



HAL
open science

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

Eleonora Salakos, Tioka Rabeony, Marie Courbebaisse, Joëlle Taieb, Vassilis Tsatsaris, Jean Guibourdenche, Marie-Victoire Senat, Hazar Haidar, Jacques C. Jani, Dragos Barglazan, et al.

► To cite this version:

Eleonora Salakos, Tioka Rabeony, Marie Courbebaisse, Joëlle Taieb, Vassilis Tsatsaris, et al.. Relationship between vitamin D status in the first trimester of pregnancy and gestational diabetes mellitus - A nested case-control study. *Clinical Nutrition*, 2021, 40, pp.79 - 86. 10.1016/j.clnu.2020.04.028 . hal-03493511

HAL Id: hal-03493511

<https://hal.science/hal-03493511>

Submitted on 2 Jan 2023

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

3
4 Eleonora Salakos^a, Tioka Rabeony^c, Marie Courbebaisse^d, Joëlle Taieb^e, Vassilis Tsatsaris^{b,f},
5 Jean Guibourdenche^g, Marie-Victoire Senat^h, Hazar Haidarⁱ, Jacques C. Jani^j, Dragos
6 Barglazan^k, Emeline Maisonneuve^l, Marie-Clotilde Haguët^m, Norbert Winerⁿ, Damien
7 Masson^o, Caroline Elie^c, Jean-Claude Souberbielle^p, Alexandra Benachi^{a,b}

8
9 ^aGynécologie-Obstétrique, Hôpital Antoine-Béclère, Assistance Publique-Hôpitaux de Paris
10 (AP-HP), Université Paris-Sud, Clamart, France

11 ^bFondation PremUp, Paris, France

12 ^cURC/CIC Paris Descartes Necker Cochin, Hôpital Necker-Enfants malades, AP-HP, Paris,
13 France

14 ^dService de Physiologie-Explorations Fonctionnelles Rénales, Hôpital Européen Georges
15 Pompidou, AP-HP, Université Paris Descartes, INSERM U1151, Paris, France

16 ^eBiochimie, Hôpital Antoine-Béclère, AP-HP, Université Paris-Sud, Clamart, France

17 ^fMaternité Port-Royal, Hôpital Cochin, AP-HP, Université René Descartes, Paris, France

18 ^gBiochimie hormonale, Hôpital Cochin, AP-HP, Université René Descartes, Paris, France

19 ^hGynécologie-Obstétrique, Hôpital Bicêtre, AP-HP, Université Paris-Sud, Kremlin Bicêtre,
20 France

21 ⁱService de génétique Moléculaire, Pharmacogénétique et Hormonologie, Hôpital Bicêtre,
22 Hôpital Bicêtre, AP-HP, Université Paris-Sud, Kremlin Bicêtre, France

23 ^jDepartment of Obstetrics and Gynecology, University Hospital Brugmann, Université Libre
24 de Bruxelles, Brussels, Belgium

25 ^kDepartment of Clinical Chemistry, University Hospital Brugmann, Université Libre de
26 Bruxelles, Brussels, Belgium

27 ^lService de Médecine Fœtale, Hôpital Armand Trousseau, UPMC-Sorbonne Université, Paris,
28 France

29 ^mBiochimie, Hôpital Trousseau, Hôpital Armand Trousseau, UPMC-Sorbonne Université,
30 Paris, France

31 ⁿDepartment of Gynecology and Obstetrics, Nantes University Hospital, 44000, France

32 ^oDepartment of Biochemistry, Nantes University Hospital Hotel-Dieu, Nantes 44000, France

33 ^pLaboratoire d'Explorations Fonctionnelles, Hôpital Necker-Enfants malades, AP-HP, Paris,
34 France

35

36 **Revision number:** YCLNU-D-19-01316R3

37

38 **Corresponding author**

39 Eleonora Salakos, MD

40 Service de Gynécologie-Obstétrique

41 Hôpital Antoine Béchère

42 157, Rue de la porte de Trivaux

43 92140 Clamart, France

44 Tel : +33 1 45374476

45 Fax : +33 1 45374967

46 E-mail : e.salakos@gmail.com

47

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 ≤ 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 (<5th
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 7th 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 ≥ 25 kg/m²)
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 ≥ 35
185 years), body mass index (BMI) before pregnancy (<25 kg/m² or ≥ 25 kg/m²), 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°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 ≥ 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 ≥ 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 ≥ 7 mmol/L (126 mg/mL) or 2-
210 hour plasma glucose ≥ 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 7th 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 7th 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 26th 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.

