

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

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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
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42 43 44 45 46	Hôpital Antoine Béclère 157, Rue de la porte de Trivaux 92140 Clamart, France Tel : +33 1 45374476 Fax : +33 1 45374967 E-mail : e.salakos@gmail.com
42 43 44 45 46 47	Hôpital Antoine Béclère 157, Rue de la porte de Trivaux 92140 Clamart, France Tel : +33 1 45374476 Fax : +33 1 45374967 E-mail : e.salakos@gmail.com
42 43 44 45 46 47 48	Hôpital Antoine Béclère 157, Rue de la porte de Trivaux 92140 Clamart, France Tel : +33 1 45374476 Fax : +33 1 45374967 E-mail : e.salakos@gmail.com Abbreviations

- 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:

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

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66 Methods:

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

74

75 **Results:**

The GDM risk was significantly greater for patients with 250HD levels < 20 ng/mL (OR=1.42, 95% CI 1.06-1.91; p = 0.021). However, there was no significant relationship with other thresholds. The study of 250HD levels with the more precise cutting of 5 units intervals showed a variable relationship with GDM risk, as the risk was low for very low 250HD levels, increased for moderated levels, decreased for normal levels, and finally increased for higher levels.

82 **Conclusion:**

According to our study, there seems to be no linear relationship between GDM and 250HD levels in the first trimester of pregnancy since GDM risk does not continuously decrease as 250HD concentrations increase. Our results most probably highlight the absence of an association between 250HD 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 with onset or first recognition during pregnancy (1). The most common risk factors for GDM 92 are maternal age, a history of GDM or a family history of type 2 diabetes, obesity or 93 overweight, previous fetal death or delivery of a macrosomic infant, and ethnicity (1,2). 94 GDM is the most common complication during pregnancy in Europe (3), affecting 1 to 14% 95 of pregnancies (4), and its incidence is rising. GDM has been associated with adverse 96 pregnancy outcomes, fetal and neonatal complications, as well as with an increased risk of 97 obesity, metabolic syndrome, type 2 diabetes and cardiovascular disease in the long term for 98 99 both mother and child (5,6). Hence, the challenge is to identify the mechanisms leading to GDM in order to improve its prevention and treatment. The causes of GDM are actively 100 investigated, with a growing interest in poor vitamin D status as a potential cause. 101

102 Vitamin D is a steroid with a hormone-like activity that regulates the expression of a wide range of genes. Vitamin D status is evaluated by measuring serum concentration of 25-103 104 hydroxyvitamin D (250HD). Vitamin D deficiency, i.e., 250HD levels \leq 50 nmol/L or 20 105 ng/mL (7), is widely prevalent worldwide (8). Pregnant women present a greater risk of vitamin D deficiency; it has been estimated that 18% of pregnant women in England, 25% in 106 the United Arab Emirates, 80% in Iran, 42% in Northern India, and 60-84% of non-western 107 women in the Netherlands, have 25OHD concentrations below 25 nmol/L (9-13). A recent 108 large cohort study of pregnant women living in France showed that vitamin D insufficiency 109 was highly prevalent at the beginning of pregnancy, with 50% of women with 25OHD below 110 20 ng/mL (14). One possible explanation for high rates of vitamin D deficiency during 111 pregnancy may be the increase of vitamin D needs during pregnancy without increasing the 112 intakes. Furthermore, the reduced outdoor activity and sun exposure combined with an 113 increase in fat mass at the end of pregnancy could also explain this association. Vitamin D's 114

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

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

129 Regarding interventional studies, a recent randomized controlled trial conducted in Iran, in women at risk for developing GDM, suggested that vitamin D supplementation during 130 pregnancy reduces the incidence of GDM (36). Another meta-analysis of 87 observational 131 studies and 25 randomized controlled trials showed that vitamin D supplementation during 132 pregnancy could influence not only 250HD levels but also other biomarkers related to GDM 133 (25). However, the literature is conflicting (37,38). The aim of the present study was to assess 134 the risk of GDM according to vitamin D status during the first trimester of women with a 135 singleton pregnancy in five centers of the middle-north of France and one Belgian center. 136

137 MATERIAL AND METHODS

138 Study design

This nested case-control study was a secondary analysis of a prospective observational cohort (FEPED cohort) study, conducted from April 2012 through July 2014, which included pregnant women from six centers: one in Belgium (latitude 50·83°N) and five in France. Four of the French centers were in Paris, or its suburbs (Béclère, Bicêtre, Cochin and Trousseau university hospitals, latitude 48·86°N) and the fifth was in Nantes (latitude 47·22°N). The first aim of this study was to assess the association between maternal 250HD levels during 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 of amenorrhea, (WA)) of a singleton pregnancy. The enrolment took place at the first prenatal 147 visit in one of the maternity units participating in the study. Exclusion criteria were: 1) any 148 149 condition interfering with 25OHD levels such as a history of hypercalcemia (> 2.65 mmol/L) or any other phosphocalcic disorder, bone disease, lithium therapy, bowel malabsorption or 150 kidney stone disease; 2) any condition susceptible to interfere with the diagnosis of pre-151 eclampsia including uncontrolled hypertension (>140/90 mmHg since first trimester) and 152 renal insufficiency (creatinine >120 μ mol/L). Furthermore, patients were excluded from case 153 selection if pregestational diabetes mellitus (PGDM) had been diagnosed, if we could not 154 insure whether GDM or PGDM had previously occurred, if the pregnancy was interrupted 155 (abortion, intrauterine fetal death), if missing data on delivery and/or pre-eclampsia. Eligible 156 controls were pregnant women without GDM who had delivered after at least 37 WA, whose 157 newborn was alive in the delivery room and presented no intrauterine growth restriction (<5th 158 percentile) at birth, with first trimester 250HD measurement available and with no missing 159 data on any matching factor. 160

