

# The Association between Maternal Dietary Protein Intake and Risk of Gestational Diabetes Mellitus

## Abstract

**Background:** The amount and type of dietary protein affect glucose metabolism. However, the association between dietary protein intake and gestational diabetes mellitus (GDM) risk is vague. We examined this association. **Methods:** We included 152 GDM and 168 non-GDM participants (total 320), age 18–45 years from Arash Women's Hospital, Tehran, Iran. Protein intake was ascertained from 168-item Food Frequency Questionnaire at 24–40 weeks' gestation. GDM was defined as fasting blood sugar >95 mg/dL and/or oral glucose tolerance test >155 mg/dL. Dietary data were assessed using N4 software and statistical analysis was performed using SPSS 21. We tested the association between the amount of protein consumed from red and processed meat, poultry, dairy, egg, seafood, and vegetable plus sociodemographic and lifestyle covariates and GDM risk using multivariate logistic regression analysis. **Results:** There was a significant association between the physical activity ( $P < 0.035$ ), socioeconomic status ( $P < 0.013$ ), body mass index, age, and each trimester's weight ( $P < 0.001$ ), and risk of GDM. No significant association was observed between the intake of protein from major protein sources and risk of GDM. The only significant association was observed for egg consumption which was lower in GDM participants ( $P = 0.004$ ), yet this association turned nonsignificant after adjustment for confounders, except for the fourth quartile (odds ratio: 0.43, 95% confidence interval: 0.208, 0.893). **Conclusions:** According to our findings, dietary intake of total and major protein sources could not affect the GDM risk. Differences between Iranian and Western population and the reverse causality might be the main reasons for this nonsignificant association.

**Keywords:** Animal protein, diet, dietary protein, gestational diabetes mellitus, vegetable protein

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## Introduction

Gestational diabetes mellitus (GDM) is the state of glucose intolerance with onset or first recognition during pregnancy.<sup>[1]</sup> Various diagnostic criteria are in use in different countries because of the wide range in the GDM prevalence,<sup>[2]</sup> such as International Association of the Diabetes and Pregnancy Study Groups and World Health Organization which are commonly used to diagnose GDM in high-risk pregnant women.<sup>[3]</sup> In Iran though, GDM is diagnosed at 24–28 weeks of gestation with a fasting plasma glucose level >95 mg/dL and/or oral glucose tolerance test (OGTT) >155 mg/dL.<sup>[4]</sup> The prevalence of GDM is 1%–14% worldwide<sup>[5]</sup> and has been reported to be between 1.25% and 29.9% in Iran.<sup>[6]</sup> GDM is not only associated with adverse consequences for mothers but also causes complications for their children. Mothers are exposed to the increased risk

of delivering macrosomic babies and 7-fold risk of developing diabetes (especially type 2 diabetes) later in life<sup>[7]</sup> and their babies are at risk of increased adiposity, obesity, and metabolic syndrome in childhood, adverse cardiometabolic profile, and earlier onset of puberty among girls.<sup>[8]</sup>

Lifestyle and diet are two modifiable risk factors associated with GDM.<sup>[9]</sup> Dietary carbohydrate and fat intake have been reported to be associated with GDM;<sup>[10,11]</sup> however, a few studies have investigated the role of dietary protein intake in this matter.

Many studies have examined the dietary protein intake in relation to the risk of type 2 diabetes mellitus (T2DM). Most of those studies demonstrated that the higher animal protein intake, especially red meat, is positively associated with the risk of T2DM, yet vegetable protein sources showed a negative association.<sup>[12,13]</sup> The same result was concluded for the risk of

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GDM,<sup>[14]</sup> although different results have been observed in different populations with different dietary patterns. Adherence to a Mediterranean diet pattern of eating, which is consisted of less red meat and more fish, poultry, and vegetable protein sources, was associated with lower risk of GDM.<sup>[15]</sup> In the United States, different studies came up with different results. One study demonstrated that protein-dense foods, such as red meat and dairy, were not associated with the risk of GDM,<sup>[16]</sup> but some other studies found an association between animal protein intake and GDM.<sup>[17,18]</sup> An Asian study suggested that a higher intake of dietary protein from both animal and vegetable sources is associated with a higher risk of developing GDM.<sup>[19]</sup>

Different results in previous studies might be explained by differences in race and ethnicity between populations, controlling different confounders, retrospective or prospective design, and various sample sizes. Limited investigation on the relationship between the protein intake and risk of developing GDM plus such contradictory results among the existing studies persuaded us to examine the association of dietary intake of protein from major dietary sources during pregnancy with the risk of GDM.

