1 **Relationship between vitamin D status in the first trimester of the pregnancy**

2 **and gestational weight gain: A mediation analysis**

3 **Authors names:** Mina Amiri[a], Maryam Rostami[b], Razieh Bidhendi-Yarandi[a, c], Aida

4 Fallahzadeh[d], Masoumeh Simbar[e], Fahimeh Ramezani Tehrani[a,]*

5 **Running title:** Maternal vitamin D and gestational weight gain

6 **Affiliations:**

    a. Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

    b. Department of Social Medicine, Faculty of Medicine, Ahvaz Jundishapure University of Medical Sciences, Ahvaz Iran.

    c. Department of Biostatistics, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

    d. Department of Midwifery and Reproductive Health, Faculty of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

    e. School of Medicine, Tehran University of Medical Science, Tehran, Iran.

16 ***Correspondence Author:** Fahimeh Ramezani Tehrani, MD,

17 Professor of Obstetrics and Gynecology

18 **Address:** Research Institute for Endocrine Sciences,

19 No 24, Parvane Street, Yaman Street, Velenjak, Tehran, Iran.

20 **Tel:** +98 22432500; **Fax:** +98 22416264; Email: ramezani@endocrine.ac.ir; framezan@post.harvard.edu

21

22

Abstract

Objective

To evaluate the total, and direct effects of vitamin D, measured by circulating 25-hydroxyvitamin D [25(OH)D] levels, on GWG after adjustment for confounding variables, and then assess the indirect effects by demonstrating the role of gestational age at birth as a mediator in this association.

Design

A secondary analysis of data collected in a screening program in pregnancy.

Setting and population

Data collected in "Khuzestan Vitamin D Deficiency Screening Program in Pregnancy" was used for the present study; it was included the data of 900 pregnant women referred to the health centers of Shushtar (Khuzestan Province, Iran), whose vitamin D status during the third trimester of pregnancy was available.

Methods

A mediation analysis was applied to detect the causal relationship between serum level of 25(OH)D, covariates (maternal age, parity, and baseline maternal weight), mediator (gestational age), and outcome (GWG).

Main outcome measures

The main outcome measure of the study was gestational weight gain.

Results

The adjusted total effect of vitamin D on GWG was estimated 0.0699 (95%CI: 0.0537, 0.0849; P=0.000). Although, an adjusted direct effect of vitamin D on GWG was not statistically

significant, the adjusted indirect effect of this micronutrient on GWG by considering gestational age as a mediator was found to be significant [0.059 (95%CI: 0.048, 0.0708; P=0.000)]. Women with severe vitamin D deficiency had the lowest speed as compared to moderate and normal levels.

Conclusion

This study shows that maternal vitamin D status affects the gestational weight gain by reducing the risk of preterm delivery.

Keywords: Vitamin D, gestational weight gain, preterm delivery

Tweetable abstract

The maternal vitamin D status can affect the gestational weight gain by reducing the risk of preterm delivery.

Introduction

Vitamin D, a fat-soluble vitamin and a pro-hormone, which can be synthesized from a steroid precursor other or a dietary source, is essential for calcium and phosphorus homeostasis, and bone mineralization. [1,2] In addition to vitamin D function to maintaining bone health, this micronutrient plays a crucial role in normal metabolism, and cellular growth, puberty, reproduction, immune system regulation, and prevention of some medical conditions, such as infectious, and cardio-metabolic disorders, cancers, depression, and cognitive deficits. [1-6]

Vitamin D insufficiency measured by circulating 25-hydroxyvitamin D [25(OH)D] levels, the best biomarker of vitamin D status, is considered a frequent disturbance among young women

68 particularly during pregnancy even in sunny regions, such as Iran, despite the intense sunlight in

69 these countries. [7-10] The prevalence of maternal vitamin D deficiency is estimated to be 54%

70 worldwide. [11]

71 Sufficient 25(OH)D concentrations are vital during pregnancy due to the increasing demand for

72 calcium during the growth and development of the fetus. [12, 13] Several studies have shown that

73 vitamin D deficiency in pregnant women is associated with adverse pregnancy outcomes, such as

74 preterm delivery, recurrent pregnancy losses, gestational diabetes [14-16], preeclampsia [17-19], primary

75 cesarean section rate [20, 21], depression [22, 23], and small-for-gestational-age (SGA) infants. [12, 24, 25]

76 Moreover, a limited number of studies have suggested that 25(OH)D concentration can be

77 related to gestational weight gain (GWG), although the results of these studies are still debated

78 and conflicting [26-28]. For example, a recent study showed that the associations between circulating

79 levels of 25(OH)D and GWG only among pre-gestational overweight women [26], whereas other

80 studies revealed no relationship between maternal vitamin D status and GWG. [27, 28]

81 Generally, mechanisms involved in this association are complex and discussed; however, based

82 on the available documents, 25(OH) D influences GWG may influence through both direct and

83 indirect effects. [29-34] Earlier studies have suggested that the effects of 25(OH) D on GWG may be

84 explained by biologic activities of this micronutrient on adipose tissue. Indeed, vitamin D

85 receptors (VDR) are present on human adipocytes and 25(OH) D appears to influence

86 lipogenesis, lipolysis, adipogenesis, and reducing adipose tissue inflammation. [29-32] Also due to

87 the anabolic effect of 25(OH)D on growth, vitamin D deficiency can be associated with impaired

88 maternal weight gain and fetal growth among vitamin D deficient mothers. [33] On the other hand,

89 it has shown that insufficient GWG is associated with an increased risk of adverse pregnancy

90 outcomes, such as preterm delivery. [35-37] There is strong evidence demonstrating an increased risk

91 of preterm delivery in mothers with vitamin D deficiency [34, 38] and dilution of this risk in women

92 treated with supplementation [39]; hence, preterm delivery due to insufficient serum level of

93 25(OH) D may mediate the association between this micronutrient and GWG. According to this

94 hypothesis, we aimed to evaluate the total, and direct effects of 25(OH)D level on GWG after

95 adjusting for confounding variables, and then assess the indirect effects by demonstrating the

96 role of gestational age at birth as a mediator in this association.

