Association of Maternal Serum Zinc, Copper and Magnesium Levels, Pre-pregnancy Body Mass Index and Gestational Weight Gain Patterns With the Risk of Gestational Diabetes Mellitus Among Third-trimester Pregnant Women: a Cross-sectional Study

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Research

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Abstract

**Background:** This study sought to evaluate the association between selected micronutrients (zinc, copper, and magnesium), pre-pregnancy BMI, and weight gain during pregnancy with the risk of gestational diabetes mellitus third-trimester pregnant women Urmia, Iran.

**Methods:** This analytic cross-sectional study included 400 pregnant women. The nutritional, demographic, clinical data, and fasting blood samples (selected micronutrients and blood glucose) were evaluated. The data were analyzed using chi-square, independent t-test, and logistic regression tests.

**Results:** The prevalence of gestational diabetes mellitus (GDM) was 18%. The OR for GDM was (OR: 0.329; 95% CI: 0.156-0.696) in normal-weight compared to mothers who were obese before pregnancy. Normal serum zinc concentration was associated with 0.413-fold lower rates of developing GDM (95% CI: 0.227-0.750). Magnesium supplementation was inversely associated with the risk of GDM (OR: 0.986; 95% CI: 0.979-0.994). Inadequate and excessive gestational weight gain was significantly associated with developing GDM in lean and obese women before pregnancy, respectively (p=0.01, p=0.003).

**Conclusions:** Gestational diabetes is highly prevalent in Urmia, and it is likely related to excessive serum zinc concentrations, elevated pre-pregnancy BMI, and gestational weight gain.

Background

Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications and was historically defined as any degree of impaired glucose tolerance diagnosed in the third trimester of pregnancy[1]. The incidence of GDM in 2017 was about 14% of all pregnancies worldwide [2, 3]. The prevalence of GDM in Iran was 3.41% (the highest and lowest prevalence reported were 18.6% and 1.3%, respectively) [4]. Gestational diabetes may lead to adverse fetal, infant, and maternal outcomes [5, 6]. Also, women with unmanaged GDM remain at a higher risk for developing type 2 diabetes and long-term obesity later in life [7, 8]. The GDM etiology is multifactorial. Generally known risk factors for gestational diabetes, including Maternal age, overweight/obesity, family history of diabetes, behaviors, and dietary habits [9]. Furthermore, evidence suggests that changes in serum micronutrient levels may be somewhat responsible for GDM [10].

Changes in trace elements like zinc (Zn), copper (Cu), and magnesium (Mg) are critical in gestational diabetes because of their possible teratogenicity associations [10, 11]. Low serum of Zn and Mg in pregnancy is likely to be associated with the onset of gestational diabetes [11, 12]. Zn is a vital micronutrient necessary for insulin homeostasis in human pancreatic β cells [13]. Pregnant women affected by GDM showed less intracellular Mg [11]. Some studies have shown a significant inverse relationship between dietary Mg intake and glucose homeostasis in patients with GDM [14, 15]. Recently, both human and animal studies have reported a significant association between excessive Cu and abnormal glucose metabolism [16]. Other studies have found a positive correlation between Cu and glycated hemoglobin (HbA1c) [17, 18]. Therefore, maintaining Cu homeostasis seems essential for
preventing and treating abnormal glucose metabolism [19]. However, research on the relationship between Cu concentration and GDM has been limited, and their results are inconsistent [20].

Excessive gestational weight gain and obesity increase the risk of adverse pregnancy outcomes for mothers and infants [21–23]. The effect of weight gain during pregnancy on GDM is unknown [24, 25]. According to reports from the Institute of Medicine and the Agency for Healthcare and Quality Research, there is no evidence of the effect of pregnancy weight gain on GDM [23, 25]. However, some studies have shown that excessive weight gain during pregnancy may cause GDM [26, 27]. Most studies have proven the effect of maternal BMI in women with GDM [21, 22, 28]. Few reports have warned about a relationship between overweight or obese women with normal glucose tolerance [29, 30].

