Effects of High-Dose Vitamin D Supplementation on Metabolic Status and Pregnancy Outcomes in Pregnant Women at Risk for Pre-Eclampsia

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Abstract

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This study was designed to assess the beneficial effects of high-dose (cholecalciferol) vitamin D supplementation on metabolic profiles and pregnancy outcomes among pregnant women at risk for pre-eclampsia. This randomized double-blind placebo-controlled clinical trial was performed among 60 pregnant women at risk for pre-eclampsia according to abnormal uterine artery Doppler waveform. Subjects were randomly divided into 2 groups to receive 50 000 IU vitamin D supplements (n=30) or receive placebo (n=30) every 2 weeks from 20 to 32 weeks of gestation. Fasting blood samples were taken at baseline study and 12 weeks after the intervention to quantify relevant variables. Newborn's anthropometric measurements were determined. Pregnant women who received cholecalciferol supplements had significantly increased serum 25-hydroxyvitamin D concentrations ($+17.92\pm2.28$ vs. $+0.27\pm3.19$ ng/ml, $p<0.001$) compared with the placebo. The administration of cholecalciferol supplements, compared with the placebo, resulted in significant differences in serum insulin concentrations ($+1.08\pm6.80$ vs. $+9.57\pm10.32$ µIU/ml, $p<0.001$), homeostasis model of assessment-insulin resistance (HOMA-IR) ($+0.19\pm1.47$ vs. $+2.10\pm2.67$, $p<0.001$), homeostatic model assessment-beta cell function (HOMA-B) ($+5.82\pm29.58$ vs. $+39.81\pm38.00$, $p<0.001$) and quantitative insulin sensitivity check index (QUICKI) score (-0.009 ± 0.03 vs. -0.04 ± 0.03, $p=0.004$). Furthermore, cholecalciferol-supplemented pregnant women had increased HDL-cholesterol concentrations ($+2.67\pm8.83$ vs. -3.23 ± 7.76 mg/dl, $p=0.008$) compared with the placebo. Finally, cholecalciferol supplementation led to a significant rise in plasma total antioxidant capacity (TAC) concentrations ($+79.00\pm136.69$ vs. -66.91 ± 176.02 mmol/l, $p=0.001$) compared with the placebo. Totally, the administration of cholecalciferol supplements among pregnant women at risk for pre-eclampsia for 12 weeks had favorable effects on insulin metabolism parameters, serum HDL-cholesterol, and plasma TAC concentrations.

Supporting Information for this article is available online at http://www.thieme-connect.de/products.

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Abbreviations

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CHD	Coronary heart disease
DBP	Diastolic blood pressure
FPG	Fasting plasma glucose
FRAP	Ferric reducing antioxidant power
GSH	Glutathione
GDM	Gestational diabetes
GPX	Glutathione peroxidase
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
IOM	Institute of Medicine
LBW	Low birth weight
IL-	Interleukin-6
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acid
NO	Nitric oxide
NAFLD	Nonalcoholic fatty liver disease
OSI	Oxidative stress index
PTH	Parathyroid hormone
PUFA	Polyunsaturated fatty acid
QUICKI	Quantitative insulin sensitivity check index
ROS	Reactive oxygen species
SFA	Saturated fatty acid
SOD	Superoxide dismutase
SBP	Systolic blood pressure
TAC	Total antioxidant capacity
TBARs	Thiobarbituric acid reactive substance
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor alpha
TDF	Total dietary fiber

Introduction
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Pre-eclampsia is a disorder characterized by pregnancy-induced hypertension and new-onset proteinuria occurring after 20 weeks of gestation in a previously normotensive women [1]. The prevalence of pre-eclampsia has reported between 1.3–6.7% of all pregnancies [2]. Despite numerous attempts at early diagnosis and treatment, to date, no method has been found that prevents the development of pre-eclampsia [3]. Increased biomarkers of oxidative stress are considered to be a key factor in pre-eclampsia process [4]. In addition, decreased maternal plasma ascorbic acid concentrations [4], increased synthesis of free radicals and reactive oxygen species (ROS) in the placenta [5], and reduced activities of antioxidant enzymes [6] may lead to the development of pre-eclampsia.

