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The effects of vitamin D and omega-3 fatty acid co-supplementation on glycemic control and lipid concentrations in patients with gestational diabetes

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KEYWORDS:
Vitamin D;
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Supplementation;
Glycemic control;
Lipid concentrations;
Gestational diabetes

OBJECTIVE: This study was performed to evaluate the effects of vitamin D and omega-3 fatty acids co-supplementation on glucose metabolism and lipid concentrations in gestational diabetes (GDM) patients.

METHODS: This randomized double-blind placebo-controlled clinical trial was done among 140 GDM patients. Participants were randomly divided into 4 groups to receive: (1) 1000 mg omega-3 fatty acids containing 360 mg eicosapentaenoic acid and 240 mg docosahexaenoic acid (DHA) twice a day + vitamin D placebo ($n = 35$); (2) 50,000 IU vitamin D every 2 weeks + omega-3 fatty acids placebo ($n = 35$); (3) 50,000 IU vitamin D every 2 weeks + 1000 mg omega-3 fatty acids twice a day ($n = 35$), and (4) vitamin D placebo + omega-3 fatty acids placebo ($n = 35$) for 6 weeks.

RESULTS: After 6 weeks of intervention, patients who received combined vitamin D and omega-3 fatty acids supplements compared with vitamin D, omega-3 fatty acids, and placebo had significantly decreased fasting plasma glucose (-7.3 ± 7.8, -6.9 ± 6.6, -4.0 ± 2.5, and $+1.0 \pm 11.4$ mg/dL, respectively, $P < .001$), serum insulin levels (-1.9 ± 1.9, -1.3 ± 6.3, -0.4 ± 6.3, and $+2.6 \pm 6.5$ μIU/mL, respectively, $P = .005$), homeostatic model of assessment for insulin resistance (-0.7 ± 0.6, -0.5 ± 1.4, -0.2 ± 1.5, and $+0.6 \pm 1.5$, respectively, $P < .001$) and increased quantitative insulin sensitivity check index ($+0.01 \pm 0.01$, $+0.008 \pm 0.02$, $+0.002 \pm 0.02$, and -0.005 ± 0.02, respectively, $P = .001$). In addition, changes in serum triglycerides (-8.2 ± 41.0, $+7.6 \pm 31.5$, $+3.6 \pm 29.9$, and $+20.1 \pm 29.6$ mg/dL, respectively, $P = .006$) and

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very low–density lipoprotein cholesterol (−1.6 ± 8.2, +1.5 ± 6.3, +0.8 ± 6.0, and +4.0 ± 5.9 mg/dL, respectively, $P = .006$) in the vitamin D plus omega-3 fatty acids group were significantly different from the changes in these indicators in the vitamin D, omega-3 fatty acids, and placebo groups.

CONCLUSION: Overall, vitamin D and omega-3 fatty acids co-supplementation for 6 weeks among GDM patients had beneficial effects on fasting plasma glucose, serum insulin levels, homeostatic model of assessment for insulin resistance, quantitative insulin sensitivity check index, serum triglycerides, and very low–density lipoprotein cholesterol levels.

Introduction

Gestational diabetes (GDM) is defined as any carbohydrate intolerance and impaired insulin metabolism with the onset or first recognition during pregnancy.[1] GDM affects 1% to 14% of pregnancies depending on the diagnostic criteria, gestational age, and characteristics of the study population.[2] GDM is associated with a marked effect on the future health of both mother and offspring including pre-eclampsia,[3] shoulder dystocia, higher rates of cesarean section,[4] macrosomia, neonatal hypoglycemia, and respiratory distress syndrome.[5] In addition, hyperinsulinemia and hyperglycemia during pregnancy have the potential to be detrimental to growth and metabolism in the offspring.[6] Dyslipidemia is associated with type II diabetes mellitus,[7] endothelial dysfunction, atherosclerosis, and intrauterine growth retardation.[8]

Prior studies have suggested that circulating levels of vitamin D[9] and omega-3 fatty acids[10] were low in GDM subjects than those healthy pregnant women. In addition, data on the effect of vitamin D or omega-3 fatty acids supplementation alone on metabolic profiles are conflicting. Nowadays, there is a growing interest to use vitamin D and omega-3 fatty acids during pregnancy. The basis of this interest is because of both vitamin D deficiency during pregnancy[11] and the results of epidemiologic observations exhibiting the significant inverse association between vitamin D or omega-3 fatty acids and pregnancy complications.[12,13] Furthermore, few studies have reported the beneficial effects of vitamin D or omega-3 fatty acids supplementation alone among GDM women. We have previously shown that vitamin D treatment at dosage of 50,000 IU every 3 weeks for 6 weeks in women with GDM improved glycemia, total cholesterol, and LDL cholesterol concentrations but did not influence other lipid profiles.[14] Improved insulin resistance was also observed after the intake of 1000 mg omega-3 fatty acids per day for 6 weeks in GDM subjects, but unchanged plasma glucose, insulin sensitivity, and lipid profiles.[15] In another study by Baidal et al.[16] was seen that combined high-dose omega-3 fatty acids and high-dose vitamin D3 therapy improved beta-cell function in patients with new onset type I diabetes mellitus.