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

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

After delivery, sociodemographic and clinical data were abstracted from medical records by 169 personnel trained for the study. Sociodemographic maternal characteristics included parity, 170 maternal age, height, pre-pregnancy weight, ethnicity and smoking status before, and during 171 pregnancy. Information was also collected on medical and obstetrical history (diabetes, 172 173 chronic hypertension, auto-immune diseases, obstetrical history of SGA infant, stillbirth and other pregnancy complications), ultrasound, complications of the current pregnancy (pre-174 175 eclampsia, HELLP syndrome, gestational diabetes), the delivery, and the infant's health status at birth. The phototype of each subject was determined according to the Fitzpatrick skin type 176 classification (41). Pre-pregnancy body mass index (BMI), calculated from height and pre-177 178 pregnancy weight, was classified using the WHO cut-off for overweight (<25 or \geq 25 kg/m²) (42). 179

180 The primary objective of our nested case-control study was to assess the risk of GDM181 according to vitamin D status during the first trimester, before 15 WA.

182

183 Selection of cases and controls

For each case, four controls were matched for parity (primipara or more), age (<35 or \ge 35 years), body mass index (BMI) before pregnancy (<25 kg/m² or \ge 25 kg/m²), season of 186 conception (autumn/winter or spring/summer) and phototype ($\langle V \text{ or } \geq V \text{ Fitzpatrick scale}$). 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

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

GDM was diagnosed according to World Health Organization guidelines (43). A fasting oral glucose tolerance test (OGTT) was performed after ingestion of 75 g glucose between 24 and 28 WA. GDM was diagnosed if one or more of the following criteria were met: fasting plasma glucose $5 \cdot 1 - 6 \cdot 9 \text{ mmol/L} (92 - 125 \text{ mg/dL})$, 1-hour plasma glucose $\geq 10.0 \text{ mmol/L}$ (180 mg/dL) and/or 2-hour plasma glucose $8 \cdot 5 - 11 \cdot 0 \text{ mmol/L} (153 - 199 \text{ mg/dL})$ following a 75 g oral glucose load. Patients with fasting plasma glucose $\geq 7 \text{ mmol/L} (126 \text{ mg/mL})$ or 2hour plasma glucose $\geq 11 \cdot 1 \text{ mmol/L} (200 \text{ mg/dL})$ were considered to have a type 2 diabetes prior to pregnancy (PGDM). Seventy-eight-point four percent of patients were screened for
GDM. Among them, 90,3% had an OGTT and 9,7% had another type of screening. Were
qualified as PGDM, both patients with type 2 diabetes prior to pregnancy as well patients who
were diagnosed with GDM with early onset (before 22 WA).

215

216 Statistical analysis

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

225 **RESULTS**

226 Demographic and clinical characteristics of cases and controls

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

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

240

241 Routine vitamin D supplementation

In agreement with French national guidelines, 83.2% of women received vitamin D after inclusion. The time at first vitamin D supplementation did not differ between cases and controls (p=0.76). In both groups, some women received a vitamin D supplementation in the 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

In our cohort, mean 250HD 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

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

261 **DISCUSSION**

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

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

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

previous analyses, in an attempt to draw a definitive conclusion regarding the relationship between vitamin D and GDM. The results were consistent with the previous meta-analyses: patients with vitamin D deficiency or insufficiency have a higher risk of GDM (OR 1.85, 95% CI 1.471-2.328) and 250HD levels are significantly lower in patients with GDM (p<0.001) (25). Lastly, a 2018 meta-analysis by Hu et al. indicates a significant association between vitamin D insufficiency and increased risk of GDM (46).

