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The effects of vitamin D plus calcium supplementation on metabolic profiles, biomarkers of inflammation, oxidative stress and pregnancy outcomes in pregnant women at risk for pre-eclampsia

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Keywords
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Abstract

Background: The present study was designed to examine the effects of vitamin D plus calcium administration on metabolic profiles and pregnancy outcomes among women at risk for pre-eclampsia.
Methods: In a prospective, double-blind, placebo-controlled trial, 60 women at risk for pre-eclampsia were randomised to take either 50 000 IU vitamin D_3 every 2 weeks plus 1000 mg day^{-1} calcium supplements (as calcium carbonate) ($n = 30$) or to receive placebos at the same times ($n = 30$) from 20 to 32 weeks of gestation. Fasting blood samples were taken at baseline and 12 weeks after intervention to determine related variables. Newborn anthropometric measurements were determined.
Results: Taking combined cholecalciferol and calcium supplements, compared to placebo, led to significant reductions in fasting plasma glucose (FPG) [mean (SD)] [−5.7 (5.5) versus −0.6 (12.6) mg dL^{-1}, $P = 0.04$], serum insulin concentrations [−2.8 (6.0) versus +7.7 (9.8) μIU mL^{-1}, $P < 0.001$], homeostasis model of assessment-insulin resistance [−0.8 (1.3) versus +1.6 (2.2), $P < 0.001$], homeostatic model assessment-beta cell function [−8.2 (25.8) versus +32.6 (41.3, $P < 0.001$] and a significant rise in quantitative insulin sensitivity check index score [+0.02 (0.02) versus −0.02 (0.02, $P < 0.001$]. Additionally, pregnant women who received cholecalciferol plus calcium supplements had increased serum high-density lipoprotein (HDL)-cholesterol [+4.6 (8.3) versus −2.9 (7.7) mg dL^{-1}, $P = 0.001$] and plasma total glutathione (GSH) concentrations [+23.4 (124.0) versus −94.8 (130.2) μM, $P = 0.001$] compared to placebo. However, after adjustment for the baseline levels, maternal age and baseline body mass index, the effects on FPG levels ($P = 0.13$) and systolic blood pressure ($P = 0.13$) disappeared.
Conclusions: Vitamin D plus calcium administration for 12 weeks had beneficial effects on glycaemic status, HDL-cholesterol, GSH and blood pressure among women at risk for pre-eclampsia.

Introduction

Pre-eclampsia, defined by hypertension and proteinuria occurring after 20 weeks of gestation, is a multifactorial disorder that is associated with maternal and perinatal morbidity and mortality worldwide, as well as with neurological disorders, liver disease and abnormal renal function [1–3]. Its prevalence is 2–10% of all pregnancies and this may be higher in low resource settings [4]. Previous studies have reported that pre-eclampsia may increase the prevalence of various cardiovascular disease risk factors, including metabolic syndrome, impaired in insulin metabolism, microalbuminuria, endothelial dysfunction [5], inflammatory factors and oxidative stress[6] .

Effective management of pre-eclampsia may be divided into three categories including the prevention of pre-eclampsia, early detection and treatment [7]. Earlier studies have shown that maternal vitamin D and calcium deficiency during pregnancy may be an independent risk factor for pre-eclampsia [8,9]. In a study by Haugen *et al.* [10], a 27% reduction in risk of pre-eclampsia was observed among women taking 400–600 IU vitamin D supplements per day compared to those taking no supplements. In another study, calcium supplementation ($\geq$1 g day^{-1}) resulted in a significant reduction in the risk of pre-eclampsia, particularly for women with low-calcium diets [11]. These findings resulted in the hypothesis that early supplementation with cholecalciferol or calcium could be useful in decreasing markers of oxidative stress, thereby preventing or ameliorating the course of pre-eclampsia. Recent evidence suggested that combined vitamin D and calcium intake may be associated with better metabolic health [12,13]. In a previous study, 1000 mg calcium per day + 50 000 IU week^{-1} vitamin D supplementation for 8 weeks among vitamin D deficient patients with polycystic ovary syndrome (PCOS) had beneficial effects on markers of insulin metabolism and triglycerides concentrations compared to only calcium or the vitamin D group [13]. Moreover, 1000 mg calcium day^{-1} and two pearls (soft gel) containing 50 000 IU of cholecalciferol supplementation for 6 weeks among women with gestational diabetes (GDM) resulted in a decreased caesarean section rate and maternal hospitalisation, as well as decreased macrosomia, hyperbilirubinaemia and hospitalisation in newborns [14].