There are speculations that vitamin D and omega-3 fatty acids may improve metabolic status because of their shared functions and each nutrient-specific role that complements the other nutrient's functions.[17,18] This trial was, therefore, conducted to investigate the effects of vitamin D and omega-3 fatty acids co-supplementation on parameters of glucose homeostasis and lipid concentrations in GDM women.

Methods

Trial design

The present study was a 6-week prospective randomized double-blind placebo controlled clinical trial.

Participants

This study was done among 140 GDM subjects aged 18-40 years without prior diabetes that have been diagnosed with GDM by "one-step" 2-h 75-g oral glucose tolerance test (OGTT) at 24-28 weeks' gestation referred to Kosar Clinic in Arak, Iran from March 2016 to July 2016. We diagnosed GDM based on the American Diabetes Association guidelines[19]: those whose plasma glucose met 1 of the following criteria were considered as having GDM: fasting plasma glucose (FPG) ≥ 92 mg/dL, 1-hour OGTT ≥ 180 mg/dL, and 2-hour OGTT ≥ 153 mg/dL.[19] Exclusion criteria were taking vitamin D and/or omega-3 fatty acids supplements, taking insulin, placenta abruption, pre-eclampsia, eclampsia, hypothyroidism and hyperthyroidism, smokers, those with kidney or liver diseases.

Ethics statements

This research was conducted according to the principles of the Declaration of Helsinki, and the study protocol was approved by the ethics committee of Arak University of Medical Sciences (reference number IR.ARAK-MU.REC.1394.373). The study protocol was carefully explained to all subjects before obtaining informed consent form. This trial was registered in the Iranian Web site (www.irct.ir) for registration of clinical trials (http://www.irct.ir: IRCT201605135623N78).

Study design

Patients were initially randomized to "intervention vs Placebo," and then divided into the 4 groups to intake omega-3 fatty acids ($n = 35$), vitamin D ($n = 35$), vitamin

D plus omega-3 fatty acids supplements ($n = 35$), or placebo ($n = 35$) for 6 weeks. At the onset of the study, patients were requested not to change their routine physical activity or usual dietary intakes throughout the study and not to consume any supplements other than the one provided to them by the investigators as well as not to take any medications that might affect findings during the 6-week intervention. Dietary macro- and micro-nutrients intakes were assessed using the 3-day food records (comprising 2 working days and 1 weekend) at the onset, weeks 3, 5, and end of the intervention. All participants' physical activity records were completed at weeks 0, 3, and 6 of the intervention. Modified Nutritionist-4 software program (First Databank, San Bruno, CA) was used to estimate the energy and nutrient intakes. In the present study, physical activity was described as metabolic equivalents (METs) in hours per day. To determine the METs for each patient, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables.[20]

Intervention

In the intervention groups, participants were received (1) 1000 mg omega-3 fatty acids containing 360 mg eicosapentaenoic acid (EPA) and 240 mg docosahexaenoic acid (DHA) twice a day + vitamin D placebo ($n = 35$); (2) 50,000 IU vitamin D every 2 weeks + omega-3 fatty acids placebo ($n = 35$); and (3) 50,000 IU vitamin D every 2 weeks + 1000 mg omega-3 fatty acids twice a day ($n = 35$) for 6 weeks. The appearance of the placebo capsule was indistinguishable in color, shape, size, and packaging, smell, and taste from vitamin D and omega-3 fatty acids capsules. Vitamin D and omega-3 fatty acids capsules were produced by Zahravi Pharmaceutical Company, Tabriz, Iran, that approved by Food and Drug Administration.

Treatment adherence

Every 2 weeks, individuals were taken enough supplements and placebos to last 3 days after their next scheduled visit and were instructed to return all unused supplements at each visit. The remaining supplements were counted and subtracted from the number provided to determine the number taken. To increase the compliance, all persons were receiving short messages on their cell phones to take the supplements every day.

Assessment of anthropometric measurements

Weight and height (Seca, Hamburg, Germany) were taken by a nutritionist before and after treatment in a fasting status without shoes and a minimal clothing state by a trained midwife. Body mass index (BMI) was calculated using the height and weight measurements (weight in kg/[height in meters]^2).

Assessment of outcomes

The primary outcome measurements were markers of insulin metabolism in the present study. The secondary outcome measurements were lipid concentrations.