Materials and Methods

Study design and participants

99 This study was carried out on the data collected from the Khuzestan Vitamin D Deficiency

100 Screening Program in Pregnancy. The details of the study procedure have been reported

101 previously. [39] Briefly, this study was a stratified randomized field trial, consisting of two phases.

102 In the first phase, 1600 and 900 first-trimester pregnant women, referred to the health centers of

103 Masjed-Soleyman and Shushtar (Khuzestan Province, Iran), were recruited, respectively, and

104 fasting blood samples were collected. The serum samples of the participants in Shushtar were

105 stored and kept frozen at -80°C until further assays at the end of the study, whereas the vitamin

106 D status of participants in Masjed-Soleyman was immediately determined. In the second phase

107 of this study, the subjects with vitamin D deficiency from Masjed-Soleyman were assigned a

108 treatment regimen and received vitamin D3 supplementation until delivery. Other samples were

109 collected in the third trimester of pregnancy from all participants. Since participants from

110 Masjed-Soleman were treated with vitamin D supplementation, this study was conducted only on

111 participants referred to the health centers of Shushtar.

112 Participants received standard prenatal care, and both maternal and neonatal outcomes were

113 recorded. The adverse pregnancy outcomes included preterm delivery (birth at <37 weeks of

114   gestation), PE (systolic blood pressure >140 mmHg or diastolic blood pressure ≥90 mmHg and

115   24-hour proteinuria ≥ 0.3 g, initiated at >20 weeks of gestation), and GDM (glucose intolerance

116   first detected during pregnancy, based on the criteria of the International Association of Diabetes

117   and Pregnancy Study Groups). The study participants were classified into three groups according

118   to their serum concentration of 25(OH)D as severely deficient (<10 ng/mL), moderately deficient

119   (10 to 20 ng/mL), and >20 ng/mL.

120   **Clinical and laboratory measurements**

121   Trained examiners assessed the clinical and anthropometric measurements for all participants at

122   baseline and third trimester of pregnancy. All participants were interviewed for

123   sociodemographic, their history of pregnancies, and to obtain medical, obstetrics, and family

124   histories using pretested questionnaires. Adverse pregnancy outcomes were defined based on the

125   standard diagnostic criteria. At the time of data collection, women were asked about their history

126   of preeclampsia, based on a self-reporting questionnaire at each follow-up, details of which have

127   been previously published. [39]

128   Serum levels of 25(OH)D were assayed for all participants at baseline and third trimester of

129   pregnancy. Circulating 25(OH)D levels were assayed using the ELISA method and a kit of

130   Immunodiagnostics Systems by Auto Analyzer (Human Corporation, Germany). The inter-assay

131   and intra-assay coefficients of variation were 3.891% and 3.37%, respectively (sensitivity of 5

132   nmol/L). Calibration of the instruments was done as per the manufacturer's instructions, and

133   validation studies were done before the test. Samples were analyzed by a single technician using

134   the same equipment throughout the study in a reference laboratory and were measured according

135   to standard operating procedures.

136   **Outcome of interest**

137 The gestational weight gain was considered as the outcome of interest of the study, which was

138 assessed at the end of each trimester of the pregnancy.

139 **Statistical analysis**

140 A mediation analysis was applied to detect the casual relationship between covariates, mediator,

141 and outcome variables. In this mediation analysis, gestational weight gain was considered as the

142 outcome of interest, vitamin D in the first trimester of pregnancy as the main exposure,

143 gestational age as a mediator, and maternal age, parity, and baseline maternal weight as the

144 potential confounding variables.

145 Once the crude analysis was applied to estimate the total, direct and indirect effects. The

146 estimated regression coefficient (95%CI) for total effect showed the overall mean of gestational

147 weight gain per increase of one unit vitamin D, regardless of any other covariates as the

148 mediators. The estimated regression coefficient (95%CI) for direct effect showed the mean of

149 weight gain during pregnancy per increase of one unit vitamin D, considering gestational age as

150 the confounding variable. Also, the estimated regression coefficient (95%CI) for indirect effect

151 showed the mean of weight gain during pregnancy per increase of one unit vitamin D,

152 considering gestational age as a mediator. The analysis was repeated to adjust maternal age,

153 parity, and baseline weight as the potential confounding variables. Bootstrap confidence intervals

154 and standard error were estimated through the bootstrap approach with replacement sampling of

155 10000. A direct acyclic graph (DAG) as the casual diagram was drawn to show the casual

156 relationships.

157   A trajectory plot with a fitted regression model was also used to show the trend of weight during

158 pregnancy trimesters for women with three group levels of vitamin D in the first trimester of

159 pregnancy (normal, moderate, and severe deficiency).

7

160   The analyses were conducted by SPSS software version 21 (SPSS Inc., Chicago, IL); PROCESS

161   v3.5 for the SPSS package was used to estimate total, direct, and indirect effects.