The favourable effects of combined cholecalciferol and calcium intake on metabolic status and pregnancy outcomes might a result of their effects on the activation of antioxidant enzymes [15] and suppression of parathyroid hormone [16], influencing skeletal composition and smooth muscle strength [17]. We are unaware of any trial examining the favourable effects of combined cholecalciferol and calcium supplementation on markers of insulin metabolism, lipid concentrations, biomarkers of inflammation, oxidative stress and pregnancy outcomes in women at risk for pre-eclampsia. The present study aimed to investigate the beneficial effects of taking cholecalciferol plus calcium supplements on metabolic status and pregnancy outcomes in women at risk for pre-eclampsia.

Materials and methods

Participants

The present study was a parallel, balanced randomisation (1 : 1), randomised double-blind placebo-controlled clinical trial performed between September 2014 to February 2015, in Kashan, Iran. The pregnant women included in the present study comprised primigravida pregnant women, aged 18–40 years old, who were at risk for pre-eclampsia, and lived approximately 20 km or less from the clinic and hospital. Women 'at-risk' for pre-eclampia were recognised by laboratory tests including free β-human chorionic gonadotrophin, inhibin α dimeric, unconjugated oestriol and maternal serum α-foetoprotein, and haemodynamic assessment of uterine artery Doppler waveform at 16–20 weeks of gestation [18]. Participants were excluded if they had hypertension, renal diseases, GDM or an abnormal foetal anomaly scan. For estimating sample size, we used a randomised clinical trial sample size formula where type 1 (α) and type 2 errors (β) were 0.05 and 0.20 (power = 80%), respectively. According to a previous study [14], we considered 355.6 as the SD and 286 g as the difference in mean (d) of newborn weight at birth as a key variable. Accordingly, we required 25 pregnant women in each group to have 80% power of the study. However, we recruited 30 patients in each group (60 patients in total) to compensate for any probable loss to follow-up. The present study was approved by the Ethics Committee of Kashan University of Medical Sciences (KUMS). All pregnant women provided their written informed consent. This trial was registered on the Iranian website (www.irct.ir) for registration of clinical trials (IRCT code: IRCT201102135444N3).

Study design

Initially, pregnant women, after stratification for pre-intervention body mass index (BMI) (<25 and $\geq$25 kg m^{-2}) and maternal age (<30 and $\geq$30 years), were randomly allocated into two groups to take either 50 000 IU vitamin D_3 every 2 weeks plus 1000 mg day^{-1} calcium supplements (as calcium carbonate) (n = 30) or to receive placebos at the same times (n = 30) from 20 to 32 weeks of gestation. The randomised allocation sequence from a

computer random number generator was obtained out by a trained midwife at the maternity clinic. Randomisation and allocation were concealed from both researchers and pregnant women until the statistical analysis was completed. Vitamin D_3 and its placebo (from Zahravi Pharm Co, Tabriz, Iran) and calcium and its placebo (from Tehran Shimi Pharm Co.) were provided. An investigator with no clinical involvement in the present study packed cholecalciferol, calcium supplements and placebos into numbered bottles based on the random list. With respect to colour, shape, size and package, placebo pearls and placebo tablets were similar to the vitamin D_3 and calcium tablets and contained edible paraffin (for vitamin D_3) and starch (for calcium). Pregnant women were requested not to change their regular physical activity or normal dietary intakes throughout the study and not to take any supplements other than that provided to them by the investigators. All pregnant women were also taking 400 μg day^{-1} folic acid from the beginning of gestation, 60 mg day^{-1} ferrous sulphate from the second trimester and a multivitamin mineral capsule (containing 400 IU vitamin D and 500 mg calcium) from the second half of pregnancy. Participants' nutritional status and physical activity were assayed by 3-day food records and a pregnancy physical activity questionnaire, respectively. Pregnant women were requested to record all foods and beverages consumed throughout the day at weeks 3, 6 and 9 of intervention for 2 days of the week and 1 day of the weekend. Then, to analyse nutrient intakes of patients, we used NUTRITIONIST IV (First Databank, San Bruno, CA, USA).

Assessment of anthropometric variables

Anthropometric measurements of pregnant women at maternity clinic were measured by a trained midwife at baseline and then after 12 weeks of intervention. Weight (to the nearest 0.1 kg) and height (to the nearest 0.5 cm) were measured without shoes using a scale (Seca, Hamburg, Germany) and a wall-meter, respectively. Finally, BMI was determined for each patient using the formula: weight (kg)/height^2 (m^2).

Primary and secondary outcomes

In the present study, primary outcomes were the rate of pre-eclampsia, low birth weight (LBW) (<2500 g), newborn's birth size (newborn's weight, length and head circumference) and prevalence of preterm delivery (<37 weeks). Secondary outcomes were markers of insulin metabolism, lipid concentrations, biomarkers of inflammation, oxidative stress and blood pressures.

