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Association between Early Pregnancy Vitamin D Status and Changes in Serum Lipid Profiles throughout Pregnancy

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Abstract

Objective: To evaluate the associations between first trimester 25-hydroxyvitamin D [25(OH)D] status and changes in high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total cholesterol (TC), triglyceride (TG) concentrations, TG/HDL-c, and TC/HDL-c ratios throughout pregnancy. We hypothesized that first trimester 25(OH)D inadequacy is associated with lower concentrations of HDL-c and higher LDL-c, TC, TG, TG/HDL-c, and TC/HDL-c ratios throughout pregnancy.

Methods: A prospective cohort study with 3 visits at 5-13 (baseline), 20-26, and 30-36 gestational weeks, recruited 194 pregnant women attending a public health care center in Rio de Janeiro, Brazil. Plasma 25(OH)D concentrations were measured in the first trimester using liquid chromatography-tandem mass spectrometry. 25(OH)D concentrations were classified as adequate (≥ 75 nmol/L) or inadequate (< 75 nmol/L). Serum TC, HDL-c, and TG concentrations were measured enzymatically. Crude and adjusted longitudinal linear mixed-effects models were employed to evaluate the association between the first trimester 25(OH)D status and changes in serum lipid concentrations throughout pregnancy. Confounders adjusted for in the multiple analysis were age, homeostatic model assessment (HOMA), early pregnancy BMI, leisure time physical activity before pregnancy, energy intake, and gestational age.

Results: At baseline, 69% of the women had inadequate concentrations of 25(OH)D. Women with 25(OH)D inadequacy had higher mean LDL-c than those with adequate concentrations (91.3 vs 97.5 mg/dL; $P = 0.064$) at baseline. TC, HDL-c, LDL-c TG, TG/HDL-c ratios, and TC/HDL-c ratios, increased throughout pregnancy independently of 25(OH)D concentrations (ANOVA for repeated measures $P < 0.001$). The adjusted

models showed direct associations between the first trimester 25(OH)D status and changes in TC (β = 9.53; 95%CI = 1.12-17.94), LDL-c (β = 9.99; 95% CI = 3.62-16.36) concentrations, and TC/HDL-c ratios (β = 0.16; 95% CI = 0.01-0.31) throughout pregnancy.

Conclusions: Inadequate plasma 25(OH)D concentrations during early pregnancy were associated with more pronounced changes of TC, LDL-c concentrations, and TC/HDL-c ratios throughout pregnancy. Changes in these cardiovascular markers suggest the importance of ensuring adequate vitamin D status at the beginning of pregnancy.

Keywords: 25-hydroxyvitamin D; lipids; pregnancy; cohort; cardiovascular disease

1. Introduction

During pregnancy, physiological changes in carbohydrate and lipid metabolism are associated with increases in TC, TG, HDL-c, and LDL-c. [1,2]. Although these changes in the maternal serum lipid profile are thought to be normal adaptations to support fetal development, studies have shown that some factors such as being overweight [1], sedentary behavior [3], smoking [4], vitamin B1 (thiamine) and B2 (riboflavin) deficiency [5] and lifestyle [6] negatively influence this increase.

There is increasing evidence shown that an abnormal lipid profile during gestation may increase the risk of cardiovascular disease and result in undesirable outcomes for the mother and the fetus, including preeclampsia [7,8], gestational diabetes mellitus [9,10], intrauterine growth restriction and premature birth [11,12]. Recent clinical trials and observational studies have shown that adequate serum concentrations of 25(OH)D are related to a better lipid profile in children, adolescents, and adults [13,14,15]. In contrast, low vitamin D status has been associated with an

increase in blood pressure (BP) and markers of cardiovascular disease risk [16,17]. Vitamin D is thought to modify cardiometabolic processes, either directly by the actions of its nuclear vitamin D receptor [18] or indirectly by regulation of calcium homeostasis [19].

Vitamin D inadequacy has been described as a pandemic condition and a serious public health problem, with a high prevalence worldwide in adult women, including 77% in Brazil, 73% in Australia, 84% in Scotland, and 83% in Nigeria [20]. Similar prevalences were found in pregnant women in other parts of the world, such as Germany (77%), Belgium (74%), and Pakistan (79%) [20]. The lack of prospective studies investigating the association between vitamin D status and lipid changes throughout pregnancy, combined with the high prevalence of vitamin D inadequacy among women of reproductive age, [20,21] highlight the importance of the current study.

The purpose of this study was to evaluate the associations between first trimester 25(OH)D status and subsequent changes in HDL-c, LDL-c, TC, TG concentrations, and TG/HDL-c and TC/HDL-c ratios during the course of pregnancy. We hypothesize that first trimester 25(OH)D inadequacy is associated with lower concentrations of HDL-c, higher LDL-c, TC, and TG concentrations, and higher TG/HDL-c and TC/HDL-c ratios throughout pregnancy.

2. Methods

2.1 Study design and eligibility criteria

This is a prospective cohort study that recruited women from a public health

prenatal care center in Rio de Janeiro, Brazil, between November 2009 and October 2011. The eligibility criteria to participate comprised recruitment between the fifth and thirteenth gestational weeks, age of 20-40 years, lack of chronic diseases (except obesity) and infectious diseases, singleton pregnancy, and residence in the catchment area of the prenatal care center.

Interviews and blood samples were taken at 3 time points: 5-13 (baseline), 20-26, and 30-36 weeks gestation. A total of 322 women met the eligibility criteria and were invited to participate. From those 322 women, 23 chose not to participate, and 105 were excluded after enrollment for the following reasons: diagnosed with chronic diseases (n = 12) or with infectious diseases (n = 9), advanced pregnancy at baseline (≥ 14 weeks of gestation) (n = 15), multiple pregnancies (n = 4), missed the baseline interview (n = 5) or the baseline blood collection (n = 6) or suffered early miscarriages (n = 25), missing information on 25(OH)D concentration (n = 4), or missing information on the lipid profile (n = 19) or confounders at baseline (n = 6). The baseline sample comprised 194 pregnant women. From the baseline to the second trimester visit, 10 follow-up losses occurred; women who moved out of the program (n = 5) and women who abandoned prenatal care (n = 5) were excluded, leaving 184 women at the second trimester visit. Between the second and third visits, women moved out of the program catchment area (n = 1), abandoned prenatal care (n = 1), or had missing information on their lipid profile (n = 3) or confounders (n = 18) and so were excluded from the analyses. The final sample in the third trimester was 161 women (**Supplemental Figure 1**).

