Effects of vitamin D supplementation on metabolic indices and hs-CRP levels in gestational diabetes mellitus patients: a randomized, double-blinded, placebo-controlled clinical trial

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BACKGROUND/OBJECTIVES: Vitamin D plays an important role in the etiology of gestational diabetes mellitus (GDM). This study evaluated the effect of vitamin D supplementation on metabolic indices and hs-C-reactive protein (CRP) levels in GDM patients.

SUBJECTS/METHODS: The study was a randomized, placebo-controlled, double-blinded clinical trial. Seventy-six pregnant women with GDM and gestational age between 24-28 weeks were assigned to receive four oral treatments consisting of 50,000 IU of vitamin D₃ (n = 38) or placebo (n = 38) once every 2 weeks for 2 months. Fasting blood glucose (FG), insulin, HbA1c, 25-hydroxyvitamin D, lipid profile, hs-CRP, and homeostasis model assessment-insulin resistance (HOMA-IR) were measured before and after treatment. Independent and paired t-tests were used to determine intra- and intergroup differences, respectively. ANCOVA was used to assess the effects of vitamin D supplementation on biochemical parameters.

RESULTS: Compared with the placebo group, in the vitamin D group, the serum level of 25-hydroxyvitamin D increased (19.15 vs. -0.40 ng/ml; P < 0.01) and that of FG (-4.72 vs. 5.27 mg/dl; P = 0.01) as well as HbA1c (-0.18% vs. 0.17%; P = 0.02) decreased. Improvements in the lipid profiles were observed in the vitamin D group, but without statistical significance. Significant increases in concentrations of hs-CRP, FG, HbA1c, total cholesterol, and LDL cholesterol were observed in the placebo group. No significant change in fasting insulin and HOMA-IR was observed in either group.

CONCLUSIONS: In GDM patients, vitamin D supplementation improved FG and HbA1c but had no significant effects on lipid profile or hs-CRP.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance occurring in or first recognizable during pregnancy. GDM is regarded as a complication of pregnancy [1]. Biochemical abnormalities including higher fasting glucose, greater insulin resistance, dyslipidemia (higher triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol), and increased inflammatory biomarkers such as C-reactive protein (CRP) can be observed in GDM patients [2]. Approximately 3%-8% of all pregnancies in the USA are affected by GDM, but its global prevalence varies depending on the population studied and GDM diagnostic criteria used. Worldwide, GDM prevalence varies from 1% to 14% of all pregnancies. GDM affects 7% of pregnant women in Iran [3].

GDM has serious implications on maternal, fetal, and neonatal well-being [4]. Among mothers, it is associated with preeclampsia, higher rates of cesarean section, and increased long-term risks for development of metabolic syndrome and type 2 diabetes mellitus [5]. GDM is also involved in fetal morbidity and mortality [3] from hyperglycemia, hyperinsulinemia, hyperlipidemia, endothelial cell dysfunction [6], and heart dysfunction [7]. In addition, GDM increases the risks of adverse fetal outcomes including macrosomia, neonatal hypoglycemia, infant respiratory distress syndrome, shoulder dystocia, intraterine growth retardation [8], birth trauma, and hyperbilirubinemia [9]. A systematic review and meta-analysis showed an increased risk of later-life obesity and glucose intolerance in the offspring of mothers with GDM [5].

Certain factors may indicate an increased risk of GDM, including high maternal age, ethnicity, obesity, prior history of

This article was written based on a dataset of MS thesis, registered in Tabriz University of Medical Sciences, Tabriz, Iran (Grant number: 5/97/1160-1392/4/30)

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Received: April 13, 2015, Revised: October 22, 2015, Accepted: January 7, 2016

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GDM, and a family history of type 2 diabetes [5]. Recently, poor vitamin D status was also proposed as a contributor to increased GDM risk [3-5,10-12].

Vitamin D, a steroid hormone, is present in animal-based foods and mainly generated from 7-dehydrocholesterol in a sunlight-induced pathway in the skin [12]. Vitamin D has classical effects on calcium homeostasis and bone mineralization. In addition to these classical processes, vitamin D is involved in many other processes, including insulin secretion, cellular proliferation and differentiation, and modulation of immune function [13].

Poor vitamin D status during pregnancy increases risks for infectious diseases, cesarean delivery, preeclampsia, maternal insulin resistance, preterm birth, low birth weight, skeletal abnormalities, and developmental disorders [14]. The prevalence of vitamin D deficiency, defined as 25-hydroxyvitamin D concentrations < 10 ng/ml (25 nmol/l), varies from 18% to 89.5% during pregnancy, depending on the region and the types of clothing usually worn by women. Vitamin D deficiency has been reported in 80% of pregnant Iranian women [15].

Pregnant women are at high risk for vitamin D deficiency, leading to a nearly twofold increased risk of GDM [3,4,16], by factors regulating the production of vitamin D in the skin, including skin pigmentation, skin covered by clothes, season, aging, latitude, sunscreen use, and air pollution [5,14], and by factors that affect its absorption or metabolism [5,14], such as obesity [15] and fetal development [17].