## Materials and Methods

### Participants

During the period of August through December 2017, this case–control study was performed at Arash’s Women Hospital, Tehran, Iran, to which women from all over the country are referred to receive advanced and cheap services of this governmental hospital. This study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC 1396.9411323007.9/18/2017). A total sample size of 320 individuals was calculated using data from Zhang *et al.*’s<sup>[20]</sup> study and using STATA14 software with 80% power and 95% confidence interval. Mothers age 18–40 years were assigned to the case ( $n = 152$ ) and control ( $n = 168$ ) groups at 24–40 weeks of gestation. Women who were waiting in the hospital’s clinic to be visited by the doctors were asked to participate. To prevent selection bias, the control group was recruited from the same catchment area as the cases. All participants signed a written informed consent and went through face-to-face interview by trained interviewers using written questionnaires. Women were excluded from this study if they had a family history of diabetes, a history of GDM, stillbirth, macrosomia, and congenital anomalies or they have had abortion in their previous pregnancies, hypothyroidism, smoking or drinking habit, height <148 cm, multiple pregnancy, pregnancy through *in vitro* fertilization, and consumption of drugs affecting serum glucose such as progesterone, glucocorticoids, chemotherapy, or psychotropic drugs. Noncompliance to the exclusion criteria was self-reported. Also, we have excluded women who provided incomplete Food Frequency Questionnaire (FFQ)

data or over- or underestimated the energy intake (<800 or >4,500 kcal/day) [Figure 1]. Overall, a total of 320 participants (152 with and 168 without diabetes) remained for the final analyses.

GDM was defined as fasting glucose level >95 mg/dL and/or OGTT >155 mg/dL.<sup>[4]</sup>

### Demographic and anthropometric measures

Trained interviewers collected the information, including age, height, weight, body mass index (BMI), gestational weight gain in each trimester, socioeconomic status, education, career, child’s birth order, drug and supplement consumption, and fasting blood sugar (FBS) and OGTT values. Weight was measured to the nearest 100 g using digital scales, while the participants were minimally clothed, without shoes. Height was measured to the nearest 0.5 cm, in a standing position without shoes, using a tape meter. BMI was calculated as weight (kg) divided by the square of the height (m<sup>2</sup>). Data on gestational weight gain in each trimester, socioeconomic status, education, career, child’s birth order, drug and supplement consumption, and FBS and OGTT values were also provided by the mothers themselves, or derived from their medical record. We also controlled physical activity as a confounder using the International Physical Activity Questionnaire which is validated for the Iranian population.<sup>[21]</sup> This questionnaire is consisted of four sections: sedentary, moderate, intense activities, and jogging. Each section is scored according to its frequency and dedicated time during the past week.

### Assessment of dietary intake

Dietary data were collected using a 168-item FFQ. Reliability and validity of this questionnaire were assessed