The International Association of Diabetes and Pregnancy Study Groups (IADPSG) was formed in 1998 as an umbrella organization to serve as the basis for internationally endorsed criteria for diagnosing and classification diabetes in pregnancy. The principal objectives of IADPSG are to foster an international approach to enhancing the quality of care, facilitating research, and advancing education in the field of diabetes in pregnancy [31].

According to the contradictions mentioned in the current study, the first aim was to highlight the prevalence of GDM in Urmia city using the new diagnostic criteria of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidelines. The second purpose was to characterize the association between selected micronutrients (Zn, Cu, and Mg) and body weight (pre-pregnancy BMI and weight gain during pregnancy) with the risk of gestational diabetes mellitus using cross-sectional data.

**Materials And Methods**

**Study design and participants**

This analytical cross-sectional study was performed in urban health care centers and their subset health stations in Urmia, Iran. The study has been approved and reviewed by the ethics committee of Urmia University of Medical Sciences. This study's sample size was calculated based on a study (4) that the highest prevalence rate of GDM was 18.6% in Iranian pregnant women. The formula for cross-sectional studies was performed to calculate the sample size of the current study $N = \frac{(Z^2\alpha/2P (1-P))/d^2}{32}$; where $P$ and $d$ were the prevalence of GDM and accuracy, respectively. Given the $Z \alpha/2 = 1.96$, $\alpha=0.05$, $d = 0.05$ and $P = 18.6$ percent, the calculated sample size was 233 subjects. In total, to increase the accuracy of the study results, 400 pregnant women entered the study.

We assessed the prevalence of GDM in the population of pregnant women over six months. Seven hundred ninety pregnant women were referred to health care facilities for routine care from July to December 2018. Based on the number of pregnant women covered by each center, we selected 400 healthy pregnant women with gestational age $\geq 27$ weeks by simple random sampling. Informed consent was obtained from all individual participants included in the study. Then pregnant women were asked to
refer to the Nutrition Department's clinical laboratory for blood sampling and screen for GDM and interview the following day, after a 10-hour fast.

**Information and blood sampling**

A questionnaire was completed for each participant by a nutritionist through medical records, physical exams, and face-to-face interviews. Pregnant women's questionnaire including questions about demographic characteristics (age and education) and lifestyle during pregnancy (smoking, alcohol consumption), socioeconomic status (employment, adequacy of family income, place of residence), pre-pregnancy BMI, medical history of women, and delivery (maternal gravidity, maternal parity, number of abortions, stillbirths, and history of previous gestational diabetes), pre-pregnancy weight and questions related to dietary intakes and supplement. A smoker was defined as a woman who smoked at least one cigarette during pregnancy. In this study, gestational age was calculated based on ultrasound estimated. Maternal weight was measured at the time of blood sampling, with the subjects wearing light clothing and no footwear. Each participant's weight was measured using a calibrated digital weighing scale at a precision of 0.1 kg (Seca 813; Germany). Gestational weight gain was calculated by the difference between pre-pregnancy weight and gestational weight, measured via a digital scale, at blood sampling. Women were divided into weight gain categories (Inadequate, normal, and excessive) according to the Institute of Medicine's (IOM) recommendations for total gestational weight gain based on their pre-pregnancy BMI. Specifically, the normal weight gain during gestation for women with pre-pregnancy underweight, normal weight, overweight and obesity were respectively considered 0.51 (0.44–0.58), 0.42 (0.35–0.50), 0.28 (0.23–0.33) and 0.22 (0.17–0.27) kg/week [23]. Pre-pregnancy BMI was classified as underweight (BMI < 18.5 kg/m2), normal weight (BMI = 18.5-24.9 kg/m2), overweight (25 ≤ BMI < 30 kg/m2), or obese (BMI ≥ 30 kg/m2) [33].