Biochemical assessment

Blood sample was drawn at weeks 0 and 6 after at least 12-h fasting. All blood samples were centrifuged at 3000*g* for 10 minutes, and serum were separated into the clean tube aliquots and were stored at −80°C until analysis at the Arak University of Medical Sciences Reference Laboratory. To determine FPG, serum triglycerides, very low–density lipoprotein (VLDL) cholesterol, total cholesterol, low-density lipoprotein and high-density lipoprotein (HDL)-cholesterol concentrations, we used enzymatic kits (Pars Azmun, Tehran, Iran). All inter-assay and intra-assay coefficient variances (CVs) for FPG and lipid concentrations were lower than 5%. Serum 25-hydroxyvitamin D concentrations were quantified using a commercial enzyme-linked immunosorbent assay kit (IDS, Boldon, UK) with the intra-assay and inter-assay CVs 5.1% and 7.1%, respectively. Circulating levels of serum insulin were determined by use of the enzyme-linked immunosorbent assay kit (Monobind, CA) with the intra-assay and inter-assay CVs 3.0% and 4.7%, respectively. The homeostatic model of assessment for insulin resistance (HOMA-IR), homeostatic model assessment for B-cell function (HOMA-B), and the quantitative insulin sensitivity check index (QUICKI) were determined according to suggested formulas.[21]

Sample size

To calculate sample size, we used the standard formula suggested for parallel clinical trials by considering type I error (α) of 0.05 and type II error (β) of 0.20 (power = 80%). Based on a previous study,[14] we used 1.41 as standard deviation and 1.03 as the difference in mean (d) of HOMA-IR as key variable. Based on this, we needed 30 persons in each group. Assuming 5 dropouts in each group, the final sample size was determined to be 35 persons per group.

Randomization

Randomization assignment as blinding was done using computer-generated random numbers by a trained staff at the gynecology clinic.

Statistical methods

The Kolmogorov–Smirnov test was used to examine the normal distribution of variables. The analyses were done based on intention-to-treat approach. One-way analysis of variance (ANOVA) was used to detect differences in

general characteristics, dietary intakes, and metabolic profiles at the study baseline between the 4 groups. To determine the effects of vitamin D plus omega-3 fatty acids supplementation on markers of insulin metabolism and lipid profiles, we used repeated measures ANOVA. Changes in metabolic profiles across 4 groups were compared using repeated measure ANOVA. To assess if the magnitude of the change depended on the baseline values, we adjusted all analyses for the baseline values, maternal age, and baseline BMI to avoid the potential bias that might have resulted. P values $<.05$ were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc, Chicago, IL).

Results

Among individuals in the omega-3 fatty acids group, 3 patients (withdrawal because of personal reasons [$n = 3$]) were excluded. The exclusions in the placebo group were 3 persons (withdrawal because of personal reasons [$n = 3$]). Finally, 140 subjects (vitamin D [$n = 35$], omega-3 fatty acid [$n = 35$], vitamin D plus omega-3 fatty acids [$n = 35$], and placebo [$n = 35$]) completed the trial (Figure 1). However, as the analysis was done based on intention-to-treat approach, all 140 subjects (35 in each group) were included in the final analysis. No side effects were reported after supplementation of vitamin D and omega-3 fatty acids in GDM women throughout the study as well as vitamin D and omega-3 fatty acids were tolerated in particular in terms gastrointestinal tolerance.

Mean age, height, baseline, and end-of-intervention weight and BMI of study participants were not statistically different between the 4 groups (Table 1).

Based on the 3-day dietary records obtained throughout the intervention, no statistically significant difference was seen between the 4 groups in terms of dietary intakes of macronutrients and micronutrients (data not shown).

No significant differences were observed between the 4 groups in terms of baseline values of markers of insulin metabolism and lipid profiles (Table 2).

Co-supplementation with vitamin D and omega-3 fatty acids resulted in a significant increase in serum 25-hydroxyvitamin D concentrations ($+21.5 \pm 3.4$, $+19.2 \pm 3.9$, $+0.9 \pm 2.7$, and -0.1 ± 1.7 mg/dL, respectively, $P < .001$) compared with vitamin D, omega-3 fatty acids, and placebo (Table 3). After 6 weeks of intervention, patients who received combined vitamin D and omega-3 fatty acids supplements compared with vitamin D, omega-3 fatty acids, and placebo had significantly decreased FPG (-7.3 ± 7.8, -6.9 ± 6.6, -4.0 ± 2.5, and $+1.0 \pm 11.4$ mg/dL, respectively, $P < .001$), serum