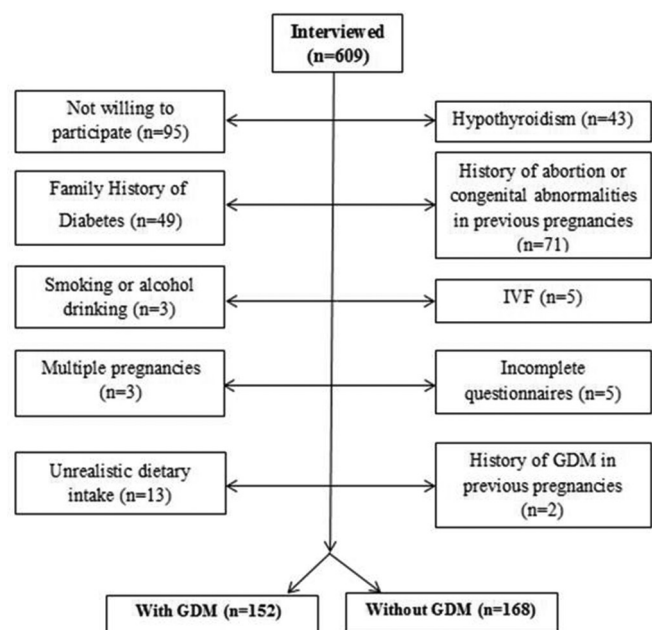


Figure 1: Excluded participants

in 2012 by Asghari *et al.*<sup>[22]</sup> The questionnaires that were administered by trained interviewers represent their dietary intake over the past year (including both pregnancy and prepregnancy time duration). Serving size of each food item was reported by the mothers using the common kitchen utensils (e.g. bowl, spoon). Then, these serving sizes were converted to the amount (g) consumed using the conversion factors for each food item. Entering the amounts into the Nutritionist4 (N4) software, we calculate the amount of macro- and micronutrients received. Dietary intake of protein from red and processed meat, poultry, dairy, egg, seafood, and vegetable plus total protein intake was calculated and analyzed separately [Table 1].

**Statistical analysis**

Normality of the data was investigated by Kolmogorov–Smirnov test. The data were reported as mean ± SD for variables with normal distribution and median (interquartile range) for non-normal ones. The independent-samples *t*-test (normal distribution) or Mann–Whitney *U*-test (non-normal distribution) was used to compare continuous variables between the two groups. Chi-square and Fisher’s exact test were used to compare categorical variables between the two groups. Besides, multivariate logistic regression analysis was used to examine the association between GDM and dietary protein intake with adjustment for confounding variables which were selected from a bivariate analysis. *P* value <0.05 was considered statistically significant. All analyses were performed using the Statistical Package for Social Sciences (version 21.0; SPSS Inc., Chicago, IL, USA).

**Results**

Comparison between continuous demographic characteristics of the case and control groups is shown in Table 2. Based on our results, overweight and obese women tend to overtake GDM during pregnancy. Age and each trimester’s weight were also significantly higher in GDM women (*P* < 0.001). Women with GDM had significantly lower physical activity compared with the control group (*P* < 0.03). Dietary confounders including energy, carbohydrate, fat, fiber, fruit, and vegetable intake were not significantly different between the two groups. Except for egg whose intake was significantly lower in the

GDM group, other protein sources’ intake was not different between GDM and non-GDM participants.

As the table shows, there were no significant differences between participants with and without GDM controlling for mother’s educational level or the child’s birth order. However, there was a significant association between the GDM and non-GDM groups in socioeconomic status (*P* < 0.013), although it does not follow a specific pattern. This variable was defined by the self-report of participants.

In this study, the mean energy provided from protein was 13.7%, 10.6% of this percentage was from animal and 1.6% from vegetable protein. The median intake of total dietary protein was 11.98%, 12.88%, 13.77%, and 15.62% of energy from the lowest to the highest quartile, respectively. After adjustment for age, BMI, mother’s education, socioeconomic status, birth order of the child, and gestational age, total protein intake was not significantly associated with GDM risk. Similarly, we observed no significant association between the amount of protein met from major protein sources including red and processed meat, poultry, dairy, egg, seafood, and vegetable and risk of GDM [Table 3]. Among the major protein sources, highest protein intake was from dairy versus the lowest level of intake from seafood protein. Although it was not statistically significant, the higher intake of protein from egg seems to protect against GDM [from odds ratio (OR): 0.677, 95% confidence interval (CI): 0.35, 1.27, in quartile 2, to OR: 0.43, 95% CI (0.20, 0.89) in quartile 4]. This association was significant in the fourth quartile (*P* < 0.024).

