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Effects of selenium supplementation on glucose homeostasis, inflammation, and oxidative stress in gestational diabetes: Randomized, double-blind, placebo-controlled trial

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ABSTRACT

Objective: To our knowledge, no reports are available indicating the effects of selenium supplementation on metabolic parameters, inflammatory factors, and oxidative stress in gestational diabetes mellitus (GDM). The aim of this study was to assess the effects of selenium supplementation on metabolic status in pregnant women with GDM who were not on oral hypoglycemic agents.
Methods: This randomized, double-blind, placebo-controlled clinical trial was performed with 70 women with GDM. Patients were randomly assigned to receive either 200 μg selenium supplements as tablet (n = 35) or placebo (n = 35) for 6 wk from weeks 24 to 28 of gestation. Fasting plasma samples were taken at study baseline and after 6 wk of intervention to quantify related variables.
Results: Selenium supplementation, compared with placebo, resulted in a significant reduction in fasting plasma glucose (−10.5 ± 11.9 versus +4.5 ± 12.9 mg/dL; $P < 0.001$), serum insulin levels (−1.98 ± 11.25 versus +5.26 ± 9.33 μIU/mL; $P = 0.005$), homeostasis model of assessment (HOMA)-insulin resistance (−0.84 ± 2.76 versus +1.47 ± 2.46; $P < 0.001$) and a significant increase in quantitative insulin sensitivity check index (+0.008 ± 0.03 versus −0.01 ± 0.01; $P = 0.009$). Additionally, a significant decrease in serum high-sensitivity C-reactive protein (hs-CRP) levels (−791.8 ± 2271.8 versus +500.5 ± 2563.3 ng/mL; $P = 0.02$) was seen after the administration of selenium supplements compared with placebo. Additionally, we observed a significant elevation in plasma glutathione (+52.14 ± 58.31 versus −39.93 ± 153.52 μmol/L; $P = 0.002$) and a significant reduction in plasma malondialdehyde levels (−0.01 ± 0.36 versus +0.67 ± 1.90 μmol/L; $P = 0.04$) after consumption of selenium supplements compared with placebo. We did not find any significant effect of taking selenium supplements on HOMA β-cell function, lipid profiles, plasma nitric oxide, or total antioxidant capacity concentrations.
Conclusion: Selenium supplementation in pregnant women with GDM demonstrated beneficial effects on glucose metabolism, hs-CRP levels, and biomarkers of oxidative stress.

Introduction

Gestational diabetes mellitus (GDM) is a common complication of pregnancy that has been defined as impaired insulin metabolism and carbohydrate intolerance of varying degrees of severity with onset or first recognition during pregnancy [1]. Requirement for selenium is increased during pregnancy [2]. Selenium is involved in inhibiting the expression of

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cyclooxygenase (COX)-2 and P-selectin [3], therefore, its nutritional inadequacy during pregnancy might increase the risk for GDM. Mean serum levels of selenium are less than normal in healthy Iranian women [4]. On the other hand, the prevalence of GDM in Iranian pregnant women is 4.7% [5]. It is associated with short- and long-term health complications for the mother, fetus, and the neonate [6]. Additionally, previous studies have reported that hyperglycemia in patients with diabetes could result in increased oxidative stress and nitrosative stress [7]. Poor glycemic control in these patients has been associated with the depletion of serum antioxidant activity [8].

Several dietary [9,10] and nondietary [11,12] strategies have been proposed for management of GDM. Some studies have reported a significant inverse association between body selenium status and plasma glucose levels in patients with GDM [13,14]. Others have shown that selenium supplementation in pregnant women might result in decreased oxidative stress and improved pregnancy outcomes [15,16]. Dietary sources of selenium are nuts, cereals, meat, mushrooms, fish, and eggs [17]. Selenium is an important component of the enzymes that protect cells from the adverse effects of free radicals [18]. Selenium is also involved in the production of thyroid hormones and has been shown to contain anti-inflammatory effects [19]. Current data supports the beneficial effect of selenium on hypertension [20], coronary artery disease [21], certain cancers [22], and inflammatory diseases [23]. Others have examined the efficacy of selenium supplementation in cancers [24]. Some studies have indicated [25] a significant decrease in serum insulin levels and insulin resistance in centrally obese women after supplementation with 200 μg/d selenium added to a hypocaloric diet enriched with legumes; however, no significant effect on lipid profiles, inflammatory factors, and biomarkers of oxidative stress have been reported. Additionally, selenium supplementation has resulted in increased erythrocyte and plasma total antioxidant status (TAS), erythrocyte-reduced glutathione (GSH) and glutathione peroxidase (GPx) in patients with epilepsy and refractory epilepsy [26].

Selenium supplementation might affect glucose homeostasis, inflammation, and oxidative stress by inhibiting the production of advanced glycation end products [3] and decreased free radical production and lipid hydroperoxides [27]. Therefore, we hypothesized that selenium supplementation might affect the metabolic status of pregnant women with GDM. We are not aware of any studies that examined the effect of selenium supplementation on glucose homeostasis, lipid profiles, inflammatory factors, and biomarkers of oxidative stress in women with GDM. This study aimed to investigate the effect of selenium supplementation on the metabolic profile of women with GDM who were not on oral hypoglycemic agents (OHAs).

