

Longitudinal Association of 25-Hydroxyvitamin D with Adipokines and Markers of Glucose Metabolism among Brazilian Pregnant Women

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Abstract

This study aimed to evaluate the longitudinal association of vitamin D status with glycaemia, insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), adiponectin and leptin. A prospective cohort with 181 healthy, pregnant Brazilian women was followed at the 5th–13th, 20th–26th, and 30th–36th gestational weeks. In this cohort, 25(OH)D plasma concentrations were analysed using liquid chromatography-tandem mass spectrometry. Vitamin D status was categorized as sufficient or insufficient using the Endocrine Society Practice Guidelines (ES) (≥ 75 / < 75 nmol/L) and the Institute of Medicine (IOM) (≥ 50 / < 50 nmol/L) thresholds. Linear mixed-effect regression models were employed to evaluate the association between vitamin D status and each outcome, considering the interaction terms between vitamin D status and gestational age ($P < 0.1$). At baseline, 70.7% of pregnant women had 25(OH)D levels < 75 nmol/L and 16% had levels < 50 nmol/L. Women with sufficient vitamin D status at baseline, using both the ES and IOM thresholds, presented lower glycaemia than those with insufficient 25(OH)D. Pregnant women with 25(OH)D concentrations < 75 nmol/L showed lower insulin ($\beta = -0.12$; 95% CI -0.251, 0.009; $P = 0.069$) and adiponectin ($\beta = -0.070$; 95% CI -0.150, 0.010; $P = 0.085$) concentrations throughout pregnancy than those with 25(OH)D levels ≥ 75 nmol/L. Pregnant women with 25(OH)D < 50 nmol/L at baseline presented significantly higher leptin concentrations than those with 25(OH)D levels ≥ 50 nmol/L ($\beta = -0.253$, 95% CI: -0.044; 0.550, $P = 0.095$). The baseline status of vitamin D influences the biomarkers involved in glucose metabolism. Vitamin D sufficient women at baseline had higher increases of insulin and adiponectin changes throughout gestation than those who were insufficient.

Introduction

Prevention of gestational diabetes mellitus (GDM) is a key intervention to circumvent the maternal and foetal consequences of this disease. The primary method of preventing GDM is by lifestyle changes, such as physical activity and diet⁽¹⁾. One important modifiable risk factor is vitamin D deficiency among pregnant women. Vitamin D deficiency is considered highly prevalent worldwide and often persists during pregnancy^(2,3). Studies have suggested that low concentrations of vitamin D may be associated with alterations in glucose metabolism⁽⁴⁾, can be a risk factor for GDM⁽⁵⁻⁷⁾ and are characterized by glucose intolerance or glycaemia values higher than the normal range when detected during pregnancy⁽⁸⁾.

One possible mechanism by which vitamin D may act on glucose metabolism is by increasing adiponectin secretion^(9,10). This adipokine has insulin sensitization properties of the target cells (liver, skeletal muscle and adipose tissue) and can enhance glucose uptake in muscle tissue⁽¹¹⁻¹³⁾. Adiponectin also protects β -cells from immune attack and reduces insulin resistance through immunoregulatory and anti-inflammatory effects⁽¹¹⁻¹⁴⁾. In pregnant women, adiponectin concentrations have been inversely associated with adverse maternal outcomes, such as GDM^(15,16), overweight, and obesity⁽¹⁷⁾. During pregnancy, a reduction in maternal plasma adiponectin concentrations is expected^(18,19). Many factors can influence the expression of this adipokine. Vitamin D concentration is one example^(13,20), as it down-regulates pro-inflammatory cytokines and up-regulates anti-inflammatory cytokines. Vitamin D appears to be positively associated with adiponectin gene expression, and suppresses the renin-angiotensin-aldosterone system (RAS) components secreted by adipocytes, thus contributing to increased adiponectin secretion⁽²⁰⁻²²⁾. Low 25(OH)D concentrations may then reduce the secretion of adiponectin and could be considered a risk factor for GDM and insulin resistance^(13,23,24).

Leptin is a hormone that regulates lipid metabolism and inflammation by influencing glucose metabolism in an inverse manner to adiponectin, i.e., it reduces insulin sensitivity⁽²⁵⁾. Leptin is directly associated with preeclampsia, GDM and macrosomia^(26,27). Studies have shown an inverse association between vitamin D and leptin concentrations⁽²⁸⁻³⁰⁾. The mechanism by which vitamin D metabolites impact leptin levels remains unclear. One possible mechanism is that the fat solubility of vitamin D can cause it to be sequestered in fat tissues, leading to a high level of intracellular calcium, which increases lipogenesis and decreases lipolysis⁽³¹⁾. A lower

serum vitamin D may lead to a higher serum leptin since its concentration is related to greater body fat mass^(32,33). However, randomized clinical trials have found no association between vitamin D supplementation and changes in leptin concentrations^(31,34).

There are few studies evaluating the association between vitamin D status, glycaemia and adipokines during pregnancy, particularly among healthy pregnant women without comorbidities such as GDM^(31,35,36). The aim of this study was to evaluate the vitamin D status of pregnant women in the first trimester and, if variation in vitamin D status during pregnancy was associated with changes in adiponectin, leptin and glycaemia concentrations throughout pregnancy among a cohort of healthy Brazilian women.

Methods

Study design

This study consists of a prospective cohort with healthy pregnant adult women from a public health care centre in Rio de Janeiro, Brazil, carried out from November 2009 to October 2011.

Two hundred and ninety-nine women were invited to participate in the study in accordance with the following eligibility criteria: (a) being between 5-13 weeks of gestation, (b) aged between 20 and 40 years, and (c) without any known infectious or chronic non-communicable diseases (except obesity). Criteria for exclusions after baseline clinical evaluation consisted of the following: miscarriages ($n=25$), chronic arterial hypertension ($n=3$), multiple gestation ($n=4$), diagnosis of infectious or non-communicable disease after starting the study ($n=11$), advanced pregnancy (≥ 14 weeks of gestation, $n=15$), prenatal care withdrawn ($n=7$), stillbirth ($n=5$), no vitamin D measured at baseline ($n=30$), self-reported glycaemia alteration ($n=12$), and no biochemical analyses performed ($n=1$).