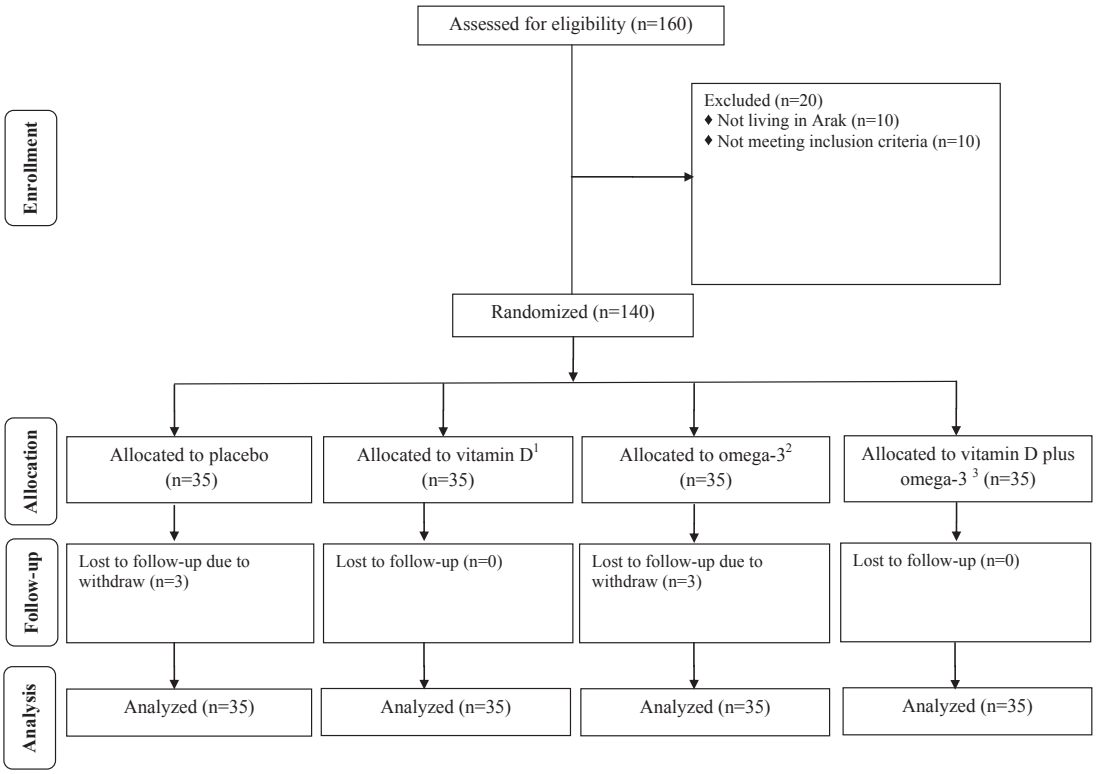

Figure 1 Summary of patient flow diagram. [1]Individuals received 50,000 IU vitamin D every 2 weeks plus placebo for omega-3 fatty acids twice a day; [2]individuals received 1000 mg omega-3 fatty acids (360 mg eicosapentaenoic acid and 240 mg docosahexaenoic acid) twice a day plus placebo for vitamin D every 2 weeks; [3]individuals received 50,000 IU vitamin D every 2 weeks plus 1000 mg omega-3 fatty acids twice a day.

Table 1 General characteristics of study participants*

General characteristics	Placebo (n = 35)	Vitamin D† (n = 35)	Omega-3‡ (n = 35)	Vitamin D + omega-3§ (n = 35)	P¶
Age (y)	30.7 ± 4.1	31.5 ± 7.0	30.7 ± 3.5	31.2 ± 4.3	.87
Height (cm)	161.5 ± 5.0	162.3 ± 5.8	161.6 ± 3.5	161.4 ± 4.2	.86
Weight at study baseline (kg)	75.9 ± 7.1	78.4 ± 15.2	75.0 ± 5.8	77.3 ± 9.9	.52
Weight at end-of-trial (kg)	77.9 ± 7.8	80.6 ± 14.9	77.1 ± 5.7	79.5 ± 9.9	.48
Weight change (kg)	2.0 ± 0.7	2.2 ± 1.1	2.1 ± 0.6	2.2 ± 0.6	.74
BMI at study baseline (kg/m^2)	29.2 ± 3.4	29.7 ± 5.1	28.8 ± 2.4	29.7 ± 3.9	.67
BMI at end-of-trial (kg/m^2)	29.9 ± 3.4	30.5 ± 5.0	29.6 ± 2.4	30.5 ± 3.9	.64
BMI change (kg/m^2)	0.7 ± 0.3	0.8 ± 0.4	0.8 ± 0.2	0.8 ± 0.2	.76

BMI, body mass index.

*Data are means± standard deviations.

†Receiving 50,000 IU vitamin D every 2 weeks plus placebo for omega-3 fatty acids twice a day.