Materials and methods

Participants

This randomized, double-blind, placebo-controlled trial was conducted in Arak, Iran, from February to April 2014. For estimating sample size, we considered type 1 (α) and type 2 errors (β) of 0.05 and 0.20 (power = 80%), respectively, and homeostasis model of assessment-insulin resistance (HOMA-IR) as a key variable. Based on a previous study [25], SD of HOMA-IR was 0.4 and the difference in mean (d) of HOMA-IR was 0.3. We reached the sample size of 28 women for each group using the suggested formula for parallel clinical trials. In the present study, we included pregnant women, primigravida, ages 18 to 40 y who were carrying singleton pregnancies who had been diagnosed with GDM by "one-step" 2-h 75-g oral glucose tolerance test (OGTT) at 24 to 28 wk gestation. None of the participants were taking OHAs. Gestational age was assessed from the date of last menstrual period and concurrent clinical assessment [28]. Pregnant women without a previous diagnosis of glucose intolerance were screened. Diagnosis of GDM was done based on the American Diabetes Association criteria [29]: Those whose plasma glucose met one of the following criteria were considered to have GDM: fasting ≥92 mg/dL, 1-h ≥180 mg/dL, or 2-h ≥153 mg/dL. In all, 1200 pregnant women attending maternity clinics affiliated with the Arak University of Medical Sciences, Arak, Iran, were screened for GDM. Seventy pregnant women met the inclusion criteria (1110 were excluded because they did not have GDM and 20 others were not included because they were diagnosed with GDM class A2, and required insulin therapy: fasting plasma glucose [FPG] >105 and blood sugar 2-h postprandial >120 mg/dL). Women with intrauterine fetal death (IUFD), premature preterm rupture of membrane (PPROM), placenta abruption, preeclampsia, eclampsia, chronic hypertension, hypo- or hyperthyroidism, urinary tract infection, smokers, and those with kidney or liver diseases or those taking estrogen therapy and stressful life conditions were not included in the present study. We excluded women who required commencing insulin therapy during intervention (FPG >105 and blood sugar 2-h postprandial >120 mg/dL). In all, 70 pregnant women were recruited in the present study and after stratification for preintervention body mass index (BMI; <30 and ≥30 kg/m^2) and weeks of gestation (<26 or ≥26 wk), they were randomly assigned to consume either selenium supplements (n = 35) or placebo (n = 35) for 6 wk. Random assignment was done by the use of computer-generated random numbers. Randomization and allocation were concealed from the researcher and participants until the main analyses were completed. A trained midwife at the maternity clinic did the randomized allocation sequence, enrolled participants, and assigned participants to interventions. The study was conducted according to the guidelines of the Declaration of Helsinki. The present study was approved by the ethics committee of Arak University of Medical Sciences and registered on the Iranian registry of clinical trials website (IRCT201403175623 N18). All participants provided informed written consent before recruitment.

Study design

Women were randomly assigned to take either 200 μg selenium supplements as tablet or placebo daily for 6 wk during weeks 24 to 28 of gestation. Selenium supplements and placebos were manufactured by Nature Made Pharmaceutical Company (Northridge, CA, USA) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. The appearance of the placebo, such as color, shape, size, and packaging, were identical to the selenium capsules. Selenium supplements and placebos were packed in identical packages and coded by the producer to guarantee blinding. Participants were asked not to alter 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. All the women were also consuming 400 μg/d folic acid from the beginning of pregnancy and 60 mg/d ferrous sulfate from the second trimester. To assess compliance, participants were asked to bring the medication containers. Compliance was checked through counting unused capsules. To increase compliance, all patients received short messages on their cell phones reminding them to take the supplements daily. All participants provided three dietary records (one weekend day and two weekdays) and three physical activity records to ensure they maintained their usual diet and physical activity during the intervention. Both dietary and physical activity records were taken at weeks 2, 4, and 6 of the intervention. The dietary records were based on estimated values of household measurements. To obtain nutrient intakes of participants based on these 3-d food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods. After being diagnosed with GDM, the women were first instructed about a healthy diet; however, they were not given a specific menu and they were only participating in a nutritional education class that focused on the basics of healthy diet.

Assessment of anthropometric and biochemical variables

Data on prepregnancy weight and height (measured values) were taken from the women's preexisting medical records. A trained midwife at the maternity clinic performed the anthropometric measurements at study baseline and 6 wk after the intervention. Body weight was measured in an overnight fasting state, without shoes, and while minimally clothed by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kg divided by height in m^2. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with a 10-min interval between the two measurements via a sphygmomanometer (ALPK2, Zhejiang, China). The average of these two measurements was considered as the participant's final BP. As the intervention was completed at least 6 wk before expected delivery, required information on cesarean delivery, newborns' hyperbilirubinemia and newborns' weight, height, and head circumference were collected using data from their medical records at the center or the hospital.