**Dietary assessment**

Dietary assessment was fulfilled by the three-day 24-hour recall method for three consecutive days, including two weekdays and one day of the weekend. The intake on the first day was conducted as a personal interview, whereas the dietary assessment was done on the two other days as a telephone call interview[34]. A modified version of Nutritionist IV software for the Iranian community was used to analyze nutritional data [35]. Micronutrients (Zn, Cu, and Mg) and total dietary fiber intakes of the three days were calculated and then averaged. Dietary adequacy for Zn, Cu, Mg, and dietary fiber was determined based on Recommended Dietary Allowances (RDAs) [36].

**GDM definition**

Gestational diabetes mellitus was assessed by glucose tolerance test (OGTT) (consumption of 75 g oral glucose). Women were diagnosed with GDM according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations [37]: fasting plasma glucose (FPG) ≥ 5.1 mmol/ L (≥ 92 mg/dL), or 1-hour plasma glucose (1 h-PG) ≥ 10.0 mmol/L (≥ 180 mg/dL), or 2-hour plasma glucose (2 h-PG) ≥ 8.5 mmol/L ≤ 153 mg/dL). A diagnosis of GDM was given if one or more tests had abnormal value.
Laboratory tests

Gestational diabetes mellitus screening was done just after sampling in a clinical nutrition laboratory using the Pars Azmoon test kit (Pars Azmoon Co., Iran). Other laboratory parameters were serum Zn, Cu, and Mg levels. For this purpose, fasting blood samples were collected and, after clotting at room temperature, centrifuged at 3000 rpm for 5 minutes. Then, the serum was removed from the blood clot and kept at -80 °C until analysis. After completion of sampling, all serum samples were analyzed by using standard kits (Dialab Co., Australia) and the BT-1500 auto-analyzer (Biotechnical, Rome, Italy) [38, 39]. The serum Zn, Cu, and Mg levels were analyzed on the same day of sampling. Serum Zn: 50 to 77 (µg) / dl, serum Cu: 130–240 µg / dl and serum Mg level: 1.1–2.2 mg / dl were considered as normal range [38].

Statistical analysis

The data of prevalence were reported as percentages and 95% confidence intervals. The normality of distributions of variables was assessed by the Kolmogorov–Smirnov equality of distribution test. Descriptive statistics were conducted for the study population characteristics (women with GDM or not). Continuous and categorical variables were respectively expressed as mean ± SD and frequency (percentage). Independent t-test was used for continuous variables, and Pearson chi-square and Fisher’s exact test were applied for categorical variables to compare the characteristics between groups. A p-value of less than 0.05 was considered statistically significant in the single variable analysis. Logistic regression with 95% confidence intervals was used to calculate odds ratios (OR) for predictor variables in developing GDM. Variables with a p-value of less than 0.05 in the single variable analysis were included in the multivariable analysis. The data were analyzed with SPSS version 16.

Results

The mean ± standard deviation of a total of 400 pregnant women's age, gestational week, gestational weight gains, and pre-pregnancy BMI were 27.5 ± 6.6, 28.2 ± 2.7, 5.9 ± 3.8 and 25.9 ± 4.8, respectively.

Seventy-two pregnant women (18%) (95% CI, 14.2–21.7%) were confirmed as having GDM. None of the participants had cigarette and alcohol consumption, multiple pregnancies, previous GDM diagnosis, history of diabetes type 1 or type 2 in their first-degree family. Variables of maternal age, pre-pregnancy BMI, pregnancy weighting pattern, serum Cu pattern, serum Zn pattern, dietary Mg intake, and mean Mg supplementation were statistically significantly different between the two groups with and without GDM (Tables 1 and 2). The low mean of weight gain in pregnant women with low pre-pregnancy BMI was directly related to GDM. In contrast, there was a direct association between GDM and the high mean of weight gain in pregnant women who were obese considering pre-pregnancy BMI (Fig. 1).