2.2 Blood samples

Maternal blood sample collection (5 mL) was performed between 6:50 am and 7:50 am by a trained technician (nurse) after 12 hours of fasting. Blood samples were collected into vacutainer tubes at 3 different clinic visits (first, second, and third gestational trimesters). Maternal blood samples were immediately centrifuged (5,000 rpm for 5 minutes), and aliquots of serum (prepared from blood collected in tubes with gel separator) and plasma (prepared from blood collected in tubes containing EDTA) were stored at -80°C until analysis.

2.3 Vitamin D status

The first trimester plasma concentrations of 25(OH)D were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at Quest Diagnostics Nichols Institute (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 laboratory is part of the Hormone Standardization Program of the Centers for Disease Control and Prevention. LC-MS/MS is the best assay available and has high sensitivity and specificity with analytical measurement ranging from 10 to 640 nmol/L. [22]. Interclass and intraclass variability were < 10%.

Women were classified as having either adequate, 25(OH)D ≥ 75 nmol/L, or inadequate, 25(OH)D < 75 nmol/L, vitamin D status as recommended for pregnant women by the Endocrine Society Practice Guidelines [22].

2.4 Lipid profile

The lipid profile outcomes in this study were HDL-c, TC, TG, LDL-c, TC/HDL, and TG/HDL. Serum samples were used to determine the concentrations of HDL-c, TC,

and TG concentrations using an enzymatic colorimetric method in an automated analyzer (Labmax Plenno, Labtest Diagnostica, Minas Gerais, Brazil) and commercial kits (Labtest Diagnostica, Minas Gerais, Brazil). LDL-c was calculated using the Friedewald, Levy, and Fredrickson (1972) formula. [23].

2.5 Covariates assessment

Early maternal body weight was measured to the nearest 0.1 kg between the fifth and thirteenth gestational weeks using an electronic scale (Filizzola Ltd., São Paulo, Brazil). Height was measured in duplicate using a portable stadiometer (Seca Ltd., Hamburg, Germany) at baseline to the nearest 0.1 cm. Early BMI was calculated using the formula weight (kg)/height $(m)^2$ The cut points proposed by the World Health Organization and endorsed by the Institute of Medicine (IOM) (2009) [24] were used to classify the women's BMI in early pregnancy. All anthropometric measurements were obtained according to standardized procedures and recorded by trained interviewers. [25].

In the first trimester, a validated semi-quantitative Food Frequency Questionnaire (FFQ) was administered by trained interviewers; this FFQ is an updated version of the most commonly used FFQ in Rio de Janeiro, Brazil [26]. Total vitamin D (mg/day) and energy (kcal/day) intakes were estimated based on the daily frequency of intake and portion sizes reported for each FFQ food item. A Brazilian household measures table was used to quantify portion sizes that were converted into grams or milliliters. [27]. The nutrient database was constructed primarily with the nutritional composition of food consumed [28] and complemented by the database developed by the U.S. Department of Agriculture [29].

Gestational age was measured via ultrasound performed before 24 weeks of gestation (n = 171, 88.1%). If this information was not available in the obstetric record, gestational age was calculated based on the reported date of the last menstrual period (n = 23, 11.9%).

Plasma insulin concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) (Millipore, St. Charles, MO, USA) with sensitivity of 2 μU/mL. Insulin resistance was estimated by calculating the homeostasis model assessment-insulin resistance (HOMA-IR = fasting insulin [U/ml] x fasting glucose [mmol/L]/22.5) [30].

2.6 Statistical analysis

We compared mean concentrations of 25(OH)D, lipid outcomes, and relevant confounders measured at baseline between the women who reached the final follow-up (n = 161) and those who were lost to follow-up or excluded from the analysis (n = 33). The chi-squared test was applied for categorical variables and Student's t-test for continuous variables.

The characteristics of pregnant women were described as means and standard deviation (SD) for continuous variables and as absolute (n) and relative frequency (%) for categorical variables according to first trimester 25(OH)D status. Means were compared using Student's t-test and proportions using the chi-squared test. The trend of the lipid profile outcomes throughout pregnancy was compared using ANOVA for repeated measures.

Crude and adjusted longitudinal linear mixed-effect (LME) regression models were performed to evaluate the association between 25(OH)D status at baseline and

changes in lipid profiles during pregnancy. An independent model for each outcome (TC, LDL-c, HDL-c, TG, TC/HDL, and TG/HDL) was performed. Confounders were added to the model based on biological plausibility and statistical significance in bivariate regression models ($P < 0.2$) with both the main exposure, 25(OH)D status, and the lipid profile outcomes (HDL-c, TC, TG, LDL-c, TC/HDL-c, and TG/HDL-c). The following variables reached this condition and were included in the adjusted models: age, early pregnancy BMI, total energy intake, parity, leisure time physical activity before pregnancy, HOMA-IR, and gestational age. Quadratic gestational age was further included in adjusted models (except for TG) as the association between TC and fractions during pregnancy with time showed a non-linear function. The season of measurement of 25(OH)D, although associated with the main exposure, had no relationship with the lipid profile outcomes. Therefore, seasonality was not considered as a confounder and was not included in the adjusted models.

The maximum likelihood ratio and Akaike information criterion were considered to select the most parsimonious model, and the unstructured covariance matrix was used in all models.

Effect plots containing data scatter, longitudinal prediction, and 95% confidence intervals (CIs) were constructed to illustrate the variation in lipid concentrations during pregnancy according to 25(OH)D status in the first trimester.

Statistical analyses were performed using Stata Data Analysis and Statistical Software (STATA), version 12.0 [31] and R statistical software package, version 3.1.2 [32].

2.7 Ethics

The Rio de Janeiro Municipal Health Secretary Research Ethics Committee approved the study procedures (reference number: 0139.0.314.000-09). Written informed consent was obtained from each volunteer before entry into the study.

3. Results

No differences were found regarding 25(OH)D or lipid profile concentrations at baseline between women who completed the follow-up and to those classified as lost to follow-up or excluded (**Supplemental Table 1**).

The mean age of the women was 26.7 years (SD = 5.5). Twenty five percent (n = 49) of the women reported leisure time physical activity before pregnancy and 38.6% (n = 75) were nulliparous. Sixty-nine percent (n = 135) of women had inadequate concentrations of 25(OH)D at baseline. The majority of women (59.8%) had normal weight, 26.8% were overweight, and 13.4% were obese according to early pregnancy BMI (kg/m²). TC, HDL-c, LDL-c, TG, and TC/HDL-c ratios increased throughout pregnancy independently of 25(OH)D concentrations (ANOVA for repeated measures $P < 0.001$) (**Table 1**).