**Discussion**

In our case–control study, we observed no significant relationship between the maternal protein intake and risk of GDM. This nonsignificant relationship applies not only to total protein but also to the red and processed meat, poultry, dairy, and seafood protein as well. Meanwhile, in the nonadjusted model, egg consumption had a negative significant association with GDM risk. In the adjusted model, there was a trend of decreasing risk of GDM by increasing egg consumption, and those in the fourth quartile had a lower risk of GDM by 57%.

We did not observe a significant association between animal protein intake and risk of GDM. Our findings are

**Table 1: Food items taken into account for each food group**

Food group	Food item
Vegetable protein	Vegetable Legumes (beans, peas, split peas, broad beans, lentils, soy), nuts [peanuts, pistachios, hazelnuts, sunflower seeds, walnuts, chickpea (roasted)]
Animal protein	Red and processed meat Beef, hamburger, lamb, canned tuna fish, beef, sausages, cold cut
	Poultry Chicken with or without skin
	Seafood Different sorts of fish
	Dairy Whey, cheese, ice cream, low- and high-fat milk, chocolate milk, low- and high-fat yoghurt, dough (an Iranian drink made of yoghurt)
	Egg Chicken egg

**Table 2: Demographic characteristics**

		GDM (n=152)	Without GDM (n=168)	P
Age (years)		30.81±5.28	28.77±5.42	0.003
Age of pregnancy (week)		33.58±3.73	34.61±3.61	0.011
Weight (kg)	Prepregnancy	71.77±14.07	65.44±12.02	0.0001
	First trimester	72.94±13.42	67.43±11.72	0.002
	Second trimester	78.46±13.31	72.32±11.37	0.0001
	Third trimester	82.77±13.54	78.67±11.94	0.017
Height (cm)		161.17±9.93	161.72±5.41	0.955
BMI		28.35±11.81	25.04±4.54	0.0001
Physical activity		198.00 (477.00)	234.50 (693.00)	0.035
Education level*	Illiterate	4 (2.6%)	2 (1.2%)	0.33
	Under diploma	39 (25.8%)	38 (22.6%)	
	Diploma	77 (51.0%)	103 (61.3%)	
	B.S.	27 (17.9%)	23 (13.7%)	
	M.S. and higher	4 (2.6%)	2 (1.2%)	
Socioeconomic* status	Poor	10 (6.6%)	3 (1.8%)	0.013
	Moderately poor	102 (67.1%)	94 (57.3%)	
	Moderately rich	37 (24.3%)	60 (36.6%)	
	Rich	3 (2.0%)	7 (4.3%)	
Birth order*	First child	70 (46.4%)	82 (48.8%)	0.081
	Second child	52 (34.4%)	69 (41.1%)	
	Third child	24 (15.9%)	16 (9.5%)	
	Fourth child and above	5 (3.3%)	1 (0.6%)	
Energy (kcal)		3028.46 (926.43)	3158.08 (1150.45)	0.14
Carbohydrate (g)		407.87±140.15	439.50±156.69	0.10
Fat (g)		123.53 (57.45)	118.59 (67.85)	0.94
Fiber (g)		40.19 (23.25)	41.75 (27.52)	0.80
Fruit (g)		564.49 (444.98)	572.37 (373.60)	0.80
Vegetable (g)		337.7047 (306.17)	279.58 (206.90)	0.19
Total protein (g)		101.29 (47.40)	105.19 (54.41)	0.33
Vegetable protein (g)		10.58 (9.49)	11.31 (11.46)	0.26
Red and processed meat (g)		13.98 (16.10)	12.35 (17.85)	0.87
Poultry (g)		6.49 (9.41)	6.49 (8.08)	0.42
Seafood protein (g)		0.60 (1.31)	0.66 (2.00)	0.57
Dairy protein (g)		29.18 (25.73)	31.00 (21.05)	0.17
Egg (g)		2.16 (2.16)	3.24 (2.16)	0.004