Biochemical analysis

Blood samples (10 mL) were taken at baseline and after 12 weeks of intervention at Kashan reference laboratory after overnight fasting. Blood samples were immediately centrifuged at 1465 g for 10 min at room temperature. Then, the samples were frozen in −70 °C before analysis at the KUMS reference laboratory. However, fasting plasma glucose (FPG) and lipid profiles were immediately conducted on the day of sampling. Serum 25-hydroxyvitamin D concentrations was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (IDS, Boldon, UK) with inter- and intra-assay coefficients of variation (CVs) of 4.5–7.0%, respectively. In addition, commercial kits were used to measure FPG, triglycerides, cholesterol, very low-density lipoprotein (VLDL)-, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol concentrations (Pars Azmun, Tehran, Iran). The intra- and inter-assay CVs for FPG and lipid concentrations were <5%. Serum insulin was measured by ELISA kit (Monobind, California, USA) with intra- and inter-assay CVs of 2.5% and 5.6%, respectively. The homeostasis model of assessment-insulin resistance (HOMA-IR), β-cell function (HOMA-B) and quantitative insulin sensitivity check index (QUICKI) were calculated based on suggested formulas [19]. Serum high sensitivity C-reactive protein (hs-CRP) was quantified using an ELISA kit (LDN, Nordhorn, Germany) with intra- and inter-assay CVs of 2.5% and 4.5%, respectively. Plasma nitrite/nitrate, taken as an index of nitric oxide concentration, was measured using the Giess method modified by Tatsh *et al.* [20] Plasma total antioxidant capacity (TAC) was quantified by the use of ferric reducing antioxidant power method developed by Benzie and Strain [21]. The plasma total glutathione (GSH) and malondialdehyde were measured by the method of Beutler *et al.* [22] and the thiobarbituric acid reactive substance spectrophotometric test [23].

Statistical analysis

Distribution of data in terms of normality was evaluated by the Shapiro–Wilk test [24]. All data had a normal distribution. To detect changes in anthropometric characteristics and dietary intakes between the two groups, we applied an independent samples Student's *t*-test. Intention-to-treat (ITT) analysis of the primary study endpoint was performed for all the randomly assigned participants. Comparisons of changes (end-point minus baseline) between the two groups were performed by one-way repeated measures analysis of variance. In addition, to control confounding variables (baseline variables, maternal age and baseline BMI), we used analysis of covariance.

The Pearson chi-squared test was used to compare categorical variables. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS, version 18 (SPSS Inc., Chicago, IL, USA).

Results

In the present study, 60 pregnant women at risk for pre-eclampsia met the inclusion criteria based on laboratory and haemodynamic criteria and were enrolled in the study (Fig. 1). Among individuals in the cholecalciferol plus calcium group, one pregnant women (withdrawn for personal reasons; $n = 1$) and, in the placebo group, one women (withdrawn for personal reasons; $n = 1$) did not complete the trial. However, because the analysis was conducted according to an ITT protocol, all 60 patients at risk for pre-eclampsia were included in the end analysis. On average, the rate of compliance in the present study was high, such that >90% of pearls and tablets were taken throughout the trial in both groups.

At baseline and end-point, the trial means of weight and BMI were not different between combined cholecalciferol plus calcium and placebo groups (Table 1).

No significant change was observed between the two groups in terms of dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, cholesterol, total dietary fibre, vitamin D, calcium and phosphors according to the 3-day dietary records obtained throughout the trial (Table 2).

After 12 weeks, mean (SD) serum 25(OH)D concentrations had significantly increased in the vitamin D plus calcium group [+8.2 (7.7) versus +0.1 (3.2) ng mL^{-1}, $P < 0.001$] compared to placebo (Table 3). In addition, taking combined cholecalciferol and calcium supplements, compared to placebo, led to significant reductions in FPG [−5.7 (5.5) versus −0.6 (12.6) mg dL^{-1}, $P = 0.04$], serum insulin concentrations [−2.8 (6.0) versus +7.7 (9.8) μIU mL^{-1}, $P < 0.001$], HOMA-IR [−0.8 (1.3) versus +1.6 (2.2), $P < 0.001$], HOMA-B [−8.2 (25.8) versus +32.6 (41.3), $P < 0.001$] and a significant elevation in QUICKI score [+0.02 (0.02) versus −0.02 (0.02), $P < 0.001$]. Additionally, pregnant women who received cholecalciferol plus calcium supplements had increased serum HDL-cholesterol [+4.6 (8.3) versus −2.9 (7.7) mg dL^{-1}, $P = 0.001$] and plasma GSH concentrations