At baseline and after 6 wk of intervention, 10 mL venous blood samples were taken after overnight fasting at Arak reference laboratory. FPG levels were measured on the day of blood collection. Blood samples were immediately

centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500*g* for 10 min to separate serum. Serum lipid profiles were also quantified on the day of blood collection. The samples were stored at −70°C before analysis at the AUMS reference laboratory. Commercial kits were used to measure FPG, serum cholesterol, triacylglycerols (TGs), and low- (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations (Pars Azmun, Tehran, Iran). The intra- and interassay coefficient of variations (CVs) for FPG were 1.7 and 3%, respectively. All intra- and interassay CVs for lipid profile measurements were <5%. Serum insulin levels were assayed by enzyme-linked immunosorbent assay (ELISA) kit (Mononbind, Lake Forest, CA, USA). The intra- and interassay CVs for serum insulin were 2.9% and 5.8%, respectively. HOMA-IR and β-cell function (HOMA-B) and quantitative insulin sensitivity check index (QUICKI) were calculated based on suggested formulas [30]. Serum high sensitivity C-reactive protein (hs-CRP) was quantified using ELISA kit (LDN, Nordhorn, Germany) with intra- and interassay CVs of 2.8% and 4.4%, respectively. Plasma nitrite/nitrate (NOx), taken as an index of nitric oxide (NO) concentration, was determined using the Giess method as previously modified [31]. Plasma total antioxidant capacity (TAC) was assessed by the ferric-reducing ability of plasma *method* previously developed [32]. Plasma total GSH was examined using a previously described method [33]. We measured plasma malondialdehyde (MDA) levels using the thiobarbituric acid reactive substance spectrophotometric test. CVs for plasma TAC, GSH, and MDA were 0.8%, 2.6%, and 3.4%, respectively. Measurements of lipid profiles, insulin function, inflammatory factors, and biomarkers of oxidative stress were performed in a blinded fashion, in duplicate, in pairs (pre- and postintervention) at the same time, in the same analytical run, and in random order to reduce systematic error and interassay variability.

Statistical analysis

We used the Kolmogrov-Smirnov test to examine the normal distribution of variables. Log transformation was applied for non-normally distributed variables. The analyses were done based on an intention-to-treat approach. Missing values were treated based on last observation carried forward method. Independent samples Student's *t* test was used to detect differences in general characteristics and dietary intakes between the two groups. To determine the effects of selenium supplementation on glucose homeostasis, lipid profiles, inflammatory factors, and biomarkers of oxidative stress, we used one-way repeated measures analysis of variance. In this analysis, the treatment (selenium versus placebo) was regarded as between-subject factor and time with two time points (baseline and week 6 of intervention) was considered as within-subject factor. To assess whether the magnitude of the change in dependent variables depended on the baseline values, maternal age, and baseline weight, we controlled all analyses for these variables to avoid any potential bias. In the case of plasma glucose and serum lipid profiles, we further adjusted for changes in serum hs-CRP, NO, and MDA to determine if the effect of supplementation on plasma glucose and lipid profiles were mediated through these indicators. These analyses were done using analysis of covariance. $P < 0.05$ was considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, IL, USA).

Results

Randomization characteristics

Three women from the selenium group were excluded: one because of IUFD; two were hospitalized. Two women in the placebo group were excluded: one for placenta abruption and one because she required insulin therapy. Finally, 65 participants (selenium group n = 32; placebo group n = 33) completed the trial (Fig. 1). However, as the analysis was done based on an intention-to-treat approach, all 70 women (35 in each group) were included in the final analysis. On average, the rate of compliance in the study was high, such that all of the capsules were taken throughout the study in both groups. No side effects were reported after consumption of selenium supplements; however, the side effects could not be examined in infants because all participants completed the trial ≥6 wk before delivery.

Participants' mean age, prepregnancy weight, and BMI were 28.6 ± 4.6 y, 68.2 ± 10.6 kg, and 26.3 ± 3.7 kg/m^2, respectively. Gestational age, 2-h OGGT, baseline and end-of-trial means of weight, and BMI did not significantly differ between the two groups (Table 1). No significant differences in physical activity levels were observed between the two groups (data not shown).

Based on 3-d dietary records obtained throughout the intervention, no statistically significant difference was seen between the two groups in terms of dietary intakes of energy, carbohydrates, proteins, fats, saturated fatty acids, polyunsaturated fatty