Studies assessing the effects of vitamin D supplementation on the metabolic features of diabetes patients have reported controversial outcomes. In a study conducted by Talaei et al., vitamin D3 supplementation (50,000 IU/week) for 8 weeks resulted in significantly decreased serum insulin concentration and homeostasis model assessment-insulin resistance (HOMA-IR) in type 2 diabetes patients [18]. Asemi et al. showed that vitamin D supplementation, with administration of 50,000 IU vitamin D3 for 21 days over 6 weeks, had beneficial effects for GDM patients, including improvements in glycemic, total cholesterol, and LDL cholesterol concentrations, but had no effects on inflammation [17]. However, in a study conducted by Mozaffari-Khosravi, a single postpartum injection of 300,000 IU of vitamin D3 in mothers with GDM did not affect fasting plasma glucose, HbA1c, or CRP [19].

There is a need to address the scarcity of data on this subject, the controversial results, and the limitations of the aforementioned studies, including low sample sizes and low doses of vitamin D [17]. Therefore, this study was designed to assess the effects of vitamin D supplementation on the metabolic profiles and hs-CRP statuses of pregnant women with GDM.

SUBJECTS AND METHODS

Study participants

GDM patients were recruited from Al-Zahra Hospital, the academic outpatient center of the Tabriz University of Medical Sciences, Tabriz, Iran. Using a type I error of 5% (α = 0.05), type II error of 20% (β = 0.20, power = 80%), HbA1c concentration as a key factor, and 20% attrition during the study period, it was calculated that 36 subjects would be required for each study condition [20]. The following inclusion criteria were used:

- Diagnosis of GDM: Pregnant women without a prior diagnosis of glucose intolerance in the first trimester were asked to participate in a 75-g OGTT at 24-28 weeks of gestation. Diagnosis of GDM was based on the International Association of Diabetes and Pregnancy Study Groups criteria. Individuals whose plasma glucose met one or more values in the following criteria were considered to have GMD: fasting plasma glucose, ≥ 92 mg/dl; 1 h, ≥ 180 mg/dl; 2 h, ≥ 153 mg/dl [1,21].
- Age between 15-45 years.
- Gestational age between 24-28 weeks.

The exclusion criteria were presence of thyroid or parathyroid disorder, kidney or liver diseases, cardiovascular disease, or hypertension, taking vitamin D supplements within 6 months prior to the study, and smoking. Of 230 GDM patients, 76 patients met the inclusion and exclusion criteria, and were selected for the study. The patients were randomly assigned to the vitamin D supplement (n = 38) or placebo (n = 38) conditions, while considering pre-pregnancy weight, and gestational diabetes management method (diet therapy, oral hypoglycemic agents, and insulin therapy). Random assignment was performed using random computer-generated numbers. A trained midwife conducted the randomized allocation sequence and assigned participants to the groups. The study protocol was accepted by the ethics committee of the Tabriz University of Medical Sciences. The IRB approval number of the research was 5/97/1160, 1392/4/30. All subjects provided written informed consent prior to participation. The trial was registered at the Iranian website (www.irct.ir) for the registration of clinical trials as IRCT201306253140N11.

Study design

The study was conducted using a randomized, placebo-controlled, double-blinded approach. The intervention ran from July 2013 through September 2014. Subjects were selected during the summer and spring; therefore, seasonal variation of ultraviolet radiation was deemed not to be a confounding factor.

Participants were advised to maintain their routine diets and lifestyle parameters, including levels of sunlight exposure and physical activity, which could affect vitamin D levels and metabolic factors.

Demographic data and pre-pregnancy weight were collected by interview. Weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were measured in a standard position using a scale (Seca) and an unstretched measuring tape. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m).

The dietary intakes of patients were assessed by a 3-day, 24-hour dietary recall before and after the study (2 weekdays, 1 weekend day). Nutritionist IV software, with modifications for Iranian foods, was used for the calculations of average daily nutrient intake (version 3.5.2, First DataBank; Hearst Corp, San Bruno, CA, USA).

Participants in the vitamin D group received oral capsules containing 50,000 IU vitamin D3 (D-Vitin50000; Zahravi Pharm Co., Iran), once every 2 weeks for 2 months, for a total of 4 capsules. Those in the placebo group received placebos.
composed of paraffin oil (Dana Pharm Co., Iran) using the same schedule, for a total of 4 placebos. Therefore, the vitamin D group received a total dose of 200,000 IU vitamin D₃. The safety of this dose in pregnant women was demonstrated in previous studies [22-24].

All features of the placebo and vitamin D₃ capsules were identical. Participants were followed up with a telephone call every 2 weeks to ensure adherence. Participants were asked to take their vitamin D or placebo capsules after a meal with water. All subjects consumed 400 micrograms/day of folic acid from the beginning of pregnancy and 60 mg/day of ferrous sulfate from the second trimester, on the basis of self-reported data, in accordance with the Iranian Ministry of Health guidelines for pregnant women.