Biochemical analyses

Venous blood samples were taken after a 12 h overnight fasting at three different visits (first, second, and third gestational trimesters). Samples were immediately centrifuged (5,000 rpm for 5 minutes), aliquoted and stored at -80°C until further analysis. Plasma samples were used to determine 25-hydroxyvitamin D [25(OH)D] (nmol/L), leptin (ng/dL) and adiponectin (ng/mL) concentrations.

Main independent variable

Plasma 25(OH)D concentrations were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Quest Diagnostics Nichols Institute laboratory (San Juan Capistrano, CA, USA) using the LC Thermo Cohesive System coupled to a Thermo Quantum Ultra Mass Spectrometer (Thermo Fisher; San Jose, CA, USA). This method is the gold standard with analytical measurement range of 10-640 nmol/L and the coefficient of variation <10%.

The 25(OH)D concentrations at baseline (5th–13th gestational weeks) were used to determine vitamin D status. Pregnant women were categorized as being vitamin D sufficient or insufficient, using two cut-points based on the Endocrine Society Practice Guidelines (ES) and the Institute of Medicine (IOM). According to the ES, vitamin D sufficiency and insufficiency are defined as having 25(OH)D concentrations ≥ 75 nmol/L and < 75 nmol/L, respectively⁽²⁾. Using the IOM cut-points, sufficiency and insufficiency were defined as 25(OH)D concentrations ≥ 50 nmol/L or < 50 nmol/L, respectively⁽³⁷⁾. There are controversies in the literature, as to which cut-point of vitamin D for insufficiency is the most appropriate. There is also no specific guideline set forth for pregnant women. We have decided to report both cut-points, to increase transparency and to allow for comparability between various studies in the literature.

Dependent variables

Serum fasting glycaemia was estimated by enzymatic colorimetric methods, using commercial kits (Linco Research, St. Charles, Missouri, USA and Wiener Lab., Rosario, Argentina, respectively) and insulin concentrations were assessed by enzyme-linked immunosorbent assay (ELISA) using specific commercial kits designed for humans (Millipore, St. Charles, MO, USA), with sensitivity of 2 μ U/ml. HOMA-IR was calculated based on the product of fasting insulin (μ U/mL) and glucose (mmol/L) divided by 22.5⁽³⁸⁾. The adipokines were evaluated using ELISA, with sensitivities of 0.78 ng/mL for adiponectin and 0.5 ng/dL for leptin.

Covariate assessment

A structured questionnaire administered at baseline was used to collect information on study population characteristics including: age (y), per-capita monthly household income (USD), parity (nulliparous or primiparous/multiparous) and self-

reported skin colour (white, black or mixed). Date of first blood collection was used to define the season at baseline (summer, autumn, winter and spring).

Anthropometric measurements were collected according to standardized procedures at baseline⁽³⁹⁾. Weight was measured using a digital scale (Filizzola PL 150, Filizzola Ltda., São Paulo, Brazil) at each gestational trimester and height was measured in duplicate using a portable stadiometer (Seca Ltda., Hamburg, Germany). The early pregnancy Body Mass Index (BMI) was calculated using the formula: weight at baseline (kg)/height² (m²). The gestational age was obtained from the first ultrasonography (USG) exam (92.3%; $n = 167$) or using the reported date of the last menstrual period if the USG was not performed before the 24th week of gestation (7.7%; $n = 14$).

The total dietary vitamin D intake (IU/day) was calculated using Nutritional Composition Tables from the Brazilian Institute of Geography and Statistics⁽⁴⁰⁾ according to a semi-quantitative food frequency questionnaire (FFQ)⁽⁴¹⁾. The FFQ was validated for the adult population of Rio de Janeiro⁽⁴¹⁾, and administered in the first trimester of gestation by trained interviewers and referring to intake of the last six months. Data on vitamin D supplementation was self-reported throughout pregnancy (first, second and third trimesters).

Statistical analysis

Data distribution was analysed according to the Shapiro-Wilk test. Maternal age, vitamin D intake, per-capita family income, early pregnancy BMI, glycaemia, insulin, HOMA-IR, adiponectin and leptin were not normally distributed (Shapiro-Wilk test p -value <0.05). The sample characteristics are described using medians and interquartile ranges (IQR). Categorical data are presented as absolute values (n) and relative frequencies (%). Baseline characteristics were stratified according to vitamin D status (sufficiency *v.* insufficiency). To compare medians and frequencies, the Mann-Whitney U test and the chi-squared test were used, respectively.

Dropout analyses were performed to verify the presence of selection bias comparing those who completed the study to those who were lost to follow-up. The following variables were considered: vitamin D status according to thresholds, dietary vitamin D intake, age, early pregnancy BMI, per-capita family income, glycaemia, insulin, HOMA-IR, adiponectin and leptin concentrations, parity, self-reported skin colour and season at recruitment.

Scatter plots containing longitudinal prediction, and 95% confidence intervals (CIs) were constructed to illustrate the variation in glycaemia and adipokine concentrations during pregnancy according to vitamin D baseline status.

Linear mixed effect (LME) models were performed in order to assess the longitudinal associations of vitamin D baseline status with the outcomes throughout pregnancy. The modelling process allows the inclusion of time-dependent and time-independent variables, it is robust to deal with unbalanced time intervals, and considers the correlation between repeated measures^(42,43). Interactions between vitamin D baseline status and gestational age were considered to explore the longitudinal behaviour of the evaluated outcomes. The LME models were adjusted for confounders selected by biological plausibility and statistical significance ($p < 0.2$) in the bivariate analysis for each of the five outcomes (glycaemia, insulin, HOMA-IR, adiponectin and leptin). The test to detect interactions was low powered. We considered $P < 0.1$ ⁽⁴⁴⁾ as a threshold to define statistical significance for the interaction terms and $P < 0.05$ for the main effect variables.