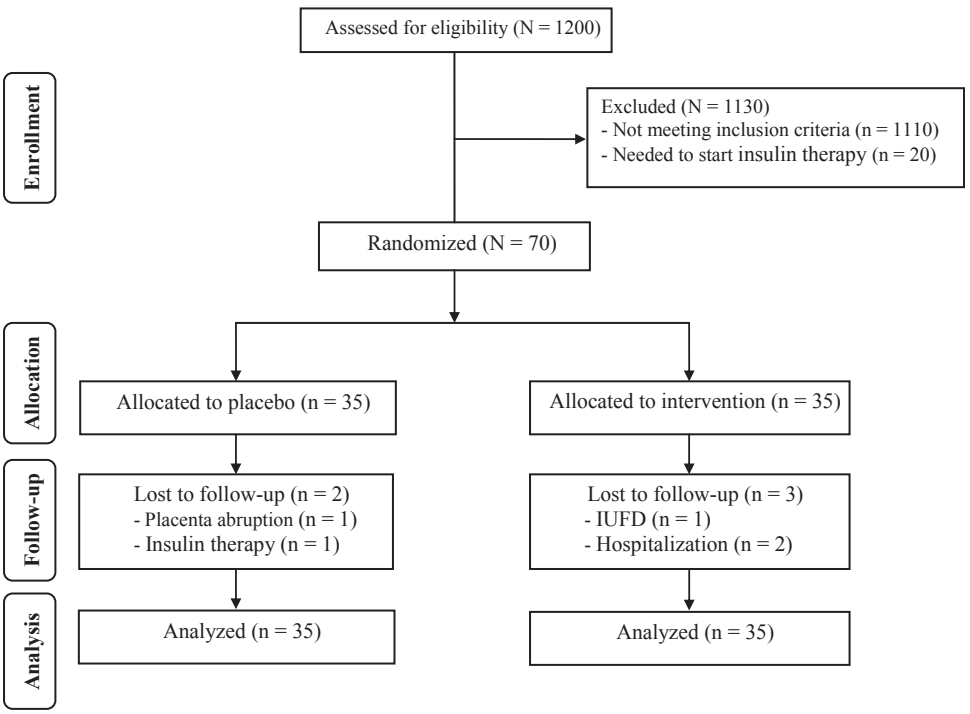

Fig. 1. Summary of patient flow diagram. IUFD, intrauterine fetal death.

Table 1
General characteristics of pregnant women with GDM who received selenium supplements or placebo

	Placebo group (n = 35)	Selenium group (n = 35)	*P* value*
Maternal age (y)	29.6 ± 3.6	27.6 ± 5.3	0.07
Height (cm)	160.5 ± 3.8	161.4 ± 4.9	0.37
Prepregnancy weight (kg)†	69 ± 9.2	67.3 ± 12	0.51
Weight at study baseline (kg)	73.5 ± 10.5	71.4 ± 12.3	0.44
Weight at end of trial (kg)	75.3 ± 10.5	73.4 ± 12.4	0.49
Weight change (kg)	1.8 ± 1.2	2 ± 0.9	0.41
Prepregnancy BMI (kg/m^2)†	26.8 ± 3.3	25.8 ± 4	0.26
BMI at study baseline (kg/m^2)	28.5 ± 3.8	27.3 ± 4.2	0.22
BMI at end of trial (kg/m^2)	29.2 ± 3.8	28.1 ± 4.2	0.25
BMI change (kg/m^2)	0.7 ± 0.5	0.8 ± 0.4	0.49
Gestational age (wk)	25.9 ± 1.4	25.6 ± 1.4	0.53
2-h OGTT (mg/dL)	121.1 ± 23	124 ± 23.5	0.61

BMI, body mass index; GDM, gestational diabetes; OGTT, oral glucose tolerance test.
Data are means ± SD.
* Obtained from independent *t* test.
† Based on participants' measured weight and height as per their records in the maternity clinics.

acid, monounsaturated fatty acids, cholesterol, total dietary fiber, magnesium, selenium, and vitamins C, E, and A (Table 2).

Selenium supplementation resulted in a significant reduction in FPG (−10.5 ± 11.9 versus +4.5 ± 12.9 mg/dL; $P < 0.001$), serum insulin levels (−1.98 ± 11.25 versus +5.26 ± 9.33 μIU/mL; $P = 0.005$), HOMA-IR (−0.84 ± 2.76 versus +1.47 ± 2.46; $P < 0.001$) and a significant increase in QUICKI score (+0.008 ± 0.03 versus −0.01 ± 0.01; $P = 0.009$) compared with placebo (Table 3). Additionally, a significant decrease in serum hs-CRP levels (−791.8 ± 2271.8 versus +500.5 ± 2563.3 ng/mL; $P = 0.02$) was seen after administration of selenium supplements compared with placebo. Additionally, we observed a significant elevation in plasma GSH (+52.14 ± 58.31 versus −39.93 ± 153.52 μmol/L; $P = 0.002$) and a significant reduction in plasma MDA levels (−0.01 ± 0.36 versus +0.67 ± 1.90 μmol/L; $P = 0.04$) after consuming selenium supplements but not placebos. We did not find any significant effect of taking selenium supplements on HOMA-B, lipid profiles, plasma NO, TAC concentrations, and SBP and DBP.

Table 2
Dietary intakes of pregnant women with GDM who received selenium supplements or placebo

	Placebo group (n = 35)	Selenium group (n = 35)	*P* value*
Energy (kcal/d)	2405 ± 267	2366 ± 299	0.56
Carbohydrates (g/d)	329.5 ± 54.1	326.6 ± 56.9	0.82
Protein (g/d)	88 ± 13.3	87.0 ± 17.2	0.78
Fat (g/d)	85.3 ± 12.4	82.8 ± 15.3	0.44
SFA (g/d)	25.2 ± 5.2	25.9 ± 5	0.52
PUFA (g/d)	26.1 ± 6.2	26.1 ± 7.1	0.97
MUFA (g/d)	23.5 ± 6.7	23.2 ± 6.1	0.82
Cholesterol (mg/d)	214.2 ± 108.2	198.4 ± 96.3	0.52
TDF (g/d)	19.5 ± 5.2	18.7 ± 4.8	0.48
Magnesium (mg/d)	277.5 ± 70.1	281.5 ± 59.7	0.80
Selenium (μg/d)	114.9 ± 35.1	114.9 ± 45.5	0.99
Vitamin C (mg/d)	184.4 ± 80.8	166.4 ± 91.9	0.38
Vitamin E (mg/d)	11.7 ± 2.4	12.2 ± 2.8	0.50
Vitamin A (μg/d)	698 ± 341.7	670.2 ± 230.3	0.69

GDM, gestational diabetes; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber.
Data are means ± SD.
* Obtained from independent *t* test.