Results

163

164   Table 1 present the baseline characteristics and pregnancy outcomes of the study population. The

165   study participants had a mean age ± SD of 29 ± 5 years and mean weight at baseline (SD) of 66.2

166   ± 7.1 kg. The median and IQR of the vitamin D level at baseline were 11.3 (8, 16.5) ng/ml. Table

167   2 shows the results of the crude mediation model. The total effect of vitamin D on GWG was

168   estimated 0.070 (95% CI: 0.055, 0.086; P= 0.000), which means the overall mean of gestational

169   weight gain was increased by 0.07 gram per each one-unit increase of vitamin D. After

170   adjustment for gestational age as confounding variable, vitamin D had no significant direct effect

171   on GWG [0.009 (95%CI: -0.007, 0.027; P=0.256)]. By considering the gestational age as a

172   mediator and estimating the indirect relationships through casual paths vitamin D→GA→WG,

173   vitamin D showed a significant effect on WG by 0.06 (95%CI: 0.049, 0.072; P=0.000); and the

174   mean of gestational weight gain was increased by 0.06 gram per each one-unit increase of

175   vitamin D. The causal relationships vitamin D in the first trimester of pregnancy and GWG is

176   presented in Figure 1.

177   Results of the mediation analysis adjusted for maternal age, parity, and baseline maternal weight

178   were presented in table 3. The adjusted total effect of vitamin D on WG was estimated 0.0699

179   (95%CI: 0.0537, 0.0849; P=0.000). Although, adjusted direct effect of vitamin D on WG was not

180   statistically significant [0.0105(95%CI: -0.007, 0.028; P=0.236)], the adjusted indirect effect of

181   this pro-hormone on WG considering GA as mediator was found to be significant [0.059(95%CI:

182   0.048, 0.0708; P=0.000)]. Figure 2 shows the causal relationships for this adjusted mediation

183   analysis.

184   Figure 3 shows the trajectory plot with the fitted regression model; it illustrates an increasing

185   trend of weight gain during pregnancy trimesters for women with different levels of 25(OH)D;

186   women with severe vitamin D deficiency had the lowest increasing speed as compared to

187   moderate and normal levels.

188   **Discussion**

189   This study was conducted to demonstrate the causal pathways between maternal vitamin D status

190   and gestational weight gain. Our findings demonstrate that 25(OH)D level in the first trimester of

191   pregnancy had a significant positive total effect on GWG, finding that remained significant after

192   adjustment for confounders, such as maternal age, parity, and baseline weight. While we found

193   no direct effect between 25(OH)D and GWG after adjustment for gestational age at birth (with or

194   without adjusting other confounders), when the gestational age was considered as a mediator, a

195   significant indirect effect was detected.

196   Overall, our study results showed the positive total effect of 25(OH)D on the GWG. We also

197   observed an increasing trend of GWG during pregnancy trimesters in both groups of women with

198   normal 25(OH)D level, and those with insufficiency, although mothers with severe vitamin D

199   deficiency had a lower GWG, compared to women with moderate deficiency and normal levels.

200   Our findings are in line with previous studies. A randomized controlled trial (RCT) conducted by

201   Hashemipour et al. showed that treatment of pregnant women with vitamin D deficiency resulted

202   in greater maternal weight gain during pregnancy. Another study conducted by Brooke et al.

203   showed that women treated with vitamin D supplementation gained weight faster in the late

204   trimester than those in the control group. [40] A meta-analysis of randomized controlled trials and

205   observational studies conducted by Thorne-Lyman and Fawzi [25] showed a greater average daily

206   weight gain in the third trimester of gestation among women supplemented with vitamin D. In

207   contrast to our results, a cohort study conducted by Figueiredo et al. showed that women who

208   had vitamin D inadequacy presented a higher increase in total gestational weight gain compared

209   to those with vitamin D adequacy and this association was present only in overweight women [26];

210   they explained their results by the fact that vitamin D modulates adipogenesis and apoptosis and

211   thus regulates adipose tissue growth and also inflammation. [41, 42] A possible explanation for the

212   difference between the results of our study and those of Figueiredo et al. is that in this study we

213   measured vitamin D once at 14 gestational weeks, while Figueiredo et al. measured it three times

214   throughout pregnancy and also the vitamin D inadequacy group in their study had a small sample

215   size. Also, in contrast with our results, Shakeri et al. [27] and Nobles et al. [28] found no relationship

216   between maternal vitamin D status and gestational weight gain. This discrepancy could be due to

217   differences in studies designs. For example, Shakeri et al. [27] measured vitamin D levels in the

218   third trimester, while our study measured it at the first trimester of the pregnancy. Also, the study

219   of Nobles et al. [28] conducted on women at risk for gestational diabetes mellitus, whereas this

220   study has conducted on healthy women. None of these studies adjusted the results for

221   confounding factors, while we adjusted our results by maternal age, parity, and baseline weight.

222   Although some previous studies have been reported overall effects of 25(OH)D level on GWG,

223   the exact mechanisms through which vitamin D may affect GWG have not yet clarified. [26-28] In

224   other words, it has not understood whether this prohormone has a direct, indirect, or both effects

225   on GWG. It is well documented that adipose tissue, the main storage site for vitamin D,

226   expresses vitamin D receptors (VDR) and enzymes involved in vitamin D metabolism. [43-45]

227   Vitamin D has both stimulating and inhibiting effect on adipogenesis and modulatory effect on

228   adipose tissue inflammation and energy homeostasis. [46] Moreover, vitamin D deficiency is

229   associated with anorexia and malaise which may explain the poor weight gain among pregnant

230   women with vitamin D inadequacy. [47] Also due to the anabolic effect of vitamin D on growth,

231   vitamin D deficiency is associated with impaired maternal weight gain and fetal growth among

232   vitamin D deficient mothers. [33] Despite the mentioned mechanisms, this study showed no direct

233   effect of 25(OH)D on GWG. Since several factors could affect the GWG, we adjusted our results

234   for important potential confounders, such as maternal age, parity, and baseline weight; our

235   results remained unchanged after these adjustments.