Various therapeutic strategies for prevention of pre-eclampsia are proposed including the use of aspirin [7], antioxidant supplementation, especially vitamins C and E [8], and the use of low molecular weight heparin [9]. Previous studies have reported that intake of cholecalciferol supplements can rise glutathione levels and reduce the production of lipid peroxidation products [10,11], which in turn, would result in decreased oxidative stress. These observations result in the hypothesis that early supplementation with high-dose cholecalciferol could be useful in decreased biomarkers of oxidative stress as well as improving vascular endothelial function, thereby preventing or ameliorating the course of pre-eclampsia. Taking 100 000 IU vitamin D supplements among patients with gestational diabetes (GDM) showed improved insulin function, decreased total- and LDL-cholesterol concentrations after 6 weeks [12]. In addition, in a meta-analysis study it has been shown that pregnant women who received vitamin D supplements in early pregnancy had lower odds of pre-eclampsia [13]. Vitamin D supplementation in healthy nulliparous women did affect pregnancy outcome with regard to pre-eclampsia [14].

The favorable effects of cholecalciferol supplementation on metabolic profiles and pregnancy outcomes might result from its effect on apolipoprotein gene expression [15], parathyroid hormone (PTH) suppression [16], improved insulin sensitivity [17], and decreased production of lipid peroxidation products [11]. We are aware that no study had examined the beneficial effects of high-dose cholecalciferol supplementation on metabolic profiles, biomarkers of inflammation, oxidative stress, and pregnancy outcomes in pregnant women at risk for pre-eclampsia. The current study was, therefore, performed to investigate the favorable effects of cholecalciferol administration on metabolic status and pregnancy outcomes in pregnant women at risk for pre-eclampsia.

Subjects and Methods
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Participants

Between July 2014 and October 2014, we did a randomized double-blind placebo-controlled clinical trial in Arak, Iran. For estimating sample size, we used a randomized clinical study 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 [18], we also considered 0.9 as SD and 0.55 cm as the difference in the mean (d) of newborns' length at birth as the key variable. Based on this, we needed 25 pregnant women in each group to have 80% power of the study. However, we recruited 30

subjects in each group (totally, 60 patients) to compensate for the probable loss to follow-up. In the present study, our inclusion criteria were pregnant women primigravida, aged 18–40 years old and at risk for pre-eclampsia. Women were identified as "at-risk" by abnormal uterine artery Doppler waveform (18–20 weeks' gestation, mean resistance index > 0.67 or pulsatility index > 1.65 with or without the presence of unilateral or bilateral diastolic notches) [19]. We excluded pregnant women who were unable or unwilling to give written informed consent, having abnormal fetal anomaly scan, or were being treated with warfarin. Gestational age was assessed from the date of last menstrual period and concurrent clinical assessment [20]. The present trial protocol was approved by the Ethics Committee of Arak University of Medical Sciences (AUMS) (Registration no. 92-12-161). All pregnant women signed the written informed consent to take part in the current study. This study was registered in the Iranian website (www.irct.ir) for registration of clinical trials (IRCT code: IRCT201410035623N27).