GDM=Gestational diabetes mellitus; BMI=Body mass index; SD=Standard deviation. Mann-Whitney for physical activity and dietary intake and independent sample *T*-test for demographic data. \*Chi-square or Fisher's exact test, data presented as *n* (%). Data presented as median (interquartile range) except for the carbohydrate intake and demographic data which had a normal distribution and are presented as mean±SD

different from that of Nurses' Health Study II cohort which found significant association between total meat intake and the risk of GDM,<sup>[17]</sup> and a meta-analysis of cohort studies which reported 41% and 21% increased risk of T2DM for the highest versus lowest intake of processed meat and red meat, respectively.<sup>[23]</sup> This inconsistency might be due to the red and processed meat consumption in the United States, which is consumed at more than three times the global average,<sup>[24]</sup> adding pork meat intake to their calculations, and their participants to have been older than ours. However, Kurotani *et al.* did not find any significant relationship between red meat consumption and T2DM in women.<sup>[25]</sup> A mechanism to explain the adverse association

of red meat intake and diabetes is the effect of heme-iron derived from it. Iron is a strong prooxidant and increases the level of oxidative stress which can damage many tissues, including the pancreatic beta cells.<sup>[26]</sup> In our study though, all mothers received the iron supplement. In fact, iron deficiency is of high prevalence in Iranian pregnant women.<sup>[27]</sup> Therefore, this might be a reason for us not to have observed a significant relationship. In addition, the mean intake of meat in our study population was very scant (17.52 ± 14.92 g/day). This low intake inhibits both red and processed meat to affect GDM significantly.

Poultry intake was also not significantly associated with the risk of GDM. Some studies reported an increased risk,<sup>[28]</sup> some



**Table 3: ORs for GDM according to intake of dietary protein during pregnancy**

Protein source	Q1 (n=81)	Q2 (n=81)	Q3 (n=82)	Q4 (n=80)
Total protein	Reference	1.148 (0.559, 2.357)	0.800 (0.389, 1.647)	1.008 (0.486, 2.091)
<i>P</i>	-	0.707	0.545	0.984
Vegetable protein	Reference	1.137 (0.554, 2.337)	0.940 (0.459, 1.925)	0.875 (0.419, 1.830)
<i>P</i>	-	0.726	0.867	0.723
Red and processed meat	Reference	0.699 (0.333, 1.468)	1.592 (0.741, 3.417)	0.987 (0.471, 2.070)
<i>P</i>	-	0.344	0.233	0.973
Poultry	Reference	0.551 (0.229, 1.327)	0.830 (0.341, 2.016)	0.763 (0.339, 1.715)
<i>P</i>	-	0.184	0.680	0.512
Seafood protein	Reference	1.191 (0.572, 2.482)	1.423 (0.677, 2.988)	0.623 (0.292, 1.327)
<i>P</i>	-	0.641	0.352	0.220
Dairy protein	Reference	0.623 (0.300, 1.294)	0.502 (0.244, 1.034)	0.746 (0.362, 1.536)
<i>P</i>	-	0.205	0.062	0.427
Egg	Reference	0.677 (0.359, 1.275)	0.565 (0.230, 1.387)	0.431 (0.208, 0.893)
<i>P</i>	-	0.227	0.213	0.024

OR=Odds ratio; GDM=Gestational diabetes mellitus; BMI=Body mass index; CI=Confidence interval. Logistic regression with adjustment for age, BMI, mother's education, socioeconomic status, birth order of the child, and age of pregnancy. Data presented as OR [95% CI (upper, lower)]

reported a decreased risk,<sup>[29]</sup> and some observed no significant association between the poultry intake and risk of T2DM similar to our findings.<sup>[30]</sup> Poultry seems to have both promotive and protective correlation with diabetes. Polyunsaturated fatty acid (PUFA) in poultry can improve insulin sensitivity,<sup>[31]</sup> and it may not significantly increase iron storage;<sup>[26]</sup> on the other hand, it contains branched chain amino acids which cause insulin resistance. This may lead to the neutral association between the poultry intake and risk of GDM.