236   Interestingly, when gestation age at birth was considered as a mediator factor, our results

237   detected an indirect association between 25(OH)D level and GWG. Indeed, the role of

238   gestational age as a mediator can be explained by the possible effect of vitamin D on decreasing

239   the risk of preterm delivery. It has been shown that vitamin D supplementation could decrease

240   the risk of preterm delivery up to 40%. [39] Other reports also found an inverse association between

241   maternal vitamin D and preterm delivery. [34, 38] Vitamin D is a potent regulator of inflammation in

242   the placenta, this may explain the linkage between vitamin D with pathologic conditions such as

243   pre-eclampsia [48]. Vitamin D also regulates target genes associated with proper implantation of

244   the placenta and is important for pregnancy maintenance through being related to calcium

245   metabolism in myometrium. [49, 50] In addition, vitamin D influences other aspects of immunity,

246   especially the stimulation of antimicrobial innate immune response [51, 52] and direct role in the

247   production of antimicrobial peptides such as cathelicidin [53] thus play an important role in

248   preventing infection during pregnancy. Intrauterine infections lead to preterm delivery through

249   mechanisms related to activation of the innate immune system. [54] The mechanism through which

250   vitamin D reduces the risk of preterm delivery could be explained due to the effect of vitamin D

251   on innate immune and antimicrobial responses in placental cells and preventing infections during

252 pregnancy. These mechanisms emphasize on the indirect effects of vitamin D on the GWG,

253 which are mediated by gestational age at birth.

254 To the best of our knowledge, this is the first population-based study to determine the total,

255 direct, and indirect effects of serum level of 25(OH)D and GWG. The main strengths of this

256 study were the population-based design, the relatively large sample size, and the use of

257 appropriate statistical methods for data analysis, with adjustments for important confounders

258 such as age, parity, and maternal weight at baseline. It is important to emphasize that the present

259 study has some limitations. First, as data collection was carried out throughout the year, we were

260 unable to adjust for seasonal variance; second, we were not able to recruit liquid chromatography

261 technique to quantify 25(OH)D values; however, the ELISA technique is considered as a reliable

262 method when performed by experienced staff. [55] Finally, due to the diversity of food supplies, we

263 had no information on vitamin D dietary intakes in our participants.

264 **Conclusion**

265 In conclusion, this study shows that maternal vitamin D status affects the gestational weight gain

266 by reducing the risk of preterm delivery. Therefore, the detection and treatment of women with

267 vitamin D inadequacy can improve the trend of their weight gain by reducing the risk of preterm

268 delivery. However, further studies with a prospective design and more comprehensive measures

269 are warranted to disentangle the association between vitamin D status and total gestational

270 weight gain.

271 **Disclosure of interests**

272 The authors declare no competing interests. Completed dis-closure of interest forms are available

273 to view online assupporting information.

274 **Contribution to authorship**

275 MA was involved in the study design, managed the literature search, interpretation of data, and

276 manuscript drafting. MR was involved in the study design and data collection, carried out the

277 sample analysis, and manuscript drafting. RBY was contributed in statistical analyses,

278 interpreting of data, and manuscript drafting. AF was involved in searching literature,

279 interpretation of data, and manuscript drafting. MS was contributed in the study design,

280 interpretation of data, and manuscript drafting. FRT was involved in the study conception and

281 design and carried out the analysis and interpretation of data, managed the literature search, and

282 manuscript drafting.

293
294 **References:**

295 1.   Brown AJ, Dusso A, Slatopolsky E. Vitamin D. Am J Physiol. 1999 Aug;277(2):F157-

296 75.

297 2.   Holick MF. Vitamin D and bone health. J Nutr. 1996 Apr;126(4 Suppl):1159s-64s.

298   3.   Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskeletal health and the

299   need for supplementation. Nutrients. 2013 Jan 10;5(1):111-48.

300   4.   Skaaby T, Husemoen LLN, Pisinger C, Jørgensen T, Thuesen BH, Fenger M, et al.

301   Vitamin D status and incident cardiovascular disease and all-cause mortality: a general

302   population study. Endocrine. 2013;43(3):618-25.

303   5.   Barnard K, Colón-Emeric C. Extraskeletal effects of vitamin D in older adults:

304   cardiovascular disease, mortality, mood, and cognition. Am J Geriatr Pharmacother. 2010

305   Feb;8(1):4-33.

306   6.   Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D

307   in reducing cancer risk and progression. Nature reviews cancer. 2014;14(5):342-57.

308   7.   Hovsepian S, Amini M, Aminorroaya A, Amini P, Iraj B. Prevalence of vitamin D

309   deficiency among adult population of Isfahan City, Iran. Journal of health, population, and

310   nutrition. 2011;29(2):149.