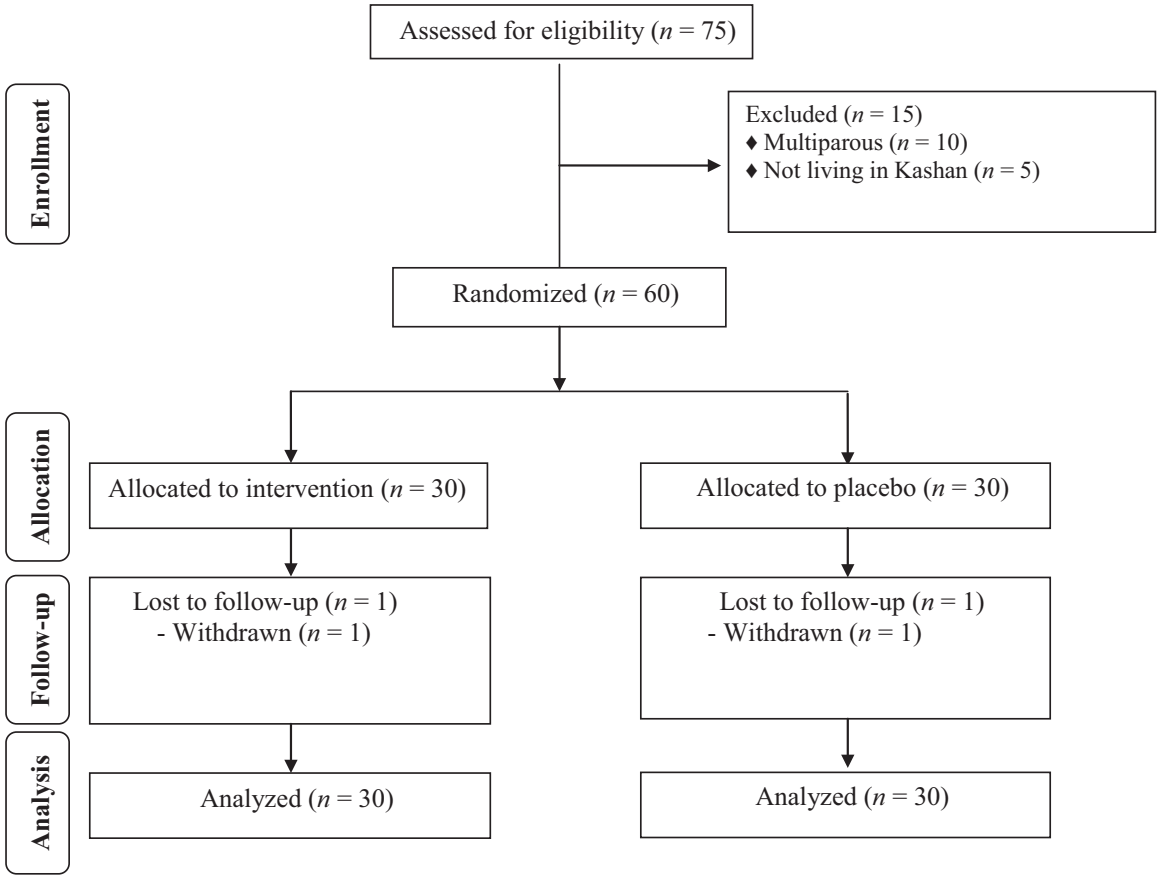

Figure 1 Summary of the patient flow diagram.

Table 1 General characteristics of the study participants

	Placebo group (n = 30)	Vitamin D plus calcium group (n = 30)	P*
Maternal age (years)	27.1 (5.2)	27.3 (3.7)	0.84
Height (cm)	159.8 (5.2)	158.9 (3.2)	0.43
Weight at study baseline (kg)	65.5 (11.1)	69.2 (9.4)	0.16
Weight at end-of-trial (kg)	70.6 (10.3)	73.8 (9.7)	0.21
BMI at study baseline (kg m^{-2})	25.6 (4.0)	27.4 (3.3)	0.06
BMI at end-of-trial (kg m^{-2})	27.6 (3.7)	29.2 (3.4)	0.10

Data are the mean (SD).
*Obtained from an independent *t*-test.BMI, body mass index.