‡Receiving 1000 mg omega-3 fatty acids (360 mg eicosapentaenoic acid and 240 mg docosahexaenoic acid) twice a day plus placebo for vitamin D every 2 weeks.

§Receiving 50,000 IU vitamin D every 2 weeks plus 1000 mg omega-3 fatty acids twice a day.

¶Obtained from analysis of variance test.

insulin levels (−1.9 ± 1.9, −1.3 ± 6.3, −0.4 ± 6.3, and +2.6 ± 6.5 μIU/mL, respectively, P = .005), HOMA-IR (−0.7 ± 0.6, −0.5 ± 1.4, −0.2 ± 1.5, and +0.6 ± 1.5, respectively, P < .001), and increased QUICKI (+0.01 ± 0.01, +0.008 ± 0.02, +0.002 ± 0.02, and −0.005 ± 0.02, respectively, P = .001). In addition, changes in serum triglycerides (−8.2 ± 41.0, +7.6 ± 31.5, +3.6 ± 29.9, and +20.1 ± 29.6 mg/dL, respectively, P = .006) and VLDL-cholesterol (−1.6 ± 8.2, +1.5 ± 6.3, +0.8 ± 6.0, and +4.0 ± 5.9 mg/dL, respectively, P = .006) in the vitamin D plus omega-3 fatty acids group were significantly different from the changes in these indicators in the vitamin D, omega-3 fatty acids, and placebo groups. Co-supplementation with vitamin D and omega-3 fatty acids had no significant effects on HOMA-B and other lipid profiles levels.

When the analyses were adjusted for baseline levels, maternal age, and baseline BMI, no significant changes in our findings occurred except for HOMA-B (P < .001; Table 4).

Table 2 The baseline metabolic profiles of study participants*

Metabolic profiles	Placebo (n = 35)	Vitamin D† (n = 35)	Omega-3‡ (n = 35)	Vitamin D + omega-3§ (n = 35)	P¶
Vitamin D (ng/mL)	16.6 ± 2.6	15.2 ± 3.8	16.9 ± 3.5	15.5 ± 3.1	.07
FPG (mg/dL)	93.6 ± 8.9	96.0 ± 3.9	96.5 ± 3.9	94.1 ± 9.6	.24
Insulin (μIU/mL)	13.2 ± 4.7	13.2 ± 6.4	12.9 ± 4.7	11.9 ± 2.5	.62
HOMA-IR	3.1 ± 1.2	3.1 ± 1.5	3.1 ± 1.1	2.8 ± 0.7	.60
HOMA-B	47.4 ± 17.5	46.4 ± 24.7	44.7 ± 16.4	42.4 ± 9.4	.67
QUICKI	0.32 ± 0.02	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	.90
Triglycerides (mg/dL)	182.6 ± 63.6	172.6 ± 72.3	205.6 ± 76.2	201.0 ± 92.5	.23
VLDL-cholesterol (mg/dL)	36.5 ± 12.7	34.5 ± 14.5	41.1 ± 15.2	40.2 ± 18.5	.23
Total cholesterol (mg/dL)	208.7 ± 43.5	197.1 ± 37.3	217.5 ± 48.3	204.3 ± 41.7	.25
LDL-cholesterol (mg/dL)	112.0 ± 30.1	104.1 ± 39.3	118.1 ± 42.8	107.5 ± 47.3	.50
HDL-cholesterol (mg/dL)	60.1 ± 14.2	58.5 ± 7.2	58.2 ± 10.2	56.6 ± 8.3	.55
Total-/HDL-cholesterol	3.6 ± 0.9	3.4 ± 0.8	3.8 ± 1.1	3.7 ± 1.0	.34

FPG, fasting plasma glucose; HDL-cholesterol, high-density lipoprotein cholesterol; HOMA-B, homeostatic model assessment-Beta cell function; HOMA-IR, homeostasis model of assessment-insulin resistance; LDL-cholesterol, low-density lipoprotein cholesterol; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low–density lipoprotein cholesterol.

*Data are means± standard deviations.

†Receiving 50,000 IU vitamin D every 2 weeks plus placebo for omega-3 fatty acids twice a day.

‡Receiving 1000 mg omega-3 fatty acids (360 mg eicosapentaenoic acid and 240 mg docosahexaenoic acid) twice a day plus placebo for vitamin D every 2 weeks.

§Receiving 50,000 IU vitamin D every 2 weeks plus 1000 mg omega-3 fatty acids twice a day.

¶Obtained from analysis of variance test.

