

Vitamin D Supplementation Affects Serum High-Sensitivity C-Reactive Protein, Insulin Resistance, and Biomarkers of Oxidative Stress in Pregnant Women^{1,2}

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Abstract

Unfavorable metabolic profiles and oxidative stress in pregnancy are associated with several complications. This study was conducted to determine the effects of vitamin D supplementation on serum concentrations of high-sensitivity C-reactive protein (hs-CRP), metabolic profiles, and biomarkers of oxidative stress in healthy pregnant women. This randomized, double-blind, placebo-controlled clinical trial was conducted in 48 pregnant women aged 18–40 y old at 25 wk of gestation. Participants were randomly assigned to receive either 400 IU/d cholecalciferol supplements ($n = 24$) or placebo ($n = 24$) for 9 wk. Fasting blood samples were taken at study baseline and after 9 wk of intervention to quantify serum concentrations of hs-CRP, lipid concentrations, insulin, and biomarkers of oxidative stress. After 9 wk of intervention, the increases in serum 25-hydroxyvitamin D and calcium concentrations were greater in the vitamin D group (+3.7 $\mu\text{g/L}$ and +0.20 mg/dL, respectively) than in the placebo group (–1.2 $\mu\text{g/L}$ and –0.12 mg/dL, respectively; $P < 0.001$ for both). Vitamin D supplementation resulted in a significant decrease in serum hs-CRP (vitamin D vs. placebo groups: –1.41 vs. +1.50 $\mu\text{g/mL}$; P -interaction = 0.01) and insulin concentrations (vitamin D vs. placebo groups: –1.0 vs. +2.6 $\mu\text{IU/mL}$; P -interaction = 0.04) and a significant increase in the Quantitative Insulin Sensitivity Check Index score (vitamin D vs. placebo groups: +0.02 vs. –0.02; P -interaction = 0.006), plasma total antioxidant capacity (vitamin D vs. placebo groups: +152 vs. –20 mmol/L; P -interaction = 0.002), and total glutathione concentrations (vitamin D vs. placebo groups: +205 vs. –32 $\mu\text{mol/L}$; P -interaction = 0.02) compared with placebo. Intake of vitamin D supplements led to a significant decrease in fasting plasma glucose (vitamin D vs. placebo groups: –0.65 vs. –0.12 mmol/L; P -interaction = 0.01), systolic blood pressure (vitamin D vs. placebo groups: –0.2 vs. +5.5 mm Hg; P -interaction = 0.01), and diastolic blood pressure (vitamin D vs. placebo groups: –0.4 vs. +3.1 mm Hg; P -interaction = 0.01) compared with placebo. In conclusion, vitamin D supplementation for 9 wk among pregnant women has beneficial effects on metabolic status. *J. Nutr.* 143: 1432–1438, 2013.

Introduction

Due to several metabolic alterations, pregnancy is associated with inflammation, insulin resistance, oxidative stress, and dyslipidemia (1–3). Increased inflammatory factors, insulin resistance, biomarkers of oxidative stress, and lipid concentrations during pregnancy can result in preeclampsia (4), preterm delivery (5), intrauterine growth retardation (6), increased risk of low birth weight (7), DNA damage (fragmentation, apoptosis, base modi-

fications, and strand breaks), and lipid, protein, and polysaccharide oxidation (8) as well as gestational diabetes mellitus (GDM)⁷ (9).

To reduce complications in pregnant women, different strategies have been suggested, including antioxidant supplementation (10), the use of lipid-lowering medications (11), and oxidative stress-lowering agents (12) as well as antiinflammatory agents (13). Recently, a few studies have shown that vitamin D supplementation might decrease inflammation, biomarkers of oxidative

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⁷ Abbreviations used: DBP, diastolic blood pressure; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; GSH, total glutathione; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; QUICKI, Quantitative Insulin Sensitivity Check Index; SBP, systolic blood pressure; TAC, total antioxidant capacity; TNF- α , tumor necrosis factor α ; 25(OH)D, 25-hydroxyvitamin D.

stress, and metabolic profiles (14,15). Due to its effect on insulin sensitivity (16), apolipoprotein gene expression (17), and parathyroid hormone suppression (18), vitamin D intake might control the metabolic alterations in pregnancy. Furthermore, it has been shown that vitamin D may reduce lipid peroxidation (19,20) and improve vascular endothelial function through modulating the expression of radical generating and scavenging enzymes (21).