Since several variables have influenced the onset of gestational diabetes mellitus, we used logistic regression model with the LR method with PE = 0.05 and PR = 0.1 to estimate the coefficients of effectiveness variables as predictors of gestational diabetes mellitus. The women younger than 25 years
have a lower chance of GDM (OR: 0.263; 95% CI: 0.114–0.607) compared to pregnant women older than 35 years, and women aged 25 to 29 are also less likely to have GDM (OR: 0.320; 95% CI: 0.137–0.747). The mothers who had normal weight were less likely to have GDM than mothers who were obese before pregnancy (OR: 0.329; 95% CI: 0.156–0.696). The probability of developing gestational diabetes in participants who had adequate dietary Mg intake, as recommended by the RDA, was 0.346 than those with inadequate Mg intake. Binary logistic regression showed a negative relationship between mean Mg supplementation with gestational diabetes mellitus (OR: 0.986; 95% CI: 0.979–0.994). Pregnant women who had normal serum zinc had a lower probability of developing GDM (OR: 0.413; 95% CI: 0.227–0.750), and women with low serum zinc were more likely to develop GDM than women who had higher serum zinc (OR: 1.2466; 95% CI: 0.295–5.258) (Table 3).

Discussion

The present study showed pregnant women who had excessive or inadequate weight gain were more likely to develop diabetes than women who had normal weight gain. Also, excessive weight gains in obese women and inadequate weight gain in underweight women are associated with gestational diabetes. In parallel with our study, Gibson et al. reported that weight gain during pregnancy in obese and overweight women was associated with gestational diabetes mellitus [40]. In a cohort study, increased BMI classification from normal to overweight or overweight to obesity was associated with the increased risk of GDM [41]. However, in several studies, there was no association between "excessive mid-trimester weight gain" and abnormal glucose tolerance test results [42, 43]. According to a meta-analysis study, excessive gestational weight gain does not affect the risk of gestational diabetes in normal weight and overweight/obese women [44]. Gestational weight gain early in pregnancy is often related to increased plasma volume; However, the increase in adipose tissue starts from 12 to 14 weeks of gestation in overweight and obese women and potentially earlier in underweight and normal-weight women [45]. Gestational weight gain has also been shown to be inversely proportional to insulin sensitivity changes [46]. Gestational diabetes develops when pancreatic β cell function cannot adequately maintain normoglycemia due to increased insulin resistance. Since overweight and obese women have increased insulin resistance before conception; therefore, excessive weight gain may "push them over the edge" toward GDM development [40].

So far, limited studies have examined the relationship between Cu and GDM risk. This study found that the frequency of pregnant women with high serum Cu concentration in the GDM group was higher than the control group. Also, plasma Cu concentrations were not significantly associated with GDM risk. In the previous case-control studies conducted by Wang et al. and Li et al., Cu concentrations in the GDM group were higher than the control group, consistent with our results. [20, 47]. However, in a study, no difference was observed in Cu concentrations in women with gestational diabetes than in healthy pregnant women [48]. Recently, a meta-analysis including 910 GDM and 1760 healthy pregnant women discovered that in the Asian people during the third trimester, Cu concentrations in women with GDM were higher than the control group. Nonetheless, in the Caucasian people, no significant difference was between the two groups [49]. Therefore, this inconsistency of results could be related to different ethnicity, geographical
locations, sample size, and confounding factors. Cu may be involved in glucose metabolic disorders' pathogenesis through reactive oxygen species (ROS) production. ROS is thought to be involved in the development of insulin resistance. Cu ions can facilitate ROS production by mediating the transfer of electrons[50]. Cu can increase the production rate of advanced glycation end products (AGEs) associated with diabetes and its complications[51]. Cu ions can stimulate the accumulation of human Amylin peptide in amyloid fibrils associated with decreased β cell mass and progressive β cell failure[52, 53]. Also, the production of H2O2 byproducts can induce oxidative damage, cytotoxicity, and the progressive destruction of β cells[54].