Biochemical analysis

Blood samples (5 ml) were obtained after 12 h of overnight fasting at baseline and after the 8-week intervention. The samples were divided into two parts, one aliquot, which was poured for measurement of HbA1c in heparinized tubes, and the remaining amount was centrifuged immediately (Hettich; ROTOTOFIX 32 A) at 4,000 rpm for 5-10 min to separate the serum. Both serum and blood samples were stored at -70°C until assayed at the Tabriz University of Medical Sciences Reference Laboratory.

A chemiluminescence immunoassay method was used to determine serum 25-hydroxyvitamin D concentrations (DiaSorin, Stillwater, USA), and insulin levels (DiaSorin, Saluggia, Italy). The fasting plasma glucose level was measured using a spectrophotometric method using a Hitachi Automatic Analyzer 911 (Pars Azmun kit; Hitachi, Tokyo, Japan). A CERA-STAT kit was used for measurement of HbA1c. The HOMA-IR method \[\text{fasting insulin (mU/l) } \times \text{fasting glucose (mg/dl)} / 405\] was used for calculation of insulin resistance [25]. An enzymatic method was utilized to determine serum total cholesterol, triglyceride, and LDL- and HDL-cholesterol concentrations (Pars Azmun kit). The hs-CRP level was measured using the immunoturbidimetry method (Bio system kit).

Biochemical measurements were performed in a blinded fashion in duplicate, evaluating paired pre- and post-intervention samples at the same time, in the same analytic run, and in a random order to reduce systematic error and interassay variability.

Statistical analysis

The Kolmogorov-Smirnov test was used to examine the normal distribution of variables. Mean ± SD or median (IQR) was used for the population characteristics. For normally distributed data, an independent samples Student’s t-test was performed for determination of basic differences between 2 groups, and a paired t-test to determine differences in each group before and after the intervention.

The Mann-Whitney U test and the Wilcoxon paired rank test were used for nonparametric distributions. To assess the effects of vitamin D supplementation on biochemical parameters between the 2 groups after the intervention, ANCOVA was used by adjusting for the baseline measurements and covariates, including: GDM duration, GDM management method, type and dosage of drugs or insulin, baseline metabolic parameters, age, pre-pregnancy weight, weight gain, and energy and other macronutrient intake (Carbohydrate, Fat, and Protein). Data analysis was performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at a P-value \(\leq 0.05\).

RESULTS

Baseline characteristics

Of the 76 participants, 72 completed the trial (vitamin D

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**Fig. 1.** Flow chart illustrating the recruitment of patients for the study
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Table 1. General characteristics of participants

| Characteristics               | Placebo group (n = 36) | Vitamin D group (n = 36) | P-value^1 |
|------------------------------|------------------------|--------------------------|-----------|
| Maternal age (yrs)           | 32.11 ± 3.61           | 31.64 ± 4.40             | 0.62      |
| Height (cm)                  | 160.55 ± 4.22          | 160.72 ± 4.01            | 0.86      |
| Weight                       |                        |                          |           |
| Pre-pregnancy weight (kg)    | 74.63 ± 10.21          | 74.80 ± 10.91            | 0.94      |
| Weight at study baseline (kg)| 81.09 ± 9.80           | 81.48 ± 10.79            | 0.87      |
| Weight at end of study (kg)  | 85.73 ± 9.93           | 85.93 ± 10.53            | 0.93      |
| weight change (kg)           | 4.44 ± 0.97            | 4.63 ± 0.99              | 0.40      |
| BMI                          |                        |                          |           |
| Pre-pregnancy BMI (kg/m²)    | 28.97 ± 3.88           | 28.92 ± 3.75             | 0.95      |
| baseline BMI (kg/m²)         | 31.47 ± 3.71           | 31.51 ± 3.74             | 0.96      |
| after trial BMI (kg/m²)      | 33.28 ± 3.80           | 33.24 ± 3.63             | 0.96      |
| BMI change                   | 1.8 ± 0.40             | 1.72 ± 0.38              | 0.37      |
| Gestational diabetes management method* |                   |                          |           |
| Diet therapy                 | 8 (22.2)               | 8 (22.2)                 | 1         |
| Metformin                    | 4 (11.7)               | 4 (11.7)                 |           |
| Insulin                      | 24 (66.7)              | 24 (66.7)                |           |
| Type of insulin*             |                        |                          |           |
| Rapid acting                 | 19 (52.8)              | 19 (52.8)                | 1         |
| Long time acting             | 5 (13.9)               | 5 (13.9)                 |           |
| Middle time acting           | 9 (25)                 | 9 (25)                   |           |
| Dosage                       |                        |                          |           |
| Metformin (mg/day)           | 1,125 ± 250            | 1,125 ± 250              | 1         |
| Insulin (IU/day)             |                        |                          |           |
| Rapid acting                 | 13.37 ± 8.29           | 13.37 ± 8.71             | 0.97      |
| Long time acting             | 18.40 ± 2.19           | 19.20 ± 3.34             | 0.66      |
| Middle time acting           | 4.56 ± 0.52            | 5.33 ± 2.55              | 0.38      |
| Serum 25 (OH)D*              |                        |                          | 0.47      |
| Before                       | 33 (91.6)              | 34 (94.4)                |           |
| < 20 ng/ml                   | 1 (2.8)                | 2 (5.6)                  |           |
| ≥ 30 ng/ml                   | 2 (5.6)                | 0                       |           |
| After                        | 33 (91.7)              | 0                       | 0.01      |
| < 20 ng/ml                   | 8 (22.2)               | 18 (50)                  |           |
| ≥ 30 ng/ml                   | 0                     | 18 (50)                  |           |
| Duration of sun exposure (min/day) | 22.5 ± 9.82       | 20.42 ± 8.89             | 0.34      |
| Before                       | 21.3 ± 9.54            | 19.98 ± 8.11             | 0.28      |