311   8.   Heshmat R, Mohammad K, Majdzadeh S, Forouzanfar M, Bahrami A, Ranjbar Omrani

312   G. Vitamin D deficiency in Iran: A multi-center study among different urban areas. Iran J Public

313   Health. 2008;37(1):72-8.

314   9.   Badfar G, Shohani M, Mansouri A, Soleymani A, Azami M. Vitamin D status in Iranian

315   pregnant women and newborns: a systematic review and meta-analysis study. Expert Rev

316   Endocrinol Metab. 2017 Sep;12(5):379-89.

317   10.   Tolppanen A-M, Fraser A, Fraser WD, Lawlor DA. Risk factors for variation in 25-

318   hydroxyvitamin D3 and D2 concentrations and vitamin D deficiency in children. The Journal of

319   Clinical Endocrinology & Metabolism. 2012;97(4):1202-10.

320   11.   Saraf R, Morton SM, Camargo CA, Jr., Grant CC. Global summary of maternal and

321   newborn vitamin D status - a systematic review. Matern Child Nutr. 2016 Oct;12(4):647-68.

322   12.   Ponsonby AL, Lucas RM, Lewis S, Halliday J. Vitamin D status during pregnancy and

323   aspects of offspring health. Nutrients. 2010 Mar;2(3):389-407.

324   13.   Fernández-Alonso AM, Dionis-Sánchez EC, Chedraui P, González-Salmerón MD, Pérez-

325   López FR. First-trimester maternal serum 25-hydroxyvitamin D_3 status and pregnancy outcome.

326   Int J Gynaecol Obstet. 2012 Jan;116(1):6-9.

327   14.   Burris HH, Rifas-Shiman SL, Kleinman K, Litonjua AA, Huh SY, Rich-Edwards JW, et

328   al. Vitamin D deficiency in pregnancy and gestational diabetes mellitus. American Journal of

329   Obstetrics and Gynecology. 2012 2012/09/01/;207(3):182.e1-.e8.

330   15.   Bener A, Al-Hamaq AO, Saleh NM. Association between vitamin D insufficiency and

331   adverse pregnancy outcome: global comparisons. Int J Womens Health. 2013;5:523-31.

332   16.   Lacroix M, Battista MC, Doyon M, Houde G, Ménard J, Ardilouze JL, et al. Lower

333   vitamin D levels at first trimester are associated with higher risk of developing gestational

334   diabetes mellitus. Acta Diabetol. 2014 Aug;51(4):609-16.

335   17.   Scholl TO, Chen X, Stein TP. Vitamin D, secondary hyperparathyroidism, and

336   preeclampsia. Am J Clin Nutr. 2013 Sep;98(3):787-93.

337   18.   Bodnar LM, Simhan HN, Catov JM, Roberts JM, Platt RW, Diesel JC, et al. Maternal

338   vitamin D status and the risk of mild and severe preeclampsia. Epidemiology. 2014

339   Mar;25(2):207-14.

340   19.   Ullah MI, Koch CA, Tamanna S, Rouf S, Shamsuddin L. Vitamin D deficiency and the

341   risk of preeclampsia and eclampsia in Bangladesh. Horm Metab Res. 2013 Sep;45(9):682-7.

342   20.   Merewood A, Mehta SD, Chen TC, Bauchner H, Holick MF. Association between

343   vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab. 2009

344   Mar;94(3):940-5.

345   21.   Scholl TO, Chen X, Stein P. Maternal vitamin D status and delivery by cesarean.

346   Nutrients. 2012 Apr;4(4):319-30.

347   22.   Brandenbarg J, Vrijkotte TG, Goedhart G, van Eijsden M. Maternal early-pregnancy

348   vitamin D status is associated with maternal depressive symptoms in the Amsterdam Born

349   Children and Their Development cohort. Psychosom Med. 2012 Sep;74(7):751-7.

350   23.   Cunha Figueiredo AC, Trujillo J, Freitas-Vilela AA, Franco-Sena AB, Rebelo F, Cunha

351   GM, et al. Association between plasma concentrations of vitamin D metabolites and depressive

352   symptoms throughout pregnancy in a prospective cohort of Brazilian women. J Psychiatr Res.

353   2017 Dec;95:1-8.

354   24.   Pérez-López FR. Vitamin D: the secosteroid hormone and human reproduction. Gynecol

355   Endocrinol. 2007 Jan;23(1):13-24.

356   25.   Thorne-Lyman A, Fawzi WW. Vitamin D during pregnancy and maternal, neonatal and

357   infant health outcomes: a systematic review and meta-analysis. Paediatr Perinat Epidemiol. 2012

358   Jul;26 Suppl 1(0 1):75-90.

359   26.   Figueiredo ACC, Carrilho TRB, Batalha MA, Farias DR, Barros EG, Kac G. Association

360   between vitamin D status during pregnancy and total gestational weight gain and postpartum

361   weight retention: a prospective cohort. European Journal of Clinical Nutrition. 2020

362   2020/01/01;74(1):126-34.

363 27.   Shakeri M, Jafarirad S. The relationship between maternal vitamin D status during third

364 trimester of pregnancy and maternal and neonatal outcomes: A longitudinal study. Int J Reprod

365 Biomed (Yazd). 2019 Jan;17(1):33-40.

366 28.   Nobles CJ, Markenson G, Chasan-Taber L. Early pregnancy vitamin D status and risk for

367 adverse maternal and infant outcomes in a bi-ethnic cohort: the Behaviors Affecting Baby and

368 You (B.A.B.Y.) Study. Br J Nutr. 2015 Dec 28;114(12):2116-28.

369 29.   Cianferotti L, Demay MB. VDR-mediated inhibition of DKK1 and SFRP2 suppresses

370 adipogenic differentiation of murine bone marrow stromal cells. 2007;101(1):80-8.

