  
341 Obstetricians recommends a single 100 000 IU dose of vitamin D administered at the  
342 beginning of the 7<sup>th</sup> month of pregnancy (40). The World Health Organization recommends a  
343 vitamin D intake of 200 IU per day for pregnant women (54).

344 Considering there might be a link between vitamin D and GDM, vitamin D supplementation  
345 during pregnancy could represent a simple and safe intervention to reduce the incidence of  
346 GDM. Several randomized clinical trials on the effect of vitamin D supplementation on  
347 pregnancy outcomes have been conducted. Some demonstrate a reduced incidence of GDM,  
348 as well as correction of vitamin D deficiency, with sufficient vitamin D supplementation.  
349 Shahgheibi et al., in a double-blind placebo-controlled randomized study including 90 Iranian  
350 pregnant women at high risk of GDM, found a significantly higher incidence of GDM in the  
351 placebo group than in the group receiving 5 000 IU per week of vitamin D until the 26<sup>th</sup> WA  
352 (36). In their meta-analysis, Zang et al. found that vitamin D supplementation during  
353 pregnancy seemed to reduce the incidence of GDM, but the results were not statistically  
354 significant (RR 0.718, 95% CI 0.392-1.314). Their results also showed that vitamin D  
355 supplementation influenced other biomarkers of GDM. It elevated blood 25OHD significantly  
356 ( $p < 0.001$ ), glutathione ( $p = 0.003$ ) and HDL cholesterol concentrations ( $p = 0.04$ ) as well as  
357 reduced fasting insulin levels ( $p = 0.001$ ), fasting plasma glucose ( $p < 0.001$ ), insulin resistance  
358 index ( $p < 0.001$ ), blood CRP levels ( $p = 0.02$ ), blood total cholesterol concentration ( $p = 0.003$ )  
359 and blood LDL cholesterol concentrations ( $p = 0.003$ ) (25). Other authors, such as Sablok et al.

360 and Tehrani et al., have failed to prove such an effect (37,38). Regarding our results, it seems  
361 most unlikely that achieving a vitamin D sufficient status during early pregnancy is an  
362 effective way to reduce the incidence of GDM.

363 **CONCLUSION**

364 The literature on the potential link between vitamin D deficiency and GDM is inconsistent.  
365 The use of different thresholds for vitamin D (<30, <20, <10 ng/mL) suggests that the  
366 strength and consistency of the association between vitamin D status in early pregnancy and a  
367 greater risk of GDM should be questioned. Indeed, the GDM risk does not continuously  
368 decrease as 25OHD concentrations increase. Thus, our results do not enable us to establish a  
369 relationship between vitamin D and GDM risk. We suggest that among existing publications,  
370 authors retrospectively access their data and analyze the vitamin D level per subgroup.  
371 Furthermore, large studies are necessary in order to confirm our results and further support the  
372 exclusion of vitamin D insufficiency as a potential GDM risk factor in early pregnancy.

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379 **STATEMENT OF AUTHORSHIP**

380 ES interpreted the data and wrote the manuscript.

381 TR and CE analyzed and interpreted the data.

382 AB, CE and JCS conceived and designed the study.

383 JCS performed 25OHD measurements.

384 JT, VT, JG, MVS, HH, JJ, DB, EM, MCH, NW, DM, and MC included patients.

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

390 **DECLARATION OF INTERESTS**

391 JC. Souberbielle reports lecture fees and/or travel/hotel expenses from DiaSorin, Roche

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393 The other authors declare no conflicts of interest.