Vitamin D3 and placebo group: received four doses of vitamin D3 (50,000 IU) or placebo as 1 capsule, once every 2 weeks, respectively. Values are expressed as mean ± SD; ^1 Independent samples t-test; ^2 Based on participants’ measured weights and heights from maternity clinic records; None of the participants showed changes in their disease management methods or dosages during the study.

Table 2. Dietary characteristics of participants

| Variable                    | Placebo group (n = 36) | Vitamin D group (n = 36) | P-value^1 |
|-----------------------------|------------------------|--------------------------|-----------|
| Energy (kcal/day)           | 2,016.16 ± 428.21      | 1,982.83 ± 344.70        | 0.71      |
| Carbohydrate (g/d)          |                        |                          |           |
| Before                      | 306.62 ± 60.93         | 303.66 ± 67.91           | 0.84      |
| After                       | 329.38 ± 65.54         | 327.97 ± 57.52           | 0.96      |
| Fat (g/d)                   |                        |                          |           |
| Before                      | 57.74 ± 22.86          | 3.38 ± 15.44             | 0.34      |
| After                       | 57.87 ± 18.42          | 63.85 ± 21.03            | 0.09      |
| Protein (g/d)               |                        |                          |           |
| Before                      | 73.92 ± 19.45          | 79.53 ± 22.29            | 0.25      |
| After                       | 77.61 ± 20.60          | 83.22 ± 17.15            | 0.36      |
| SFA (g/d)                   |                        |                          |           |
| Before                      | 14.19 ± 6.96           | 14.53 ± 5.86             | 0.82      |
| After                       | 14.47 ± 6.25           | 17.15 ± 7.33             | 0.09      |
| MUFA (g/d)                  |                        |                          |           |
| Before                      | 14.99 (11.42-20.64)    | 18.81 (13.91-24.86)      | 0.28      |
| After                       | 14.11 ± 6.07           | 12.89 ± 6.79             | 0.42      |
| Cholesterol (mg/d)          |                        |                          |           |
| Before                      | 190.72 (95.50-368.80)  | 217.95 (87.37-340.57)    | 0.67      |
| After                       | 195.50 (102.62-273.45) | 269.65 (108.17-355.20)   | 0.29      |
| Dietary fiber (g/d)         |                        |                          |           |
| Before                      | 14.63 ± 3.94           | 15.35 ± 4.80             | 0.48      |
| After                       | 13.44 ± 2.88           | 15.17 ± 4.35             | 0.06      |
| Calcium (mg/d)              |                        |                          |           |
| Before                      | 773.58 ± 256.75        | 918.34 ± 294.91          | 0.03      |
| After                       | 839.49 ± 309.94        | 1003.88 ± 356.63         | 0.07      |

Vitamin D3 and placebo group: received four doses of vitamin D3 (50,000 IU) or placebo as 1 capsule, once every 2 weeks, respectively. We used mean ± SD for normally distributed and median (25th and 75th percentiles) for nonparametric distribution data; ^1 Independent samples t-test; ^2 Based on participants’ measured weights and heights from maternity clinic records; None of the participants showed changes in their disease management methods or dosages during the study.
the 72 patients were 31.88 ± 4.00 years, 74.72 ± 10.49 kg, 28.94 ± 3.79 kg/m², and 11.3 ± 6.25 ng/mL, respectively. The baseline details for each group are shown in Table 1.

The types of drugs used (metformin and insulin (NovoRapid, Lantus and NPH) used were similar in both groups. None of the participants showed changes in their disease management methods or dosages during the study. Baseline and post-intervention means of sun exposure duration did not show significant change during the study.

Dietary Intakes before and after the Intervention Period

No significant differences in dietary intake at baseline, with the exception of calcium intake, were observed between the 2 groups. After the intervention, the vitamin D group showed significant increases in the dietary intake of energy, fat, SFA, MUFA, and vitamin D. However, there were no statistically significant changes in dietary intake within the placebo group.