Table 2 Dietary intakes of study participants throughout the study

	Placebo group (n = 30)	Vitamin D plus calcium group (n = 30)	P*
Energy (MJ day^{-1})	10.0 (1.1)	10.2 (1.0)	0.50
Carbohydrates (g day^{-1})	327.9 (54.9)	342.9 (52.2)	0.28
Protein (g day^{-1})	86.9 (13.7)	86.1 (12.1)	0.79
Fat (g day^{-1})	84.5 (13.1)	83.5 (14.4)	0.79
SFA (g day^{-1})	24.5 (4.9)	25.1 (4.9)	0.67
PUFA (g day^{-1})	25.6 (6.3)	26.8 (7.3)	0.50
MUFA (g day^{-1})	23.3 (7.2)	22.5 (5.4)	0.61
Cholesterol (mg day^{-1})	214.5 (115.9)	196.1 (90.7)	0.49
TDF (g day^{-1})	19.4 (5.7)	20.2 (5.2)	0.57
Vitamin D (μg day^{-1})	2.7 (0.8)	2.7 (0.7)	0.94
Calcium (mg day^{-1})	1127.9 (195.9)	1152.9 (183.2)	0.61
Phosphors (mg day^{-1})	1164.5 (196.6)	1184.1 (187.4)	0.69

SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; TDF, total dietary fibre.
Data are the mean (SD).
*Obtained from an independent *t*-test.

[+23.4 (124.0) versus −94.8 (130.2) μM, P = 0.001] compared to placebo. Finally, administration of cholecalciferol plus calcium supplements has resulted in significant decreases in systolic blood pressure (SBP) [−3.8 (5.8) versus+1.7 (8.7) mmHg, P = 0.006] and diastolic blood pressure (DBP) [−2.0 (6.6) versus +3.7 (6.3) mmHg, P = 0.001] compared to placebo. Supplementation showed no detectable changes in other lipid profiles and inflammatory markers.

Baseline levels of vitamin D [16.2 (3.5) versus 13.1 (6.4) ng mL^{-1}, P = 0.02] and calcium [9.2 (0.6) versus 8.7 (0.3) mg dL^{-1}, P = 0.002] were significantly different between the two groups. Therefore, we controlled the analyses for the baseline levels of the corresponding biochemical measure, maternal age and baseline BMI. However, after this adjustment for baseline levels, maternal age and baseline BMI, no detectable changes in our findings occurred, except for FPG levels (P = 0.13) and SBP (P = 0.13) (Table 4).

The present study has not shown any significant effect for pregnancy outcomes including caesarean section rate, gestational age, preterm delivery, newborn's birth size, Apgar scores, pre-eclampsia rate and LBW on comparing the two groups (Table 5).

Discussion

The present study demonstrated, for the first time, that joint vitamin D_3 and calcium supplementation for 3 months in women at risk for pre-eclampsia resulted in a significant decrease in FPG, serum insulin levels, HOMA-IR, HOMA-B, SBP and DBP, as well as a significant increase in QUICKI score, serum HDL-cholesterol and plasma GSH concentrations. It must be noted that, in the present study, we used a dose of approximately 3600 IU day^{-1} of vitamin D supplements, which is higher than the current recommended dietary allowance (600 IU day^{-1}) or the standard prenatal supplement dose (400 IU day^{-1}). However, this dose is lower than the tolerable upper intake levels advised by the Institute of Medicine [25]. The prevalence of vitamin D deficiency is high among Iranian pregnant women. Our previous study has shown that 95.8% of pregnant women in Kashan, Iran, had serum vitamin D concentrations <20 ng mL^{-1} [26]. In addition, previous studies have reported that intramuscular injection of 600 000 IU vitamin D supplements was associated with significant increases in serum vitamin D levels at 1.5, 3, and 6 months, but not 9 and 12 months later [27,28]. Moreover, in the present study, we checked serum calcium levels at the beginning of the study and every 2 weeks aiming to avoid vitamin D toxicity. It should be noted that under- and over-reporting of dietary intakes is an important point in epidemiologic observational studies. Because this was a clinical trial and its sample size was small, we did not assess the prevalence of under- and over-reporting of dietary intakes. In addition, dietary intakes were only compared to examine the probable differences between the two groups. It was an accessory variable and not the main exposure variable in the present study.

Pregnant women are sensitive to foetal and maternal complications [29,30]. The present study revealed that joint cholecalciferol and calcium administration for 12 weeks in pregnant women at risk for pre-eclampsia led to significant reductions in FPG, serum insulin concentrations, HOMA-IR, HOMA-B and a significant elevation in QUICKI score compared to placebo. However, after controlling for baseline levels, maternal age and BMI at study baseline, the changes in FPG were not significantly different between the two groups. Limited data are available

Table 3 Mean (SD) of metabolic profiles, biomarkers of inflammation and oxidative stress at baseline and after the intervention