Decreased proinflammatory factors in patients with colorectal adenoma (22) and reduced biomarkers of oxidative stress in diabetic rats (21) were seen with vitamin D supplementation. Vitamin D supplementation resulted in increased insulin secretion (23) and improved lipid concentrations in diabetic patients (24). Although the effects of vitamin D on human health have received great attention in recent years, limited data are available examining its effects in pregnancy. Our previous study revealed that vitamin D deficiency is highly prevalent among pregnant women in Iran (25). Furthermore, vitamin D deficiency during pregnancy is associated with several adverse complications including GDM and preeclampsia (26,27). Maternal vitamin D deficiency was also prospectively associated with low bone mineral density, type 1 diabetes, and eczema in the offspring (28,29). The adverse effects of vitamin D deficiency may be mediated through its influence on inflammation and oxidative stress. We are not aware of any study of the effects of vitamin D supplementation on inflammation and oxidative stress in pregnant women. This study was therefore performed to investigate the effects of vitamin D supplementation on serum high-sensitivity C-reactive protein (hs-CRP), metabolic profiles, and biomarkers of oxidative stress in Iranian pregnant women.

Participants and Methods

Participants. This randomized, double-blind, placebo-controlled clinical trial was performed in Kashan, Iran, during March 2012 to September 2012. On the basis of sample-size formulas suggested for randomized clinical trials and considering a type I error of 5% ($\alpha = 0.05$), a type II error of 20% ($\beta = 0.20$; power = 80%), and serum hs-CRP concentration as a key variable (22), we reached the sample size of 20 persons for each group. Pregnant women, primigravida, aged 18–40 y old with a singleton pregnancy at 25 wk of gestation were recruited for this study. Gestational age was assessed from the date of last menstrual period and concurrent clinical assessment (30). Individuals with the above-mentioned inclusion criteria who attended maternity clinics affiliated with Kashan University of Medical Sciences, Kashan, Iran, were recruited for participation in the study. We did not include those with preeclampsia, hypertension, GDM, intrauterine fetal death, or those with a history of rheumatoid arthritis, hepatic or renal failure, metabolic bone disease and malabsorption, or thyroid, parathyroid, or adrenal diseases. Furthermore, smokers and those taking medications including nonsteroidal antiinflammatory drugs and aspirin were not included. A total of 90 pregnant women aged 18–35 y old were screened; of these, 54 met the inclusion criteria. Participants were randomly assigned to receive either cholecalciferol supplements ($n = 27$) or placebo ($n = 27$) for 9 wk. Among individuals in the vitamin D group, 3 women [preterm delivery ($n = 1$), intrauterine fetal death ($n = 1$), and placental abruption ($n = 1$)] were excluded. Three women in the placebo group were also excluded [GDM ($n = 1$), preterm delivery ($n = 1$), and severe preeclampsia ($n = 1$)]. A total of 48 participants [vitamin D ($n = 24$) and placebo ($n = 24$)] completed the trial (Fig. 1). The study was performed according to the guidelines in the Declaration of Helsinki. The ethical committee of Kashan University of Medical Sciences approved the study, and informed written consent was obtained from all participants.