In our study, normal serum Zn concentration was inversely associated with the cause of GDM. Also, the frequency of pregnant women with high serum Zn concentration in the GDM group was higher than the control group. In parallel with our study Zhou et al. [55] found Zn increases OGTT. However, Behboudi-Gandevani et al. [56] did not find any differences in Zn serum concentration between GDM and non-GDM groups. Unlike our findings, in Wang et al. study [47], serum Zn concentrations in the GDM group were lower than the healthy group. Another study mentioned that Zn supplementation increased insulin sensitivity and decreased fasting plasma glucose [57]. The disagreement among results could be attributed to different modes of Zn exposure and specific physiological requirements in different individuals. Although Zn plays a vital role in insulin secretion and production, at high levels, it could have adverse effects on FPG. It should be noted that since a large amount of Zn is required for pancreatic islet β-cells, zinc suddenly released under specific conditions might affect the function or survival of islet cells. Therefore, paracrine effects of endogenous Zn cause β cells' death and excess serum zinc may be toxic to the pancreas. Increased Zn in the pancreas has been suggested to cause toxicity to β-cells [58]. Therefore, trace elements toxicity because of different exposure and environmental conditions in future studies should be determined and studied.

According to our study results, gestational diabetes mellitus was more likely to occur in participants who received inadequate RDA-based dietary Mg than in those who had sufficient intake. Furthermore, we found that a negative relationship between mean Mg supplementation and GDM risk. Mg supplementation has been reported to reduce the risk of GDM in hypomagnesemia pregnant women [59]. A recent meta-analysis indicated that Mg supplementation was far more effective than other nutrients supplementation in maintaining glucose metabolic homeostasis and decreasing serum insulin in women with GDM [60]. Mg is required in multiple steps of the insulin-signaling pathways, and the depletion of it can affect the process of glucose metabolism. Mg deficiency could change pancreatic cells' structure, reduce β cell particles, and result in impaired insulin production and secretion [61]. Deficiency of Mg also can mitigate insulin receptor activity and lead to insulin resistance. Hypomagnesemia impedes the glucose uptake in the insulin-stimulated and basal states. Consequently, Mg has an essential role in glucose homeostasis [62].

Our study showed various strengths. We measured gestational weight gain before the diagnosis of GDM, so the confounding effect of GDM treatment on gestational weight gain was eliminated. Also, dietary intake data were assessed in our study, which enabled us to analyze dietary factors and serum trace
elements concentrations. Several limitations of our study should also be mentioned. First, the atomic absorption method was not used due to the lack of accessibility. Second, the nature of the case-control study limited us to find causal associations.

Conclusions

In conclusion, gestational diabetes is highly prevalent in Urmia. These findings indicate that gestational diabetes may, in part, be associated with excessive serum zinc concentrations, elevated pre-pregnancy BMI, and gestational weight gain as a significant risk factor for GDM. Disturbances in trace element metabolism may play a role in the pathogenesis and progression of complex disorders associated with gestational diabetes. Trace elements and antioxidant supplements may help treat complex disorders and help prevent complications, but they should be used in people with a low serum concentration. Therefore, careful monitoring of these parameters in pregnant women is recommended. Being overweight and underweight before pregnancy increases the risk of GDM; therefore, women should be encouraged to maintain a normal BMI before pregnancy. Excessive and inadequate weight gain also increases the risk of GDM, which is best to train and encourage normal weight gain during pregnancy.

Abbreviations

GDM: Gestational diabetes mellitus; BMI: Body mass index; Cu: Copper; Zn: Zinc; Mg: Magnesium; HbA1C: Hemoglobin A1c; IADPSG: International Association of Diabetes and Pregnancy Study Groups; IOM: Institute of Medicine; RDAs: Recommended dietary allowances; OGTT: Oral glucose tolerance test; FPG: Fasting plasma glucose; OR: Odds ratio; ROS: reactive oxygen species; AGEs: Advanced glycation end products.