	Placebo group (n = 30)			Vitamin D plus calcium group (n = 30)			
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	P*
Vitamin D (ng mL^{-1})	16.2 (3.5)	16.3 (4.9)	0.1 (3.2)	13.1 (6.4)	21.3 (8.4)†	8.2 (7.7)	<0.001
Calcium (mg dL^{-1})	9.2 (0.6)	8.9 (0.4)	−0.3 (0.7)	8.7 (0.3)	8.8 (0.4)	0.1 (0.4)	0.08
FPG (mg dL^{-1})	86.4 (8.2)	85.8 (11.4)	−0.4 (12.6)	87.7 (6.4)	82.0 (4.7)†	−5.7 (5.5)	0.04
Insulin (μIU mL^{-1})	12.1 (5.9)	19.8 (7.7)†	7.7 (9.8)	14.3 (5.2)	11.5 (4.6)†	−2.8 (6.0)	<0.001
HOMA-IR	2.6 (1.2)	4.2 (1.8)†	1.6 (2.2)	3.1 (1.1)	2.3 (0.9)†	−0.8 (1.3)	<0.001
HOMA-B	47.6 (25.9)	80.2 (31.3)†	32.6 (41.3)	55.5 (22.1)	47.3 (21.0)	−8.2 (25.8)	<0.001
QUICKI	0.33 (0.02)	0.31 (0.01)†	−0.02 (0.02)	0.32 (0.02)	0.34 (0.01)†	0.02 (0.02)	<0.001
Triglycerides (mg dL^{-1})	184.8 (51.0)	211.9 (62.9)†	27.1 (52.1)	188.5 (74.3)	224.5 (52.4)†	36.0 (80.1)	0.61
VLDL-cholesterol (mg dL^{-1})	37.0 (10.2)	42.4 (12.6)†	5.4 (10.4)	37.7 (14.9)	44.9 (10.5)†	7.2 (16.0)	0.61
Total cholesterol (mg dL^{-1})	226.6 (39.1)	233.8 (34.9)	7.2 (30.7)	215.9 (41.8)	234.6 (42.8)†	18.7 (38.4)	0.20
LDL-cholesterol (mg dL^{-1})	119.6 (31.9)	124.3 (30.8)	4.7 (23.8)	108.9 (32.4)	115.8 (39.2)	6.9 (24.7)	0.72
HDL-cholesterol (mg dL^{-1})	70.0 (10.4)	67.1 (11.4)	−2.9 (7.7)	69.3 (11.2)	73.9 (11.5)	4.6 (8.3)	0.001
Total: HDL cholesterol ratio	3.3 (0.8)	3.6 (0.8)†	0.3 (0.4)	3.1 (0.6)	3.2 (0.7)	0.1 (0.5)	0.13
hs-CRP (ng mL^{-1})	7598.5 (3738.2)	6976.7 (3606.8)	−621.8 (3058.4)	7146.3 (3453.6)	5859.8 (3108.3)†	−1286.5 (3213.3)	0.38
NO (μM)	48.5 (7.1)	50.8 (15.2)	2.3 (12.7)	43.5 (4.1)	41.7 (8.4)	−1.8 (10.6)	0.18
TAC (mM)	673.8 (125.5)	599.4 (93.4)†	−74.5 (140.6)	726.6 (123.5)	732.2 (199.4)	5.6 (184.0)	0.06
GSH (μM)	538.8 (128.9)	444.0 (110.4)†	−94.8 (130.2)	517.6 (75.7)	541.0 (97.4)	23.4 (124.0)	0.001
MDA (μM)	4.8 (0.6)	5.3 (1.0)†	0.5 (1.1)	4.8 (1.3)	4.7 (1.3)	−0.1 (1.64)	0.13
SBP (mmHg)	108.8 (6.7)	110.5 (6.2)	1.7 (8.7)	114.6 (9.4)	110.8 (8.9)†	−3.8 (5.8)	0.006
DBP (mmHg)	72.8 (5.5)	76.5 (7.7)†	3.7 (6.3)	72.0 (8.0)	70.0 (9.1)	−2.0 (6.6)	0.001

*Obtained from repeated measures analysis of variance.
†Different from baseline study, $P < 0.05$.

Table 4 Adjusted changes in metabolic variables in women at risk for pre-eclampsia who received either vitamin D plus calcium supplements or placebo