ANOVA, adjusted for BMI and baseline values, showed that the differences of dietary factors before and after intervention could have affected the metabolic risk factors in the vitamin D intervention group, therefore the effects of energy intake and other macronutrients before and after the intervention were considered in the analysis of metabolic risk factors.

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ANOVA, adjusted for BMI and baseline values, showed that only phosphorous intake was significantly different between the two groups after the intervention. There were no other significant differences in dietary intake (Table 2).

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Metabolic Profiles and hs-CRP Level before and after the Intervention Period

At baseline, 66 patients (93.1%) had 25-hydroxyvitamin D levels less than 20 ng/mL. At the end of the study, 100% of the patients in the vitamin D group had serum 25-hydroxyvitamin D levels higher than 20 ng/mL compared with 3 patients (8.3%) in the placebo group. However, vitamin D supplementation increased serum 25-hydroxyvitamin D concentrations compared to placebo (19.15 (14.15-29.12) vs. -0.40 (-0.82 - 0.30) ng/mL; P < 0.01). Serum 25-hydroxyvitamin D levels ≥ 50 ng/mL were not observed in any patient after vitamin D supplementation.

Vitamin D supplements were associated with a significant decrease in fasting glucose (4.72 ± 13.99 vs. 5.27 ± 9.93 mg/dL;
vitamin D3 supplementation (two doses of 50,000 IU) during
placebo group, but did not change in the vitamin D group
levels were observed in the placebo group. The results of clinical trials
between 25-hydroxyvitamin D and individual components of metabolic syndrome [27]. An inverse association
between 25-hydroxyvitamin D and glycemic control was also
reported in women with GDM [28,29].

In the current study, administration of 4 oral doses of
cholecalciferol (50,000 IU) to GDM patients during a period of
2 months resulted in significantly improved vitamin D status,
compared to placebo. In the placebo group, despite constant
intake of vitamin D during the intervention, vitamin D concen-
trations decreased significantly. Similar significant reductions in
serum 25-hydroxyvitamin D levels in the placebo group have
been reported in other studies [19,36]. Decreases in 25-hydroxyvitamin
D level in the placebo group in this study and previous research
may be related to rapid fetal development, particularly bone
calcification at the end of pregnancy [17].

In the current study, significant reductions in FPG and HbA1c
without changes in insulin concentrations or HOMA-IR were
observed in the vitamin D group. The results of clinical trials
of the effects of vitamin D supplementation on glucose
homeostasis have been controversial. Asemi et al. reported that
vitamin D$_3$ supplementation (two doses of 50,000 IU) during
6 weeks in 52 GDM patients resulted in significantly decreased
FPG as well as insulin and HOMA-IR [17]. Similar to our findings,
administration of 88,865 IU/week vitamin D for one year in 109
prediabetes and vitamin D hypovitaminosis patients had no
effect on HOMA-IR. However, lower HbA1c levels were reported
in the vitamin D group [30]. In another study in 46 PCOS
patients, taking 20,000 IU/week vitamin D$_3$ orally for 24 weeks
resulted in significantly decreased FPG without changes in
fasting insulin level or HOMA-IR [31]. In studies conducted by
Witham et al. [32] and Ryu et al. [33] in type 2 diabetes patients,
vitamin D$_3$ had no significant effect on HOMA-IR. Ardabili
et al. reported that, among 50 PCOS patients with vitamin D
deficiency, receiving 3 oral capsules of 50,000 IU D$_3$ for 2 months
did not result in change of fasting insulin levels or HOMA-IR
[34]. In contrast, Mozaffari-Khosravi found that a single postpartum
injection of a high dose of vitamin D$_3$ (300,000 IU) in women
with GDM did not affect fasting glucose, HbA1c, or HOMA-IR
[19]. Taking 400 IU/day vitamin D$_3$ for 14 weeks showed no
alterations on HbA1c for 51 type 2 diabetic patients [35]. In
70 type 2 diabetes patients, insulin and HbA1c levels increased
significantly in both groups who received either 0.5 μg/day
calcitriol or placebo for 12 weeks [36].

FPG and HbA1c showed a remarkable increase in the placebo
group compared to the vitamin D group. Similar significant
increases in FPG and HOMA-IR among GDM patients [19], as
well as increased HbA1c among type 2 diabetes patients [36],
has been reported.

Vitamin D may have beneficial effects on insulin secretion,
either directly, by enhancing β-cell biosynthetic activity and
accelerating the transformation of pro-insulin to insulin [37], or
indirectly, via its role in regulating calcium flux through β-cells.
Insulin secretion is a calcium-dependent process; hence, alterations
in calcium flux can have adverse effects on β-cell secretory
functions [36]. Vitamin D may also have a beneficial effect on
insulin action either directly, through stimulating the expression
of insulin receptors and activating peroxisome proliferator
activated receptors [38], or indirectly, via its role in regulating
extracellular and intracellular calcium [27].