Table 3 The effect of vitamin D plus omega-3 supplementations on glucose metabolism and lipid profiles in GDM patients*

	End-of-trial					Changes from baseline				
Metabolic profiles	Placebo (n = 35)	Vitamin D† (n = 35)	Omega-3‡ (n = 35)	Vitamin D + Omega-3§ (n = 35)	P¶	Placebo (n = 35)	Vitamin D† (n = 35)	Omega-3‡ (n = 35)	Vitamin D + Omega-3§ (n = 35)	P¶
Vitamin D (ng/mL)	16.5 ± 2.6	34.4 ± 6.1	17.8 ± 3.7	37.1 ± 4.0	<.001	−0.1 ± 1.7	19.2 ± 3.9‖	0.9 ± 2.7#	21.5 ± 3.4‖	<.001
FPG (mg/dL)	94.6 ± 10.3	89.1 ± 6.8	92.5 ± 4.2	86.8 ± 6.4	<.001	1.0 ± 11.4	−6.9 ± 6.6‖	−4.0 ± 2.5‖	−7.3 ± 7.8‖	<.001
Insulin (μIU/mL)	15.8 ± 7.9	13.2 ± 6.4	12.5 ± 4.1	11.9 ± 2.5	<.001	2.6 ± 6.5	−1.3 ± 6.3‖	−0.4 ± 6.3	−1.9 ± 1.9‖	.005
HOMA-IR	3.7 ± 1.9	2.6 ± 1.1	2.8 ± 0.9	2.1 ± 0.5	<.001	0.6 ± 1.6	−0.5 ± 1.4‖	−0.2 ± 1.5	−0.7 ± 0.6‖	<.001
HOMA-B	56.9 ± 31.0	44.9 ± 22.5	45.1 ± 15.9	38.4 ± 10.3	.005	9.5 ± 24.4	−1.5 ± 25.6	0.4 ± 24.2	−4.0 ± 6.1	.05
QUICKI	0.32 ± 0.002	0.33 ± 0.01	0.32 ± 0.01	0.34 ± 0.009	<.001	−0.005 ± 0.02	0.008 ± 0.02‖	0.002 ± 0.02	0.01 ± 0.01‖	.001
Triglycerides (mg/dL)	202.7 ± 68.8	180.2 ± 79.8	209.2 ± 71.4	192.8 ± 85.6	.41	20.1 ± 29.6	7.6 ± 31.5	3.6 ± 29.9	−8.2 ± 41.0‖	.006
VLDL-cholesterol (mg/dL)	40.5 ± 13.8	36.0 ± 16.0	41.9 ± 14.3	38.6 ± 17.1	.41	4.0 ± 5.9	1.5 ± 6.3	0.8 ± 6.0	−1.6 ± 8.2‖	.006
Total cholesterol (mg/dL)	212.0 ± 40.3	196.6 ± 41.8	218.1 ± 50.2	201.7 ± 36.1	.14	3.3 ± 18.6	−0.5 ± 23.3	0.6 ± 27.0	−2.6 ± 27.8	.78
LDL-cholesterol (mg/dL)	112.6 ± 30.6	101.6 ± 39.3	117.2 ± 45.9	106.1 ± 43.0	.37	0.6 ± 14.8	−2.5 ± 17.7	−0.9 ± 24.4	−1.4 ± 23.6	.94
HDL-cholesterol (mg/dL)	58.9 ± 12.4	58.9 ± 8.5	59.1 ± 10.7	60.0 ± 9.2	.80	−1.2 ± 4.9	0.4 ± 4.5	0.8 ± 4.2	0.4 ± 6.3	.30
Total-/HDL-cholesterol	3.7 ± 1.0	3.4 ± 0.9	3.8 ± 1.2	3.6 ± 0.9	.34	0.1 ± 0.4	−0.03 ± 0.3	−0.01 ± 0.7	−0.06 ± 0.5	.29

FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; HDL-cholesterol, high-density lipoprotein cholesterol; HOMA-B, homeostatic model assessment-Beta cell function; HOMA-IR, homeostasis model of assessment-insulin resistance; LDL-cholesterol, low-density lipoprotein cholesterol; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low–density lipoprotein cholesterol.

*Data are means± standard deviations.

†Receiving 50,000 IU vitamin D every 2 weeks plus placebo for omega-3 fatty acids twice a day.

‡Receiving 1000 mg omega-3 fatty acids (360 mg eicosapentaenoic acid and 240 mg docosahexaenoic acid) twice a day plus placebo for vitamin D every 2 weeks.

§Receiving 50,000 IU vitamin D every 2 weeks plus 1000 mg omega-3 fatty acids twice a day.

¶Obtained from analysis of variance test.

‖Significant difference with the placebo group.

#Significant difference with the vitamin D and vitamin D plus omega-3 fatty acids groups.