Study design. At study baseline, participants were randomly assigned to receive either vitamin D supplements (400 IU/d cholecalciferol) or placebo for 9 wk. Random assignment was performed by the use of

computer-generated random numbers. A trained midwife at the maternity clinic performed the randomized allocation sequence and assigned participants to the groups. Vitamin D supplements and placebo were provided by Shahre Daru Company. Placebo pills contained microcrystalline cellulose and were packed in identical tablets and coded by the producer to guarantee blinding. Quality control of vitamin D supplements was performed in the laboratory of the Iranian Food and Drug Administration in Tehran, Iran, by the HPLC method. After quality control, we found that the amount of cholecalciferol in the prescribed tablets was in the range of 380–480 IU. Participants were asked not to alter their routine physical activity or usual diets throughout the study and not to consume any supplements other than the one provided to them by the investigators. Participants were also consuming 400 $\mu\text{g}/\text{d}$ folic acid from the beginning of pregnancy and 60 mg/d ferrous sulfate from the second trimester. Compliance with the consumption of cholecalciferol supplements was monitored once a week through phone interviews. Dietary intakes of participants were assessed by means of 3-d dietary records (2 weekdays at weeks 3 and 6 and 1 weekend day at week 9 of the intervention) completed throughout the study. The dietary records were based on estimated values in household measurements. To obtain nutrient intakes of participants on the basis of these 3-d food diaries, we used Nutritionist IV software (First Databank) modified for Iranian foods.

Assessment of variables. Data on prepregnancy weight and height (measured values) were obtained from the records of pregnant women existed in the clinic. A trained midwife at the maternity clinic performed anthropometric measurements at study baseline and 9 wk after the intervention. Body weight was measured to the nearest 0.1 kg in an overnight fasting state, without shoes, and while wearing minimal clothing by the use of a digital scale (Seca). Height was measured to the nearest 0.1 cm by using a nonstretched tape measure (Seca). BMI was calculated as weight in kilograms divided by height in meters squared. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a sphygmomanometer (ALPK2). Fasting blood samples (10 mL) were taken early in the morning at baseline and after the 9-wk intervention at the Kashan reference laboratory. Blood samples were immediately

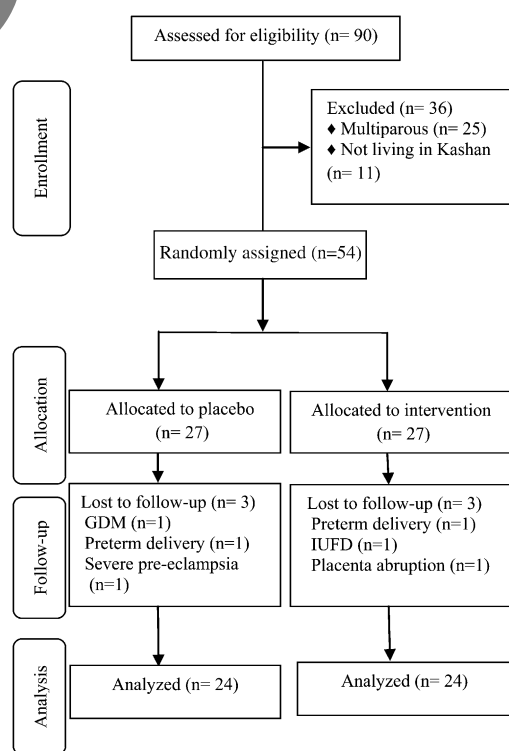


FIGURE 1 Summary of patient flow. The intervention was supplementation of 400 IU/d cholecalciferol. GDM, gestational diabetes; IUFD, intrauterine fetal death.