The trend of changes throughout pregnancy was positive and statistically significant for all lipid outcomes except for the TG/HDL ratio. This holds true even after adjustment for a wide range of confounders. The increment was independent of first trimester 25(OH)D status. The dots representing each observation are concentrated toward the regression line, meaning a good fit of the models (**Figures 1-6**).

A direct and significant association was observed between baseline 25(OH)D status and changes in TC ($\beta = 9.53$; 95% CI = 1.12-17.94; *P* value = 0.026) and LDL-c ($\beta = 9.99$; 95% CI = 3.62-16.36; *P* value = 0.002) concentrations during pregnancy

when analyses were adjusted for confounders (**Table 2**).

Inadequate 25(OH)D status at baseline was not associated with HDL-c (β = 0.08; 95% CI = 2.33-2.49; *P* value = 0.946) and TG concentrations (β = -6.09; 95% CI = -15.97-3.78; *P* value = 0.227) or with TG/HDL-c ratios (β = -0.15; 95% CI = -0.37-0.07; *P* value = 0.186) throughout pregnancy **(Tables 3 and 4)**. However, 25(OH)D inadequacy was directly and significantly associated with TC/HDL-c ratio (β = 0.16; 95% CI = 0.01-0.31; *P* value = 0.040) changes during pregnancy, in both crude and adjusted models **(Table 4)**.

4. Discussion

To the best of our knowledge, this is the first longitudinal study to evaluate the association between 25(OH)D concentrations and lipid profile changes during pregnancy. Our findings show that women with inadequate 25(OH)D status in the first trimester of pregnancy presented significantly higher changes in concentrations of TC, LDL-c, and TC/HDL-c ratios throughout pregnancy compared to women with adequate vitamin D status at baseline after adjustment for a wide range of confounders. It is interesting to note that when analyses were performed on the absolute values for each lipid outcome in the point-by-point evaluation (that is, for each trimester assessment), no differences were found for any of the lipid outcomes according to baseline vitamin D status. This means that vitamin D status at baseline plays a role in the longitudinal changes of the lipid profile and not on the absolute values observed at each time point in pregnancy.

The mean 25(OH)D concentration in the first trimester was 65.5 nmol/L and 69.6% of the pregnant women were found to have vitamin D inadequacy in our study.

The high prevalence of vitamin D inadequacy may be regarded as a serious public health problem. Song et al. (2013) also found a high prevalence of vitamin D inadequacy in a study of 125 healthy pregnant women from China and observed that 96.8% of the women in the sample presented serum 25(OH)D concentrations < 50 nmol/L and none had serum 25(OH)D levels ≥ 75 nmol/L in early pregnancy [33]. Our results also corroborate a recent study conducted in North India with 418 healthy pregnant women. They report that 93.5% of the women were vitamin D deficient (defined as a 25(OH)D concentration between 25-80 nmol/L) and 34.4% were severely vitamin D deficient: 25(OH)D < 25 nmol/L [34]. Furthermore, a high prevalence of vitamin D inadequacy (< 75 nmol/L) during pregnancy was also observed in other tropical or subtropical countries such as Australia and China [35,36].

The relationship between lipid profiles, vitamin D, and biomarkers of cardiovascular disease risk have clear clinical and public health implications. Studies have shown that an altered lipid profile characterized by high concentrations of atherogenic fractions such as high LDL-c and TG and low HDL-c during gestation might increase the risk of cardiovascular diseases and may also result in undesirable outcomes for the mother and the fetus. [7,11]. Vitamin D may be an important modifiable risk factor and thus should be considered as a potential target for interventions. Studies in humans have shown that vitamin D adequacy might be related to improvements in the lipid profile [13,14,15]. For instance, a recent study of 195 adults without cardiovascular disease found that participants with coronary artery calcification were 3.3 times more likely (OR = 3.31, 95% CI: 1.12-9.77) to be vitamin D deficient, 25(OH)D < 50 nmol/L, after adjusting for age, BMI, smoking, alcohol intake,

C-reactive protein, and TG when compared to those without calcification [15]. Consistent evidence suggests that vitamin D receptors are present in tissues, including the vascular endothelium [37], and myocardium [17] and have been shown to decrease proliferation of vascular cell smooth muscle and to improve vasodilation of the endothelium. Additionally, studies have suggested that low concentrations of vitamin D are related to cardiovascular disease, myocardial infarction, stroke, and congestive heart failure [38,39,40]. It is known that vitamin D plays an important role in the regulation of cholesterol biosynthesis. Vitamin D inhibits coenzyme A reductase (HMG CoA reductase) and thus inhibits cholesterol synthesis [41,42]. Furthermore, it has a potent anti-lipolytic action, increasing intracellular calcium levels, regulates the renin-angiotensin system, and suppresses lipolysis in human adipocytes [43]. Nevertheless, little is known about these associations during gestation.

Few studies have addressed the association between 25(OH)D and lipid concentrations during pregnancy. A cross-sectional study conducted with 515 Saudi Arabian women in the first trimester of pregnancy observed a direct correlation between vitamin D status and TC ($r = 0.172$; P value < 0.01) and TG ($r = 0.184$; P value < 0.01). Our results also show a positive and significant association between 25(OH)D inadequacy and changes in TC concentrations during pregnancy. Additionally, first trimester inadequacy of 25(OH)D was positively and significantly associated with LDL-c concentrations and changes during pregnancy, in both crude and adjusted models.

Recent clinical trials have evaluated the effects of supplementation with vitamin D during pregnancy and several maternal and fetal outcomes. [45,46,47]. Asemi et al. (2013), in a randomized single-blind controlled clinical trial performed with pregnant women, showed that those supplemented with vitamin D and calcium (500 mg

carbonate calcium plus 200 IU vitamin D3) presented lower LDL concentrations compared to the placebo group (-10.8; SD = 22.4 vs 10.4; SD = 28.0; *P* value = 0.003) and a significantly higher reduction in TC concentrations (-11.0; SD = 23.5 vs 9.5; SD = 36.5 mg/dL; *P* value = 0.01). However, no significant differences were found regarding serum HDL-c and TG concentrations. [45]. Samimi et al. (2016), in a prospective double-blind placebo-controlled trial with 60 pregnant women, found that women who received 50.000 IU of vitamin D3 every 2 weeks plus 1,000 mg/day of calcium supplements (as calcium carbonate) from 20 to 32 weeks of gestation had significantly increased serum HDL-c when compared to women who received a placebo (4.6; SD = 8.3 vs -2.9; SD = 7.7 mg/dL; *P* value = 0.001) [48].