Study design

After stratification for pre-intervention BMI (< 25 and ≥ 25 kg/m^2) and maternal age (< 30 and ≥ 30 years), pregnant women were randomly allocated into 2 groups to take either cholecalciferol supplements (n=30) or placebo (n=30). A trained midwife at maternity clinic did the randomized allocation sequence with a computer random number generator. An investigator with no clinical involvement in our study packed cholecalciferol and placebos in numbered bottles based on the random list. Randomization and allocation were hidden from the researchers and pregnant women until the statistical analysis was completed. Pregnant women either received one oral pearl containing of 50 000 IU vitamin D3 (D-Vitin 50 000; Zahravi Pharm Co, Tabriz, Iran) or a placebo (Barij Essence Co, Kashan, Iran) every 14 days for 12 weeks from 20 to 32 weeks of gestation. Placebo pearls were similar in color, shape, size, and package to the vitamin D3 ones and contained edible paraffin. Subjects were requested not to alter their regular physical activity or normal dietary intakes throughout the study and not to take any supplements other than the one provided to them by the investigators. All pregnant women were also taking 400 µg/d folic acid from the start of pregnancy, 60 mg/d ferrous sulfate from the second trimester, and a multivitamin mineral capsule (containing 400 IU vitamin D) from the second half of pregnancy. Both dietary and physical activity records were taken at week 3, 6, and 9 of intervention. The dietary records were based on estimated values in household measurements. To obtain nutrient intakes of participants according to these 3-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods.

Assessment of anthropometric variables

Information on pre-pregnancy weight and BMI were obtained from the records of pregnant women existed in the clinic. A trained midwife at maternity clinic did anthropometric measurements at the beginning of the study and the end of the intervention. Height was measured without shoes using stadiometer with a precision of 0.1 cm. Weight was measured in light clothing to the nearest 0.1 kg. BMI was determined as weight (kg) divided by squared height (m^2).

Primary and secondary outcomes

Primary outcomes were pre-eclampsia rate, low birth weight (LBW) (< 2 500 g), newborn's birth size, and preterm delivery

(<37 weeks). Secondary outcomes were metabolic concentrations, biomarkers of inflammatory factors, oxidative stress, and blood pressures.

Biochemical analysis

Fasting blood samples (10 ml) were taken at baseline and 12 weeks after the intervention at Arak reference laboratory at early morning after an overnight fast. Blood samples were immediately centrifuged (Hettich 78532, Tuttlingen, Germany) at 3 500 rpm for 10 min to separate serum. Then, the samples were stored at −70 °C before analysis at the AUMS reference laboratory. Serum 25-hydroxyvitamin D concentrations was assayed by a commercial ELISA kit (IDS, Boldon, UK). The inter- and intra-assay CVs for serum 25-hydroxyvitamin D assays ranged from 4.9 to 7.2 %. Commercial kits were used to measure fasting plasma glucose (FPG), triglycerides, cholesterol, VLDL-, LDL- and HDL-cholesterol concentrations (Pars Azmun, Tehran, Iran). The intra- and inter-assay CVs for FPG and lipid concentrations were <5 %. Serum insulin was assayed by ELISA kit (Monobind, CA, USA). The intra- and inter-assay CVs for serum insulin were 2.9 and 5.9 %, respectively. Homeostasis model of assessment-insulin resistance (HOMA-IR) and β-cell function (HOMA-B) and quantitative insulin sensitivity check index (QUICKI) was calculated based on suggested formulas [21]. Serum high sensitivity C-reactive protein (hs-CRP) was quantified using ELISA kit (LDN, Nordhorn, Germany) with intra- and inter-assay CVs of 2.4 and 3.9 %, respectively. Plasma nitrite/nitrate (NOx), taken as an index of nitric oxide (NO) concentration, was determined using the Giess method modified by Tatsh et al. [28]. Plasma total antioxidant capacity (TAC) was assessed by the use of ferric reducing antioxidant power (FRAP) method developed by Benzie and Strain [22]. The plasma total glutathione (GSH) and malondialdehyde (MDA) were measured by the method of Beutler et al. [23] and thiobarbituric acid reactive substance (TBARs) spectrophotometric test [24].