373 **ACKNOWLEDGMENTS**

374 We would like to thank S. Albert, V. Buth, V. da Costa, S. Larrède, E. Etienne, L. Peudecerf
375 and I. Rieger for monitoring the data and A. Bellino and M. Delattre for coordinating the
376 study. We are grateful to the doctors, midwives and nurses for their management of patients.
377 We would also like to thank the “Centre de Ressources Biologiques” at the Centre Hospitalier
378 Intercommunal de Créteil for the management and storage of biospecimens.

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

392 Diagnosis, Abbott, Amgen, Shire, MSD, Lilly, and Rottapharm/Meda.

393 The other authors declare no conflicts of interest.

394 **ROLE OF THE FUNDING SOURCES**

395 The study was sponsored by the Assistance Publique - Hôpitaux de Paris (AP-HP) and it was
396 funded by a grant from the Programme Hospitalier de Recherche Publique – PHRC national
397 2010 (Ministry of Health – AOM10113). It is registered under ClinicalTrials.gov identifier
398 NCT01648842. Samples were stored in the Perinat Collection (ANR-10-EQPX-0010).

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.

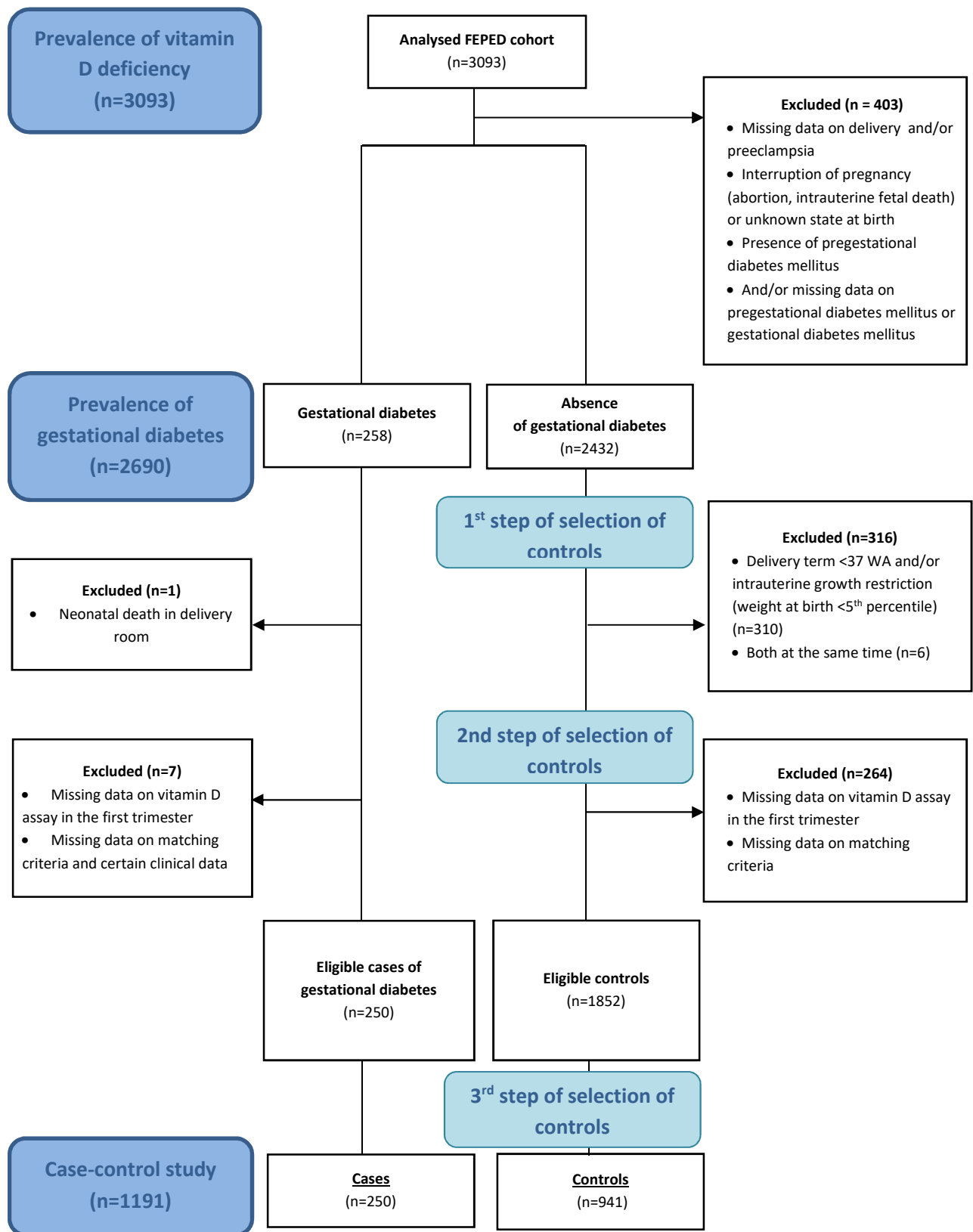
- 436 14. Courbebaisse M, Souberbielle J-C, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, et
437 al. Vitamin D status during pregnancy and in cord blood in a large prospective French
438 cohort. *Clin Nutr Edinb Scotl* 2018; <https://doi.org/10.1016/j.clnu.2018.08.035>.
- 439 15. Aghajafari F, Nagulesapillai 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, Lanzzone 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 fetomaternal 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