centrifuged (Hettich) at $1465 \times g$ for 10 min to separate serum. Then, the samples were stored at -70°C before analysis at the Kashan University of Medical Sciences reference laboratory. A commercial kit was used to measure serum calcium concentrations (Calcium 1400007; Pars Azmun). Serum 25-hydroxyvitamin D [25(OH)D] was assayed by using a commercial ELISA kit (25-Hydroxyvitamin D ELISA kit; Immuno Diagnostic Systems). The inter- and intraassay CVs for serum 25(OH)D assays ranged from 5 to 7.5%. Serum hs-CRP was quantified by using an ELISA kit (hs-CRP ELISA kit; Labor Diagnostika Nord) with intra- and interassay CVs of 2.5 and 3.8%, respectively. Commercial kits were used to measure fasting plasma glucose (FPG) (FPG 1012117; Pars Azmun), serum cholesterol (Cholesterol 1500010; Pars Azmun), TG (Triglycerides 1006132; Pars Azmun), LDL-cholesterol (LDL-C 92001; Pars Azmun), and HDL-cholesterol concentrations (HDL-C 92002; Pars Azmun). The intra- and interassay CVs for FPG were 2.0 and 3.5%, respectively. All inter- and intraassay CVs for lipid profile measurements were $<5\%$. Serum insulin was assayed by ELISA kit (Insulin ELISA kit; DiaMetra). The intra- and interassay CVs were 2.9 and 5.9%, respectively. Homeostatic model assessment (HOMA) for insulin resistance (HOMA-IR) and for β -cell function (HOMA-B) and the Quantitative Insulin Sensitivity Check Index (QUICKI) score were calculated on the basis of suggested formulas (31). Plasma total antioxidant capacity (TAC) was assessed by the use of the FRAP (ferric reducing antioxidant power) method developed by Benzie and Strain (32). The plasma total glutathione (GSH) was measured by the method of Beutler et al. (33). Measurements of vitamin D, glucose, lipids, insulin, hs-CRP, TAC, GSH, and calcium were performed in a blinded fashion, in duplicate, in pairs (before/after intervention) at the same time, in the same analytical run, and in random order to reduce systematic error and interassay variability.

Statistical analysis. To ensure normal distribution of variables, the Kolmogorov-Smirnov test was applied. Log transformation was conducted for nonnormally distributed variables. Independent-samples Student's *t* test was used to detect differences in general characteristics and dietary intakes between the 2 groups. To determine the effects of vitamin D supplementation on hs-CRP, metabolic profiles, and biomarkers of oxidative stress, we used 1-factor repeated-measures ANOVA. In this analysis, the treatment (vitamin D vs. placebo) was regarded as the between-subjects factor and time with 2 time points (baseline and week 9 of the intervention) was considered as the within-subjects factor. $P < 0.05$ was considered significant. All statistical analyses were performed by using the Statistical Package for Social Sciences, version 17 (SPSS, Inc.).

Results

The mean (\pm SD) age, prepregnancy weight, and BMI of the study participants were 25.0 ± 3.9 y, 65.8 ± 10.5 kg, and 25.2 ± 3.6 kg/m², respectively. Baseline and end-of-trial weight and BMI were not significantly different between vitamin D and placebo groups (Table 1). Baseline serum 25(OH)D concentrations were 17.8 ± 1.3 $\mu\text{g/L}$ in the vitamin D group and 14.5 ± 1.2 $\mu\text{g/L}$ in the placebo group. Among those in the vitamin D group, 12 participants (50%) had serum 25(OH)D concentrations <20 $\mu\text{g/L}$, and among those in the placebo group this rate was 83.3% ($n = 20$).

Dietary intakes of energy, carbohydrate, fat, protein, dietary fiber, vitamin D, calcium, phosphorus, magnesium, and zinc did not differ between the groups (Table 2).

On average, the rate of compliance in our study was high, such that $>90\%$ of pills were taken throughout the study in both groups. After 9 wk of intervention, the increase in serum 25(OH)D and calcium concentrations was greater in the vitamin D group than in the placebo group, in whom these biomarkers were decreased (Table 3). Individuals who took vitamin D supplements had a significant decrease in their serum hs-CRP (P -interaction = 0.01) and insulin (P -interaction = 0.04) concentrations as well as a significant increase in QUICKI score (P -interaction = 0.006) and plasma TAC (P -interaction = 0.002) and GSH concentrations (P -interaction = 0.02) compared with those who took

TABLE 1 General characteristics of healthy pregnant women who received either vitamin D supplements or placebo¹