Declarations

Ethics approval and consent to participate

The study has been approved and reviewed by the ethics committee of Urmia University of Medical Sciences (IR.UMSU.REC.1397.130).

Consent for publication

Not applicable.

Availability of data and materials

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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**Authors’ contributions**

S.Gh. and M.Gh. performed the research. S.Gh. and M.Gh. designed the study. S.Gh. and M.Gh. were involved in the clinical aspects of the study. T.R. and E.D. coordinated dietary assessment. T.R. and M.Gh. were involved in laboratory aspects of the study. E.D. and T.R. analyzed the data. S.Gh. and M.Gh. wrote the first draft of the manuscript; all authors have read and approved the final manuscript.

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Tables
| Characteristic                | Total (N =400) Frequency (%) | Non-GDM (82%) Frequency (%) | GDM (18%) Frequency (%) | P-value |
|-------------------------------|------------------------------|-----------------------------|-------------------------|---------|
| Age (year)                    |                              |                             |                         |         |
| < 25                          | 148(37)                      | 130 (39.6)                  | 18(25)                  |         |
| 25–29                         | 114(28.5)                    | 97 (29.6)                   | 17(23.6)                | 0.001   |
| 30–34                         | 88(22)                       | 69(21)                      | 19(26.4)                |         |
| ≥35                           | 50(12.5)                     | 32(9.8)                     | 18(25)                  |         |
| Education                     |                              |                             |                         | 0.099   |
| More than high school         | 110(25.3)                    | 83(25.3)                    | 18(25)                  |         |
| High school                   | 172(43)                      | 148(45.1)                   | 24(33.3)                |         |
| Less than high school         | 127(31.8)                    | 97(29.6)                    | 30(41.7)                |         |
| Mother's occupation           |                              |                             |                         |         |
| Housewife                     | 371(92.7)                    | 306(93.3)                   | 65(90.3)                | 0.372   |
| Employed                      | 29(7.3)                      | 22(6.7)                     | 7(9.7)                  |         |
| Adequacy of income            |                              |                             |                         |         |
| Completely enough             | 111(27.8)                    | 93(28.4)                    | 18(25)                  | 0.209   |
| Somewhat enough               | 232(58)                      | 193(58.8)                   | 39(54.2)                |         |
| Not enough                    | 57(14.2)                     | 42(12.8)                    | 15(20.8)                |         |
| Housing status                |                              |                             |                         | 0.866   |
| Householder                   | 259(64.8)                    | 213(64.9)                   | 46(63.9)                |         |
| Rental house                  | 141(35.3)                    | 115(35.1)                   | 26(36.1)                |         |
| Maternal parity               |                              |                             |                         |         |
| 0                             | 152(38)                      | 128(39)                     | 24(33.3)                |         |
| 1                             | 134(33.5)                    | 114(34.8)                   | 20(27.8)                | 0.01    |
| 2                             | 84(21)                       | 68(20.7)                    | 16(22.2)                |         |
| ≥3                            | 30(7.5)                      | 18(5.5)                     | 12(16.7)                |         |
| Maternal gravidity | 1 | 2 | ≥3 |
|-------------------|---|---|----|
|                   | 127(31.8) | 108(32.9) | 19(26.4) |
|                   | 129(32.3) | 108(32.9) | 21(29.2) | 0.274 |
|                   | 144(36) | 112(34.1) | 32(44.4) |

| Abortion | Yes | No |
|----------|-----|----|
|          | 82(20.5) | 318(79.2) |
|          | 265(80.8) | 63(19.2) | 19(26.4) | 0.172 |
|          | 53(73.6) | 19(26.4) |