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399 **REFERENCES**

- 400 1. Metzger BE, Coustan DR. Summary and recommendations of the Fourth International  
401 Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee.  
402 *Diabetes Care*. 1998;21:B161-167.
- 403 2. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble  
404 ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J*  
405 *Med*. 2008;358:1991–2002.
- 406 3. Buckley BS, Harreiter J, Damm P, Corcoy R, Chico A, Simmons D, et al. Gestational  
407 diabetes mellitus in Europe: prevalence, current screening practice and barriers to  
408 screening. A review. *Diabet Med J Br Diabet Assoc*. 2012;29:844–54.
- 409 4. American Diabetes Association. Diagnosis and classification of diabetes mellitus.  
410 *Diabetes Care*. 2010;33:S62-69.
- 411 5. Silverman BL, Rizzo TA, Cho NH, Metzger BE. Long-term effects of the intrauterine  
412 environment. The Northwestern University Diabetes in Pregnancy Center. *Diabetes*  
413 *Care*. 1998;21:B142-149.
- 414 6. Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen  
415 TD. Gestational diabetes mellitus and long-term consequences for mother and offspring:  
416 a view from Denmark. *Diabetologia*. 2016;59:1396–9.
- 417 7. Ross A, Manson J, Abrams S, Brannon P, Clinton S, Durazo-Arvizu R, et al. The 2011  
418 report on dietary reference intakes for calcium and vitamin D from the Institute of  
419 Medicine: what clinicians need to know. - PubMed - NCBI. *J Clin Endocrinol Metab*.  
420 2011;96:53–8.
- 421 8. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–81.
- 422 9. Dawodu A, Wagner CL. Mother-child vitamin D deficiency: an international  
423 perspective. *Arch Dis Child*. 2007;92:737–40.
- 424 10. Van der Meer IM, Karamali NS, Boeke AJP, Lips P, Middelkoop BJC, Verhoeven I, et  
425 al. High prevalence of vitamin D deficiency in pregnant non-Western women in The  
426 Hague, Netherlands. *Am J Clin Nutr*. 2006;84:350–3.
- 427 11. Datta S, Alfaham M, Davies DP, Dunstan F, Woodhead S, Evans J, et al. Vitamin D  
428 deficiency in pregnant women from a non-European ethnic minority population--an  
429 interventional study. *BJOG Int J Obstet Gynaecol*. 2002;109:905–8.
- 430 12. Nicolaidou P, Hatzistamatiou Z, Papadopoulou A, Kaleyias J, Floropoulou E, Lagona E,  
431 et al. Low vitamin D status in mother-newborn pairs in Greece. *Calcif Tissue Int*.  
432 2006;78:337–42.
- 433 13. Dent CE, Gupta MM. Plasma 25-hydroxyvitamin-D-levels during pregnancy in  
434 Caucasians and in vegetarian and non-vegetarian Asians. *Lancet Lond Engl*.  
435 1975;2:1057–60.