Statistical analysis

Distribution of data related to normality was assessed by Kolmogorov-Smirnov test. Independent sample Student's *t*-test was used to detect changes in general characteristics and dietary intakes between the 2 groups. Comparisons of changes (endpoint minus baseline) 12 weeks after the intervention between the 2 groups were done by two-way repeated measures analysis of variance. In this analysis, the treatment (cholecalciferol vs. placebo) was regarded as between-subject factor and time with 2 time-points (baseline and 12 weeks after the intervention) was considered as within-subject factor. To control for confounding variables, analysis of covariance (ANCOVA) test was used to determine the differences between the 2 groups post-intervention, while adjusting for baseline measurements, maternal age and baseline BMI. A p-value of <0.05 was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, IL, USA).

Results
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Totally, 60 pregnant [cholecalciferol (n = 30) and placebo (n = 30)] completed the trial. On average, the rate of compliance in our study was high, such that 100 % of pearls were taken throughout the trial in both groups. Compliance with the consumption of

vitamin D supplements and placebos was monitored every 2 weeks through telephone interviews and by the use of 3-day dietary records completed at week 3, 6, and 9 of intervention. Mean age, pre-pregnancy weight and BMI of pregnant women was 27.4±5.2 years, 64.5±10.6 kg and 25.9±4.6 kg/m², respectively. Baseline and end-of-trial means of weight and BMI were not significantly different between cholecalciferol and placebo groups (Data not shown).

Based on the three-day dietary records obtained throughout the intervention, no significant change was seen between the two groups in terms of dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), cholesterol, dietary fiber, vitamin D, calcium, phosphors, magnesium, zinc, manganese, selenium, and vitamin C (Data not shown).

Pregnant women who received cholecalciferol supplements had significantly increased serum 25-hydroxyvitamin D concentrations (+17.92±2.28 vs. +0.27±3.19 ng/ml, p<0.001) compared with the placebo (○ Table 1). The administration of cholecalciferol supplements, compared with the placebo, resulted in significant differences in serum insulin concentrations (+1.08±6.80 vs. +9.57±10.32 μIU/ml, p<0.001), HOMA-IR (+0.19±1.47 vs. +2.10±2.67, p<0.001), HOMA-B (+5.82±29.58 vs. +39.81±38.00, p<0.001) and QUICKI score (−0.009±0.03 vs. −0.04±0.03, p=0.004). Furthermore, cholecalciferol-supplemented pregnant women had increased HDL-cholesterol concentrations (+2.67±8.83 vs. −3.23±7.76 mg/dl, p=0.008) compared with the placebo. Finally, cholecalciferol supplementation led to a significant rise in plasma TAC concentrations (+79.00±136.69 vs. −66.91±176.02 mmol/l, p=0.001) compared with the placebo. There were no significant differences between the cholecalciferol and placebo groups in terms of changes in FPG, other lipid profiles, biomarkers of inflammation and oxidative stress and blood pressures.

Baseline levels of SBP were significantly different between the 2 groups. Therefore, we controlled the analyses for the baseline levels. However, after this adjustment no significant changes in our findings occurred, except for plasma NO levels (p<0.001) **Table S1**. Further adjustments for maternal age and baseline BMI did not affect our findings, except for serum HDL-cholesterol levels (p=0.06) and systolic blood pressure (SBP) (p=0.04).

We did find no significant change in cesarean section rate, gestational age, preterm delivery, newborn's birth size, Apgar scores, pre-eclampsia rate and LBW comparing the 2 groups (○ Table 2).

Discussion
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The current study demonstrated that high-dose vitamin D administration among women at risk for pre-eclampsia had beneficial effects on insulin metabolism parameters, serum HDL-cholesterol, and plasma TAC concentrations, but did not affect FPG, other lipid profiles, inflammatory factors and other biomarkers of oxidative stress. To the best of our knowledge, the current study is the first evaluating the effects of high-dose cholecalciferol administration on metabolic status and pregnancy outcomes in pregnant women at risk for pre-eclampsia according to abnormal uterine artery Doppler waveform. It must be kept in mind that participants in the current study were pregnant women who have vitamin D deficiency by definition and in addition they have a very low vitamin D intake. Therefore, vitamin D supplementation with appropriate dosage is suggested in

Table 1 Means (±standard deviation) of metabolic profiles, inflammatory factors, and biomarkers of oxidative stress at baseline and after the intervention.