| Stillborn** | Yes | No |
|-------------|-----|----|
|             | 10(2.5) | 390(97.5) |
|             | 10(3) | 318(97) |
|             | 0(0) | 72(100) | 0.220 |
|             | 10(3) | 318(97) |

| Gestational age* week (mean ± SD) | 28.5 ±2.1 | 28.5±2.1 | 28.6±2.05 | 0.720 |

| Pre-pregnancy BMI, kg/m2 (n, %) | Under weight | Normal | Overweight | Obese |
|--------------------------------|--------------|--------|------------|-------|
|                                | 18(4.5)      | 13(4)  | 161(40.3)  | 144(43.9) |
|                                | 390(97.5)    | 318(97)| 252(76.8)  | 17(23.6) |
|                                | 139(34.8)    | 114(34.8) | 57(17.4) | 25(34.7) |
|                                | 82(20.5)     | 57(17.4) | 25(34.7) | 25(34.7) |

| IOM recommendations | Adequate WG | Excessive WG | Inadequate WG |
|---------------------|-------------|--------------|---------------|
|                     | 297(74.3)   | 252(76.8)    | 21(62.5)      | 0.040 |
|                     | 25(6.3)     | 19(5.8)      | 6(8.3)        |      |
|                     | 78(19.5)    | 57(17.4)     | 45(29.2)      |      |

Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index; IOM, Institute of Medicine; WG, weight gain. Maternal gravidity (number of pregnancy or frequent reproductive cycling). Maternal parity (number of stillborn and child birth).

* Gestational age at blood drawing time.

Values are means ± SDs for normally distributed data or n (%) for categorical data. Comparison between GDM and Non-GDM groups, were performed with the independent t-test for continuous variables and the chi-square test for categorical variables.

**P value; for a comparison of frequencies, fishers exact test was used.
Differences between GDM and Non-GDM groups are statistically significant $p < 0.05$. 
Table 2
Nutritional data of the participants

| Characteristic          | Total (N=400) | Non-GDM (82%) (N=328) | GDM (18%) (N=72) | P-value |
|-------------------------|---------------|------------------------|------------------|---------|
|                         | Frequency (%) | Frequency (%) | Frequency (%) |         |
| **Cu (µg/dl)**          |               |                       |                  |         |
| Excessive (>240)        | 52(13)        | 36(11)                 | 16(22.2)         | 0.019   |
| Normal (130-240)        | 338(84.5)     | 285(86.9)              | 53(73.6)         |         |
| Below (<130)            | 10(2.5)       | 7(2.1)                 | 3(4.2)           |         |
| **Serum Cu (µg/dl)** a  | 202.8± 34.8   | 202.7 ± 33.4           | 203.8 ± 40.9     | 0.832   |
| **Zn (µg/dl)**          |               |                       |                  |         |
| Excessive (>77)         | 107(26.8)     | 78(23.8)               | 29(40.3)         | 0.004   |
| Normal (50 to 77)       | 281(70.3)     | 242(73.8)              | 39(54.2)         |         |
| Below (<50)             | 12(3)         | 8(2.4)                 | 4(5.6)           |         |
| **Serum Zn (µg/dl)** a  | 72.7± 17.5    | 72.6 ± 18.3            | 73.2 ± 13.8      | 0.572   |
| **Mg (mg/dl)**          |               |                       |                  |         |
| Excessive (>2.2)        | 96(24)        | 79(24.1)               | 17(23.6)         | 0.891   |
| Normal (1.1-2.2)        | 303(75.8)     | 248(75.6)              | 55(76.4)         |         |
| Below (<1.1)            | 1(0.3)        | 1(0.3)                 | 0(0)             |         |
| **Serum Mg (mg/dl)** a  | 2.1± 0.19     | 2.1 ± 0.19             | 2.1 ± 0.18       | 0.803   |
| **Intake food Cu (mg/d)** |           |                       |                  |         |
| Adequate (1)            | 357(89.2)     | 292(89)                | 65(90.3)         | 0.756   |
| Inadequate (<1)         | 43(10.8)      | 36(11)                 | 7(9.7)           |         |
| **Intake food Zn (mg/d)** |          |                       |                  |         |
| Adequate (11-12)        | 365(93.3)     | 296(90.2)              | 69(95.8)         | 0.129   |
| Inadequate (<11)        | 35(8.7)       | 32(9.8)                | 3(4.2)           |         |
| **Zn supplementation(mg/d)** a | 3.4±9 | 3.5±9.3 | 2.8±7.9 | 0.515 |
| **Intake food Mg (mg/d)** |           |                       |                  |         |
| Adequate (350-400) | 363(90.8) | 304(92.7) | 59(81.9) | 0.004 |
|-------------------|-----------|-----------|----------|-------|
| Inadequate (<350) | 37(9.3)   | 24(7.3)   | 13(18.1) |       |
| **Mg supplementation a** | | | | |
| Adequate (350-400) | 87.8±33.1 | 90.4±30.5 | 76.08±41.1 | 0.001 |
| Inadequate (<350) | 55(13.7) | 44(13.4) | 11(15.3) | 0.678 |
| Intake fiber (g/d) | | | | |
| Adequate (25-30) | 345(86.3) | 284(86.6) | 61(84.7) | |