Baseline levels of FPG and plasma NO were significantly different between the two groups. Therefore, we controlled the analyses for the baseline levels. However, after this adjustment no significant changes in our findings occurred, except for finding a significant effect of selenium supplements on HOMA-B ($P = 0.004$) and plasma TAC levels ($P = 0.04$; Table 4). Additional adjustments for baseline levels, maternal age, and baseline weight only affected plasma TAC ($P = 0.02$) and MDA levels ($P = 0.05$). Additionally, adjusted glucose homeostasis parameters and lipid profiles for baseline levels, maternal age, and baseline weight as well as hs-CRP, NO, and MDA changes did not affect our findings. Additionally, further adjustment for changes in serum hs-CRP, NO, and MDA did not affect plasma glucose or lipid profiles.

Taking selenium supplements, compared with placebo, resulted in a lower percentage of cesarean deliveries in the selenium group than in the placebo group (31.4% versus 60%; $P = 0.02$). Additionally, the hyperbilirubinemia of newborns (≥15 mg/dL in infants 25–48 h old, 18 mg/dL in infants 49–72 h old, and 20 mg/dL in infants >72 h) was significantly lower in the selenium group than in the placebo group (5.7% versus 44%; $P = 0.001$). There were no significant differences in the mean weight (3321.1 versus 3573.4 g; $P = 0.14$), height (50.2 versus 51.2 cm; $P = 0.22$), or head circumference of newborns (35 versus 35.5 cm; $P = 0.25$) in the two groups.

Discussion

Our findings demonstrated that selenium supplementation in pregnant women with GDM led to improved glucose homeostasis, reduced inflammation, and improved oxidative stress; however, it did not affect lipid profiles or plasma NO. To the best of our knowledge, this was the first study to examine the effects of selenium supplementation on glucose homeostasis, inflammation, and biomarkers of oxidative stress in pregnant women with GDM.

No side effects were reported after selenium administration in the study patients. It should be noted that mean dietary plus supplemental selenium intake in the study participants was lower than the upper limits (400 μg). However, data on the toxic effects of selenium on human health are conflicting. For example, one study found that intake of moderate (200 μg/d) to large doses (600 μg/d) of selenium supplements for 16 wk was safe in volunteers ages ≥18 y [34]. Some studies have shown a small, nonsignificant increase in the risk for diabetes after selenium supplementation [35]. Others have reported hair loss, dystrophic fingernail changes, gastrointestinal symptoms, and memory difficulties as the adverse effects of selenium intake [36]. Additionally, high selenium intake might have toxic effects on growth hormone levels, insulin-like growth factor-1, and thyroid function [37]. Nonetheless, additional studies are needed to review the potential toxicity/teratogenicity of long-term increased selenium intake. It should also be noted that the rate of compliance in our study was high. Taking 100% of supplements in an interventional study might seem unusual; however, because participants were on a strict control to use the supplements, it was expected that all supplements would used. There was a significant difference at baseline on FGP and plasma NO levels between the two groups. This difference might have been occurred for several reasons. The diagnosis of GDM in this study was done according to the criteria of the American Diabetes Association (one-step). In other words, when one or more of the venous plasma concentrations (FPG, GTT1-h and GTT2-h) were abnormal, the woman was diagnosed with GDM. Therefore, the

Table 3
Metabolic profiles, inflammation, and biomarkers of oxidative stress at study baseline and after 6-wk intervention in pregnant women with GDM who received selenium supplements or placebo