The different physiological potency of vitamin D_2 and D_3 and the potential differences on the associations under study could not be investigated in our research. The vitamin D_2 concentrations were too low and could not be detected. This may be explained by the fact that no women from our study reported vitamin D supplement intake. The use of supplements is not common among the Brazilian population, especially in low-income groups. The lack of mandatory vitamin D fortification in Brazil represents a missed window of opportunity and should be reconsidered from the policymakers' perspective.

The present study has some strengths and limitations. The main strength refers to the vitamin D and lipid measurements throughout pregnancy, allowing us to establish a trend for the lipid concentrations during pregnancy according to first trimester 25(OH)D status. The longitudinal design allowed us to determine directionality. Furthermore, the association between 25(OH)D and the lipid profile during pregnancy is quite novel and has not been established before. Another important strength was the analysis of plasma

25(OH)D by LC-MS/MS, a method considered to be the gold standard. Regarding limitations in our study, information concerning an individual's sun exposure was not collected. Second, there were losses of participants during follow-up (17%) that might have reduced our power to detect significant associations. However, our losses to follow-up happened to be random. Third, although associations between changes in serum lipids and vitamin D inadequacy have been identified in early pregnancy, our findings are limited since there are no validated cutoff points to define serum lipid abnormality during pregnancy. Finally, it is worth noting that the current analysis did not include data on perinatal and maternal outcomes beyond the lipid outcomes.

In conclusion, the present study demonstrated that pregnant women with inadequate plasma 25(OH)D concentrations in the first trimester presented a significantly higher increase in TC, LDL-c, and TC/HDL-c ratios during pregnancy compared to those with adequate 25(OH)D concentrations, after adjustment for a wide range of confounders. Vitamin D deficiency is a serious public health problem observed in many populations around the world, including pregnant women, and even among people residing in sunny regions. The lipid concentrations are an important modifiable factor during gestation and are associated with several maternal and fetal outcomes. There is a need for research to investigate the associations between vitamin D status, serum lipids, and adverse pregnancy outcomes. Thus, strategies for monitoring vitamin D status in early pregnancy may help prevent important undesirable outcomes.

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6. Authorship

GK and JSV conceived and designed the project. JL, IE and DF performed the experiments. JL, IE, DF analyzed the data. GK, AA, AB assisted with the interpretation of the results. JL, IE, AF, AA, AF, DF and GK wrote the manuscript with input from AB, RM, MH, and LHA. All authors contributed to subsequent drafts of the manuscript and read and approved the final manuscript submitted for publication.

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8. Conflict of Interest

The authors declare that they have no conflicts of interest.

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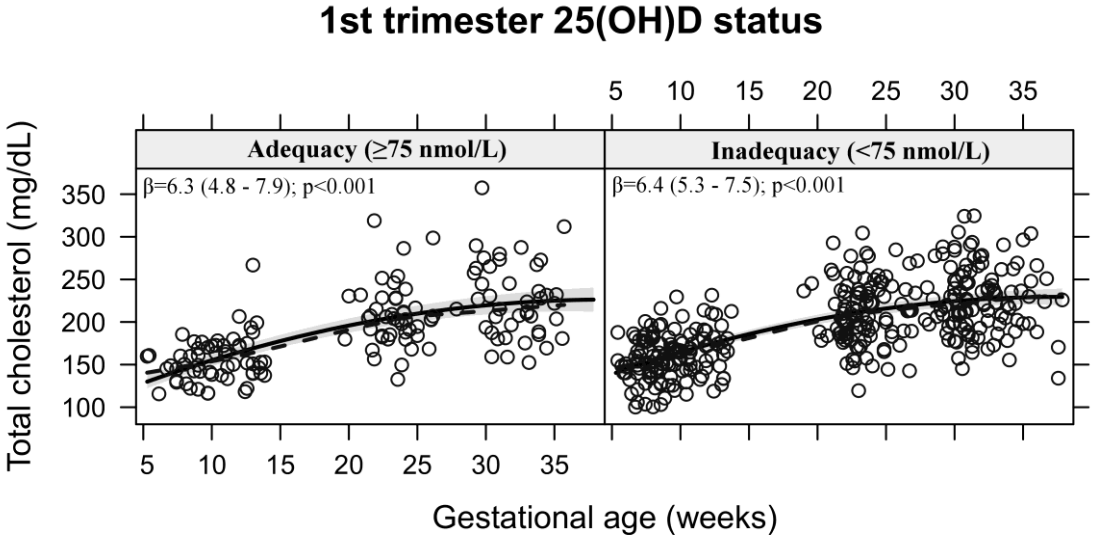

Figure 1. Changes in total cholesterol throughout pregnancy according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.

Note: Baseline 25(OH)D status was categorized in adequacy (≥75nmol/L) and inadequacy (<75nmol/L) and the adequacy was considered the reference category. The model was adjusted for age, energy intake, parity, early pregnancy BMI, leisure time physical activity before pregnancy, Homeostasis Model Assessment – Insulin Resistance and linear and quadratic gestational weeks.
Data are presented as linear mixed effect coefficient (β) and 95% CI. *p*-value refers to the maximum likelihood estimator.

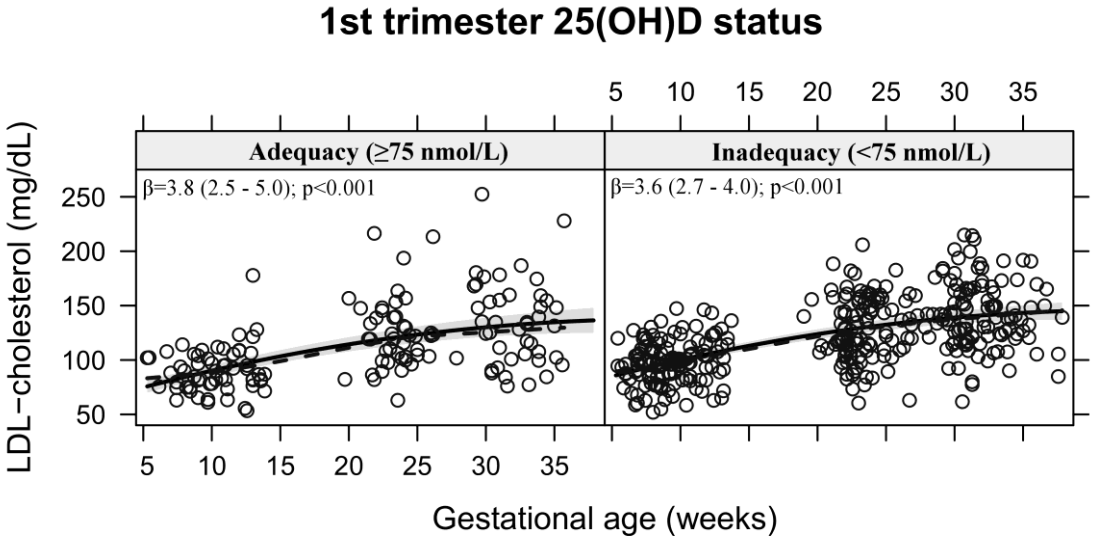

Figure 2. Changes in LDL-cholesterol throughout pregnancy according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.