Abbreviations: Cu, Copper; Zn, Zinc; Mg, Magnesium a Values are means ± SDs for normally distributed data or n (%) for categorical data. Comparison between GDM and healthy groups was performed with the independent t-test for continuous variables and the chi-square test for categorical variables.

Differences between GDM and Non-GDM groups are statistically significant p < 0.05.
Table 3

Logistic regression analysis of the factors affecting GDM

| Significant explanatory variables | Odds ratio | Lower–upper 95% CI | P-value |
|----------------------------------|------------|---------------------|---------|
| **Age, year**                    |            |                     |         |
| < 25                             | 0.263      | 0.114-0.607         | 0.002*  |
| 25–29                            | 0.320      | 0.137-0.747         | 0.008*  |
| 30–34                            | 0.478      | 0.207-0.101         | 0.083   |
| ≥35                              | Reference  | -                   | -       |
| **Pre-pregnancy BMI**            |            |                     |         |
| Underweight                      | 0.952      | 0.267-3.388         | 0.989   |
| Normal                           | 0.329      | 0.156-0.696         | 0.004*  |
| Overweight                       | 0.552      | 0.276-1.104         | 0.093   |
| Obese                            | Reference  | -                   | -       |
| **Cu (µg/dl)**                   |            |                     |         |
| Excessive (>240)                 | Reference  |                     |         |
| Normal (130-240)                 | 1.59       | 0.76-3.34           | 0.21    |
| Below (<130)                     | 1.11       | 0.53-2.29           |         |
| **Serum Zn (µg/dl)**             |            |                     |         |
| Excessive (>77)                  | Reference  | -                   | -       |
| Normal (50 to 77)                | 0.413      | 0.227-0.750         | 0.004*  |
| Below (<50)                      | 1.246      | 0.295-5.258         | 0.764   |
| **Intake food Mg (mg/d)**        |            |                     |         |
| Adequate (350-400)               | 0.346      | 0.153-0.780         | 0.011*  |
| Inadequate (<350)                | Reference  | -                   | -       |
| **Mean Supplement Mg (mg/d)**    | 0.986      | 0.979-0.994         | 0.0001* |

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; Zn, Zinc; Mg, Magnesium; OR, odds ratio; CI, confidence interval.
*Statistically significant P-value.

Figures

Figure 1

Mean weight gain ± standard deviation in participants diagnosed with gestational diabetes mellitus compared with those with normal glucose tolerance by pre-pregnancy body mass index categories. Independent T-Test P-value for underweight and obese, *P=0.01; †P=0.003.