	Placebo group (n=30)			Vitamin D group (n=30)			p**
	Wk0	Wk12	Change	Wk0	Wk12	Change	
Vitamin D (ng/ml)	17.10±2.21	17.37±4.04	0.27±3.19	16.99±1.40	34.91±2.36 *	17.92±2.88	<0.001
FPG (mg/dl)	85.80±9.47	85.86±11.52	0.06±12.57	83.73±7.77	81.96±9.75	–1.77±13.07	0.58
Insulin (µIU/ml)	10.40±6.46	19.97±9.24 *	9.57±10.32	10.95±8.31	12.03±4.87	1.08±6.80	<0.001
HOMA-IR	2.25±1.44	4.35±2.40 *	2.10±2.67	2.29±1.82	2.48±1.14	0.19±1.47	<0.001
HOMA-B	39.65±26.27	79.46±34.83 *	39.81±38.00	43.22±34.19	49.04±19.13	5.82±29.58	<0.001
QUICKI	0.35±0.04	0.31±0.02 *	–0.04±0.03	0.34±0.03	0.34±0.02	–0.009±0.03	0.004
Triglycerides (mg/dl)	177.94±56.17	201.77±70.15 *	23.83±50.11	176.49±70.85	205.68±62.80 *	29.19±47.58	0.67
VLDL-cholesterol (mg/dl)	35.58±11.23	40.35±14.03 *	4.77±10.02	35.29±14.17	41.13±12.56 *	5.84±9.51	0.67
Total cholesterol (mg/dl)	221.78±38.68	230.03±34.95	8.25±30.44	202.09±41.07	222.47±44.29 *	20.38±28.02	0.11
LDL-cholesterol (mg/dl)	116.87±31.37	123.59±29.65	6.72±23.04	101.17±30.65	112.86±39.06	11.69±41.19	0.56
HDL-cholesterol (mg/dl)	69.31±9.87	66.08±11.22	–3.23±7.76	65.61±8.70	68.28±7.78	2.67±8.83	0.008
Total: HDL cholesterol ratio	3.27±0.70	3.56±0.82 *	0.29±0.50	3.08±0.49	3.26±0.51	0.18±0.60	0.99
hs-CRP (ng/ml)	8061.56±3443.16	7582.36±3303.68	–479.20±3105.71	6927.46±4753.22	5882.75±4787.08	–1044.71±3488.00	0.51
NO (µmol/l)	54.53±10.42	59.65±10.82	5.12±13.95	50.43±5.88	49.99±6.51	–0.44±7.22	0.05
TAC (mmol/l)	690.11±141.95	623.20±126.07 *	–66.91±176.02	678.18±125.57	757.18±136.43 *	79.00±136.69	0.001
GSH (µmol/l)	581.29±165.96	475.45±165.44 *	–105.84±142.62	569.56±233.01	479.03±140.33	–90.53±242.13	0.83
MDA (µmol/l)	4.99±0.85	5.39±0.89	0.40±1.27	5.23±0.85	5.48±0.79	0.25±0.78	0.59
SBP (mm Hg)	108.33±6.52	111.33±6.14	3.00±8.27	104.66±7.53	108.00±5.95 *	3.34±7.11	0.67
DBP (mm Hg)	73.50±4.76	78.00±5.95 *	4.50±5.92	74.00±6.21	75.83±4.92	1.83±7.71	0.13

* Obtained from repeated measures ANOVA test. ** Different from baseline study, p<0.05; For abbreviations, see text