All analyses were performed in Stata 12.0 (Stata Corporation, College station, TX). The significant interactions identified in the LME models were illustrated by graphs plotted with RStudio 3.3.1 software⁽⁴⁵⁾.

Ethical approval

This study was conducted according to the guidelines of the Declaration of Helsinki. The Rio de Janeiro Federal University Maternity Hospital Ethics Committee (Protocol number: 0023.0.361.000-08), and the Municipal Secretary of Rio de Janeiro Ethics Committee (Protocol number: 0139.0.314.000-09) approved all procedures involving human subjects. All participants signed an informed consent about participation in the study.

Results

The baseline sample comprised of 181 pregnant women. From baseline to the second trimester follow-up, eleven women moved out from the prenatal program, two withdrew consent, four abandoned prenatal care at the study site, twelve missed the follow-up interview and one had no biochemical analyses performed, leaving a sample of 151 women at the second trimester. From the second to the third visit, six women were classified as lost to follow-up and seven had no biochemical analyses performed.

Eleven women missed the second trimester interview, but returned to the study in the third trimester. Therefore, our final sample at the third trimester comprised 149 women (Supplemental figure 1).

In total, 149 women completed the study and 32 were lost to follow-up. There were no significant differences between women who completed the study compared to those who were lost during follow-up (Supplemental table 1).

At baseline, women had a median age of 26 y (22.0-31.0), early pregnancy BMI of 23.7 kg/m² (21.9-27.0), per-capita family income of 292.4 USD (184.8-421.0) and reported a vitamin D intake of 134.2 IU/day (84.2-185.4), and 46.9% self-reported their skin colour to be mixed. The prevalence of vitamin D insufficiency was 70.7% (n=128/181) according to cut-offs set by ES and 16.0% (n=29/181) according the cut-offs proposed by the IOM. Women who began the study in the winter, compared to the summer, were more likely to be vitamin D insufficient (ES: 33.6% v. 15.6%, respectively, $P < 0.001$; IOM: 51.7% v. 3.5%, respectively, $P < 0.001$) (Table 1). None of the women who participated in the study received nutritional vitamin D supplementation (data not shown).

Pregnant women with vitamin D sufficiency at baseline showed lower glycaemia when compared with insufficient women (ES: median 79.5 v. 84.0 mg/L, respectively, $P=0.003$; IOM: median 83.0 v. 84.0 mg/L, respectively, $P=0.034$) (Table 2). The glycaemia decreased from the first to the second trimester, followed by a slight increase from the second to the third trimester onwards, in both women with vitamin D sufficiency and insufficiency at baseline (Fig. 1A and 1B). However, the rate of change throughout pregnancy for glycaemia did not show a significant difference between the baseline vitamin D groups (Table 3). Similar results were observed when 25(OH)D throughout pregnancy was analysed (Fig. 2A; 2B and Table 4).

The rate of change of insulin was greater among vitamin D sufficient women at baseline (ES: $\beta=0.166$; 95% CI 0.047, 0.285; $P=0.006$; IOM: $\beta=0.091$; 95% CI 0.019, 0.162; $P=0.013$) (Fig. 1C and 1D). There was a less-pronounced increase of insulin throughout pregnancy among vitamin D insufficient women compared to those who started with sufficient status according to ES (interaction $\beta=-0.12$; 95% CI -0.251, 0.009; $P=0.069$) (Table 3). We found significant results when 25(OH)D throughout pregnancy was analysed using the IOM thresholds (interaction $\beta=-0.153$; 95% CI -0.325, 0.018, $P=0.080$) (Fig. 2C and 2D and Table 4).

There was a tendency of lower HOMA-IR in the group of women classified as sufficient vitamin D status at baseline when compared with those with vitamin D insufficient status (ES: median 0.65 v. 0.93, respectively, $P=0.062$; IOM: median 0.82 v. 1.14, respectively, $P=0.071$) (Table 2). HOMA-IR increased throughout gestation among women with sufficient vitamin D status at baseline (ES: $\beta=0.019$; 95% CI 0.00001, 0.039; $P=0.050$) but did not change in the insufficient group (Fig. 1E and 1F). However, the results were not significant for this biomarker when baseline vitamin D-sufficient and insufficient women were compared (Table 3). We found significant results when 25(OH)D was analysed throughout pregnancy using the IOM thresholds (interaction $\beta=-0.030$; 95% CI -0.065, 0.005; $P=0.094$) (Fig. 2E; 2F and Table 4).

The trend in changes in adiponectin throughout pregnancy showed an increase from the beginning of pregnancy until the second trimester and was followed by a slight decrease at the end of gestation for both vitamin D status groups, whether measured at baseline or when 25(OH)D varied throughout pregnancy (Fig. 1G; 1H; 2G; 2H). Women with vitamin D insufficient status at baseline started gestation with higher median plasma levels of adiponectin, although this association was not significant (5.26 $\mu\text{g/ml}$ v. 4.49 $\mu\text{g/ml}$, $P=0.170$) (Table 2). However, the adiponectin rate of change throughout pregnancy was significantly higher in women with sufficient vitamin D levels at baseline (ES: $\beta=-0.070$; 95% CI -0.150, 0.010; $P=0.085$) (Table 3). The women with sufficient vitamin D status at baseline, according to IOM classification, tended to have a lower rate of change of adiponectin throughout pregnancy, although this did not reach significance (Table 3; Fig. 1H). The adiponectin rate of change throughout pregnancy was significantly higher in women with sufficient vitamin D levels when this marker varied throughout pregnancy (ES: $\beta=-0.056$; 95% CI -0.116, 0.004; $P=0.066$) (Table 4).