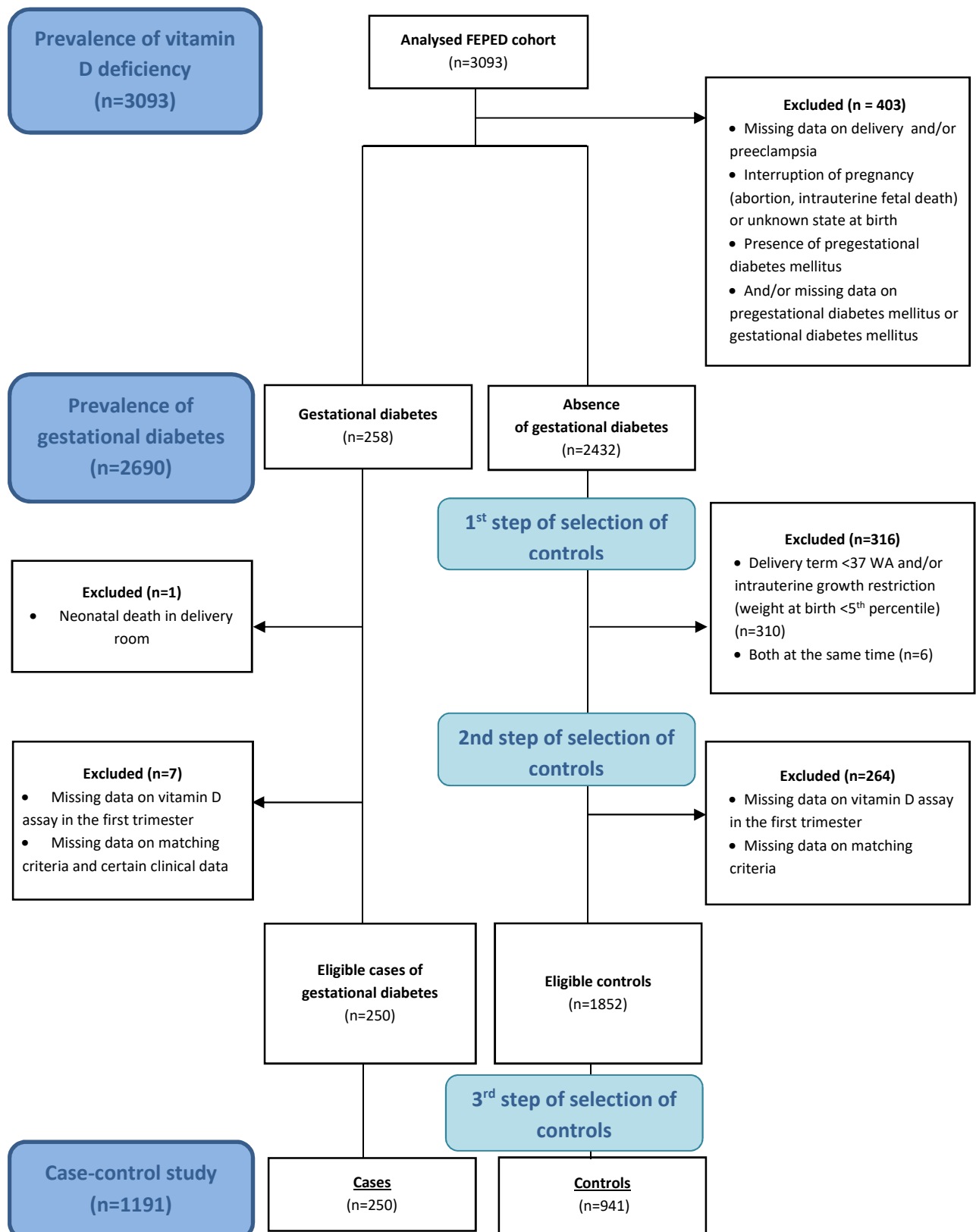
- 436 14. Courbebaisse M, Souberbielle J-C, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, et  
437 al. Vitamin D status during pregnancy and in cord blood in a large prospective French  
438 cohort. *Clin Nutr Edinb Scotl* 2018; <https://doi.org/10.1016/j.clnu.2018.08.035>.
- 439 15. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM.  
440 Association between maternal serum 25-hydroxyvitamin D level and pregnancy and  
441 neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ*.  
442 2013;346:1169.
- 443 16. Barrett H, McElduff A. Vitamin D and pregnancy: An old problem revisited. *Best Pract  
444 Res Clin Endocrinol Metab*. 2010;24:527–39.
- 445 17. Kaludjerovic J, Vieth R. Relationship between vitamin D during perinatal development  
446 and health. *J Midwifery Womens Health*. 2010;55:550–60.
- 447 18. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for  
448 glucose homeostasis. *Int J Endocrinol*. 2010;2010:351–85.
- 449 19. Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin  
450 resistance and beta cell dysfunction. *Am J Clin Nutr*. 2004;79:820–5.
- 451 20. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the  
452 metabolic syndrome among U.S. adults. *Diabetes Care*. 2005;28:1228–30.
- 453 21. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type  
454 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*.  
455 2007;92:2017–29.
- 456 22. Yoon HK. Gestational Diabetes Mellitus, Fetal Growth and Vitamin D. *J Bone Metab*.  
457 2017;24:155–9.
- 458 23. Triunfo S, Lanzone A, Lindqvist PG. Low maternal circulating levels of vitamin D as  
459 potential determinant in the development of gestational diabetes mellitus. *J Endocrinol  
460 Invest*. 2017;40:1049–59.
- 461 24. Zhang C, Qiu C, Hu FB, David RM, van Dam RM, Bralley A, et al. Maternal plasma 25-  
462 hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. *PloS  
463 One*. 2008;3:3753.
- 464 25. Zhang Y, Gong Y, Xue H, Xiong J, Cheng G. Vitamin D and gestational diabetes  
465 mellitus: a systematic review based on data free of Hawthorne effect. *BJOG Int J Obstet  
466 Gynaecol*. 2018;125(7):784-793.
- 467 26. Parlea L, Bromberg IL, Feig DS, Vieth R, Merman E, Lipscombe LL. Association  
468 between serum 25-hydroxyvitamin D in early pregnancy and risk of gestational diabetes  
469 mellitus. *Diabet Med*. 2012;29:25–32.
- 470 27. Burris HH, Rifas-Shiman SL, Kleinman K, Litonjua AA, Huh SY, Rich-Edwards JW, et  
471 al. Vitamin D deficiency in pregnancy and gestational diabetes mellitus. *Am J Obstet  
472 Gynecol*. 2012;207:182.