Note: Baseline 25(OH)D status was categorized in adequacy (≥75nmol/L) and inadequacy (<75nmol/L) and the adequacy was considered the reference category. The model was adjusted for age, energy intake, parity, early pregnancy BMI, leisure time physical activity before pregnancy, Homeostasis Model Assessment – Insulin Resistance and linear and quadratic gestational weeks.
Data are presented as linear mixed effect coefficient (β) and 95% CI. *p*-value refers to the maximum likelihood estimator.

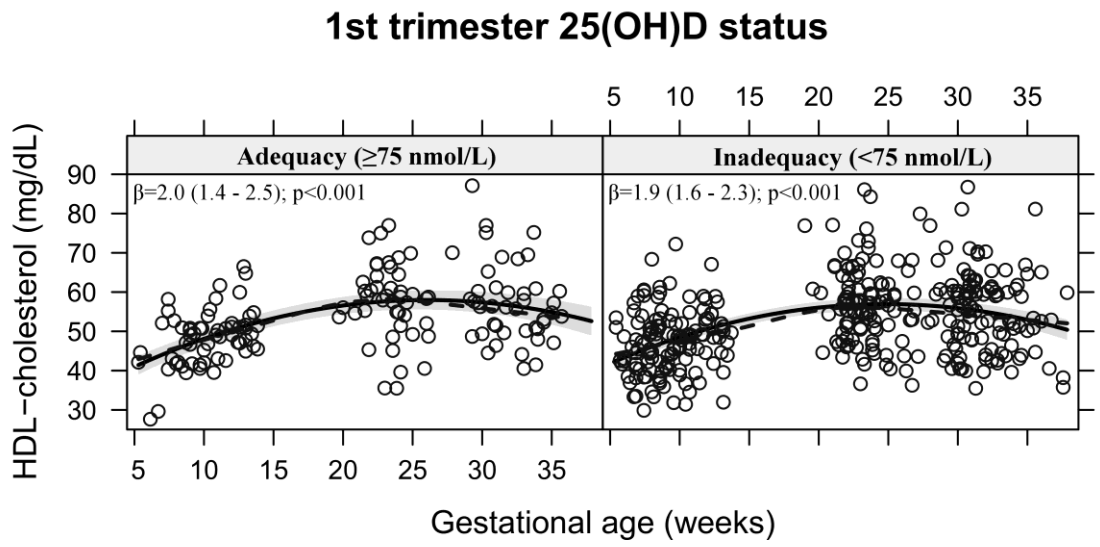

Figure 3. Changes in HDL-cholesterol throughout pregnancy according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.

Note: Baseline 25(OH)D status was categorized in adequacy (≥75nmol/L) and inadequacy (<75nmol/L) and the adequacy was considered the reference category. The model was adjusted for age, energy intake, parity, early pregnancy BMI, leisure time physical activity before pregnancy, Homeostasis Model Assessment – Insulin Resistance and linear and quadratic gestational weeks.
Data are presented as linear mixed effect coefficient (β) and 95% CI. *p*-value refers to the maximum likelihood estimator.

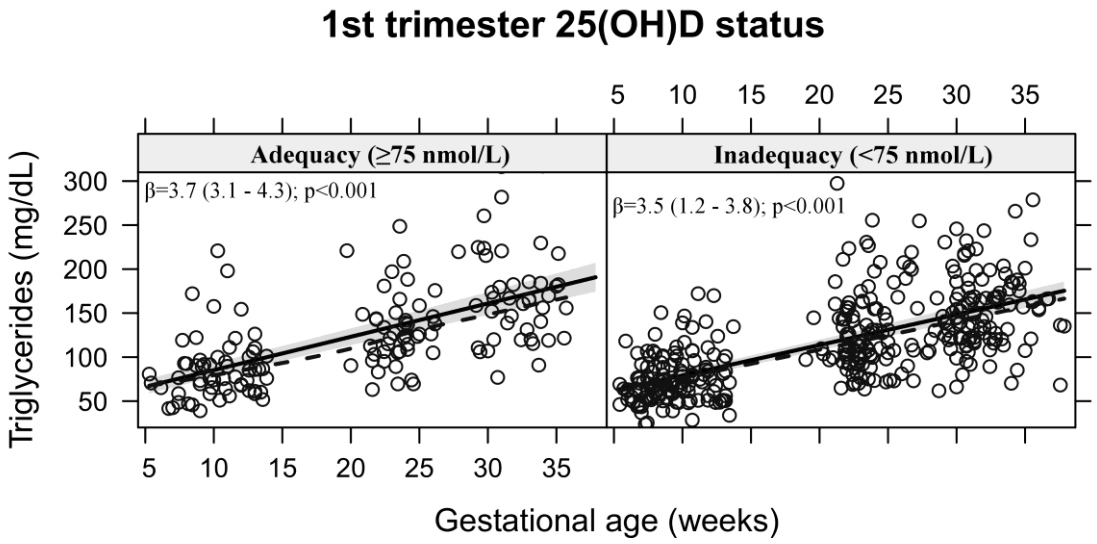

Figure 4. Changes in triglycerides throughout pregnancy according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.

Note: Baseline 25(OH)D status was categorized in adequacy (≥75nmol/L) and inadequacy (<75nmol/L) and the adequacy was considered the reference category. The model was adjusted for age, energy intake, parity, early pregnancy BMI, leisure time physical activity before pregnancy, Homeostasis Model Assessment – Insulin Resistance and linear and quadratic gestational weeks.
Data are presented as linear mixed effect coefficient (β) and 95% CI. *p*-value refers to the maximum likelihood estimator.