	Placebo group	Vitamin D group
Maternal age, y	24.8 ± 3.6	25.3 ± 4.2
Height, cm	161.2 ± 7.8	161.8 ± 4.4
Prepregnancy weight ² , kg	65.5 ± 10.2	66.0 ± 11.0
Weight at study baseline, kg	71.2 ± 11	70.3 ± 11.7
Weight at end of trial, kg	74.8 ± 11.1	73.7 ± 11.6
Weight gain after intervention, kg	3.6 ± 2.7	3.3 ± 0.9
Prepregnancy BMI ³ , kg/m ²	25.2 ± 3.5	25.2 ± 3.8
BMI at study baseline, kg/m ²	27.4 ± 4.0	26.8 ± 3.9
BMI at end of trial, kg/m ²	28.8 ± 3.7	28.0 ± 3.9

¹ Values are means \pm SDs; $n = 24$.

² Based on participants' measured weight according to their records in the maternity clinics.

³ Based on participants' measured weight and height according to their records in the maternity clinics.

placebo. The intake of vitamin D supplements led to a significant decrease in FPG (-0.65 vs. -0.12 mmol/L; P -interaction = 0.01), SBP (-0.2 vs. 5.5 mm Hg; P -interaction = 0.01), and DBP (-0.4 vs. 3.1 mm Hg; P -interaction = 0.01) compared with placebo. A trend toward a significant effect of vitamin D supplementation on reducing HOMA-IR (P -interaction = 0.06) and HOMA-B (P -interaction = 0.06) indexes was also observed. We did not find a significant effect of vitamin D supplementation on serum lipid concentrations.

Discussion

We found that vitamin D supplementation for 9 wk in pregnant women resulted in significant decreases in SBP, DBP, and concentrations of serum hs-CRP, FPG, and insulin and a significant increase in QUICKI score and concentrations of plasma TAC, serum 25(OH) D, and calcium compared with placebo. We did not find any significant effect of vitamin D supplementation on lipid concentrations. To the best of our knowledge, this study is the first that examined the effect of vitamin D supplementation on inflammation and biomarkers of oxidative stress in pregnant women. It must be noted that all participants were taking ferrous sulfate and folic acid supplements throughout the study. There is some evidence indicating an effect of ferrous sulfate and folic acid on serum hs-CRP and plasma TAC concentrations (34–37); however, because participants in both groups were taking these dietary supplements throughout the intervention, this is unlikely to have influenced our findings.

Pregnant women are susceptible to systemic inflammation, metabolic disorders, and oxidative stress, which can, in turn, can lead to complications in maternal and fetal life (38,39). We found that vitamin D supplementation for 9 wk in pregnancy resulted in a significant decrease in serum hs-CRP concentrations compared with placebo. This finding is in agreement with a previous study in men with colorectal adenoma (22). Year-long vitamin D supplementation in overweight and obese participants resulted in decreased serum interleukin (IL)-6 concentrations; however, hs-CRP concentrations were significantly increased (40). Earlier observational studies have also shown vitamin D–inflammation relationships. For instance, Eleftheriadis et al. (41) reported an inverse association between serum 25(OH) D and serum hs-CRP and IL-6 concentrations. Similar findings were also seen in diabetic patients undergoing coronary angiography (42) as well as in asymptomatic adults (43). Less production of parathyroid

TABLE 2 Dietary intakes of healthy pregnant women who received either vitamin D supplements or placebo¹