- 473 28. Poel YHM, Hummel P, Lips P, Stam F, van der Ploeg T, Simsek S. Vitamin D and  
474 gestational diabetes: a systematic review and meta-analysis. *Eur J Intern Med.*  
475 2012;23:465–9.
- 476 29. Wei S-Q, Qi H-P, Luo Z-C, Fraser WD. Maternal vitamin D status and adverse  
477 pregnancy outcomes: a systematic review and meta-analysis. *J Matern-Fetal Neonatal*  
478 *Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet.*  
479 2013;26:889–99.
- 480 30. Lacroix M, Battista M-C, Doyon M, Houde G, Ménard J, Ardilouze J-L, et al. Lower  
481 vitamin D levels at first trimester are associated with higher risk of developing  
482 gestational diabetes mellitus. *Acta Diabetol.* 2014;51:609–16.
- 483 31. Lu M, Xu Y, Lv L, Zhang M. Association between vitamin D status and the risk of  
484 gestational diabetes mellitus: a meta-analysis. *Arch Gynecol Obstet.* 2016;293:959–66.
- 485 32. Boyle VT, Thorstensen EB, Mourath D, Jones MB, McCowan LME, Kenny LC, et al.  
486 The relationship between 25-hydroxyvitamin D concentration in early pregnancy and  
487 pregnancy outcomes in a large, prospective cohort. *Br J Nutr.* 2016;116:1409–15.
- 488 33. Zhou J, Su L, Liu M, Liu Y, Cao X, Wang Z, et al. Associations between 25-  
489 hydroxyvitamin D levels and pregnancy outcomes: a prospective observational study in  
490 southern China. *Eur J Clin Nutr.* 2014;68:925–30.
- 491 34. Bal M, Şahin Ersoy G, Demirtaş Ö, Kurt S, Taşyurt A. Vitamin D deficiency in  
492 pregnancy is not associated with diabetes mellitus development in pregnant women at  
493 low risk for gestational diabetes. *Turk J Obstet Gynecol.* 2016;13:23–6.
- 494 35. Hauta-alus HH, Viljakainen HT, Holmlund-Suila EM, Enlund-Cerullo M, Rosendahl J,  
495 Valkama SM, et al. Maternal vitamin D status, gestational diabetes and infant birth size.  
496 *BMC Pregnancy Childbirth.* 2017;17:420.
- 497 36. Shahgheibi S, Farhadifar F, Pouya B. The effect of vitamin D supplementation on  
498 gestational diabetes in high-risk women: Results from a randomized placebo-controlled  
499 trial. *J Res Med Sci Off J Isfahan Univ Med Sci.* 2016;  
500 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5122001>.
- 501 37. Sablok A, Batra A, Thariani K, Batra A, Bharti R, Aggarwal AR, et al. Supplementation  
502 of vitamin D in pregnancy and its correlation with feto-maternal outcome. *Clin*  
503 *Endocrinol (Oxf).* 2015;83:536–41.
- 504 38. Tehrani HG, Mostajeran F, Banihashemi B. Effect of Vitamin D Supplementation on the  
505 Incidence of Gestational Diabetes. *Adv Biomed Res.* 2017;6:79.
- 506 39. Benachi A, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, Senat M-V, et al.  
507 Relationship between vitamin D status in pregnancy and the risk for preeclampsia: A  
508 nested case-control study. *Clin Nutr.* 2019; pii: S0261-5614(19)30069-X.
- 509 40. Collège national des gynécologues et obstétriciens français. Recommandation pour la  
510 pratique clinique. Supplémentation au cours de la grossesse. 1997:  
511 [http://www.cngof.asso.fr/D\\_TELE/RPC\\_suppl\\_97.pdf](http://www.cngof.asso.fr/D_TELE/RPC_suppl_97.pdf)