The consumption of seafood protein did not differ between the case and the control groups. The results of several epidemiologic studies and meta-analysis<sup>[19,32]</sup> on this topic were inconsistent. A Chinese study found that higher protein intake from seafood could protect against T2DM,<sup>[32]</sup> while Nurses' Health Study II found no association between fish intake and the incidence of GDM.<sup>[17]</sup> This inconsistency may reflect different preparation methods. Fried fish was not significantly associated with T2DM risk in the UK study due to production of trans fatty acids, which might modify the beneficial effect of fish, but a higher boiled fish intake was associated with a lower risk of T2D in the same population.<sup>[33]</sup> The type of fish or seafood consumed, geographical differences, and environmental contamination of the sea may also be the reason for the incompatible results.<sup>[34]</sup> Furthermore, significant association was observed mostly in the studies which introduced seafood as a source of long-chain PUFA<sup>[35]</sup> or vitamin D,<sup>[36]</sup> while few studies considered the protein content of it. Another explanation for our nonsignificant result is the low consumption of fish in our population. The amount of protein received from fish was very low ( $1.48 \pm 2.99$ ) in our participants and it may have not been enough to affect the glucose tolerance or insulin sensitivity.

Some studies found no association between the intake of vegetable protein and the risk of both T2DM<sup>[37]</sup> and

GDM,<sup>[20]</sup> yet there are studies having observed a negative<sup>[17]</sup> or positive<sup>[19]</sup> relationship. Despite the fact that vegetables contain various vitamins and minerals and are a great source of fiber and they can reduce the risk of diabetes by reducing inflammation,<sup>[38]</sup> we found a nonsignificant relationship between the GDM risk and vegetable protein consumption. In this study, vegetable protein consists of two groups of food items: legums and nuts. In Iranian population, legumes are seldom used alone and are mostly consumed as part of the stew, mixed with other vegetables and meat and are eaten with rice or bread. Previous investigations suggest that amino acids absorbed after ingestion of a mixed meal are not likely to contribute significantly to insulin secretion.<sup>[39]</sup> Nuts are also known to decrease the risk of T2DM due to their fiber, magnesium, and monounsaturated fatty acids content.<sup>[40]</sup> However, the Iranians consume nuts occasionally and roasted with a great amount of salt. Studies investigating the relationship between the salt intake and risk of GDM and T2DM indicate that higher salt intake leads to diabetes by causing increased glucose absorption, high blood pressure, obesity, and inflammatory cytokines.<sup>[41]</sup> Perhaps the effect of salt counteracted the beneficial effects of nuts in our study.

According to our results, dairy protein does not significantly affect the GDM risk. Our result contrasts with previous studies, in which dairy consumption was generally associated with a lower risk of T2DM,<sup>[42]</sup> but our findings are consistent with a cohort study in which they found no significant relationship between dairy protein intake and the risk of GDM.<sup>[43]</sup> One possible explanation for our result is that we did not separately investigate the effect of low-fat and high-fat dairy in spite of their distinct association with diabetes mellitus.<sup>[44]</sup> Adjustment for possible confounders such as saturated fat intake could have changed our results. Besides, as dairy consumption in the Iranian population is

lower than the recommended amount,<sup>[45]</sup> it is possible that we have missed the possible protective effect of dairy on GDM risk.