Table 2 The effect of vitamin D supplementation on pregnancy outcomes. *

	Placebo group (n=30)	Vitamin D group (n=30)	p**
Cesarean section (%)	10 (33.3)	9 (30.0)	0.78[†]
Gestational age (weeks)	39.1±1.3	39.4±1.3	0.31
Preterm delivery (%)	1 (3.3)	0 (0)	0.31[†]
Newborns' weight (g)	3141.0±495.9	3313.6±341.1	0.12
Newborns' length (cm)	50.4±2.1	50.9±1.5	0.29
Newborns' head circumference (cm)	34.7±1.5	34.4±0.8	0.26
1-min Apgar score	8.9±0.2	8.9±0.3	0.30
5-min Apgar score	9.9±0.2	9.9±0.3	0.30
Pre-eclampsia rate (%)	3 (10.0)	1 (3.3)	0.30[†]
LBW (%)	2 (6.7)	0 (0)	0.15[†]

* Values are means±SDs
** Obtained from independent *t*-test
[†] Obtained from Pearson Chi-square test
For abbreviation, see text

vitamin D deficient pregnant women. In addition, we used a dose of approximately 4000 IU vitamin D supplements daily in the current study, which is higher than the current recommended dietary allowance (600 IU/day) or standard prenatal supplement dose (400 IU/day). However, this dose is the tolerable upper intake levels advised by the Institute of Medicine (IOM) [25] and this is no problem in this short study of 12 weeks. Pre-eclampsia is associated with fetal and maternal complications [26]. Our study demonstrated that high-dose cholecalciferol intake for 12 weeks in women at risk for pre-eclampsia resulted in significant differences in serum insulin concentrations, HOMA-IR score, HOMA-B, and QUICKI score compared with the placebo, but did not affect FPG. Moreover, only limited data are available assessing the beneficial effects of cholecalciferol administration on metabolic status in pregnant women at risk for pre-eclampsia. Among women without risk for pre-eclampsia as well as in animal models, the favorable effects of taking vitamin D on glucose homeostasis parameters have been shown. In agreement with our study, Soheilykhah et al. [27] showed that 50000 IU cholecalciferol intake every 2 weeks from week 12 of pregnancy until delivery led to improved insulin resistance in healthy pregnant women. In our previous study among women with GDM, we also observed improved markers of insulin metabolism following the consumption of 100000 IU cholecalciferol supplements for 6 weeks [12]. However, some researchers did not see such beneficial effects of cholecalciferol administration on insulin function. For instance, taking vitamin D supplements had no beneficial effects on insulin resistance in patients with type 2 diabetes mellitus (T2DM) after 24 weeks of intervention [28] and after 6 months of therapy [29]. Furthermore, supplementation with 1000 IU vitamin D daily did not affect insulin resistance among healthy overweight or obese women for 12 weeks [30]. Impaired insulin metabolism is associated with arterial stiffness and coronary heart disease (CHD) independent of glucose tolerance status and increased hypertension [31]. Increased cholecalciferol concentration suppression of inflammatory factors and increased expression of the insulin receptor and/or proteins of the insulin-signaling cascade may result in improved insulin function [32].

Findings from the current study showed that pregnant women who received high-dose of vitamin D supplements had a significant rise in serum HDL-cholesterol concentrations compared with the placebo, but had no significant improvement in other

lipid profiles. In line with our study, a 2-month supplementation of 100 000 IU vitamin D increased HDL-cholesterol concentrations in school children [33]. In addition, vitamin D plus calcium administration (400 IU of vitamin D3 daily + 1 000 mg of elemental calcium) resulted in a significant increase in HDL-cholesterol concentrations among postmenopausal women [34]. However, some researchers did not observe any significant effect of taking cholecalciferol supplements on lipid concentrations [35,36]. Increased HDL-cholesterol concentrations in the presents study may result from form the stimulation of apolipoprotein A1 by cholecalciferol [37]. The absence of significant effect of taking cholecalciferol supplements on other lipid profiles in our study might be explained by distinct trial designs, various dosages of cholecalciferol supplementation, and subjects of the study.