	Placebo group (n = 30)	Vitamin D plus calcium group (n = 30)	P*
Vitamin D (ng mL^{-1})	0.8 (1.1)	7.6 (1.1)	<0.001
Calcium (mg dL^{-1})	−0.1 (0.1)	−0.1 (0.1)	0.56
FPG (mg dL^{-1})	−1.5 (1.5)	−4.9 (1.5)	0.13
Insulin (μIU mL^{-1})	7.0 (1.2)	−2.0 (1.2)	<0.001
HOMA-IR	1.5 (0.3)	−0.5 (0.3)	<0.001
HOMA-B	30.2 (4.9)	−5.8 (4.9)	<0.001
QUICKI	−0.02 (0.003)	0.01 (0.003)	<0.001
Triglycerides (mg dL^{-1})	30.8 (9.0)	32.4 (9.0)	0.90
VLDL-cholesterol (mg dL^{-1})	6.2 (1.8)	6.5 (1.8)	0.90
Total cholesterol (mg dL^{-1})	9.8 (5.8)	16.2 (5.8)	0.44
LDL-cholesterol (mg dL^{-1})	5.5 (4.4)	6.2 (4.4)	0.91
HDL-cholesterol (mg dL^{-1})	−3.0 (1.3)	4.7 (1.3)	<0.001
Total: HDL cholesterol ratio	0.3 (0.1)	0.04 (0.1)	0.05
hs-CRP (ng mL^{-1})	−356.7 (504.2)	−1513.7 (504.2)	0.11
NO (μM)	3.1 (2.2)	−2.5 (2.2)	0.10
TAC (mM)	−89.1 (27.2)	20.3 (27.2)	0.007
GSH (μM)	−85.4 (18.7)	13.9 (18.7)	<0.001
MDA (μM)	0.5 (0.2)	−0.1 (0.2)	0.06
SBP (mmHg)	0.2 (1.2)	−2.4 (1.2)	0.13
DBP (mmHg)	3.4 (1.2)	−1.8 (1.2	0.003

DBP, diastolic blood pressure; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment- estimated b cell function; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TAC, total antioxidant capacity.HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.
All values are the mean (SE).
*Obtained from an analysis of covariance adjusted for baseline values + maternal age and baseline body mass index.

Table 5 The effect of vitamin D plus calcium administration on pregnancy outcomes

	Placebo group (n = 30)	Vitamin D plus calcium group (n = 30)	P*
Caesarean section (%)	11 (36.7)	14 (46.7)	0.43†
Gestational age (weeks)	38.9 (1.2)	38.4 (1.1)	0.07
Preterm delivery (%)	1 (3.3)	2 (6.7)	0.55†
Newborn weight (g)	3144.0 (485.4)	3300.0 (441.0)	0.19
Newborn length (cm)	49.9 (1.5)	49.5 (1.3)	0.20
Newborn head circumference (cm)	34.5 (1.2)	34.5 (1.0)	0.86
1-min Apgar score	8.9 (0.2)	8.9 (0.4)	0.41
5-min Apgar score	9.9 (0.2)	9.9 (0.2)	1.00
Pre-eclampsia rate (%)	3 (10.0)	1 (3.3)	0.30†
LBW (%)	2 (6.7)	0 (0)	0.15†

LBW, low birth weight.
Values are the mean (SD), except where indicated as a percentage.
*Obtained from an independent t-test.
†Obtained from Pearson chi-squared test.

evaluating the favourable effects of cholecalciferol plus calcium intake on markers of insulin metabolism in women at risk for pre-eclampsia. Supporting the present study, our previous study has shown that taking 1000 mg calcium per day + 50 000 IU vitamin D every 3 weeks for 6 weeks among patients with GDM had beneficial effects on FPG, serum insulin, HOMA-IR and QUICKI score compared to the placebo [12]. In addition,

50 000 U week^{-1} vitamin D + 1000 mg day^{-1} calcium co-supplementation led to reduced serum insulin levels, HOMA-IR score and a significant increase in QUICKI score among vitamin D insufficient individuals with type 2 diabetes mellitus (T2DM) for 8 weeks [31]. However, few studies observed such beneficial effects of vitamin D–calcium co-supplementation on parameters of glucose homeostasis. For example, markers of insulin metabolism remained unchanged after the supplementation of vitamin D plus calcium for 3 months in overweight and vitamin D deficient women with PCOS [32]. Beneficial effects of vitamin D intake on pancreatic β cell function might be a result of its effect on the regulation of serum calcium, which subsequently influences insulin secretion [33]. Furthermore, the active form of vitamin D in peripheral cells may increase insulin sensitivity through transcriptional activation of the human insulin receptor gene [34].

The findings from the present study showed that pregnant women who received combined vitamin D and calcium supplements for 12 weeks had a significant increase in serum HDL-cholesterol concentrations compared to placebo, although they showed no detectable changes in other lipid profiles. A study by Schnatz *et al.* [35] showed that calcium plus vitamin D_3 supplementation (1000 mg day^{-1} of elemental calcium + 400 IU day^{-1} of vitamin D_3) among post-menopausal women for 2 years resulted in a significant increase in HDL-cholesterol and significant decreases in LDL-cholesterol and triglyceride concentrations. Furthermore, 50 000 IU week^{-1} vitamin D + 1000 mg day^{-1} calcium co-supplementation for 8 weeks among vitamin D deficient women with PCOS resulted in a significant decrease in serum triglyceride and VLDL-cholesterol levels but did not affect other lipid profiles [13]. In the present study, an HDL-cholesterol-increasing effect of cholecalciferol via the stimulation of apolipoprotein A1 [36] and calcium via a decrease in cholesteryl ester transport protein [37] has been suggested. The lack of detectable changes after supplementation with cholecalciferol and calcium on other lipid profiles in the present study might be mediated by distinct trial designs, various dosages of cholecalciferol and calcium supplementation, as well as the patients recruited to the study.