There were no significant differences in leptin concentrations, comparing each trimester individually, according to ES, but it was found that women with inadequate vitamin D status according to IOM presented higher leptin concentrations (Table 2). Women with insufficient vitamin D status according to ES had a significant decrease in leptin concentrations ($\beta=-0.026$; 95% CI -0.042, -0.009; $P=0.002$). Using the IOM cut-points, the decrease was significant only within the sufficient group ($\beta=-0.021$; 95% CI -0.036, -0.007; $P=0.004$) (Fig. 1I and 1J). Women with insufficient vitamin D levels presented a greater increase in leptin concentrations, in relation to the sufficient group,

according to IOM ($\beta=-0.253$; 95% CI -0.044, 0.550; $P=0.095$) (Table 3). These results did not hold when 25(OH)D varied throughout pregnancy (Fig. 2I; 2J; Table 4).

Discussion

The main result of this study is the difference in the variation of adiponectin and insulin concentrations throughout pregnancy according to vitamin D baseline status defined by the ES Guidelines. We observed that throughout pregnancy the rate of increase for both biomarkers was greater among women with vitamin D sufficiency compared with women who started their pregnancy in a vitamin D deficient state. Another important finding is that women with insufficient vitamin D at baseline had a higher increase in leptin levels during gestation compared to the sufficient group according to the IOM classification. Additionally, women classified with vitamin D sufficiency, independently of the cut-off criteria, had lower baseline glycaemia, although the rate of change of this biomarker did not differ between the two vitamin D status groups.

The definition of vitamin D sufficiency or insufficiency remains controversial. Currently, there are no specific cut-points for pregnant women, and the same cut-points are used for adults. It is well known that the thresholds for several biomarkers are rarely available for this period of life and the case is not different for vitamin D. Thus, more research is needed to establish the adequate cut-off point for this unique period in women's life. In the current study we opted to use two different thresholds presented by the two governing bodies of vitamin D status: the ES and the IOM. This strategy allows comparability with other studies in the literature and provides a broader picture of the studied associations in place. Another aspect of studies conducted during pregnancy that can be misleading is related to the timing vitamin D is assessed during pregnancy. The conclusions may vary if studies consider only baseline vitamin D, e.g. first trimester, what should be a representation of how preconception period affects the parameters evaluated throughout pregnancy. On the other end, if data is available, for several time points this can provide a clearer picture of the trajectory and how this may influence a specific outcome. In general, the directions of the rate of change trajectories for the studied outcomes were similar regardless of the 25(OH)D threshold used. Adiponectin was the exception. Pregnant women with sufficient vitamin D levels at baseline showed significantly higher levels of adiponectin when the ES criteria was used, while according to the IOM classification, they showed lower levels, but the results were not

significant. The results regarding the timing of vitamin D assessment yield slight conflicting results for insulin, HOMA-IR and leptin. Again, the direction of the associations remained the same, but the statistical significance of the interaction changed depending on the timing and threshold used. An important finding of this study is that differing conclusions can be made depending on the vitamin D deficiency threshold used or the timing when vitamin D status is evaluated. This is of particular importance when considering the conflicting results in the scientific literature.

The longitudinal data with three time points are an important strength of this study. We did not identify other studies assessing these associations with longitudinal data in the scientific literature. Moreover, the robust statistical analysis of the present study, taking into account the correlations between repeated measures and considering all women had information on at least two time points, is an additional strength. The method used to measure plasma 25(OH)D concentrations was LC-MS/MS, which is considered the gold standard for this marker. Furthermore, we also evaluated dietary vitamin D intake and supplementation. However, limitations, such as losses to follow-up, and the absence of information about sun exposure data, should be acknowledged. To assess whether the losses to follow-up biased our results we examined whether those women who were lost to follow-up differed from those who remained in the study. This analysis shows no significant differences in those who were lost to follow-up, which suggests that losses to follow-up occurred randomly in this study. We also adjusted all models for the season of the year at the time of blood collection, which was used as a proxy for sun exposure, since these data were not available. Additionally, we did not measure the expression of enzymes that are involved in the conversion of vitamin D and the vitamin D receptor (VDR) in the placenta to support extra-renal synthesis of 1,25(OH)₂D₃ during pregnancy⁽⁴⁶⁾. This could influence the outcomes; however, the maternal kidneys are likely to be the major source of increased maternal serum 1,25(OH)₂D₃ during pregnancy⁽⁴⁶⁾.

A high prevalence of vitamin D insufficiency according to the ES was previously reported in this cohort⁽⁴⁷⁾, which is similar to results found for pregnant women in other studies using this threshold^(48, 49). Schneuer et al. (2014) observed that 80.4% of 5,109 pregnant women from Australia had vitamin D insufficiency in the first trimester. Flood-Nichols et al. (2015) found that 70% of 235 American women had vitamin D insufficiency in early pregnancy, while 59.0% of 190 Brazilian pregnant women presented vitamin D insufficiency⁽⁵⁰⁾. We identified a low intake of vitamin D

during pregnancy (134.2 IU/day) in the present study by considering either the recommendation of the ES of 1500-2000 IU/day or the Brazilian recommendations (600 IU/day)⁽⁵¹⁾. We also observed that none of the women used vitamin D supplements, since this is not part of public prenatal care. In Brazil, only supplementation with iron (40 mg) and folic acid (400 mcg) are mandatory for pregnant women⁽⁵²⁾. Despite low vitamin D dietary intake, we have previously reported a longitudinal increase in 25(OH)D levels throughout pregnancy in this cohort of women⁽⁴⁷⁾. Additionally, the level of increase in 25(OH)D concentrations depended on seasonal variation. Pregnant women who started their pregnancy during the winter, spring or autumn seasons had a significant longitudinal increase in 25(OH)D concentrations, while women who began pregnancy during the summer season showed no significant changes in 25(OH)D concentrations throughout pregnancy⁽⁴⁷⁾.

In the current cohort, pregnant women with vitamin D insufficiency according to the ES had higher glycaemia in the first trimester compared to women with vitamin D sufficiency. This result corroborates findings from an earlier cross-sectional study with 155 Iranian pregnant women. That study showed a significant inverse association between first trimester 25(OH)D concentrations and fasting glucose ($r=0.238$; $P=0.003$)⁽⁵³⁾. On another cross-sectional study, Casey et al. (2018) evaluated the association between vitamin D and glycaemia markers at the third gestation trimester and found no significant association with fasting glucose or HOMA-IR⁽⁵⁴⁾. These results are in line with the present findings regarding the third trimester. It seems that the relationship between vitamin D and glucose concentrations may be mediated by the role of vitamin D on insulin action.