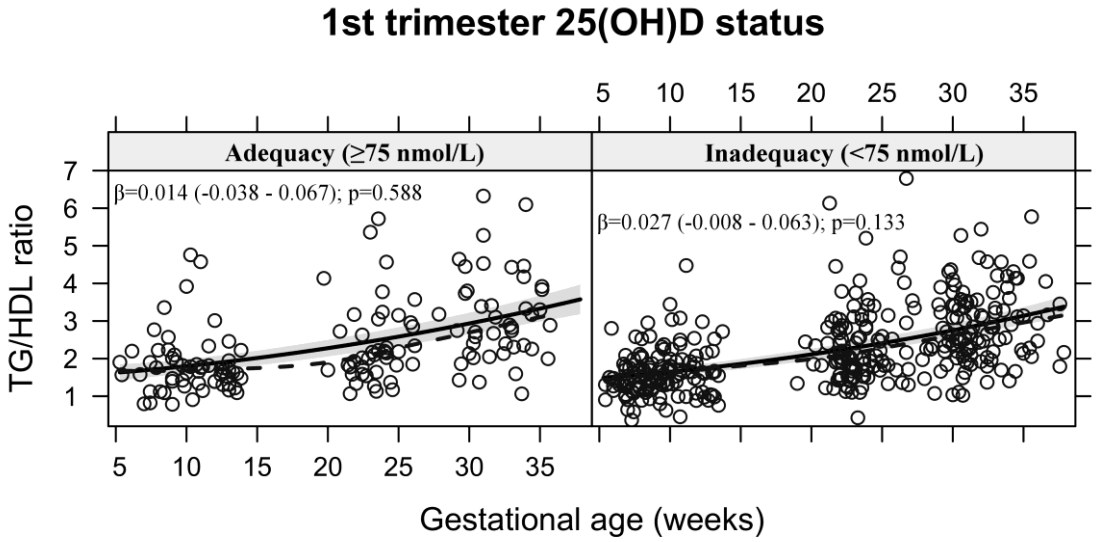

Figure 5. Changes in TG/HDL-cholesterol ratio throughout pregnancy according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.

Note: Baseline 25(OH)D status was categorized in adequacy (≥75nmol/L) and inadequacy (<75nmol/L) and the adequacy was considered the reference category. The model was adjusted for age, energy intake, parity, early pregnancy BMI, leisure time physical activity before pregnancy, Homeostasis Model Assessment – Insulin Resistance and linear and quadratic gestational weeks.
Data are presented as linear mixed effect coefficient (β) and 95% CI. *p*-value refers to the maximum likelihood estimator.

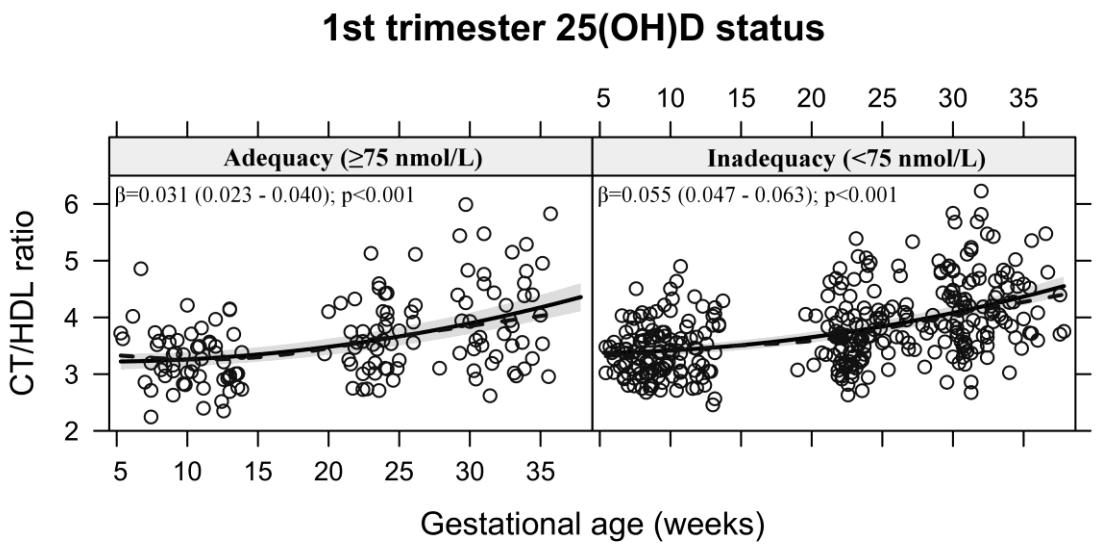

Figure 6. Changes in TC/HDL-cholesterol ratio throughout pregnancy according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.

Note: Baseline 25(OH)D status was categorized in adequacy (≥75nmol/L) and inadequacy (<75nmol/L) and the adequacy was considered the reference category. The model was adjusted for age, energy intake, parity, early pregnancy BMI, leisure time physical activity before pregnancy, Homeostasis Model Assessment – Insulin Resistance and linear and quadratic gestational weeks.
Data are presented as linear mixed effect coefficient (β) and 95% CI. *p*-value refers to the maximum likelihood estimator.

Table 1. Characteristics of pregnant women according to first trimester 25(OH)D status, Rio de Janeiro/Brazil, 2009-2012.[a]

Maternal characteristics Continuous variables [b]	All n =194	Adequate 25(OH)D (≥75 nmol/L) n = 59	Inadequate 25(OH)D (<75 nmol/L) n = 135	*P* [c]
Age (years)	26.7 (5.5)	26.8 (5.5)	26.3 (5.3)	0.565
Early pregnancy Body Mass Index (kg/m²)	25.1 (4.8)	25.6 (6.7)	25.0 (4.3)	0.331
Pre-pregnancy energy intake (kcal/day)	2,425	2,314 (811)	2,473 (940)	0.260
Pre-pregnancy vitamin D intake (mg/day)	27.2 (35.0)	21.0 (16.0)	29.9 (40.5)	0.105
Homeostasis Model Assessment – Insulin Resistance [d]	1.2 (1.0)	1.1 (0.8)	1.3 (1.1)	0.122
Total Cholesterol (mg/dL) [e]				
1st trimester	158.8 (28.4)	157.1 (26.4)	159.5 (29.2)	0.584
2nd trimester	211.3 (36.4)	208.9 (34.6)	211.8 (38.8)	0.649
3rd trimester	224.4 (42.0)	224.1 (43.8)	225.0 (41.5)	0.902
Low Density Lipoprotein (mg/dL) [e]				
1st trimester	95.6 (21.2)	91.3 (20.6)	97.5 (21.3)	0.064
2nd trimester	127.2 (28.9)	124.4 (30.4)	128.9 (29.3)	0.385
3rd trimester	137.2 (33.7)	134.1 (37.7)	140.3 (31.7)	0.301
High Density Lipoprotein (mg/dL) [e]				
1st trimester	47.4 (8.1)	48.6 (7.3)	46.9 (8.5)	0.179
2nd trimester	57.6 (10.2)	57.8 (9.0)	57.2 (10.8)	0.736
3rd trimester	55.0 (10.4)	56.3 (10.3)	54.4 (10.5)	0.321
Triglycerides (mg/dL) [e]				
1st trimester	78.7 (33.3)	85.7 (38.5)	75.8 (30.4)	0.055
2nd trimester	132.4 (49.2)	133.4 (48.5)	128.9 (50.0)	0.602
3rd trimester	160.7 (51.3)	168.6 (56.7)	151.8 (47.5)	0.058
Triglycerides/High Density Lipoprotein [e]				