In cases of hyperparathyroidism, elevated parathyroid hormone
levels and enhanced renin production resulting from hypovita-
minosis D can contribute to insulin resistance via the suppression
of insulin signaling in adipocytes [33]. Insulin resistance may
also be facilitated through the interruption of intracellular
glucose transporter translocation by angiotensin II [37].

In this study, improvements in the lipid profiles were
observed in the vitamin D group, but these changes were not
significant. Similarly, some existing literature on vitamin D and
lipids did not identify any effects of vitamin D, even when
higher doses and longer durations of treatment were used
[39-41]. In contrast with our study, 4000 IU/day vitamin D$_3$ for
6 months in 104 postmenopausal women with type 2 diabetes
decreased TG levels significantly without affecting the levels of
other lipids [42]. In another study among 50 PCOS patients with
vitamin D deficiency, administration of three oral capsules of
50,000 IU D$_3$ for 2 months resulted in significantly decreased
serum TG and TC levels [43]. Remarkable reductions in serum
total and LDL cholesterol concentrations were also observed
in 54 GDM patients who received 2 doses of 50,000 IU D$_3$ for
six weeks [17].

In the current study significant increases in total and LDL
cholesterol levels and significant decreases in HDL cholesterol
were observed within the placebo group, when their post-
treatment measurements were compared with baseline. Similar
differences were observed among GDM and PCOS patients in
other studies [17,43].

The vitamin D receptor gene regulates approximately 3% of
the human genome, including genes that are crucial for glucose
and fat metabolism [44]. A known mechanism that can provide
a probable explanation for the favorable effects of vitamin D
on serum TG levels is its effect on calcium [43]. In the liver,
increased intracellular calcium leads to stimulation of
microosomal triglyceride transfer protein, involved in the
formation and secretion of VLDL, resulting in decreased serum
triglycerides and VLDL cholesterol levels [45].

In our study, although vitamin D concentrations increased
in the vitamin D group, hs-CRP level was constant. It is possible that vitamin D may prevent increases in hs-CRP in the supplemented group. A meta-analysis of 10 randomized controlled trials suggested the significantly favorable effects of vitamin D supplementation related to the levels of circulating hs-CRP [46]. However, the results from clinical trials on vitamin D supplementation and its effects on CRP levels have been inconsistent. In agreement with our results, the studies conducted by Sadiya et al. [47] and Yiu et al. [48] with type 2 diabetes patients showed that vitamin D3 had no significant effect on CRP levels. In contrast, intake of vitamin D supplements in healthy pregnant women has been linked to significant decreases in serum hs-CRP levels [49]. In another study, hs-CRP levels were significantly increased in overweight and obese subjects who received vitamin D3 [50].

The current study, hs-CRP concentrations increased in the placebo group. Similar changes have been observed in type 2 diabetes patients and healthy pregnant women [47,49]. The diverse findings on the effects of vitamin D supplementation on metabolic features and hs-CRP levels could be due to use of low dosages of vitamin D in the studies, short durations of supplementation [30], different characteristics of the subjects such as duration of diabetes or vitamin D status, type of vitamin D used [34], baseline status of vitamin D and metabolic indices [46], and wide ranges of post-intervention serum 25-hydroxyvitamin D concentrations in the vitamin D group [39]. Thus, low baseline 25-hydroxyvitamin D levels (11.3 ± 6.25 ng/ml) in our participants and higher baseline hs-CRP levels in the vitamin D group could explain the null results of our study.

In conclusion, vitamin D3 supplementation in GDM patients can have beneficial effects on glycemic status; however, it does not affect lipid profiles or hs-CRP levels. Conduct of additional studies to better clarify the role of vitamin D supplementation in GDM patients will be necessary.

REFERENCES

1. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva A, Hod M, Kitzmiller JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt Ml; International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676-82.

2. Wen SW, Xie RH, Han H, Walker MC, Smith GN, Retnakaran R. Preeclampsia and gestational diabetes mellitus: pre-conception origins? Med Hypotheses 2012;79:120-5.

3. Alzaim M, Wood RJ. Vitamin D and gestational diabetes mellitus. Nutr Rev 2013;71:158-67.

4. Burris HH, Rifas-Shiman SL, Kleinman K, Litonjua AA, Huh SY, Rich-Edwards JW, Camargo CA Jr, Gillman MW. Vitamin D deficiency in pregnancy and gestational diabetes mellitus. Am J Obstet Gynecol 2012;207:182.e1-8.

5. Poel YH, Hummel P, Lips P, Stam F, van der Ploeg T, Simsek S. Vitamin D and gestational diabetes: a systematic review and meta-analysis. Eur J Intern Med 2012;23:465-9.

6. Peuchant E, Brun JL, Rigalleau V, Dubourg L, Thomas MJ, Daniel JY, Leng JJ, Gin H. Oxidative and antioxidative status in pregnant women with either gestational or type 1 diabetes. Clin Biochem 2004;37:293-8.

7. Chu C, Gui YH, Ren YY, Shi LY. The impacts of maternal gestational diabetes mellitus (GDM) on fetal hearts. Biomed Environ Sci 2012;25:15-22.