	Placebo group (n = 35)			Selenium group (n = 35)			*P* Value*
	Wk 0	Wk 6	Change	Wk 0	Wk 6	Change	
FPG (mg/dL)	89.51 ± 11.35	94.02 ± 13.39†	4.51 ± 12.90	99.45 ± 11.25	88.90 ± 7.14†	−10.55 ± 11.94	<0.001
Insulin (μIU/mL)	14.69 ± 7.42	19.95 ± 12.74†	5.26 ± 9.33	13.52 ± 10.51	11.54 ± 6.74	−1.98 ± 11.25	0.005
HOMA-IR	3.32 ± 1.86	4.79 ± 3.26†	1.47 ± 2.46	3.27 ± 2.78	2.43 ± 1.39†	−0.84 ± 2.76	<0.001
HOMA-B	55.16 ± 29.36	71.46 ± 46.30†	16.30 ± 36.69	43.19 ± 36.12	41.48 ± 24.91	−1.71 ± 43.62	0.06
QUICKI	0.32 ± 0.03	0.31 ± 0.03†	−0.01 ± 0.01	0.33 ± 0.03	0.34 ± 0.02	0.008 ± 0.03	0.009
Total cholesterol (mg/dL)	200.61 ± 56.33	208.69 ± 58.86	8.08 ± 31.66	210.09 ± 43.00	215.49 ± 41.10	5.40 ± 20.90	0.67
Triacylglycerols (mg/dL)	184.72 ± 69.93	203.26 ± 84.02	18.54 ± 55.42	177.53 ± 71.33	186.47 ± 69.90	8.94 ± 34.49	0.38
LDL-cholesterol (mg/dL)	108.32 ± 45.43	112.37 ± 46.87	4.05 ± 24.43	115.29 ± 33.62	118.11 ± 32.08	2.82 ± 16.25	0.80
HDL-cholesterol (mg/dL)	55.34 ± 15.69	55.67 ± 14.27	0.33 ± 8.51	59.29 ± 11.60	60.08 ± 12.13	0.79 ± 5.53	0.78
Total:HDL cholesterol ratio	3.78 ± 1.08	3.87 ± 1.02	0.09 ± 0.39	3.61 ± 0.81	3.66 ± 0.77	0.05 ± 0.29	0.65
hs-CRP (ng/mL)	6190.63 ± 4050.42	6691.18 ± 3976.97	500.55 ± 2563.34	5128.18 ± 3713.43	4336.30 ± 3275.09†	−791.88 ± 2271.84	0.02
NO (μmol/L)	91.58 ± 30.56	96.61 ± 61.91	5.03 ± 51.68	112.02 ± 39.41	132.93 ± 62.91	20.91 ± 65.32	0.26
TAC (mmol/L)	719.69 ± 168.37	737.14 ± 150.55	17.45 ± 117.33	745.08 ± 95.03	808.06 ± 137.06	62.98 ± 118.44	0.11
GSH (μmol/L)	552.37 ± 290.75	512.44 ± 215.91	−39.93 ± 153.52	452.33 ± 80.53	504.47 ± 69.57†	52.14 ± 58.31	0.002
MDA (μmol/L)	3.08 ± 1.44	3.75 ± 2.02†	0.67 ± 1.90	3.13 ± 2.19	3.12 ± 2.19	−0.01 ± 0.36	0.04
SBP (mm Hg)	105.3 ± 5.4	105.9 ± 4.9	0.6 ± 4.3	104.4 ± 7.0	105.6 ± 7.0	1.2 ± 7.5	0.69
DBP (mm Hg)	62.1 ± 4.9	62.6 ± 3.1	0.4 ± 4.9	61.7 ± 4.2	61.6 ± 3.6	−0.1 ± 4.9	0.62

DBP, diastolic blood pressure; FPG, fasting plasma glucose; GDM, gestational diabetes; GSH, glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated β-cell function; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TAC, total antioxidant capacity.

Data are means ± SD. Baseline values of FPG and NO were significantly different between the groups.

* Obtained from repeated measures analysis of variance.

† Different from week 0; $P < 0.05$.

diagnosis was not only based on FPG levels as different patients might have had different FPG levels, which could in turn lead to a different mean FPG at study baseline. Furthermore, we did not randomize participants based on baseline levels of FGP and plasma NO because all participants had GDM. Random assignment to two groups was done after stratification for pre-intervention BMI (<30 and ≥30 kg/m^2) and weeks of gestation (<26 or ≥26 wk). Therefore, the difference in baseline levels of FGP and plasma NO between the two groups might have occurred by chance.

Patients with GDM are susceptible to inflammation and oxidative stress [38]. The present study demonstrated that pregnant women with GDM who took selenium supplements for 6 wk realized a significant decrease in FPG, serum insulin levels, and HOMA-IR, and a significant increase in QUICKI compared with women in the placebo group. These findings persisted in multivariable models accounting for potential confounders. Dietary intakes of study participants did not differ between the two groups. Some studies have indicated that dietary vitamins C, A, and E might enhance selenium absorption [39,40]. Therefore, we examined the difference in the dietary intakes of these nutrients between the two groups. However, due to a nonsignificant difference between the two groups, the dietary intakes of these nutrients were not considered in the analysis. Few studies have examined the effects of selenium supplementation on glucose homeostasis. In agreement with our study, administration of 200 μg/d selenium supplements for 6 wk resulted in a significant decrease in serum insulin levels and HOMA-IR score in women with central obesity [25]. Decreased blood glucose and insulin levels were also seen after selenium administration in diabetic rats [41]. The same findings were also seen in women with polycystic ovary syndrome [42], in obese female Zucker rats, [43] and in male buffalo calves [44]. In contrast to our findings, some studies did not demonstrate any significant effect of selenium supplementation on glucose homeostasis. A 3-mo supplementation with selenium in patients with diabetes did not influence serum insulin levels and led to increased FPG levels [45]. Impaired glucose homeostasis in women with GDM can result in adverse long-term maternal outcomes, might increase perinatal morbidity, and can result in long-term aberrations in the offspring [38,46]. Beneficial effects of selenium supplementation on improved glucose homeostasis might have resulted from its effect on the inhibition of inflammatory cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-1 [47]. Amelioration of markers of insulin metabolism may be partly due to a direct effect of selenium supplements on peripheral tissues [48]. Additionally, selenium has also been shown to accelerate glucose incorporation into adipocytes [48]. Recent studies have reported that the continuous use of selenium supplements in individuals and populations with adequate-to-high selenium status cannot be justified and may increase risk for type 2 diabetes [49]. Although there is a clear association between certain selenoproteins and glucose homeostasis parameters, the relationship between selenium intake and type 2 diabetes is undoubtedly complex. It is possible that this association is U-shaped, with possible harm occurring both above and below the physiological range for optimal activity of some or all selenoproteins [49]. Therefore, examining a large number of women probably would have been more relevant to finding the exact effects of selenium supplementation.