Due to increased maternal adiposity and placental production, increased insulin resistance is expected to occur during pregnancy from the 18th gestational week until the third trimester⁽⁵⁵⁾. Peripheral insulin sensitivity decreases in the third trimester, and the hepatic glucose production increases compared to the beginning of pregnancy⁽⁵⁶⁾. Vitamin D appears to play a role in insulin sensitivity, stimulating the expression of the insulin receptors and improving glycaemic metabolism⁽⁵⁷⁻⁵⁹⁾. Thus, glucose would be better absorbed by the cells and the glycaemia would be at lower levels.

The longitudinal analyses from the present study show that pregnant women with vitamin D sufficiency at baseline had sharper increases in insulin concentration compared to those with vitamin D insufficiency according to both thresholds used. The greater increase of insulin in the group with vitamin D adequacy was not followed by

significant longitudinal increases in glycaemia or HOMA-IR during pregnancy. The active metabolite of vitamin D [1,25(OH)₂D₃] has been shown to be involved in insulin secretion by the pancreas and glucose homeostasis⁽¹⁴⁾. Additionally, pancreatic β-cells are known to have vitamin D receptors and an insulin gene promoter that is stimulated by 1,25(OH)₂D₃, thus indicating a direct association between vitamin D and insulin⁽⁶⁰⁾. This mechanism could explain the greater insulin increase among women with vitamin D adequacy compared to those with vitamin D inadequacy in our sample. This result reinforces a small study performed with adults that found that subjects with vitamin D deficiency (<50 nmol/mL) secrete less insulin than subjects with adequacy status⁽⁶¹⁾.

A recent experimental study investigated the metabolic effects of adiponectin on maternal glucose and lipid metabolism by comparing pregnant rats with and without adiponectin deficiency^(62,63). The authors observed that adiponectin stimulated the β cell proliferation and impaired the islet mass without differences in insulin sensitivity effects in comparison to the deficient group^(4,62,63). This finding allows suggesting another mechanism on insulin besides the well-established insulin-sensitizing property of this adipokine^(62,63). In humans, pregnant women present this phenomenon of β cell proliferation without increase in islet mass, but this particular study was limited because it was conducted in women who died during pregnancy⁽⁶⁴⁾. However, it is possible that adiponectin also exerts an effect on human cell proliferation⁽⁶²⁾. Vitamin D could stimulate the adiponectin secretion, which in turn, stimulates beta-cell proliferation, increasing insulin secretion. This explanation would support our results of greater increases in adiponectin and insulin in pregnant women with vitamin D adequacy status during pregnancy.

We found an association between vitamin D status and the rate of change in adiponectin during pregnancy. In the present study, pregnant women with adequate levels of 25(OH)D in the first trimester showed a sharper increase in adiponectin concentration throughout pregnancy when compared to women with inadequate levels of vitamin D. Additionally, 1,25(OH)₂D is known to regulate the renin-angiotensin system (RAS) in a negative feedback loop by binding with a transcription factor necessary for the expression of renin⁽⁶⁵⁾. Inhibition of RAS results in increased levels of adiponectin and improved insulin sensitivity, which exerts influence on glycaemia⁽²⁰⁾. This mechanism of action can explain our result of higher increase of adiponectin among pregnant women with adequate 25(OH)D levels compared to those with inadequate levels.

Previous studies found direct associations between vitamin D and adiponectin independently of BMI in both young and adult populations⁽⁶⁶⁻⁶⁸⁾, but no studies were found with pregnant women.

No differences were observed in mean adiponectin and leptin concentrations between pregnant women with vitamin D adequacy or inadequacy at each gestational trimester, i.e., cross-sectional analysis. Similarly, a case-control study conducted by McManus et al. (2014)⁽⁶⁹⁾ also found no correlation between 25(OH)D and adipokine concentrations during the third trimester in both healthy pregnant women and pregnant women with GDM.

A meta-analysis conducted in 2016 showed that vitamin D supplementation increased the serum leptin concentrations although a meta-analysis using cross-sectional studies with non-pregnant subjects found inverse associations between 25(OH)D and leptin levels (Fisher's $Z=-0.93$; 95% CI: $-0.95, -0.91$)⁽³¹⁾. In the present cohort, it was observed that pregnant women with vitamin D sufficiency showed a less pronounced increase in leptin until the second trimester and a higher decrease until the end of gestation, although this did not reach significance. Similarly, Walsh et al. (2013) evaluated the correlation between 25(OH)D and leptin levels at early pregnancy and 28 weeks of gestation and found a negative association that was not significant⁽⁷⁰⁾.

Conclusion

Pregnant Brazilian women with vitamin D adequacy [$25(\text{OH})\text{D} \geq 75 \text{ nmol/L}$] at the first trimester had a higher rate of increase in adiponectin and insulin concentrations throughout pregnancy compared to women with vitamin D inadequacy [$25(\text{OH})\text{D} < 75 \text{ nmol/L}$]. These results indicate that vitamin D status at the beginning of gestation may influence the changes in adiponectin and insulin during pregnancy. Although these women had higher values of insulin, there were no higher glycaemia values, showing that there was an adequate metabolic response. This result reinforces the importance of monitoring the vitamin D status of pregnant women in the first trimester of pregnancy in order to achieve a favourable metabolic profile for reducing the risk of undesired pregnancy outcomes. Given the scarcity of studies, there is a need for research involving pregnant women to evaluate this relationship and to provide further evidence on this topic.