- 512 41. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI.  
513 Arch Dermatol. 1988;124:869–71.
- 514 42. World Health Organisation. Physical status: the use and interpretation of anthropometry.  
515 Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995;854:1–  
516 452.
- 517 43. World Health Organisation. WHO | Diagnostic criteria and classification of  
518 hyperglycaemia first detected in pregnancy. WHO. 2013:  
519 [https://www.who.int/diabetes/publications/Hyperglycaemia\\_In\\_Pregnancy/en](https://www.who.int/diabetes/publications/Hyperglycaemia_In_Pregnancy/en).
- 520 44. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of  
521 optimal vitamin D status. Osteoporos Int J Establ Result Coop Eur Found Osteoporos  
522 Natl Osteoporos Found USA. 2005 Jul;16(7):713–6.
- 523 45. Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du  
524 travail. Vitamine D: Présentation, sources alimentaires et besoins nutritionnels. 2016:  
525 <https://www.anses.fr/fr/content/vitamine-d>.
- 526 46. Hu L, Zhang Y, Wang X, You L, Xu P, Cui X, et al. Maternal Vitamin D Status and  
527 Risk of Gestational Diabetes: a Meta-Analysis. Cell Physiol Biochem Int J Exp Cell  
528 Physiol Biochem Pharmacol. 2018;45:291–300.
- 529 47. Farrant HJW, Krishnaveni GV, Hill JC, Boucher BJ, Fisher DJ, Noonan K, et al.  
530 Vitamin D insufficiency is common in Indian mothers but is not associated with  
531 gestational diabetes or variation in newborn size. Eur J Clin Nutr. 2009;63:646–52.
- 532 48. Savvidou MD, Akolekar R, Samaha RBB, Masconi AP, Nicolaides KH. Maternal serum  
533 25-hydroxyvitamin D levels at 11(+0) -13(+6) weeks in pregnant women with diabetes  
534 mellitus and in those with macrosomic neonates. BJOG Int J Obstet Gynaecol.  
535 2011;118:951–5.
- 536 49. Makgoba M, Nelson SM, Savvidou M, Messow C-M, Nicolaides K, Sattar N. First-  
537 trimester circulating 25-hydroxyvitamin D levels and development of gestational  
538 diabetes mellitus. Diabetes Care. 2011;34:1091–3.
- 539 50. Baker AM, Haeri S, Camargo CA, Stuebe AM, Boggess KA. First-trimester maternal  
540 vitamin D status and risk for gestational diabetes (GDM) a nested case-control study.  
541 Diabetes Metab Res Rev. 2012;28:164–8.
- 542 51. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits  
543 pancreatic secretion of insulin. Science. 1980;209:823–5.
- 544 52. Vaidya A, Williams JS. The relationship between vitamin D and the renin-angiotensin  
545 system in the pathophysiology of hypertension, kidney disease, and diabetes.  
546 Metabolism. 2012;61:450–8.
- 547 53. Draznin B, Sussman KE, Eckel RH, Kao M, Yost T, Sherman NA. Possible role of  
548 cytosolic free calcium concentrations in mediating insulin resistance of obesity and  
549 hyperinsulinemia. J Clin Invest. 1988;82:1848–52.

550 54. World Health Organization. Vitamin and mineral requirements in human nutrition. 2004:  
551 <https://www.who.int/nutrition/publications/micronutrients/9241546123/en/>

553 **Figure 1: Flow chart**



555 **Table 1: Clinical and demographic characteristics at inclusion of women with**  
 556 **gestational diabetes (cases) and matched women without gestational diabetes (controls)\***