In the nonadjusted model, our results indicate that a higher intake of egg protects against GDM. There have been studies showing the adverse effect of egg consumption on T2DM,<sup>[46]</sup> but most of those studies which blame egg as an important contributor of dietary cholesterol and a serum cholesterol elevator were mostly conducted in the United States and were considered as low-quality studies due to self-reported diabetes and the follow-up rate which was inadequate or not described.<sup>[47]</sup> However, better quality studies in other populations were less likely to find an association between egg consumption and diabetes risk. Even relatively large increments in dietary cholesterol intake have shown to have little effect on total or low-density lipoprotein cholesterol concentrations.<sup>[48]</sup> In fact, dietary cholesterol affects serum cholesterol only in participants with abnormal lipid profile.<sup>[49]</sup> In our study population though, the egg is mostly regarded as a source of protein rather than cholesterol and it meets an impressive amount of protein needs in low-income families. There are also human experimental studies which showed that increased egg intake has rather a beneficial impact on several risk factors for T2DM, such as insulin resistance<sup>[50]</sup> and inflammation.<sup>[51]</sup> Furthermore, studies in which a positive significant result was observed showed a dose–response relationship and claimed that high maternal egg intake was related to diabetes risk, only if the egg consumption was higher than seven eggs/week while there was no significant association in lower amounts of egg consumption.<sup>[52,53]</sup> As our participants' average egg intake was two eggs/week, not having observed a positive association is logical.

Aligned with major protein sources, total protein intake was also not related to the GDM risk. This is against cohort and meta-analysis studies which found significant association between the intake of protein and GDM<sup>[19]</sup> and T2DM.<sup>[54]</sup> Considering the food pattern instead of one macronutrient *per se* could be a possible reason for this contradictory result.<sup>[12]</sup> According to the demographic characteristics of the participants, it is also possible that unhealthy lifestyle is more effective on the GDM risk than diet alone. A study in the United States indicated that smoking is often associated with lower physical activity and higher intake of red meat.<sup>[47]</sup> Diet acts as a marker of a healthy lifestyle too, and since the amount of protein consumed by our participants was lower than the recommended amount, the nonsignificant result was not unexpected. Most importantly, the majority of women with GDM have beta-cell dysfunction on a background of chronic insulin resistance to which the insulin resistance of pregnancy is added.<sup>[55]</sup> Therefore, protein intake during pregnancy may only slightly affect this long-term dysfunction.

In general, our study was different from studies that found a significant association in many aspects. Those studies were mostly prospective cohorts with huge sample size and long years of follow-up, while this was a retrospective case–control study with a smaller sample size. The amount of energy met by protein was also lower in our study (13.7%) in comparison to previous studies and it was less than the recommended amount. Hence, the amount of protein consumed has been so low that it only supplied the basic needs of the body and has not been able to affect the glucose metabolism. In confirmation to this fact, we observed a significant association between egg intake and GDM risk in the fourth quartile where the highest amount of energy was met by protein (15.62%). A clinical trial in which healthy diet with emphasize on 15%–20% of total energy intake from protein was prescribed reported a decrease in pregnancy-induced insulin increment and insuline resistance during pregnancy.<sup>[56]</sup>

There are limitations to this study. First, using FFQ which is prone to recall bias, since individuals are asked to report their intake retrospectively and usually refer to prolonged period of time (last year). In addition, mothers were interviewed after the 24<sup>th</sup> week of pregnancy when they were already informed whether they had GDM. This awareness could have affected the report of their dietary intake. Another defect is that although we controlled for confounding by known risk factors of GDM, not having controlled the effect of some confounders such as the amount of iron, saturated and unsaturated fat separately, and salt intake might have resulted in an attenuated association. The causality is also unclear in case–control studies, which leaves us in doubt whether the protein intake causes GDM, or the chronic insulin resistance affects the protein consumption.

The strengths of this study include the division of protein into seven different groups and interpretation of each solely. We also adjusted the results for many confounders such as physical activity which had not been taken into account in previous studies. It is also one of the pioneer studies to investigate the relationship between dietary protein intake and risk of GDM in pregnant women. As another strength, we should mention choosing the Arash's women hospital for sampling. This referral hospital allowed us to include women from different cities, with different levels of education and wealth in our study. Although our results cannot be generalized to the whole population of pregnant women because of our multiple exclusion criteria.

In conclusion, according to our findings and in contrast to that of previous studies in Western population, the intake of total and major dietary sources was not significantly associated with the risk of GDM. For future research, we suggest to study this relationship in related Iranian cohorts with bigger sample size and stronger analysis to determine whether such association exists.

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## Conflicts of interest

There are no conflicts of interest.

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