8. Sánchez-Vera I, Bonet B, Viana M, Quintanar A, Martín MD, Blanco P, Donnay S, Albi M. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. Metabolism 2007;56:1527-33.

9. Horvath K, Koch K, Jeitler K, Matyas E, Bender R, Bastian H, Lange S, Siebenhofer A. Effects of treatment in women with gestational diabetes mellitus: systematic review and meta-analysis. BMJ 2010;340:c1395.

10. Wang O, Nie M, Hu YY, Zhang K, Li W, Ping F, Liu JT, Chen LM, Xing XP. Association between vitamin D insufficiency and the risk for gestational diabetes mellitus in pregnant Chinese women. Biomed Environ Sci 2012;25:399-406.

11. Senti J, Thiele DK, Anderson CM. Maternal vitamin D status as a critical determinant in gestational diabetes. J Obstet Gynecol Neonatal Nurs 2012;41:328-38.

12. Burris HH, Camargo CA Jr. Vitamin D and gestational diabetes mellitus. Curr Diab Rep 2014;14:4-51.

13. Berti C, Biesalski HK, Gärnter R, Lapillonne A, Pietrzik K, Poston L, Redman C, Koletzko B, Cetin I. Micronutrients in pregnancy: current knowledge and unresolved questions. Clin Nutr 2011;30:689-701.

14. De-Regil LM, Palacios C, Ansary A, Kulier R, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2012;2:CD008873.

15. Wagner CL, Taylor SN, Dawodu A, Johnson DD, Hollis BW. Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients 2012;4:208-30.

16. Zhang C, Qiu C, Hu FB, David RM, van Dam RM, Bradley A, Williams MA. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PLoS One 2008;3:e3753.

17. Asemi Z, Hashemi T, Karamali M, Samimi M, Esmaillzadeh A. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. Am J Clin Nutr 2013;98:1425-32.

18. Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metab Syndr 2013;5:8.

19. Mozaffari-Khosravi H, Hosseinizadeh-Shamsi-Anar M, Salami MA, Hadinedoushan H, Mozayan MR. Effects of a single post-partum injection of a high dose of vitamin D on glucose tolerance and insulin resistance in mothers with first-time gestational diabetes mellitus. Diabet Med 2012;29:36-42.

20. Zittermann A, Frisch S, Berthold HK, Göttig C, Kuhn J, Kleesiek
21. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva A, Hod M, Kitzmiller JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt Ml, American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37 Suppl 1: S81-90.

22. Yu OX, Sykes L, Sethi M, Teoh TG, Robinson S. Vitamin D deficiency and supplementation during pregnancy. Clin Endocrinol (Oxf) 2009;70:685-90.

23. Singla P, Parkash AA, Lal H, Nanda S. Benefits of vitamin D supplementation in pregnancy for prevention of preeclampsia. Int J Pharma Bio Sci 2012;2:144-50.

24. Kalra P, Das V, Agarwal A, Kumar M, Ramesh V, Bhatia E, Gupta S, Singh S, Saxena P, Bhatia V. Effect of vitamin D supplementation during pregnancy on neonatal mineral homeostasis and anthologymetry of the newborn and infant. Br J Nutr 2012;108:1052-8.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

26. Colden-Kirk H, Overbergh L, Christensen HT, Brusgaard K, Mathieu C. Vitamin D and diabetes: its importance for beta cell and immune function. Mol Cell Endocrinol 2011;347:106-20.

27. Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, Li X, Yang X, Chen Y, Lin X. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. Diabetes Care 2009;32:1278-83.

28. El Lithy A, Abdella RM, El-Faissal YM, Sayed AM, Samie RM. The relationship between low maternal serum vitamin D levels and glycemic control in gestational diabetes assessed by HbA1c levels: an observational cross-sectional study. BMC Pregnancy Childbirth 2014;14:362.

29. Lau SL, Gunton JE, Athayde NP, Byth K, Cheung NW. Serum 25-hydroxyvitamin D and glycated haemoglobin levels in women with gestational diabetes mellitus. Med J Aust 2011;194:334-7.

30. Davidson MB, Duran P, Lee ML, Friedman TC. High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis D. Diabetes Care 2013;36:260-6.

31. Wehr E, Pieber TR, Obermayer-Pietsch B. Effect of vitamin D3 treatment on glucose metabolism and menstrual frequency in polycystic ovary syndrome women: a pilot study. J Endocrinol Invest 2011;34:757-63.

32. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 2010;53:2112-9.

33. Ryu OH, Lee S, Yu J, Choi MG, Yoo HJ, Mantero F. A prospective randomized controlled trial of the effects of vitamin D supplementation on long-term glycemic control in type 2 diabetes mellitus of Korea. Endocr J 2014;61:167-76.

34. Ardabili HR, Gargari BP, Farzadi L. Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. Nutr Res 2012;32:195-201.

35. Ghavamzadeh S, Mobasseri M, Mahdavi R. The effect of vitamin D supplementation on adiposity, blood glycated hemoglobin, serum leptin and tumor necrosis factor-a in type 2 diabetic patients. Int J Prev Med 2014;5:1091-8.

