

How Adequate are Macro- and Micronutrient Intake in Pregnant Women with Diabetes Mellitus? A Study from South India

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Abstract

Background Diabetes is the most common condition in pregnancy with a worldwide prevalence of 16.9%.

Aim To determine the adequacy of the nutrient intake of pregnant women with diabetes mellitus.

Methods This is a cross-sectional study of 85 pregnant women who met the diagnostic inclusion criteria for diabetes mellitus (gestational and pre-gestational diabetes mellitus) and who were being managed at the outpatient clinic of a tertiary care teaching hospital. Their demography, clinical characteristics (from updated medical records), anthropometric measures (using standard procedures), nutrient intake and meal pattern (obtained using

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24 h recall, food frequency and their log diaries) were collected.

Results The mean age of the group was 29.9 + 4.5 years, 54% were in the second trimester of pregnancy with a mean glycosylated haemoglobin level of 6.3 + 1.4%. The mean BMI indicated that 47% of them were in the obese grade 1 category. Insulin was used in one-third of the population. The overall macronutrient and micronutrient intakes of the population were below the recommended daily allowances for Indians (60–70% of RDA). There was a deficit in the intake of calories, fibre, proteins, iron, calcium, carotene, folic acid, thiamine, riboflavin and niacin. Between the two groups, the pre-GDM women had a significantly better nutrient intake and this could be attributed to a greater exposure to nutrition counselling that they have received during the earlier part of their diabetes care.

Conclusion The gestational period should be viewed as a window of opportunity to modify dietary patterns and introduce healthy lifestyle practices for the woman and her family.

Keywords Nutrition · Gestational diabetes · Dietary pattern · Macronutrients · Micronutrients

Introduction

Diabetes is the most common condition in pregnancy and its prevalence is increasing. The worldwide prevalence of hyperglycaemia in pregnant women (20–49 years) was 16.9% or 21.4 million live births 2013 [1]. More than 90% of the cases occur in low- and middle-income countries. Majority of these patients have gestational diabetes mellitus (GDM). The remainder has pre-existing diabetes (pre-GDM) which included mostly type 1 and type 2 DM [1].

The Diabetes Atlas (7th edition, 2015) of the International Diabetes Federation defines GDM as a glucose intolerance of varying degrees of severity which starts or is first recognized during pregnancy. One in seven births is affected by gestational diabetes [2]. The major risk factors for GDM include higher parity, advanced maternal age, family history of diabetes mellitus, non-white race, overweight and obesity [3].

The prevalence of GDM in the south-east region is 25% compared to 10.4% in the North American and Caribbean [4]. India is considered as the diabetes capital of the world

and has the largest rates of women with GDM. However, there is a wide geographical variation in the incidence of GDM [2].

Prevention of glycaemic excursions during pregnancy has several medical and economic benefits for the mother and infant on a long-term basis. Studies across the world have shown that lifestyle intervention has a positive impact on glycaemic control and pregnancy outcomes of GDM. Diet, exercise and nutrient composition play a significant role in reducing rates of adverse pregnancy outcomes [5, 6].

The primary objectives of this study were to (a) assess the adequacy of micronutrient and macronutrient intake in the diet of pregnant women with diabetes mellitus and (b) compare the intake of these macro- and micronutrients between GDM and pre-GDM women. The secondary objective was to assess the impact of the macro- and micronutrient deficiencies on maternal and foetal outcomes.

Materials and Methods

This is a cross-sectional study of pregnant women from within Tamil Nadu with diabetes mellitus being managed at the gestational diabetes mellitus clinic of the Department of Diabetes, Endocrinology and Metabolism of Christian Medical College and Hospital, Vellore, Tamil Nadu, India. This is a 2700 bedded tertiary care teaching hospital.

Pregnant women who met the IADPSG diagnostic criteria [7] for gestational diabetes, overt diabetes mellitus and those who were diagnosed with diabetes mellitus prior to pregnancy (pre-GDM) were included in the study. The women were informed about the nature of the study, and informed consent was obtained. Data were obtained over a 6 month period (August 2016–January 2017). Based on a pilot study on the energy intake of pregnant women with diabetes, the sample size was computed to be $n = 61$. Women with multiple pregnancies, IVF pregnancies, bad obstetric history and psychiatric illnesses were excluded from the study. The subjects were examined by the diabetes care team that consisted of an endocrinologist, diabetes nurse educator and a dietician.