1st trimester	1.7 (0.7)	1.8 (0.7)	1.6 (0.8)	0.201
2nd trimester	2.3 (1.1)	2.4 (1.14)	2.3 (1.1)	0.690
3rd trimester	2.9 (1.1)	3.1 (1.2)	2.8 (1.0)	0.220
Total Cholesterol/High Density Lipoprotein [e]				
1st trimester	3.4 (0.5)	3.2 (0.5)	3.4 (0.5)	**0.036**
2nd trimester	3.7 (0.6)	3.7 (0.6)	3.7 (0.6)	0.393
3rd trimester	4.1 (0.7)	4.0 (0.8)	4.2 (0.7)	0.272
Categorical variables				*P* [f]
Leisure physical activity before pregnancy				
No	143 (74.7)	40 (28.0)	103 (72.0)	0.157
Yes	49 (25.3)	19 (38.8)	30 (61.2)	
Parity (number of parturitions)				
0	75 (38.6)	20 (26.7)	55 (73.3)	0.368
≥ 1	119 (61.4)	39 (32.8)	80 (67.2)	
Early pregnancy BMI (kg/m²) [d]				
Normal weight	116 (59.8)	43 (72.9)	73 (54.1)	
Overweight	52 (26.8)	9 (15.2)	43 (38.1)	0.148
Obese	26 (13.4)	7 (11.9)	19 (7.8)	
25(OH)D (nmol/L)				
Yes	59 (30.4)	-	-	-
No	135 (69.6)	-	-	

[a] Sample size according to waves of follow-up: 1st trimester = 194; 2nd trimester = 162; 3rd trimester = 161.
[b] Continuous variables described as means (standard deviation) and categorical variables as n (%).
[c] p-values refer to Student's t test comparing adequate and inadequate 25(OH)D status.
[d] Sample reduction due to missing data for Homeostasis Model Assessment – Insulin Resistance = 193; Leisure physical activity before pregnancy = 192.
[e] p-values for ANOVA for repeated measures throughout pregnancy <0.001.
[f] p-values refer to chi-squared test comparing adequate and inadequate 25(OH)D status.

Table 2. Linear mixed effects regression models between first trimester 25(OH)D status and changes in lipids concentrations during pregnancy, Rio de Janeiro/Brazil, 2009-2012.

	Total Cholesterol (mg/dL)				**Low Density Lipoprotein (mg/dL)**			
	Crude [a]		**Adjusted [b]**		**Crude [a]**		**Adjusted [b]**	
Fixed effects	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]
Intercept	99.77 (89.80 - 109.74)	**<0.001**	75.61 (43.93 - 107.28)	**<0.001**	58.21 (50.56 - 65.87)	**<0.001**	48.92 (24.93 - 72.92)	**<0.001**
First trimester 25(OH)D status (Adequacy/inadequacy)	7.53 (-1.00 - 16.06)	0.084	9.53 (1.12 - 17.94)	**0.026**	8.58 (2.21 - 14.95)	**0.008**	9.99 (3.62 - 16.36)	**0.002**
Age (years)	-	-	0.21 (-0.57 - 0.99)	0.600	-	-	-0.08 (-0.67 - 0.51)	0.794
Early pregnancy BMI (kg/m^2)	-	-	1.06 (0.27 - 1.86)	**0.009**	-	-	0.77 (0.17 - 1.37)	**0.012**
Parity (number of labors)	-	-	2.48 (-1.19 - 6.16)	0.186	-	-	1.94 (-0.84 - 4.72)	0.171
Energy intake (kcal/d)	-	-	-0.01 (-0.01 - -0.001)	**0.020**	-	-	-0.004 (-0.01 - -0.001)	**0.016**
Leisure physical activity before pregnancy (no/ yes)	-	-	6.67 (-1.96 - 15.31)	0.130	-	-	2.21 (-4.32 - 8.74)	0.507
Homeostasis Model Assessment – Insulin Resistance	-	-	-0.63 (-1.68 - 0.41)	0.234	-	-	-0.88 (-1.7 - -0.04)	**0.039**
Gestational age (weeks)	6.51 (5.66 - 7.36)	**<0.001**	6.46 (5.57 - 7.35)	**<0.001**	3.75 (3.08 - 4.42)	**<0.001**	3.67 (2.96 - 4.37)	**<0.001**
Squared gestational age	-0.09 (-0.11 - -0.07)	**<0.001**	-0.08 (-0.11 - -0.06)	**<0.001**	-0.05 (-0.06 - -0.03)	**<0.001**	-0.04 (-0.06 - -0.03)	**<0.001**
Random effects	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]
σ Gestational age	1.06 (0.70 - 1.61)		1.07 (0.70 - 1.64)		0.76 (0.52 - 1.12)		0.76 (0.51 - 1.13)	
Covariance (gestational age)	1.80 (-5.52 - 9.11)	**<0.001**	2.24 (-5.09 - 9.56)	**<0.001**	1.27 (-3.31 - 5.85)	**<0.001**	1.97 (-2.64 - 6.57)	**<0.001**
σ Residual	201.13 (160.97 - 251.31)		207.22 (164.64 - 260.79)		126.78 (101.43 - 158.46)		131.56 (104.49 - 165.63)	

[a] 25(OH)D, total number of observations (data)=516; total number of groups (women=194), and mean of 2.7 observations per group; [b] 25(OH)D, total number of observations (data)= 497; total number of groups (women=192), and mean of 2.6 observations per group. The group refers to the number of women with at least one data point in time and observations refers to the total number of data points in time for all women; [c] p-values refer to maximum likelihood value; BMI = Body Mass Index.

Table 3. Linear mixed effects regression models between first trimester 25(OH)D status and changes in lipids concentrations during pregnancy, Rio de Janeiro/Brazil, 2009-2012.