Findings from this study demonstrated that selenium supplementation in patients with GDM did not affect lipid profiles. In line with our findings, it has been shown that 100 μg selenium supplementation in pregnant women did not affect cord-blood total cholesterol (TC), LDL-C, and HDL-C levels and led to increased serum TGs [50]. Additionally, no significant change in lipid profiles was seen after the administration of 100 μg/d selenium in pregnant women from the first trimester of pregnancy to delivery [51]. However, selenium supplementation resulted in a significant decrease in TC and TG levels in male New Zealand white rabbits [52] and male mongrel rabbits [53]. Different findings might be explained by the different study designs, discrepancy in subjects, and different dosages of selenium supplements, as well as duration of the study.

Table 4
Adjusted changes in metabolic variables in pregnant women with GDM who received selenium supplements or placebo

	Placebo group (n = 35)	Selenium group (n = 35)	*P* value*
FPG (mg/dL)			
Model 1†	1.26 ± 1.78	−7.30 ± 1.78	0.002
Model 2‡	1.54 ± 1.75	−7.58 ± 1.75	0.001
Model 3§	0.86 ± 1.79	−6.90 ± 1.79	0.006
Insulin (μIU/mL)			
Model 1	5.56 ± 1.56	−2.29 ± 1.56	0.001
Model 2	5.92 ± 1.57	−2.65 ± 1.57	<0.001
Model 3	6.21 ± 1.68	−2.93 ± 1.68	0.001
HOMA-IR			
Model 1	1.47 ± 0.38	−0.86 ± 0.38	<0.001
Model 2	1.58 ± 0.38	−0.96 ± 0.38	<0.001
Model 3	1.63 ± 0.39	−1.01 ± 0.39	<0.001
HOMA-B			
Model 1	19.95 ± 5.99	−5.37 ± 5.99	0.004
Model 2	21.32 ± 6.06	−6.73 ± 6.06	0.002
Model 3	23.52 ± 6.39	−8.94 ± 6.39	0.001
QUICKI			
Model 1	−0.01 ± 0.004	0.009 ± 0.004	0.001
Model 2	−0.01 ± 0.004	0.01 ± 0.004	<0.001
Model 3	−0.01 ± 0.005	0.01 ± 0.005	<0.001
Total cholesterol (mg/dL)			
Model 1	7.46 ± 4.44	6.01 ± 4.44	0.81
Model 2	6.46 ± 4.49	7.01 ± 4.49	0.93
Model 3	3.46 ± 4.36	10.01 ± 4.36	0.31
Triacylglycerols (mg/dL)			
Model 1	18.95 ± 7.74	8.52 ± 7.74	0.34
Model 2	17.34 ± 7.76	10.13 ± 7.76	0.51
Model 3	21.43 ± 7.88	6.04 ± 7.88	0.19
LDL-cholesterol (mg/dL)			
Model 1	3.59 ± 3.42	3.27 ± 3.42	0.94
Model 2	3.04 ± 3.50	3.82 ± 3.50	0.87
Model 3	0.05 ± 3.31	6.81 ± 3.31	0.17
HDL-cholesterol (mg/dL)			
Model 1	−0.02 ± 1.15	1.13 ± 1.15	0.48
Model 2	−0.06 ± 1.17	1.18 ± 1.17	0.46
Model 3	−0.84 ± 1.20	1.95 ± 1.20	0.12
Total:HDL cholesterol ratio			
Model 1	0.09 ± 0.05	0.03 ± 0.05	0.48
Model 2	0.08 ± 0.05	0.05 ± 0.05	0.75
Model 3	0.08 ± 0.05	0.04 ± 0.05	0.63
hs-CRP (ng/mL)			
Model 1	635.92 ± 378.22	−927.22 ± 378.22	0.005
Model 2	589.82 ± 378.60	−881.20 ± 378.60	0.009
NO (μmol/L)			
Model 1	1.67 ± 10.04	24.26 ± 10.04	0.12
Model 2	1.65 ± 10.38	24.28 ± 10.38	0.14
TAC (mmol/L)			
Model 1	13.41 ± 18.70	67.01 ± 18.70	0.04
Model 2	9.59 ± 18.12	70.83 ± 18.12	0.02
GSH (μmol/L)			
Model 1	−21.64 ± 14.86	33.85 ± 14.86	0.01
Model 2	−45.48 ± 19.64	57.69 ± 19.64	<0.001
MDA (μmol/L)			
Model 1	0.66 ± 0.23	−0.01 ± 0.23	0.04
Model 2	0.65 ± 0.23	−0.003 ± 0.23	0.05
SBP (mm Hg)			
Model 1	0.8 ± 0.9	0.9 ± 0.9	0.91
Model 2	1.0 ± 0.9	0.7 ± 0.9	0.83
DBP (mm Hg)			
Model 1	0.6 ± 0.5	−0.3 ± 0.5	0.24
Model 2	0.7 ± 0.6	−0.4 ± 0.6	0.17

DBP, diastolic blood pressure; FPG, fasting plasma glucose; GDM, gestational diabetes; GSH, glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated β-cell function; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TAC, total antioxidant capacity.
Data are means ± SE.
* Obtained from analysis of covariance.
† Adjusted for baseline values.
‡ Adjusted for baseline levels, maternal age, and baseline weight.
§ Further adjusted for hs-CRP, NO, and MDA changes.