371 30.   Blumberg JM, Tzameli I, Astapova I, Lam FS, Flier JS, Hollenberg AN. Complex role of

372 the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. J Biol Chem. 2006 Apr

373 21;281(16):11205-13.

374 31.   Abbas MA. Physiological functions of Vitamin D in adipose tissue. J Steroid Biochem

375 Mol Biol. 2017 Jan;165(Pt B):369-81.

376 32.   McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding

377 catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol

378 on body weight. Med Hypotheses. 2003 Nov-Dec;61(5-6):535-42.

379 33.   Marya RK, Rathee S, Dua V, Sangwan K. Effect of vitamin D supplementation during

380 pregnancy on foetal growth. Indian J Med Res. 1988 Dec;88:488-92.

381 34.   McDonnell SL, Baggerly, K. A., Baggerly, C. A., Aliano, J. L., French, C. B., Baggerly,

382 L. L., Ebeling, M. D., Rittenberg, C. S., Goodier, C. G., Mateus Niño, J. F., Wineland, R. J.,

383 Newman, R. B., Hollis, B. W., & Wagner, C. L. . Maternal 25(OH)D concentrations ≥40 ng/mL

384 associated with 60% lower preterm birth risk among general obstetrical patients at an urban

385 medical center. PloS one. 2017;12.

386   35.   Nohr EA, Vaeth M, Baker JL, Sørensen T, Olsen J, Rasmussen KM. Combined

387   associations of prepregnancy body mass index and gestational weight gain with the outcome of

388   pregnancy. Am J Clin Nutr. 2008 Jun;87(6):1750-9.

389   36.   Siega-Riz AM, Viswanathan M, Moos MK, Deierlein A, Mumford S, Knaack J, et al. A

390   systematic review of outcomes of maternal weight gain according to the Institute of Medicine

391   recommendations: birthweight, fetal growth, and postpartum weight retention. Am J Obstet

392   Gynecol. 2009 Oct;201(4):339.e1-14.

393   37.   Sarwer DB, Allison KC, Gibbons LM, Markowitz JT, Nelson DB. Pregnancy and

394   obesity: a review and agenda for future research. J Womens Health (Larchmt). 2006 Jul-

395   Aug;15(6):720-33.

396   38.   Amegah AK, Klevor MK, Wagner CL. Maternal vitamin D insufficiency and risk of

397   adverse pregnancy and birth outcomes: A systematic review and meta-analysis of longitudinal

398   studies. PLoS One. 2017;12(3):e0173605.

399   39.   Rostami M, Tehrani FR, Simbar M, Bidhendi Yarandi R, Minooee S, Hollis BW, et al.

400   Effectiveness of Prenatal Vitamin D Deficiency Screening and Treatment Program: A Stratified

401   Randomized Field Trial. J Clin Endocrinol Metab. 2018 Aug 1;103(8):2936-48.

402   40.   Brooke OG, Brown IR, Bone CD, Carter ND, Cleeve HJ, Maxwell JD, et al. Vitamin D

403   supplements in pregnant Asian women: effects on calcium status and fetal growth. British

404   medical journal. 1980;280(6216):751-4.

405   41.   Sun X, Zemel MB. Role of uncoupling protein 2 (UCP2) expression and 1alpha, 25-

406   dihydroxyvitamin D3 in modulating adipocyte apoptosis. Faseb j. 2004 Sep;18(12):1430-2.

407   42.   Gao D, Trayhurn P, Bing C. 1,25-Dihydroxyvitamin D3 inhibits the cytokine-induced

408   secretion of MCP-1 and reduces monocyte recruitment by human preadipocytes. Int J Obes

409   (Lond). 2013 Mar;37(3):357-65.

410   43.   Rosenstreich SJ, Rich C, Volwiler W. Deposition in and release of vitamin D3 from body

411   fat: evidence for a storage site in the rat. J Clin Invest. 1971 Mar;50(3):679-87.

412   44.   Ching S, Kashinkunti S, Niehaus MD, Zinser GM. Mammary adipocytes bioactivate 25-

413   hydroxyvitamin D_3 and signal via vitamin D_3 receptor, modulating mammary epithelial cell

414   growth. J Cell Biochem. 2011 Nov;112(11):3393-405.

415   45.   Li J, Byrne ME, Chang E, Jiang Y, Donkin SS, Buhman KK, et al. 1alpha,25-

416   Dihydroxyvitamin D hydroxylase in adipocytes. J Steroid Biochem Mol Biol. 2008 Nov;112(1-

417   3):122-6.

418   46.   Mutt SJ, Hyppönen E, Saarnio J, Järvelin M-R, Herzig K-H. Vitamin D and adipose

419   tissue—more than storage. 2014 2014-June-24;5(228).

420   47.   Specker B. Vitamin D requirements during pregnancy. Am J Clin Nutr. 2004 Dec;80(6

421   Suppl):1740s-7s.

422   48.   Liu NQ, Kaplan AT, Lagishetty V, Ouyang YB, Ouyang Y, Simmons CF, et al. Vitamin

423   D and the regulation of placental inflammation. J Immunol. 2011 May 15;186(10):5968-74.

424   49.   Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual

425   function. J Soc Gynecol Investig. 2004 Jul;11(5):263-71.

426   50.   Tribe RM. Regulation of human myometrial contractility during pregnancy and labour:

427   are calcium homeostatic pathways important? Exp Physiol. 2001 Mar;86(2):247-54.