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Benaim C, Cocate P, Kac G formulated the research question, designed the study and developed the protocol. Benaim C and Cocate P conducted the statistical analysis, and Alves-Santos NH provided support for statistical analysis. Barros E, Franco-Sena AB, Figueiredo A, Lepsch J, Adegboye AA, Holick M and Mokhtar R assisted with the literature searches and with the writing of the manuscript. All authors substantially have contributed to and have approved the final version of this manuscript.

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Table 1. Baseline characteristics of the study population according to vitamin D sufficiency status in first trimester of pregnancy.

	Endocrine Society Guidelines				Institute of Medicine		
	Total n=181	Sufficiency 25 (OH)D ≥75nmol/L n=53	Insufficiency 25 (OH)D <75nmol/L n=128	P-value ¹	Sufficiency 25 (OH)D ≥50 nmol/L n=152	Insufficiency 25 (OH)D <50 nmol/L n=29	P-value ¹
Continuous variables	Median (IQR)						
25(OH)D (nmol/L)	62.5 (52.5 - 75.0)	82.5 (77.5 - 92.5)	57.5 (50.0 - 65.0)	<0.001	67.5 (57.5 - 77.5)	40.0 (37.5 - 45.0)	<0.001
Vitamin D dietary intake (IU/day)	134.2 (84.22 - 185.4)	130.81 (67.89 - 168.5)	136.4 (89.1 - 201.2)	0.127	133.4 (82.7 - 178.9)	135.7 (98.0 - 248.1)	0.193
Age (y)	26.0 (22.0 - 31.0)	25.5 (21.0 - 31.0)	26.0 (22.0 - 31.0)	0.485	25.0 (21.5 - 31.0)	27.0 (23.0 - 31.0)	0.531
Early pregnancy BMI (kg/m ²)	23.7 (21.9 - 27.0)	23.5 (20.6 - 26.9)	23.7 (22.5 - 27.1)	0.271	23.6 (21.3 - 26.9)	24.8 (22.9 - 28.0)	0.130
Per-capita family income (USD \$)	292.4 (184.8 - 421.0)	301.17 (136.3 - 425.7)	283.6 (193.6 - 417.5)	0.448	277.8 (175.4 - 409.4)	330.4 (208.2 - 438.6)	0.288
Categorical variables	n (%)	n (%)	n (%)	P-value²	n (%)	n (%)	P-value²
Parity							
Nulliparous	70 (38.7)	34.0 (18)	52 (40.6)	0.402	56 (36.8)	14 (48.3)	0.402
Primiparous or multiparous	111 (61.3)	66.0 (35)	76 (59.4)		96 (63.2)	15 (51.7)	
Self-reported skin color							
White	47 (26.0)	26.4 (14)	33 (25.8)	0.788	43 (28.3)	4 (13.8)	0.264
Black	49 (27.1)	30.2 (16)	33 (25.8)		40 (26.3)	9 (31.0)	
Mixed	85 (46.9)	43.4 (23)	62 (48.4)		69 (45.4)	16 (55.2)	
Season ^b							
Summer	38 (21.0)	18 (34.0) *	20 (15.6)	<0.001	37 (24.3)	1 (3.5)	<0.001
Autumn	47 (26.0)	20 (37.7) *	27 (21.1)		44 (29.0)	3 (10.3)	
Winter	48 (26.5)	5 (9.4)	43 (33.6) **		33 (21.7)	15 (51.7) *	
Spring	48 (26.5)	10 (18.9)	38 (29.7)		38 (25.0)	10 (34.5)	

¹ p-value refers to Mann-Whitney test; ²p-value refers to chi-square test. *Among women with vitamin D sufficiency according to Endocrine Society at baseline, we observed significantly higher proportions of women entering pregnancy in the summer and in the autumn, compared with those who became pregnant in the winter. **Among women with vitamin D insufficiency according to Endocrine at baseline, we observed a significantly higher proportion of women entering pregnancy in the winter than in the summer. ***Among women with vitamin D insufficiency according to Institute of Medicine at baseline, we observed a significantly higher proportion of women entering pregnancy in the winter than in the summer. **Abbreviations:** 25(OH)D=25-hydroxyvitamin D, IQR: interquartile range; BMI=Body Mass Index.

Table 2. Markers of glucose and adipokines concentrations of the study population according to vitamin D sufficiency status in first trimester of pregnancy[†].