	High Density Lipoprotein (mg/dL)				Triglycerides (mg/dL)			
	Crude [a]		Adjusted [b]		Crude [a]		Adjusted [b]	
Fixed effects	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]
Intercept	33.03 (29.95 - 36.12)	**<0.001**	31.59 (22.43 - 40.75)	**<0.001**	48.61 (39.60 - 57.61)	**<0.001**	-18.46 (-54.69 - 17.77)	0.318
First trimester 25(OH)D status (Adequacy/inadequacy)	-0.51 (-2.94 - 1.92)	0.681	0.08 (-2.33 - 2.49)	0.946	-4.88 (-15.11 - 5.35)	0.350	-6.09 (-15.97 - 3.78)	0.227
Age (years)	-	-	0.18 (-0.05 - 0.40)	0.122	-	-	0.65 (-0.27 - 1.57)	0.165
Early pregnancy BMI (kg/m^2)	-	-	-0.05 (-0.28 - 0.18)	0.664	-	-	1.95 (1.02 - 2.88)	**<0.001**
Parity (number of labors)	-	-	0.28 (-0.77 - 1.34)	0.601	-	-	-0.28 (-4.61 - 4.05)	0.900
Energy intake (kcal/d)	-	-	-0.001 (-0.002 - 0.0001)	**0.076**	-	-	-0.001 (-0.01 - 0.004)	0.594
Leisure physical activity before pregnancy (no/yes)	-	-	1.77 (-0.71 - 4.25)	0.161	-	-	11.15 (1.02 - 21.28)	**0.031**
Homeostasis Model Assessment – Insulin Resistance	-	-	-0.15 (-0.48 - 0.18)	0.383	-	-	1.86 (0.34 - 3.37)	**0.016**
Gestational age (weeks)	1.94 (1.66 - 2.22)	**<0.001**	1.93 (1.64 - 2.22)	**<0.001**	3.54 (3.27 - 3.81)	**<0.001**	3.54 (3.27 - 3.82)	**<0.001**
Squared gestational age	-0.04 (-0.05 - -0.03)	**<0.001**	-0.04 (-0.05 - -0.03)	**<0.001**	-	-	-	-
Random effects	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]
σ Gestational age	0.05 (0.03 - 0.11)		0.05 (0.02 - 0.11)		1.20 (0.64 - 2.23)		1.21 (0.62 - 2.35)	
Covariance (gestational age)	-0.04 (-0.75 - 0.67)	**<0.001**	0.08 (-0.64 - 0.81)	**<0.001**	12.41 (-1.15 - 25.98)	**<0.001**	11.95 (-2.16 - 26.06)	**<0.001**
σ Residual	22.78 (18.22 - 28.49)		23.58 (18.64 - 29.83)		498.81 (402.13 - 618.73)		501.43 (398.94 - 630.25)	

[a] 25(OH)D, total number of observations (data)=516; total number of groups (women=194), and mean of 2.7 observations per group; [b] 25(OH)D, total number of observations (data)= 497; total number of groups (women=192), and mean of 2.6 observations per group. The group refers to the number of women with at least one data point in time and observations refers to the total number of data points in time for all women; [c] p-values refer to maximum likelihood value; BMI = Body Mass Index.

Table 4. Linear mixed effects regression models between first trimester 25(OH)D status and changes in lipids ratios during pregnancy, Rio de Janeiro/Brazil, 2009-2012.

	Triglycerides/High Density Lipoprotein				**Total Cholesterol/High Density Lipoprotein**			
	Crude [a]		**Adjusted** [b]		**Crude** [a]		**Adjusted** [b]	
Fixed effects	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]
Intercept	1.48 (1.17 - 1.78)	**<0.001**	0.07 (-0.78 - 0.92)	0.871	3.21 (3.03 - 3.39)	**<0.001**	2.93 (2.36 - 3.51)	**<0.001**
First trimester 25(OH)D status (Adequacy/inadequacy)	-0.10 (-0.33 - 0.13)	0.398	-0.15 (-0.37 - 0.07)	0.186	0.17 (0.02 - 0.32)	**0.030**	0.16 (0.01 - 0.31)	**0.040**
Age (years)	-	-	0.01 (-0.01 - 0.03)	0.468	-	-	-0.01 (-0.02 - 0.01)	0.292
Early pregnancy BMI (kg/m^2)	-	-	0.05 (0.02 - 0.07)	**<0.001**	-	-	0.02 (0.01 - 0.04)	**0.001**
Parity (number of labors)	-	-	-0.01 (-0.11 - 0.09)	0.854	-	-	0.03 (-0.04 - 0.09)	0.438
Energy intake (kcal/d)	-	-	-5.55e-06 (-0.0001 - 0.0001)	0.924	-	-	-0.0001 (-0.0001 - 0.00003)	0.209
Leisure physical activity before pregnancy (no/yes)	-	-	0.16 (-0.07 - 0.39)	0.165	-	-	0.002 (-0.15 - 0.16)	0.978
Homeostasis Model Assessment – Insulin Resistance	-	-	0.05 (0.01 - 0.08)	**0.009**	-	-	-0.004 (-0.02 - 0.01)	0.690
Gestational age (weeks)	0.02 (-0.01 - 0.05)	0.178	0.02 (-0.01 - 0.05)	0.180	-0.004 (-0.02 - 0.01)	0.604	-0.01 (-0.02 - 0.01)	0.445
Squared gestational age	0.001 (0.0002 - 0.002)	**0.012**	0.001 (0.0002 - 0.002)	**0.014**	0.001 (0.001 - 0.001)	**<0.001**	0.001 (0.001 - 0.001)	**<0.001**
Random effects	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]	β (95% CI)	*P* [c]
σ Gestational age	0.0005 (0.0002 - 0.001)		0.001 (0.0003 - 0.001)		0.0004 (0.0003 - 0.001)		0.0004 (0.0003 - 0.001)	
Covariance (gestational age)	0.006 (-0.0009 - 0.01)	**<0.001**	0.01 (0.005 - 0.01)	**<0.001**	-0.004 (-0.01 - -0.001)	**<0.001**	-0.002 (-0.01 - 0.001)	**<0.001**
σ Residual	0.26 (0.21 - 0.32)		0.25 (0.22 - 0.30)		0.05 (0.04 - 0.07)		0.06 (0.04 - 0.07)	

[a] 25(OH)D, total number of observations (data)=516; total number of groups (women=194), and mean of 2.7

observations per group; [b] 25(OH)D, total number of observations (data)= 497; total number of groups (women=192), and mean of 2.6 observations per group umber of observations. The group refers to the number of women with at least one data point in time and observations refers to the total number of data points in time for all women; [c] p-values refer to maximum likelihood value; BMI = Body Mass Index.