	Run-in period		Throughout the study	
	Placebo group	Vitamin D group	Placebo group	Vitamin D group
Energy, kcal/d	2362 ± 207	2372 ± 155	2323 ± 322	2435 ± 198
Carbohydrate, g/d	327 ± 47	326 ± 36	321 ± 62	338 ± 36
Fat, g/d	82 ± 18	83 ± 12	80 ± 16	88 ± 13
Protein, g/d	86 ± 20	87 ± 10	87 ± 19	90 ± 15
Dietary fiber, g/d	18.1 ± 5.3	18.5 ± 4	19.3 ± 4.6	20.2 ± 3.8
Vitamin D, µg/d	2.8 ± 0.7	2.8 ± 0.9	2.9 ± 0.8	2.9 ± 0.9
Calcium, g/d	1.10 ± 0.16	1.16 ± 0.18	1.12 ± 0.18	1.15 ± 0.20
Phosphorus, g/d	1.20 ± 0.27	1.19 ± 0.14	1.15 ± 0.20	1.22 ± 0.19
Magnesium, mg/d	279 ± 79	271 ± 47	270 ± 56	297 ± 67
Zinc, mg/d	9.8 ± 3.6	9.9 ± 2.5	10.7 ± 2.9	10.9 ± 2.5

¹ Values are means ± SDs; n = 24.

hormone with vitamin D supplementation (44), which, in turn, leads to decreased production of inflammatory factors, may explain the effects of vitamin D on hs-CRP.

We found that vitamin D supplementation during pregnancy led to significant changes in FPG and serum insulin concentrations and a significant elevation in QUICKI score compared with placebo. Naharci et al. (45) showed that vitamin D supplementation in elderly people with impaired fasting glucose resulted in a significant decrease in HOMA-IR and concentrations of serum insulin and glucose. Among patients with type 2 diabetes, vitamin D supplementation for 12 wk led to increased insulin secretion (23). The beneficial effects of vitamin D on glycemic status were also observed in polycystic ovarian syndrome (46,47). However, some studies have found contradictory conclusions (40,48). Although the exact mechanisms of the effect of vitamin D on insulin resistance are not known, multiple cellular and molecular

mechanisms have been suggested to explain this association. The biologically active form of vitamin D, 1,25-dihydroxyvitamin D, might lead to increased insulin action through enhancing insulin synthesis and release, increasing insulin receptor expression, or suppressing markers of systemic inflammation (49). Vitamin D may also result in increased insulin sensitivity due to improving calcium status and increasing local production of 25(OH)D (50).

Findings from the current study revealed that vitamin D supplementation had no significant effect on lipid concentrations compared with placebo among pregnant women. In line with this finding, some investigators did not find a significant effect of vitamin D on serum lipid concentrations (51,52). In contrast, others showed a significant reduction of serum TGs and LDL-cholesterol concentrations with consumption of calcium/vitamin D supplements in healthy, overweight, or obese women after 15 wk (53). Cholecalciferol supplementation for 18 mo has also resulted in

TABLE 3 Concentrations of serum hs-CRP, glucose and lipids and biomarkers of oxidative stress at baseline and 9 wk after an intervention in healthy pregnant women who received either vitamin D supplements or placebo¹