557

Characteristics at inclusion	Cases (N=250)	Controls <sup>a</sup> (N=941)	P- value <sup>b</sup>
<b>Center of inclusion</b>			0.011
Béclère Hospital, Paris	108 (43.2%)	420 (44.6%)	
Bicêtre Hospital, Paris	47 (18.8%)	109 (11.6%)	
Cochin Hospital, Paris	50 (20.0%)	234 (28.9%)	
Trousseau Hospital, Paris	25 (10.0%)	65 (6.9%)	
Nantes University Hospital, Nantes	7 (2.8%)	36 (3.8%)	
CHU Brugmann, Brussels	13 (5.2%)	77 (8.2%)	
<b>Pregnancy term at inclusion (weeks of amenorrhea)</b>	12.9 ± 0.8	12.7 ± 0.9	0.002
<b>Age at inclusion (years)</b>	32.8 ± 5.3	32.3 ± 5.0	0.12
<b>Body mass index before pregnancy (kg/m<sup>2</sup>)</b>	25.8 ± 5.5	24.7 ± 4.6	0.001
<b>Parity (previous deliveries)</b>			0.46
0	113 (45.2%)	424 (45.1%)	
1	96 (38.4%)	333 (35.4%)	
>1	41 (16.4%)	184 (19.6%)	
<b>Season of conception</b>			0.70
Spring/Summer	130 (52%)	502 (53.4%)	
Autumn/Winter	120 (48%)	439 (46.6%)	
<b>Skin color (Fitzpatrick scale)</b>			0.96
Skin color 1-4	192 (76.8%)	724 (76.9%)	
Skin color ≥5	58 (23.2%)	217 (23.1)	
<b>Origin</b>			0.10
Sub-Saharan Africa	22 (8.8%)	99 (10.6%)	
North Africa	59 (23.6%)	147 (15.6%)	
Asia	12 (4.8%)	28 (3%)	
French overseas departments/territories	11 (4.4%)	41 (4.4%)	
North Europe	7 (2.8%)	35 (3.7%)	
South Europe	13 (5.2%)	53 (5.7%)	
France	115 (46%)	501 (53.4%)	
Other	11 (4.4%)	34 (3.6%)	

558 \*Data are presented as number (%) or mean ± SD

559 <sup>a</sup> Parity (primipara or more), age (<35 or ≥35 years), body mass index before pregnancy (<25 kg/m<sup>2</sup> or ≥25  
 560 kg/m<sup>2</sup>), season of conception (autumn/winter or spring/summer) and phototype (<V or ≥V Fitzpatrick scale)  
 561 were matching factors.

562 <sup>b</sup> Logistic regression model

563 **Table 2: Association between vitamin D during the first trimester and gestational**

564 **diabetes mellitus\***

565

	<b>Cases</b>  <b>N=250</b>	<b>Controls</b>  <b>N=941</b>	<b>OR [95% CI]</b>  <b>P-value</b>
<b>Time of assay (WA)</b>	12.9 ± 0.8	12.7 ± 0.9	0.003
<b>Vitamin D supplementation in the month before inclusion</b>	6 (2.7%)	25 (2.8%)	1.07 [0.42-2.72]  p=0.89
<b>Vitamin D supplementation after inclusion</b>	192 (80.3%)	798 (86%)	0.64 [0.43-0.94]  p=0.022
<b>Time at first vitamin D supplementation (WA)</b>	27.8 ± 3.4	27.8 ± 3.6	0.76
<b>Vitamin D level in first trimester (ng/mL)</b>	21.1 ± 10	22.7 ± 10	p=0.028
<b>Vitamin D level classes</b>			
< 10 ng/mL	22 (8.8%)	82 (8.7%)	1.24 [0.69-2.24], p=0.47
10-30 ng/mL	176 (70.4%)	615 (65.4%)	1.35 [0.95-1.92], p=0.1
≥ 30 ng/mL	52 (20.8%)	244 (25.9%)	Ref
<b>Vitamin D deficiency</b>			
<10 ng/mL	22 (8.8%)	82 (8.7%)	0.98 [0.58-1.63], p=0.93
≥10 ng/mL	228 (91.2%)	859 (91.3%)	Ref