The present study revealed that supplementing selenium in women with GDM was associated with decreased serum hs-CRP levels, but did not influence plasma NO concentrations. In agreement with our study, high-dose selenium supplementation for 14 d (1000 μg on day 1 and 500 μg/d on days 2–14) decreased plasma CRP levels in patients with systemic inflammatory response syndrome/sepsis [54]. Additionally, a 4-wk supplementation with beta-carotene (9 mg/d), vitamin C (1500 mg/d), vitamin E (130 mg/d), zinc (45 mg/d), selenium (76 μg/d), and garlic (150 mg/d) did not influence exhaled NO levels in allergic adults [55]. In contrast, consumption of cereal biscuits with selenized onion, curcuma, and green tea for 2 mo did not affect hs-CRP levels in healthy adults [56]. The same finding was also observed in centrally obese women who took 200 μg selenium supplements for 6 wk [25]. Elevated inflammation in women with GDM makes them susceptible for future development of both metabolic and cardiovascular disease [57]. Selenium supplements may decrease serum hs-CRP levels via the inhibition of nuclear factor (NF)-κB by modulating selenoprotein gene expression [58]. NF-κB has been implicated in the production of inflammatory markers and synthesis of adhesion molecules [58]. Additionally, selenium intake in chronic inflammatory status restores the depleted hepatic and serum selenium levels by increasing selenoprotein biosynthesis, which results in suppressed CRP production and thereby attenuates the inflammatory process [23]. However, selenium supplementation may indirectly affect NO levels through effects on produced NO synthase (NOS) [59] and inducible NOS (iNOS) gene expression [60].

We found that selenium supplementation led to a significant increase in plasma GSH and a significant reduction in plasma MDA levels. In agreement with our study, decreased MDA levels have been observed after administration of 200 μg/d selenium supplements for 12 wk in hemodialysis patients [61]. Similar findings were also seen after selenium supplementation in animal models [62,63]. However, 200 μg/d selenium supplementation for 3 wk did not affect biomarkers of oxidative stress including TAS and GSH levels in overweight adults [64]. No significant change in MDA levels was seen after administering selenium supplements to rats [65]. Increased oxidative stress plays an important role in the pathogenesis of diabetes mellitus; however, there is no consensus regarding its role in GDM [66]. Reactive oxygen species (ROS), free radicals, and lipid peroxidation can alter several cellular components as well as the redox state, which in turn leads to insulin resistance, B-cell dysfunction, glucose intolerance, and, finally, type 2 diabetes mellitus (T2DM) [67]. Hyperglycemia in patients with diabetes is associated with increased glycation, oxidative stress, and nitrosative stress [7]. Additionally, poor glycemic control in T2DM has been associated with the depletion of serum antioxidant activity [8]. Selenium is an essential component of the erythrocyte GPx system [68], which functions as part of an antioxidant defense to protect polyunsaturated fatty acids and proteins from the damaging effects of free radicals, peroxides, and lipid hydroperoxides including MDA [69]. It is speculated that in some conditions like GDM where free radical levels are high, selenium supplementation may reduce free radical production and lipid hydroperoxides through increasing the activity of antioxidant enzyme GPx [27]. Additionally, selenium deficiency might increase the expression of TNF-α and IL-1β, and facilitate the activation of iNOS and COX-2 [70]. Earlier studies in diabetic mice have shown that the beneficial role of selenium might be attributed to its inhibitory effect on augmentation of proinflammatory cytokines and ROS/reactive nitrogen species [71]. Oxidative stress can be defined as increased oxidants and/or a

decreased antioxidant capacity [72]. Previous studies have reported increased oxidative stress in patients with diabetes [7]. Increased GSH and decreased MDA levels after selenium supplementation in the present study may have indirectly resulted from decreased ROS, free radicals, and lipid peroxidation. This is also the case for increased TAC levels after supplementation.

Study limitations

Some limitations need to be taken into account in the interpretation of our findings. This study involved relatively few participants. Therefore, conclusions regarding the relative beneficial effects of selenium therapy need to be confirmed in large-scale studies. As a result of limited funding, we did not examine the effects of selenium supplementation on serum or urine selenium, other biomarkers of systemic inflammation, or on other biomarkers of oxidative stress. Additionally, we did not assess the effects of selenium supplementation on all pregnancy outcomes, including infant respiratory status, hypoglycemia, and time in the neonatal intensive care unit because almost all participants completed the trial $\geq$6 wk before delivery. Future studies are needed to examine the effects of selenium supplementation on other pregnancy outcomes including week of delivery, infant respiratory status, fetal growth and heart rate, hypoglycemia, and time in the neonatal intensive care unit. Furthermore, additional studies are required to examine the effect of selenium supplementation on liver and kidney function enzymes as well as on selenium-dependent antioxidant enzymes including GPx isoforms and thioredoxin reductase. Unfortunately, because of limited funding, we did not examine the effects of selenium supplementation on these measures. Additionally, as this study was the first to examine the effects of selenium supplementation on GDM, no further information is available regarding its possible toxicity in GDM. Therefore, additional data are needed to determine the appropriate dosage of selenium supplementation in patients with GDM.

Conclusion

Selenium supplementation in pregnant women with GDM had beneficial effects on glucose homeostasis, hs-CRP levels, and biomarkers of oxidative stress.

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