We have revealed here that the administration of cholecalciferol supplements did not affect serum hs-CRP and plasma NO levels in pregnant women at risk for pre-eclampsia. In accordance with the present study, no significant change in CRP concentrations was observed after the vitamin D intake (1 000, 2 000, or 4 000 IU/day of vitamin D3 orally) for 3 months [38]. Furthermore, 1 000 mg calcium per day and 50 000 IU vitamin D3 pearl 2 times during the study (at study baseline and day 21 of intervention) co-supplementation did not affect hs-CRP and NO concentrations among GDM patients after 6 weeks [10]. In disagreement, a 9-week administration of 400 IU vitamin D supplements was associated with a significant decrease in serum hs-CRP concentrations among healthy pregnant women [39]. In addition, in an in vitro study, concomitant incubation with 1,25(OH)$_2$D reduced interleukin-6 (IL-6) by 32%, and of IL-8 levels by 34% [40].

We have demonstrated that high-dose cholecalciferol intake resulted in a significant rise in plasma TAC concentrations in pregnant women at risk for pre-eclampsia compared with the placebo, but did not influence on other biomarkers of oxidative stress. In consistent with this trial, cholecalciferol supplementation significantly decreased liver oxidative stress index (OSI) and improved serum TAC concentrations in diabetic rats [41]. In addition, our findings are in accordance with those reported by other researchers, showing decreased oxidative DNA damage in the normal human colorectal mucosa following cholecalciferol and calcium supplementation [42]. However, supplementation of 50 000 IU vitamin D3 every 14 days for 4 months among adult patients with nonalcoholic fatty liver disease (NAFLD) did not affect TAC concentrations, but led to amelioration in MDA concentrations [11]. Increased oxidative stress and free radicals is regarded as a main factor in the pathogenesis and progression of diabetes mellitus and cardiovascular complications [43]. Accurate explanation to the antioxidative effects of cholecalciferol supplements cannot be provided, but these may include stabilization of the plasma membrane against lipid peroxidation [44] or upregulation of antioxidant systems including glutathione peroxidase (GPX) and superoxide dismutase (SOD), via its nuclear receptors [45].

The current study revealed no significant effect of high-dose cholecalciferol supplementation on pregnancy outcome. Our findings are accordance with previous studies showing supplementation with cholecalciferol did show no association between maternal vitamin D status in HIV-infected pregnant women and adverse pregnancy outcomes [46]. In addition, taking 25 mg/d ergocalciferol in pregnant women did not influence mean birth weight in other studies [47,48]. Others did not find a significant effect of vitamin D effect on pregnancy outcomes [49,50]. Discrepancies between our study and others might be explained by

the different doses of cholecalciferol used as well as participants of the study.

While interpreting some limitations need to be taken into account. Due to limited funding, we in the current study did not assess the effect of cholecalciferol administration on other biomarkers of systemic inflammation including interleukin 1(IL-1), IL-6, and tumor necrosis factor alpha (TNF-α) as well as biomarkers of oxidative stress such as catalase and SOD. Furthermore, the appropriate dosage of vitamin D supplementation in pregnant women with at risk for pre-eclampsia cannot be inferred from this study and additional data would be required.

In conclusion, the administration of cholecalciferol supplementation for 12 weeks had favorable effects on insulin metabolism parameters, serum HDL-cholesterol and plasma TAC concentrations, while it did not affect FPG, other lipid concentrations, inflammation, oxidative stress, blood pressures, and pregnancy outcomes.

Author Contributions
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ZA contributed in conception, design, statistical analysis, and drafting of the manuscript. MK and EB contributed in conception, data collection, and manuscript drafting. All authors read and approved the final version of the paper. ZA is the guarantor of this work.

Conflict of Interest
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The authors declare no conflict of interest.

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