However, many authors in the medical literature did not demonstrate a significant correlation 292 between vitamin D deficiency and GDM. Farrant et al. found no significant association 293 between vitamin D insufficiency and GDM in a prospective observational cohort of 559 294 Indian mothers (47). Likewise, in their nested case-control study of 1100 patients, Savvidou et 295 al. found no significant difference in maternal 25OHD levels during the first trimester 296 between pregnant women with GDM and control patients (48). Magkoba et al., in a nested 297 298 case-control study of 90 patients with GDM and 158 healthy controls, also concluded that the increase in the risk of GDM in the case of vitamin D deficiency during first trimester of 299 pregnancy was not significant (49). Baker et al. reported similar results in a case-control study 300 301 of 60 cases of GDM and 120 controls in a North Carolina Hospital (50). In a large prospective cohort of 1953 patients in Guangzhou Hospital in south China, Zhou et al. observed a higher 302 prevalence of GDM in the vitamin D sufficient group than in the low and medium vitamin D 303 group. Thus they concluded that the risk of GDM decreased with vitamin D deficiency (33). 304 Two recent cross-sectional studies showed no significant difference in 25OHD levels between 305 women with and women without GDM, even after adjustment for confounding factors 306 (34,35). In an attempt to reach a reliable conclusion, two recent reports summarized several 307 meta-analyses, but failed to give a clear answer regarding the relationship between vitamin D 308 and the risk of GDM (22,23). 309

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

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

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

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

Recommended vitamin D supplementation varies from one country to another, and there is still no standard recommendation. The French National College of Gynecologists and Obstetricians recommends a single 100 000 IU dose of vitamin D administered at the beginning of the 7th month of pregnancy (40). The World Health Organization recommends a 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 during pregnancy could represent a simple and safe intervention to reduce the incidence of 345 GDM. Several randomized clinical trials on the effect of vitamin D supplementation on 346 pregnancy outcomes have been conducted. Some demonstrate a reduced incidence of GDM, 347 as well as correction of vitamin D deficiency, with sufficient vitamin D supplementation. 348 Shahgheibi et al., in a double-blind placebo-controlled randomized study including 90 Iranian 349 pregnant women at high risk of GDM, found a significantly higher incidence of GDM in the 350 placebo group than in the group receiving 5 000 IU per week of vitamin D until the 26th WA 351 (36). In their meta-analysis, Zang et al. found that vitamin D supplementation during 352 pregnancy seemed to reduce the incidence of GDM, but the results were not statistically 353 significant (RR 0.718, 95% CI 0.392-1.314). Their results also showed that vitamin D 354 supplementation influenced other biomarkers of GDM. It elevated blood 250HD significantly 355 (p<0.001), glutathione (p=0.003) and HDL cholesterol concentrations (p=0.04) as well as 356 reduced fasting insulin levels (p=0.001), fasting plasma glucose (p<0.001), insulin resistance 357 index (p<0.001), blood CRP levels (p=0.02), blood total cholesterol concentration (p=0.003) 358 and blood LDL cholesterol concentrations (p=0.003) (25). Other authors, such as Sablok et al. 359

and Tehrani et al., have failed to prove such an effect (37,38). Regarding our results, it seems
most unlikely that achieving a vitamin D sufficient status during early pregnancy is an
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.

- 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
- decrease as 250HD concentrations increase. Thus, our results do not enable us to establish a
- relationship between vitamin D and GDM risk. We suggest that among existing publications,
- authors retrospectively access their data and analyze the vitamin D level per subgroup.
- Furthermore, large studies are necessary in order to confirm our results and further support the
- 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.
- AB, CE and JCS conceived and designed the study.
- 383 JCS performed 250HD measurements.
- 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
- 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
- related to the accuracy or integrity of any part of the work are appropriately investigated and
- 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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552 ANNEXES

553 Figure 1: Flow chart



554

555 Table 1: Clinical and demographic characteristics at inclusion of women with

556 gestational diabetes (cases) and matched women without gestational diabetes (controls)*

557

	Cases	Controls ^a	Р-
Characteristics at inclusion	(N=250)	(N=941)	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/m ²)	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%)	
Automn/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%)	

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

^a Parity (primipara or more), age (<35 or \geq 35 years), body mass index before pregnancy (<25 kg/m2 or \geq 25

560 kg/m2), season of conception (autumn/winter or spring/summer) and phototype ($\langle V \text{ or } \geq V \text{ Fitzpatrick scale}$)

561 were matching factors.

562 ^b Logistic regression model

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

564 diabetes mellitus*

	Cases	Controls	OR [95% CI]
	N=250	N=941	P-value
Time of assay (WA)	12.9 ± 0.8	12.7 ± 0.9	0.003
Vitamin D supplementation in	6 (2.7%)	25 (2.8%)	1.07 [0.42-2.72]
the month before inclusion			p=0.89
Vitamin D supplementation	192 (80.3%)	798 (86%)	0.64 [0.43-0.94]
after inclusion			p=0.022
Time at first vitamin D	27.8 ± 3.4	27.8 ± 3.6	0.76
supplementation (WA)			
Vitamin D level in first	21.1 ± 10	22.7 ± 10	p=0.028
trimester (ng/mL)			
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
\geq 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 ≥20 ng/mL	125 (50%) 125 (50%)	394 (41·9%) 547 (58·1%)	1·42 [1·06-1·91], p=0·021 Ref
Vitamin D inadequacy <30 ng/mL ≥30 ng/mL	198 (79·2%) 52 (20·8%)	697 (74·1%) 244 (25·9%)	1·34 [0·94-1·90], p=0·1 Ref

*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 250HD was divided into 5 units intervals (ng/mL).
- 573