Discussion

We found that combined vitamin D and omega-3 fatty acids supplementation for 6 weeks among GDM patients had the beneficial effects on glycemic control, triglycerides, and VLDL-cholesterol levels. To the best of our knowledge, this study is the first that reports the effect of vitamin D and omega-3 fatty acids co-supplementation on glycemic control and lipid concentrations in GDM patients. It must be kept in mind that in the present study, observed changes in glycemic control measures in the combined vitamin D and omega-3 fatty acids group compared with other groups were clinically significant. On the other hand, the percentage of those who was treated after intervention was also significantly different between the 4 groups (91.4% for combined vitamin D and omega-3 fatty acids vs 74.3% for vitamin D, 71.9% for omega-3 fatty acids, and 59.4% for placebo groups, $P = .02$). Hyperglycemia and hyperinsulinemia in GDM patients can result in the progression to type II diabetes mellitus later in life and neonatal complications including macrosomia and hyperbilirubinemia.[22] Therefore, vitamin D and omega-3 fatty acids co-supplementation due to their decreasing effects on FPG and insulin resistance may be useful to decrease maternal and neonatal complications. However, observed difference at lipid profiles in our study was statistically significant, it was not clinically significant. Long-term interventions and higher dosage of omega-3 fatty acids might result in greater changes in lipid profiles.

The present study demonstrated that taking vitamin D and omega-3 fatty acids supplements among GDM patients for 6 weeks led to significant reductions in FPG, insulin concentrations, HOMA-IR, and a significant increase in QUICKI compared with other groups, but unchanged HOMA-B. Although few studies have evaluated the favorable effects of vitamin D or omega-3 fatty acids supplementation on glucose homeostasis parameters, to the best of our knowledge, no study has assessed the effects of vitamin D and omega-3 fatty acids co-supplementation on glycemic control resistance. We have previously shown that supplementation with 50,000 IU vitamin D every 3 weeks[14] or 1000 mg omega-3 fatty acids per day[15] for 6 weeks in pregnant women with GDM had beneficial effects on parameters of insulin metabolism. In addition, a single injection of 300,000 IU vitamin D3 at 3 to 10 days after delivery decreased indices of insulin resistance in women with recent pregnancy complicated by GDM.[23] The administration of 2.4 g/d EPA + DHA for 8 weeks also decreased serum insulin concentrations and HOMA-IR among hemodialysis subjects.[24] However, 50,000 IU vitamin D supplementation every 2 weeks for 2 months among GDM women improved FPG and HbA1c, but had no significant effect on markers of insulin metabolism.[25] Moreover, DHA supplementation at the dosage of 800 mg/d in the second half of pregnancy did not reduce the risk of GDM.[26] The beneficial effects of vitamin D on glycemic control might be explained by its effect on calcium and phosphorus metabolism and through

Table 4 Adjusted changes in metabolic variables in GDM patients who received vitamin D plus omega-3 fatty acids, omega-3 fatty acids, and vitamin D supplements or placebo*

Metabolic profiles	Placebo ($n = 35$)	Vitamin D† ($n = 35$)	Omega-3‡ ($n = 35$)	Vitamin D + omega-3§ ($n = 35$)	P¶
Vitamin D (ng/mL)	−0.1 ± 0.5	19.1 ± 0.5	1.0 ± 0.5	21.5 ± 0.5	<.001
FPG (mg/dL)	0.2 ± 1.1	−6.4 ± 1.1	−3.0 ± 1.1	−8.0 ± 1.1	<.001
Insulin (μIU/mL)	2.7 ± 0.8	−1.1 ± 0.8	−0.5 ± 0.8	−2.4 ± 0.8	<.001
HOMA-IR	0.7 ± 0.2	−0.4 ± 0.2	−0.2 ± 0.2	−0.8 ± 0.2	<.001
HOMA-B	10.5 ± 3.3	−0.6 ± 3.3	−0.2 ± 3.3	−5.3 ± 3.3	.01
QUICKI	−0.005 ± 0.003	0.008 ± 0.003	0.002 ± 0.003	0.01 ± 0.003	<.001
Triglycerides (mg/dL)	19.5 ± 5.5	5.5 ± 5.6	5.5 ± 5.6	−7.4 ± 5.5	.01
VLDL-cholesterol (mg/dL)	3.9 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	−1.5 ± 1.1	.01
Total cholesterol (mg/dL)	3.8 ± 4.0	−2.5 ± 4.0	−2.7 ± 4.0	−3.1 ± 4.0	.51
LDL-cholesterol (mg/dL)	0.9 ± 3.3	−3.5 ± 3.3	0.2 ± 3.3	−1.7 ± 3.3	.79
HDL-cholesterol (mg/dL)	−1.1 ± 0.8	0.4 ± 0.8	0.9 ± 0.8	0.1 ± 0.8	.38
Total: HDL cholesterol ratio	0.13 ± 0.07	−0.05 ± 0.08	−0.002 ± 0.08	−0.06 ± 0.07	.28

FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; HOMA-B, homeostatic model assessment-Beta cell function; HOMA-IR, homeostasis model of assessment-insulin resistance; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low–density lipoprotein cholesterol.