428 51.   Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, et al. Vitamin d-directed

429 rheostatic regulation of monocyte antibacterial responses. J Immunol. 2009 Apr 1;182(7):4289-

430 95.

431 52.   Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor

432 triggering of a vitamin D-mediated human antimicrobial response. Science. 2006 Mar

433 24;311(5768):1770-3.

434 53.   Misawa Y, Baba A, Ito S, Tanaka M, Shiohara M. Vitamin D(3) induces expression of

435 human cathelicidin antimicrobial peptide 18 in newborns. Int J Hematol. 2009 Dec;90(5):561-70.

436 54.   Romero R, Espinoza J, Kusanovic J, Gotsch F, Hassan S, Erez O, et al. The preterm

437 parturition syndrome. 2006;113(s3):17-42.

438 55.   Hollis BW, Horst RL. The assessment of circulating 25(OH)D and 1,25(OH)2D: where

439 we are and where we are going. The Journal of steroid biochemistry and molecular biology.

440 2007;103(3-5):473-6.

441

442
443
444
445
446
447
448
449

450 **Table 1.** The baseline characteristics and pregnancy outcomes of the study population.
451

Quantitative variables	Mean (SD) or Median (IQR)
Age	29 (5)
Maternal weight at baseline (kg)	66.2 (7.1)
Maternal weight at the second trimester of the pregnancy (kg)	70.9 (7.2)
Maternal weight at the third trimester of the pregnancy (kg)	73.4 (7.4)
Gravidity	2 (1, 3)
Parity	1 (0, 2)

Vitamin D level at baseline (ng/mL)	11.3 (8, 16.5)
Vitamin D level at delivery (ng/mL)	11 (8, 17)
Neonatal vitamin D level at birth (ng/mL)	10 (6.4, 15.2)
Categorized variables	**n (%)**
Education status	
Illiterate	17 (1.9)
Under diploma	671 (76.5)
Diploma or academic	189 (21.6)
Occupational status	
Household	629 (71.7)
Self-employed	113 (12.9)
Employed	135 (15.4)
Stillbirth	
No	870 (99.2)
Yes	7 (0.8)
Preeclampsia	
No	739 (84.3)
Yes	138 (15.7)
Gestational diabetes	
No	823 (93.8)
Yes	54 (6)
Preterm delivery	
No	752 (85.7)
Yes	125 (14.3)

Abbreviations: SD: standard deviation; IQR: interquartile range; n: number

452

453
454

455
456
457

458
459
460
461
462
463
464
465
466
467
468
469

470 **Table 2.** Crude mediation model to estimate the total, direct and indirect effects of Vitamin D on
471 WG and GA as a mediator.

Parameter estimated	Beta	*Boot Std. Error	*95% Wald Confidence Interval		
			Boot Lower	**Boot Upper**	**Sig.**
Total effect of Vitamin D on WG	0.070	0.0080	0.055	0.086	0.000*
Direct Effect of vitamin D by adjusting Gestational age as confounding variable	0.009	0.0087	-0.007	0.027	0.256
Indirect effect of vitamin D by considering Gestational age as a Mediator	0.06				0.000*

21

	0.0059	0.0492	0.0722

472 * Number of bootstrap samples for percentile bootstrap confidence intervals and Standard Error: 10000
473

474

475

476

477

478

479

480

481

482

483

484

485

486

487

488

489

490

491

492

493

494 **Table 3.** Adjusted the mediation model to estimate the total, the direct and indirect effect of Vitamin D on
495 WG adjusting by maternal age, parity, and baseline weight as potential confounding and GA as a
496 mediator.
497

Parameter estimated	Beta	*Boot Std. Error	*95% Wald Confidence Interval		
			Boot Lower	Boot Upper	Sig.
The total effect of Vitamin D on WG adjusted by maternal age, parity, and baseline weight as confounding variables	.0699	.0079	.0537	.0849	0.000*

Direct Effect of vitamin D by adjusting Gestational age, maternal age, maternal age, parity, and baseline weight as confounding variables	.0105	.0087	-.0068	.0275	0.236
The effect of vitamin D by considering Gestational age as a Mediator, and maternal age, maternal age, parity, and baseline weight as confounding variables	.0585	.0059	.0477	.0708	0.000*

498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538

Direct effect: 0.12(95%CI: 0.11, 0.14)   **Direct effect: 0.48(95%CI: 0.41, 0.56)**