36. Eftekhar MA, Akbarzadeh M, Dabbaghmanesh MH, Hasanzadeh J. Impact of treatment with oral calcitriol on glucose indices in type 2 diabetes mellitus patients. Asia Pac J Clin Nutr 2011;20:521-6.

37. Rammog G, Tseke P, Ziiakka S. Vitamin D, the renin-angiotensin system, and insulin resistance. Int Urol Nephrol 2008;40:419-26.

38. Pittas AG, Dawson-Hughes B. Vitamin D and diabetes. J Steroid Biochem Mol Biol 2010;121:425-9.

39. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. Br J Nutr 2010;103:549-55.

40. Madar AA, Knutsen KV, Stene LC, Brekke M, Meyer HE, Lagerløv P. Effect of vitamin D3 supplementation on glycated hemoglobin (HbA1c), fructosamine, serum lipids, and body mass index: a randomized, double-blinded, placebo-controlled trial among healthy immigrants living in Norway. BMJ Open Diabetes Res Care 2014;2:e000026.

41. Patel P, Poretsky L, Liao E. Lack of effect of subtherapeutic vitamin D treatment on glycemic and lipid parameters in Type 2 diabetes: A pilot prospective randomized trial. J Diabetes 2010;2:36-40.

42. Muñoz-Agüirre P, Flores M, Macias N, Quezada AD, Denova-Gutiérrez E, Salmerón J. The effect of vitamin D supplementation on serum lipids in postmenopausal women with diabetes: a randomized controlled trial. Clin Nutr 2015;34:799-804.

43. Rahimi-Ardabili H, Pourghassem Gargari B, Farzadi L. Effects of vitamin D on cardiovascular disease risk factors in polycystic ovary syndrome women with vitamin D deficiency. J Endocrinol Invest 2013;36:28-32.

44. Lakshman LR, Pillai BP, Lakshman R, Kumar H, Sudha S, Jayakumar RV. Comparison of vitamin D levels in obese and nonobese patients with polycystic ovarian syndrome in a South Indian population. Int J Reprod Contracept Obstet Gynecol 2013;2:336-43.

45. Asemi Z, Foroozanfard F, Hashemi T, Bahmani F, Jamilian M, Esmaillzadeh A. Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. Clin Nutr 2015;34:586-92.

46. Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: a meta-analysis of randomized controlled trials. Nutrients 2014;6:2206-16.

47. Sadiya A, Ahmed SM, Carlsson M, Tesfa Y, George M, Ali SH, Siddique RV. Comparison of vitamin D levels in obese and nonobese patients with polycystic ovary syndrome in a South Indian population. Int J Repro Contracept Obstet Gynecol 2013;2:e000026.

48. Von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. Br J Nutr 2010;103:549-55.

49. Madar AA, Knutsen KV, Stene LC, Brekke M, Meyer HE, Lagerløv P. Effect of vitamin D3 supplementation on glycated hemoglobin (HbA1c), fructosamine, serum lipids, and body mass index: a randomized, double-blinded, placebo-controlled trial among healthy immigrants living in Norway. BMJ Open Diabetes Res Care 2014;2:000026.

50. Patel P, Poretsky L, Liao E. Lack of effect of subtherapeutic vitamin D treatment on glycemic and lipid parameters in Type 2 diabetes: A pilot prospective randomized trial. J Diabetes 2010;2:36-40.

51. Muñoz-Agüirre P, Flores M, Macias N, Quezada AD, Denova-Gutiérrez E, Salmerón J. The effect of vitamin D supplementation on serum lipids in postmenopausal women with diabetes: a randomized controlled trial. Clin Nutr 2015;34:799-804.

52. Rahimi-Ardabili H, Pourghassem Gargari B, Farzadi L. Effects of vitamin D on cardiovascular disease risk factors in polycystic ovary syndrome women with vitamin D deficiency. J Endocrinol Invest 2013;36:28-32.

53. Lakshman LR, Pillai BP, Lakshman R, Kumar H, Sudha S, Jayakumar RV. Comparison of vitamin D levels in obese and nonobese patients with polycystic ovarian syndrome in a South Indian population. Int J Reprod Contracept Obstet Gynecol 2013;2:336-43.

54. Asemi Z, Foroozanfard F, Hashemi T, Bahmani F, Jamilian M, Esmaillzadeh A. Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. Clin Nutr 2015;34:586-92.

55. Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: a meta-analysis of randomized controlled trials. Nutrients 2014;6:2206-16.

56. Sadiya A, Ahmed SM, Carlsson M, Tesfa Y, George M, Ali SH, Siddique RV. Comparison of vitamin D levels in obese and nonobese patients with polycystic ovarian syndrome in a South Indian population. Int J Reprod Contracept Obstet Gynecol 2013;2:e000026.

57. Von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. Br J Nutr 2010;103:549-55.