- 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.
- 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 ^a (N=941)	P- value ^b
Center of inclusion			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%)	
Pregnancy term at inclusion (weeks of amenorrhea)	12.9 ± 0.8	12.7 ± 0.9	0.002
Age at inclusion (years)	32.8 ± 5.3	32.3 ± 5.0	0.12
Body mass index before pregnancy (kg/m²)	25.8 ± 5.5	24.7 ± 4.6	0.001
Parity (previous deliveries)			0.46
0	113 (45.2%)	424 (45.1%)	
1	96 (38.4%)	333 (35.4%)	
>1	41 (16.4%)	184 (19.6%)	
Season of conception			0.70
Spring/Summer	130 (52%)	502 (53.4%)	
Autumn/Winter	120 (48%)	439 (46.6%)	
Skin color (Fitzpatrick scale)			0.96
Skin color 1-4	192 (76.8%)	724 (76.9%)	
Skin color ≥5	58 (23.2%)	217 (23.1)	
Origin			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 ^a Parity (primipara or more), age (<35 or ≥35 years), body mass index before pregnancy (<25 kg/m² or ≥25
 560 kg/m²), season of conception (autumn/winter or spring/summer) and phototype (<V or ≥V Fitzpatrick scale)
 561 were matching factors.

562 ^b Logistic regression model

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

564 **diabetes mellitus***

565

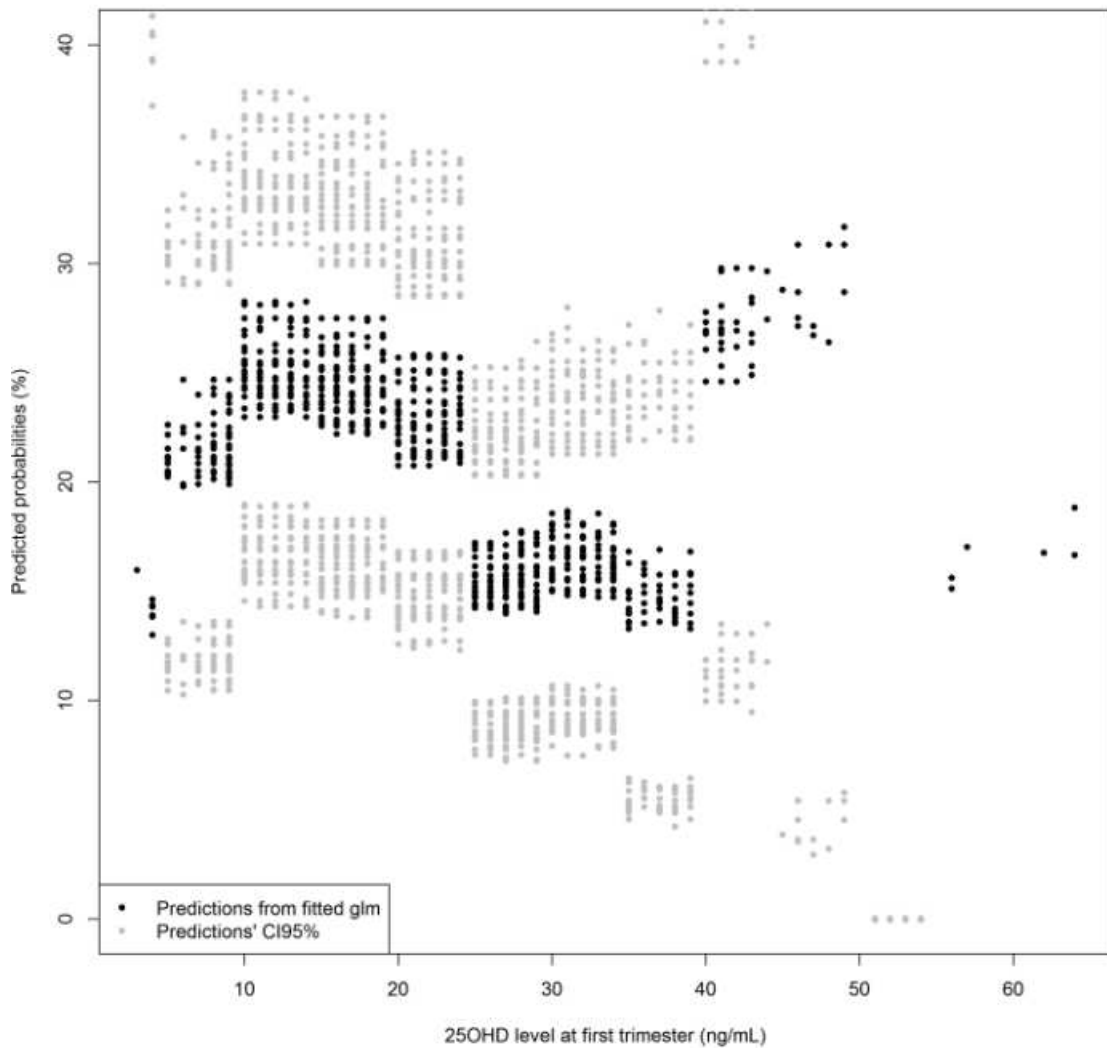
	Cases N=250	Controls N=941	OR [95% CI] P-value
Time of assay (WA)	12.9 ± 0.8	12.7 ± 0.9	0.003
Vitamin D supplementation in the month before inclusion	6 (2.7%)	25 (2.8%)	1.07 [0.42-2.72] p=0.89
Vitamin D supplementation after inclusion	192 (80.3%)	798 (86%)	0.64 [0.43-0.94] p=0.022
Time at first vitamin D supplementation (WA)	27.8 ± 3.4	27.8 ± 3.6	0.76
Vitamin D level in first trimester (ng/mL)	21.1 ± 10	22.7 ± 10	p=0.028
Vitamin D level classes			
< 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
Vitamin D deficiency			
<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

Vitamin D insufficiency			
<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
Vitamin D inadequacy			
<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