Gestational Age

Vitamin D in first Trimester of pregnancy

Weight Gain at delivery

Direct effect: 0.009(95%CI: -0.007, 0.027)
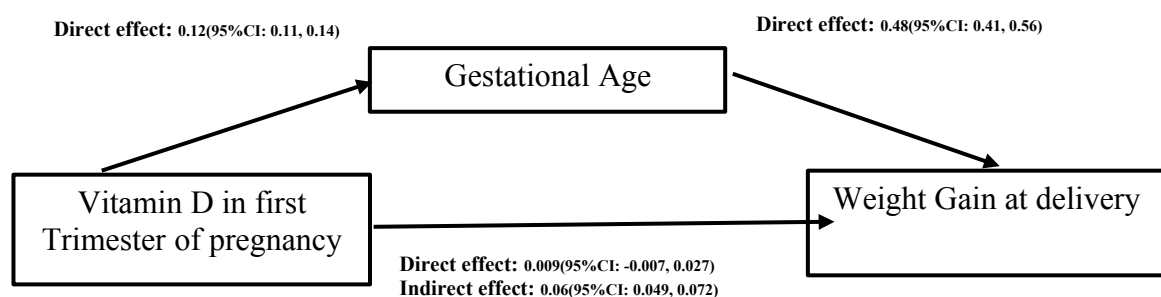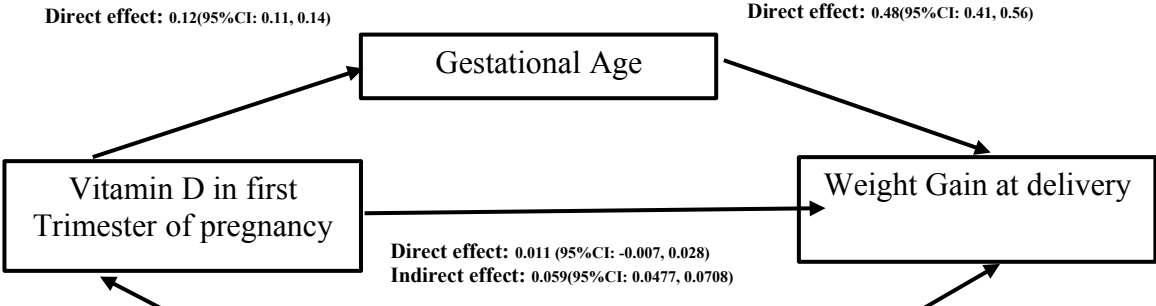**Indirect effect: 0.06(95%CI: 0.049, 0.072)**

539 **Figure 1.** A crude mediation model with a single mediator variable Gestational age causally located between
540 Vitamin D in the first trimester of pregnancy and Weight gain during pregnancy.
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585 **Direct effect: 0.12(95%CI: 0.11, 0.14)**   **Direct effect: 0.48(95%CI: 0.41, 0.56)**
586
587   Gestational Age
588
589
590
591   Vitamin D in first   Weight Gain at delivery
592   Trimester of pregnancy
593   **Direct effect: 0.011 (95%CI: -0.007, 0.028)**
594   **Indirect effect: 0.059(95%CI: 0.0477, 0.0708)**

595
596
597
598
599
600
601

Maternal age, parity, baseline weight

Figure 2. An adjusted mediation model with a single mediator variable Gestational age causally located between Vitamin D in the first trimester of pregnancy and Weight gain during pregnancy and Maternal age, Parity and baseline weight as the potential confounding variables.
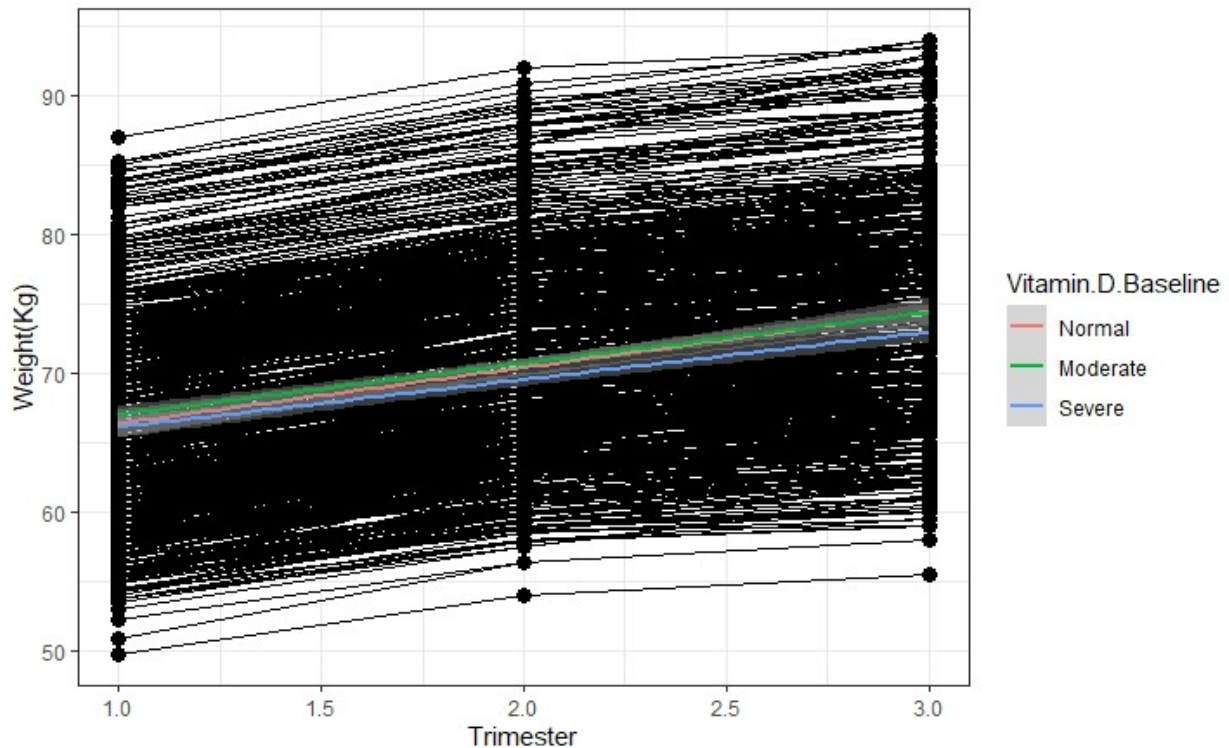
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620

Figure 3. Trajectory plot with a fitted regression model to show the trend of weight in pregnancy trimesters for women with three groups of vitamin D at baseline (Normal, moderate, and severe deficiency).

626
627
628