	Placebo group			Vitamin D group			P value ²		
	Wk 0	Wk 9	Change	Wk 0	Wk 9	Change	Time	Group	Time × Group
Serum 25(OH)D, µg/L	14.5 ± 1.2	13.3 ± 1.1*	-1.2 ± 0.4	17.8 ± 1.3	21.5 ± 1.8*	3.7 ± 1.6**	0.15	0.003	0.005
Serum 25(OH)D <12 µg/L, n (%)	11 (45.8)	13 (54.2)	—	5 (20.8)	5 (20.8)	—	—	—	—
Serum 25(OH)D <20 µg/L, n (%)	20 (83.3)	20 (83.3)	—	12 (50)	10 (41.7)	—	—	—	—
hs-CRP, µg/mL	6.96 ± 1.20	8.46 ± 1.95	1.50 ± 0.94	4.53 ± 0.64	3.12 ± 0.46*	-1.41 ± 0.55**	0.93	0.01	0.01
FPG, mmol/L	4.29 ± 0.12	4.17 ± 0.15	-0.12 ± 0.17	4.52 ± 0.08	3.87 ± 0.13*	-0.65 ± 0.11**	0.001	0.85	0.01
Insulin, µIU/mL	6.4 ± 0.6	9.0 ± 1.4	2.6 ± 1.4	6.7 ± 0.9	5.7 ± 0.4	-1.0 ± 0.9**	0.34	0.13	0.04
HOMA-IR	1.24 ± 0.15	1.84 ± 0.44	0.60 ± 0.46	1.36 ± 0.19	1.02 ± 0.10	-0.34 ± 0.19	0.60	0.20	0.06
HOMA-B	26.5 ± 2.7	38.1 ± 4.4*	11.6 ± 4.2	26.1 ± 4.1	26.0 ± 2.0	-0.1 ± 4.5	0.06	0.10	0.06
QUICKI score	0.38 ± 0.008	0.36 ± 0.007*	-0.02 ± 0.008	0.37 ± 0.007	0.39 ± 0.007	0.02 ± 0.007**	0.79	0.30	0.006
Total cholesterol, mmol/L	6.38 ± 0.23	6.61 ± 0.24	0.23 ± 0.11	6.12 ± 0.20	6.15 ± 0.27	0.03 ± 0.24	0.33	0.24	0.46
TGs, mmol/L	2.37 ± 0.15	2.82 ± 0.13*	0.45 ± 0.12	2.11 ± 0.17	2.33 ± 0.15	0.22 ± 0.14	0.001	0.06	0.24
LDL cholesterol, mmol/L	3.50 ± 0.20	3.53 ± 0.23	0.03 ± 0.11	3.44 ± 0.16	3.37 ± 0.25	-0.07 ± 0.22	0.87	0.70	0.70
HDL cholesterol, mmol/L	1.79 ± 0.09	1.78 ± 0.08	-0.01 ± 0.05	1.70 ± 0.07	1.65 ± 0.07	-0.05 ± 0.06	0.50	0.30	0.62
Total:HDL-cholesterol ratio	3.7 ± 0.2	3.8 ± 0.2	0.1 ± 0.1	3.7 ± 0.2	3.8 ± 0.2	0.1 ± 0.2	0.19	0.99	0.89
TAC, mmol/L	716 ± 31	696 ± 38	-20 ± 41	697 ± 15	849 ± 24*	152 ± 30**	0.01	0.03	0.002
GSH, µmol/L	832 ± 86	800 ± 96	-32 ± 52	604 ± 54	809 ± 92*	205 ± 87**	0.09	0.30	0.02
Calcium, mg/dL	9.17 ± 0.07	9.05 ± 0.07	-0.12 ± 0.08	8.68 ± 0.10	8.88 ± 0.13*	0.20 ± 0.08**	0.47	0.01	0.01
SBP, mm Hg	110.4 ± 1.2	115.9 ± 1.2*	5.5 ± 1.6	112.9 ± 1.1	112.7 ± 1.4	-0.2 ± 1.4**	0.01	0.80	0.01
DBP, mm Hg	67.3 ± 1.1	70.4 ± 1.2*	3.1 ± 1.1	66.0 ± 1.3	65.6 ± 1.4	-0.4 ± 1.1**	0.07	0.07	0.02

¹ Values are means ± SEs; n = 24. *Different from week 0, P < 0.05; **different from corresponding placebo, P-interaction < 0.05. DBP, diastolic blood pressure; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-B, homeostatic model assessment-β cell function; HOMA-IR, homeostasis model of assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; QUICKI, Quantitative Insulin Sensitivity Check Index; SBP, systolic blood pressure; TAC, total antioxidant capacity; 25(OH)D, 25-hydroxyvitamin D.

² Obtained from repeated-measures ANOVA.

improved lipid concentrations among diabetics (24). Discrepancies in findings might be explained by the different dosages of vitamin D used, study designs, and duration of supplementation.