		Endocrine Society Guidelines						Institute of Medicine				
		Total	Sufficiency 25 (OH)D ≥75nmol/L		Insufficiency 25 (OH)D <75nmol/L		Sufficiency 25 (OH)D ≥50 nmol/L		Insufficiency 25 (OH)D < 50 nmol/L			
Variables	n	n	Median (IQR)	n	Median (IQR)	P-value ¹	n	Median (IQR)	n	Median (IQR)	P-value	
Glycaemia (mg/dL)												
First trimester	180	83.0 (78.0 - 88.0)	54	79.5 (76.0 - 85.0)	126	84.0 (79.0 - 89.0)	0.003	151	83.0 (78.0 - 87.0)	29	84.0 (79.0 - 95.0)	0.034
Second trimester	146	78.0 (72.0 - 83.0)	42	77.5 (70.0 - 82.0)	104	78.0 (73.0 - 83.0)	0.204	122	77.5 (72.0 - 82.0)	24	78.5 (72.0 - 83.5)	0.885
Third trimester	149	79.0 (72.0 - 84.0)	40	80.5 (71.5 - 84.5)	109	78.0 (72.0 - 84.0)	0.991	123	78.0 (71.0 - 84.0)	26	80.5 (75.0 - 84.0)	0.346
Insulin (μU/ml)												
First trimester	180	4.34 (2.97 - 6.46)	53	3.37 (2.64 - 6.01)	127	4.58 (3.31 - 6.49)	0.125	152	4.04 (2.90 - 6.31)	28	4.89 (3.28 - 7.71)	0.141
Second trimester	149	4.93 (3.21 - 7.70)	44	5.07 (3.26 - 7.53)	106	4.92 (3.19 - 7.70)	0.921	125	4.90 (3.15 - 7.40)	24	6.94 (3.96 - 11.39)	0.082
Third trimester	147	5.75 (3.90 - 8.75)	40	6.89 (4.46 - 11.88)	108	5.49 (3.71 - 8.26)	0.093	123	5.65 (4.08 - 8.28)	24	7.63 (3.69 - 11.70)	0.484
HOMA-IR												
First trimester	180	0.86 (0.57 - 1.36)	54	0.65 (0.50 - 1.23)	126	0.93 (0.65 - 1.41)	0.062	152	0.82 (0.56 - 1.30)	28	1.14 (0.80 - 1.73)	0.071
Second trimester	144	0.95 (0.60 - 1.53)	42	0.94 (0.59 - 1.41)	102	0.96 (0.60 - 1.55)	0.812	120	0.95 (0.59 - 1.39)	24	1.16 (0.70 - 2.18)	0.106
Third trimester	144	1.12 (0.74 - 1.69)	38	1.38 (0.78 - 1.94)	106	1.08 (0.70 - 1.65)	0.176	120	1.10 (0.74 - 1.57)	24	1.60 (0.72 - 2.72)	0.352
Adiponectin (μg/ml)												
First trimester	181	4.96 (3.57 - 7.19)	53	4.49 (3.12 - 6.55)	128	5.26 (3.61 - 7.28)	0.170	152	4.95 (3.52 - 7.08)	29	5.68 (4.07 - 7.91)	0.254
Second trimester	146	5.05 (3.52 - 8.46)	42	5.06 (3.65 - 10.91)	104	5.04 (3.36 - 7.80)	0.469	123	4.75 (3.65 - 10.91)	23	6.21 (3.92 - 12.17)	0.123
Third trimester	148	4.59 (3.42 - 6.97)	41	4.69 (3.43 - 7.32)	107	4.45 (3.32 - 6.91)	0.750	123	4.45 (3.32 - 6.63)	125	5.21 (3.67 - 7.22)	0.494
Leptin (ng/dl)												
First trimester	181	16.7 (9.9 - 26.2)	54	13.4 (8.3 - 24.2)	127	17.4 (10.8 - 28.0)	0.093	152	16.1 (9.8 - 26.8)	29	17.4 (11.7 - 24.7)	0.588
Second trimester	151	24.8 (16.6 - 41.8)	44	20.9 (15.9 - 35.4)	107	26.8 (17.3 - 43.2)	0.196	126	23.4 (16.2 - 41.8)	25	32.7 (19.3 - 40.0)	0.228
Third trimester	149	24.9 (15.9 - 38.8)	40	24.1 (14.4 - 38.3)	109	25.66 (15.9 - 38.9)	0.472	124	23.6 (14.9 - 38.1)	25	33.3 (25.7 - 47.3)	0.021

¹p-value refers to Mann-Whitney test. **Abbreviations:** 25(OH)D=25-hydroxyvitamin D; IQR: interquartile range.

Table 3. Markers of glucose metabolism and adipokines changes during pregnancy according to vitamin D sufficiency status in first trimester, Rio de Janeiro, Brazil, 2009-2011.

Fixed-effect	Endocrine society ¹			Institute of Medicine ²		
	β^3	95% CI	<i>P</i> -value ⁴	β^3	95% CI	<i>P</i> -value ⁴
Glycaemia (mg/dL)						
Gestational age	-0.937	-1.347; -0.526	<0.001	-1.013	-1.405; -0.620	<0.001
Quadratic gestational age	0.019	0.009; 0.028	<0.001	0.019	0.009; 0.028	<0.001
Vitamin D sufficiency status ⁵	3.770	-0.128; 7.667	0.058	3.189	-1.473; 7.851	0.180
Interaction terms						
Gestational age # vitamin D status ⁵	-0.109	-0.273; 0.055	0.192	-0.052	-0.245; 0.140	0.593
Insulin (μU/ml)						
Gestational age	0.167	0.048; 0.287	0.006	0.085	0.017; 0.153	0.014
Vitamin D sufficiency status ⁵	2.357	-0.344; 5.058	0.087	1.309	-1.886; 4.503	0.422
Interaction terms						
Gestational age # vitamin D status ⁵	-0.121	-0.251; 0.009	0.069	-0.024	-0.180; 0.132	0.762
HOMA-IR						
Gestational age	0.018	-0.005; 0.041	0.116	0.007	-0.007; 0.020	0.320
Vitamin D sufficiency status ⁵	0.458	-0.053; 0.970	0.079	0.241	-0.366; 0.848	0.437
Interaction terms						
Gestational age # vitamin D status ⁵	-0.016	-0.042; 0.010	0.232	0.0002	-0.031; 0.031	0.992
Adiponectin (μg/ml)						
Gestational age	0.303	0.194; 0.478	<0.001	0.281	0.148; 0.415	<0.001
Quadratic gestational age	-0.006	-0.009; -0.002	0.001	-0.005	-0.009; -0.002	0.001
Vitamin D sufficiency status ⁵	0.737	-0.565; 2.038	0.267	0.991	-0.536; 2.520	0.203
Interaction terms						
Gestational age # vitamin D status ⁵	-0.070	-0.150; 0.010	0.085	0.008	-0.065; 0.081	0.822
Leptin (ng/dl)						
Gestational age	1.012	0.430; 1.594	0.001	0.952	0.406; 1.498	0.001
Quadratic gestational age	-0.021	-0.034; -0.007	0.002	-0.020	-0.033; -0.007	0.003
Vitamin D sufficiency status ⁵	1.207	-3.580; 5.994	0.621	-4.874	-10.460; 0.712	0.087
Interaction terms						
Gestational age # vitamin D status ⁵	0.042	-0.211; 0.295	0.745	0.253	-0.044; 0.550	0.095