*All values are means± standard errors. Values are adjusted for baseline values, age and baseline weight.

†Receiving 50,000 IU vitamin D every 2 weeks plus placebo for omega-3 fatty acids twice a day.

‡Receiving 1000 mg omega-3 fatty acids (360 mg eicosapentaenoic acid and 240 mg docosahexaenoic acid) twice a day plus placebo for vitamin D every 2 weeks.

§Receiving 50,000 IU vitamin D every 2 weeks plus 1000 mg omega-3 fatty acids twice a day.

¶Obtained from analysis of covariance test.

upregulation of the insulin receptor genes[27] and increased transcription of insulin receptor genes.[27] Inhibiting proinflammatory factors and nuclear factor kappa-light-chain-enhancer of activated B cells protein expression by omega-3 fatty acids may result in improvement of markers of insulin metabolism.[28] We hypothesize that combination therapy with vitamin D and omega-3 fatty acids in patients with GDM may work better than a single supplementation alone. Combined vitamin D and omega-3 fatty acids supplementation might also have a strong synergistic effect on glucose metabolism and lipid profiles. In a study by Gurol et al.[29] was seen that omega-3 fatty acids and vitamin D had a synergistic effect on glycemia in islet transplantation. In addition, omega-3 fatty acids supplementation may result in increased levels of vitamin D. In a study by An et al.[30] was observed that $1,25(OH)_2D$ levels significantly increased in dialysis patients compared with baseline after 3 months of omega-3 fatty acids administration without vitamin D. Treatment with omega-3 fatty acids may also overcome the inverse association of vitamin D deficiency with inflammation.[31] As most patients of our study had vitamin D deficiency, decreased inflammation may decrease insulin resistance.

We demonstrated that vitamin D plus omega-3 fatty acids supplementation in GDM women for 6 weeks resulted in significant reductions in serum triglycerides and VLDL-cholesterol concentrations compared with other groups, but did not influence serum lipid profiles. In a study by Davis et al.[32] was observed that vitamin D and omega-3 fatty acids co-supplementation for 18 months among asymptomatic adult's decreased triglycerides, total cholesterol and LDL-cholesterol, and increased HDL-cholesterol concentrations. Supplementation with high-dose vitamin D (50,000 IU/wk) for 4 months in patients with metabolic syndrome also decreased triglycerides levels, but it did not had any beneficial effects on other lipid profiles.[33] In addition, a 3-month fish oil supplementation in young healthy men decreased serum triglycerides, whereas the proportion of HDL-cholesterol (relative to total cholesterol) increased significantly.[34] Both increased maternal triglycerides and free fatty acids concentrations in GDM women correlated with fetal growth during pregnancy and with neonatal anthropometric measures.[35] Furthermore, prior studies have demonstrated that increased maternal lipid profiles are associated with complications such as macrosomia,[36] pre-eclampsia,[37] and preterm birth.[38] Different study designs, lack of considering baseline levels of dependent variables along with characteristics of study participants, different dosages of vitamin D and omega-3 fatty acids supplements as well as duration of the intervention might provide some reasons for discrepant findings. Vitamin D intake might improve lipid profiles by increased calcium absorption[39] and improved insulin sensitivity.[40] Moreover, omega-3 fatty acids intake may decrease triglycerides and VLDL-cholesterol levels through improved postprandial clearance of chylomicrons[41] and decreasing hepatic production of VLDL-cholesterol.[42]

Few limitations must be considered in the interpretation of our findings. The main limitation of our study is the lack of measurements of fatty acids fractions at study baseline and at the end-of-trial because of budget limitations. Further studies are also needed to evaluate the expressed levels of related variables with signaling pathway of insulin and lipids to explore the plausible mechanism and confirm our findings. In addition, this study was a single-center study, and therefore may not be generalizable to the overall population of women with GDM. Further studies are needed to confirm our findings. It must be kept in mind that in the present study, we used the dose of 2000 mg omega-3 fatty acids based on observed beneficial effects of omega-3 fatty acids supplementation on insulin and HOMA levels in a previous study in patients with polycystic ovary syndrome.[43] However, several studies used higher doses up to 4 grams in polycystic ovary syndrome women[44] and 2.4 g/d EPA + DHA among hemodialysis subjects,[24] we agree that future studies are needed with higher dosage of omega-3 fatty acids in GDM women.

Conclusion

Overall, vitamin D and omega-3 fatty acids co-supplementation for 6 weeks among GDM patients had beneficial effects on FPG, serum insulin levels, HOMA-IR, QUICKI, serum triglycerides, and VLDL-cholesterol levels, but did not affect HOMA-B and other lipid profiles.

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