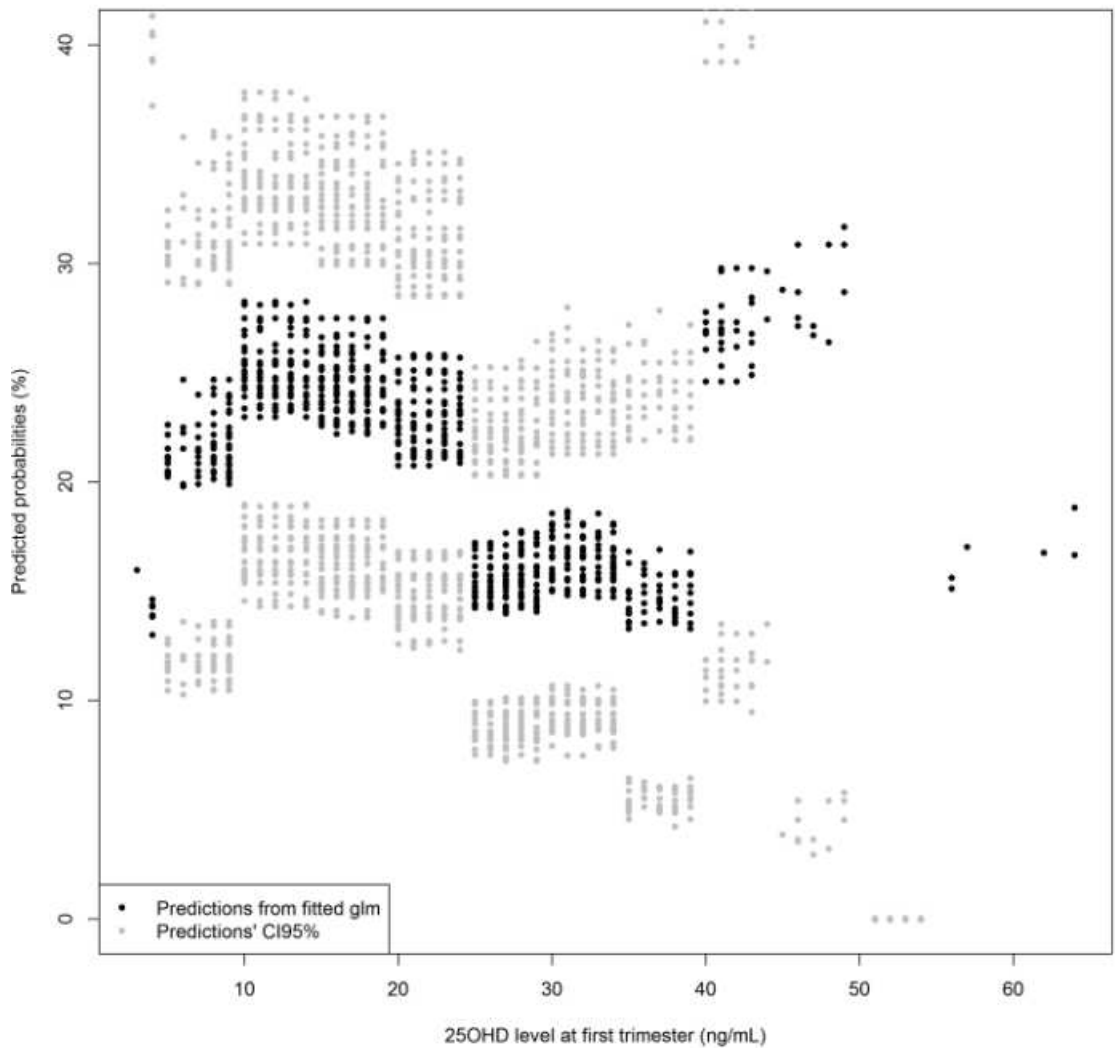


<b>Vitamin D insufficiency</b>			
<20 ng/mL	125 (50%)	394 (41.9%)	1.42 [1.06-1.91], p=0.021
≥20 ng/mL	125 (50%)	547 (58.1%)	
			Ref
<b>Vitamin D inadequacy</b>			
<30 ng/mL	198 (79.2%)	697 (74.1%)	1.34 [0.94-1.90], p=0.1
≥30 ng/mL	52 (20.8%)	244 (25.9%)	
			Ref

566 \*Data are presented as number (%) or mean ± SD

567 **Figure 2: Association between 5 units intervals of maternal serum 25OHD in the first**  
568 **trimester of gestation and the predicted probability of gestational diabetes mellitus.**

569 Predicted probabilities of gestational diabetes mellitus (●) and 95% confidence interval (◉) were calculated with  
570 a logistic model adjusted for matching factors (parity, maternal age, pregnancy BMI, season of conception,  
571 phototype) and are represented between 0 and 0.4.  
572 Serum 25OHD was divided into 5 units intervals (ng/mL).  
573



574