¹Sufficiency [reference category, 25(OH)D \geq 75nmol/L]/Inadequacy status [25(OH)D < 75nmol/L]; ² Sufficiency [reference category, 25(OH)D \geq 50nmol/L]/Insufficiency status [25(OH)D < 50nmol/L]; ³ β = longitudinal linear regression coefficient; ⁴*p*-value refers to maximum likelihood estimator; ⁵The reference category was adequate status of 25(OH)D. **Abbreviations:** 25(OH)D= 25-hidroxi-vitamin D; CI = confidence interval. **Note:** The adiponectin, leptin and glycaemia models were adjusted for gestational age and quadratic gestational age in weeks and for women's age, Body Mass Index and seasons (Spring/Summer/Autumn/Winter); the insulin and HOMA-IR models were adjusted for gestational age in weeks and for women's age, Body Mass Index and seasons (Spring/Summer/Autumn/Winter). Glycaemia model: Number of observations = 474; Number of groups = 182; Average of 2.6 observations per group. Insulin model: Number of observations = 475; Number of groups = 180; Average of 2.6 observations per group. HOMA-IR model: Number of observations = 467; Number of groups = 182; Average of 2.6 observations per group. Adiponectin model: Number of observations = 474; Number of groups = 182; Average of 2.6 observations per group. Leptin model: Number of observations = 480; Number of groups = 181; Average of 2.7 observations per group.

Table 4. Markers of glucose metabolism and adipokines according to vitamin D sufficiency status changes during pregnancy, Rio de Janeiro, Brazil, 2009-2011.

Fixed-effect	Endocrine Society ¹			Institute of Medicine ²		
	β^3	95% CI	<i>P</i> -value ⁴	β^3	95% CI	<i>P</i> -value ⁴
Glycaemia (mg/dL)						
Gestational age	-0.915	-1.345; -0.484	<0.001	-0.989	-1.369; -0.609	<0.001
Quadratic gestational age	0.017	0.007; 0.026	0.001	0.018	0.009; 0.027	<0.001
Vitamin D sufficiency status ⁵	2.624	-1.244; 6.492	0.184	3.516	-1.317; 8.350	0.154
Interaction terms						
Gestational age # vitamin D status ⁵	-0.049	-0.215; 0.116	0.560	-0.029	-0.260; 0.202	0.805
Insulin (μU/ml)						
Gestational age	0.107	0.014; 0.201	0.024	0.095	0.029; 0.160	0.004
Vitamin D sufficiency status ⁴	0.988	-1.677; 3.654	0.468	2.344	-1.073; 5.760	0.179
Interaction terms						
Gestational age # vitamin D status ⁵	-0.076	-0.199; 0.047	0.226	-0.153	-0.325; 0.018	0.080
HOMA-IR						
Gestational age	0.013	-0.006; 0.031	0.178	0.009	-0.004; 0.022	0.178
Vitamin D sufficiency status ⁴	0.269	-0.260; 0.797	0.319	0.499	-0.181; 1.178	0.150
Interaction terms						
Gestational age # vitamin D status ⁵	-0.014	-0.038; 0.011	0.270	-0.030	-0.065; 0.005	0.094
Adiponectin (μg/ml)						
Gestational age	0.291	0.145; 0.436	<0.001	0.270	0.148; 0.392	<0.001
Quadratic gestational age	-0.005	-0.008; -0.002	0.003	-0.005	-0.008; -0.002	0.001
Vitamin D sufficiency status ⁵	0.847	-0.351; 2.045	0.166	0.186	-1.265; 1.637	0.801
Interaction terms						
Gestational age # vitamin D status ⁵	-0.056	-0.116; 0.004	0.066	0.006	-0.068; 0.080	0.878
Leptin (ng/dl)						
Gestational age	1.459	0.829; 2.089	<0.001	1.379	0.814; 1.945	<0.001
Quadratic gestational age	-0.031	-0.045; -0.017	<0.001	-0.030	-0.044; -0.017	<0.001
Vitamin D sufficiency status ⁵	1.131	-4.114; 6.376	0.673	-4.046	-10.512; 2.421	0.220
Interaction terms						
Gestational age # vitamin D status ⁵	0.046	-0.205; 0.297	0.719	0.258	-0.089; 0.604	0.145

¹Sufficiency [reference category, 25(OH)D \geq 75nmol/L]/ Insufficiency status [25(OH)D < 75nmol/L]; ² Sufficiency [reference category, 25(OH)D \geq 50nmol/L]/ Insufficiency status [25(OH)D < 50nmol/L]; ³ β = longitudinal linear regression coefficient; ⁴*p*-value refers to maximum likelihood estimator; ⁵The reference category was adequate status of 25(OH)D. **Abbreviations:** 25(OH)D= 25-hydroxi-vitamin D; CI = confidence interval. **Note:** The adiponectin, leptin and glycaemia models were adjusted for gestational age and quadratic gestational age in weeks and for women's age, Body Mass Index and seasons (Spring/Summer/Autumn/Winter); the insulin and HOMA-IR models were adjusted for gestational age in weeks and for women's age, Body Mass Index and seasons (Spring/Summer/Autumn/Winter). Glycaemia model: Number of observations = 503; Number of groups = 207; Average of 2.4 observations per group. Insulin model: Number of observations = 509; Number of groups = 205; Average of 2.5 observations per group. HOMA-IR model: Number of observations = 498; Number of groups = 207; Average of 2.4 observations per group. Adiponectin model: Number of observations = 506; Number of groups = 207; Average of 2.4 observations per group. Leptin model: Number of observations = 512; Number of groups = 206; Average of 2.5 observations per group.

Figure 1. Changes in glucose metabolism markers and adipokines throughout pregnancy according to vitamin D baseline status, Rio de Janeiro/Brazil, 2009-2012.

A) and **B)** Glycaemia according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

C) and **D)** Insulin according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

E) and **F)** HOMA-IR according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

G) and **H)** Adiponectin according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

Figure 2. Changes in glucose metabolism markers and adipokines according to vitamin D status throughout pregnancy, Rio de Janeiro/Brazil, 2009-2012.

A) and **B)** Glycaemia according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

C) and **D)** Insulin according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

E) and **F)** HOMA-IR according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;

G) and **H)** Adiponectin according to vitamin D status Endocrine Society Practice Guideline and the Institute of Medicine cut-off points, respectively;