The present study demonstrated that using cholecalciferol plus calcium supplements did not influence serum hs-CRP and plasma nitric oxide levels in pregnant women at risk for pre-eclampsia compared to placebo. In accordance with our study, no significant difference in serum hs-CRP levels was seen after the administration of 1000 mg calcium per day + 50 000 IU vitamin D every 3 weeks for 6 weeks among patients with GDM [12] Similar findings were observed after vitamin-D co-supplementation among patients with T2DM for 8 weeks [38], as well as after vitamin D intake among public housing communities for 3 months [39]. By contrast, a 9-week intake of 400 IU vitamin D supplements among healthy pregnant women [40] and 30-day supplementation with 112 mg day^{-1} calcium fructoborate among healthy subjects [41] resulted in a significant reduction of CRP levels.

The present study demonstrated that joint cholecalciferol and calcium intake resulted in a significant rise in plasma GSH levels in pregnant women at risk for pre-eclampsia compared to placebo but had no effect on other biomarkers of oxidative stress. Consistent with the present study, our previous study among women with GDM for 6 weeks showed that cholecalciferol and calcium co-supplementation significantly increased plasma GSH concentrations but did not influence plasma TAC levels [12]. In addition, a 14-week supplementation of vitamin D and calcium led to a significant decrease in oxidative stress among African Americans with congestive heart failure [42]. The exact mechanisms by which vitamin D and calcium administration might influence biomarkers of oxidative stress are unclear. Increased GSH levels, decreased reactive oxygen species and pro-inflammatory cytokines as a result of vitamin D [43] and the participation of calcium in the signalling pathway [44] may all contribute to its beneficial effects on oxidative stress.

The present study demonstrated that joint vitamin D and calcium supplementation for 12 weeks is associated with significant decreases in SBP and DBP among women at risk for pre-eclampsia compared to placebo. However, after controlling for baseline levels, maternal age and BMI at study baseline, the changes in SBP were not significantly different between the two groups. In line with our studies, 50 000 IU week^{-1} cholecalciferol supplementation for 12 weeks had beneficial effect on the levels of blood pressure in patients with T2DM [45] In addition, 50 000 IU week^{-1} vitamin D_3 plus 1000 mg day^{-1} calcium administration among T2DM patients led to a significant reduction in SBP but did not affect DBP [46] An accurate mechanism decreasing blood pressure by vitamin D and calcium may be explained through their functions on the renin–angiotensin system, vascular endothelial function or vascular smooth muscle intracellular calcium concentrations [45,47].

The present study showed no significant effect of vitamin D–calcium co-supplementation on pregnancy outcomes. A meta-analysis study by Perez-Lopez *et al.* [48] reported that vitamin D administration during pregnancy was associated with increased birth weight and birth length and was not associated with other maternal and neonatal outcomes. However, our previous study showed that calcium plus vitamin D supplementation for 6 weeks among women with GDM led to a decreased caesarean section rate, macrosomia and hyperbilirubinaemia, but did not influence other pregnancy outcomes [14] Discrep-

ancies between the present study and other previous studies might be a result of the different doses of cholecalciferol and calcium used, as well as the participants recruited to the study.

One of the major limitations of the present study is the differences in FPG levels and SBP after adjustment for baseline levels, maternal age and baseline BMI. This means that baseline differences in these measures, along with other factors such as BMI and maternal age, might influence these factors much more than the results of treatment. Therefore, joint vitamin D and calcium supplementation may not have had a significant effect on FPG levels and SBP.

Overall, joint vitamin D and calcium administration for 12 weeks had beneficial effects on glycaemic status, HDL-cholesterol, GSH levels and blood pressure among pregnant women at risk for pre-eclampsia, although it did not affect other lipid profiles, inflammatory markers, other biomarkers of oxidative stress and pregnancy outcomes.

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Conflict of interests, source of funding and authorship

The authors declare that there are no conflicts of interest.
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ZA contributed to the conception, design, statistical analysis and drafting of the manuscript. MS, MK, FF, MK, FB, YH, H-RT and AE contributed to the conception, data collection and drafting of the manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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