In the current study, we observed beneficial effects of vitamin D supplementation on biomarkers of oxidative stress compared with placebo in pregnant women. Although oxidative stress is involved in the pathophysiology of several chronic conditions, limited data are available examining the effect of vitamin D supplementation on oxidative stress. Ekici et al. (54) showed that the combination of cholecalciferol and dehydroascorbic acid administration increased GSH activity in both the cortex and corpus striatum of rats. Decreased oxidative DNA damage in the normal human colorectal mucosa has been observed as a result of vitamin D and calcium supplementation (55). The exact mechanisms through which vitamin D supplementation may affect oxidant-antioxidant balance are unknown. Several studies have reported that vitamin D may reduce lipid peroxidation (19,20) and thereby lead to increased concentrations of TAC and GSH. Furthermore, vitamin D has been shown to improve vascular endothelial function by modulating the expression of radical generating and scavenging enzymes (21). Furthermore, vitamin D may lead to stabilization of the plasma membrane against lipid peroxidation or upregulation of antioxidant systems via its nuclear receptors (19,56). Taken together, it seems that vitamin D could be considered as a potent strengthening factor of the body's antioxidant system, at least in pregnant women.

We demonstrated that vitamin D supplementation for 9 wk in pregnant women led to a significant reduction in SBP and DBP compared with placebo. In line with our findings, earlier studies have also shown the beneficial effects of vitamin D on blood pressure. A significant reduction in SBP by 2.44 mm Hg was seen with vitamin D supplementation in normotensive or hypertensive participants compared with calcium or placebo (57). The same findings have also been reported with vitamin D supplementation in hypertensive patients after 3 mo (58). However, among postmenopausal women, who received 1000 mg of elemental calcium plus 400 IU cholecalciferol, no significant effect on blood pressure or risk of developing hypertension over 7 y of follow-up was observed (59). Several mechanisms can explain the beneficial effects of vitamin D supplementation on blood pressure. Vitamin D has a critical role in decreasing renin gene expression, the regulation of renin-angiotensin system (58,60), and inhibition of vascular smooth muscle cell proliferation (61). Moreover, vitamin D, through increasing calcium absorption, can affect blood pressure regulation via altering cellular concentrations of sodium and calcium ions (58).

Some limitations must be considered in the interpretation of our findings. Although assessment of supplementation on other biomarkers of systemic inflammation, including IL-1, IL-6, and tumor necrosis factor α (TNF- α) as well as biomarkers of oxidative stress such as catalase and superoxide dismutase, would be of value, unfortunately, due to budget limitations, we were unable to assess the effect of vitamin D supplements on these biomarkers. In addition, we could not assess the effect of supplementation on pregnancy outcomes in the current study. Future studies are needed to investigate the potential health outcomes of the changes in metabolic variables with vitamin D supplementation. It must also be kept in mind that for some biochemical indicators, such as FPG, as well as for blood pressure, the changes made by vitamin D supplementation were in the normal range. Therefore, future studies can assess the effect of vitamin D supplementation in pregnant women with GDM or those with hypertension. It seems that in individuals with higher

concentrations of plasma glucose or in hypertensive pregnant women, supplementation might provide further benefit. Because the study was done through spring and summer (March through September), one might be surprised to see a decrease in serum vitamin D concentrations in the placebo group. However, it must be kept in mind that due to cultural issues, the time span of outdoor activities for women is limited. Furthermore, the study participants were pregnant women who normally have limited physical activity levels and sun exposure than others. Sunscreen use and clothing might be other reasons for this observation. Another possible limitation relates to the extrapolation of our findings. The beneficial effect seen in the current study might be explained by the fact that the mean serum 25(OH)D concentrations at baseline were low in both groups. Thus, a relatively low dose of vitamin D (400 IU), which was effective in this population, might not be in others with better status. Finally, we did not include smokers and those taking nonsteroidal anti-inflammatory drugs; however, the effect of residual confounding from other factors on dependent variables (e.g., hs-CRP) cannot be excluded.

In conclusion, vitamin D supplementation for 9 wk among healthy pregnant women results in reduced SBP, DBP, and concentrations of serum hs-CRP, FPG, and insulin and increased QUICKI score and concentrations of plasma TAC, GSH, serum vitamin D, and calcium compared with placebo.

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