The study sample consisted of 85 women who met the inclusion criteria. This included 59 women with GDM and 26 who had pre-GDM. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (and revised in 2000), and approval was obtained by the Institutional Review Board of Christian Medical College and Hospital, Vellore, Tamil Nadu, India. (IRB Min. No. 10045 dated 4/4/2016).

The demographic details and clinical characteristics were obtained from the updated medical records. The

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anthropometric measures which were recorded prior to pregnancy were taken, and the BMI was computed and interpreted according to the Asia Pacific guidelines. The nutritional data of the patients were obtained by the dietician during a one-to-one interview using a 24-h recall method and food frequency questionnaire during their first visit (prior to counselling) in the case of GDM and pre-GDM women. In order to keep the comparisons similar, the data in the pre-GDM group were validated against their medical records and only the nutritional data prior to the first counselling were included in this study. Subsequently, both the GDM and pre-GDM patients received education on diabetes care and nutrition therapy and were recording their food intake and medication details on a weekly basis in the food diary. The nutritive composition of their diet was computed from the database created by the National Institute of Nutrition, which is the apex nutrition body in India. The adequacy of their nutrient intake was compared with the recommended daily allowances for Indians. Since there are no specific nutrient recommendations for a GDM mother, the nutrient requirements for a nondiabetic pregnant woman are inferred for this population [8]. The diabetes nurse educator and the dietician conducted group counselling sessions for the GDM mothers on good diabetes care using power point presentations, foods models, standardized vessels and pictorial representation of balanced meals. This was used to educate the patients on portion sizes, meal pattern, regularity of meal timings and choice of low glycaemic foods. Further, data regarding foetal and maternal outcomes including neonatal birth weight, maternal anaemia, maternal pre-eclampsia, premature birth, stillbirth/intrauterine death, neonatal hypoglycaemia and neonatal and perinatal mortality were included in this study.

Statistical Analysis

The data were statistically analysed using SPSS 19. Percentage analysis was done to represent the data. Independent sample *t* test, Chi-square tests, one-way ANOVA and Pearson's correlation of coefficient were used to study the association between nutrient intake and maternal glycaemic control and foetal and maternal outcomes.

Results and Discussion

A total of 85 women with diabetes in pregnancy were studied of which there were GDM ($n = 59$) and pre-GDM women ($n = 26$). Amongst the GDM group, $n = 11$ had overt diabetes mellitus while in the pre-GDM group, $n = 7$ had type 1 diabetes mellitus.

Table 1 Baseline demographic and clinical characteristics of pregnant women with diabetes mellitus ($n = 85$)

Characteristics	Pregnant women with diabetes ($n = 85$)
<i>Age</i>	
(Mean \pm SD) in years	29.9 \pm 4.5
<i>Glycosylated haemoglobin</i>	
Mean \pm SD %	6.3 \pm 1.4
<i>Pre-pregnancy BMI kg/m²</i>	
Mean \pm SD	27.6 \pm 4.7
Underweight	2 (2.5)
Normal	12 (14.8)
At risk	7 (8.6)
Obese grade I	38 (46.9)
Obese grade II	22 (27.2)
<i>Family history of diabetes mellitus</i>	
Yes	59 (69.4)
No	26 (30.6)
<i>Presence of comorbidities</i>	
None	63 (74.1)
Primary hypothyroidism	18 (21.2)
Essential hypertension	4 (4.7)
<i>Medication</i>	
Insulin alone	26 (30.6)
Metformin alone	17 (20)
Insulin and metformin	22 (25.9)
Medical nutrition therapy	20 (23.5)
<i>Gestational age at presentation</i>	
First trimester	21 (24.7)
Second trimester	46 (54.1)
Third trimester	18 (21.2)
<i>Birth weight in kg</i>	
(Mean \pm SD) in kg	2.71 \pm 0.64
<i>Meal Pattern</i>	
3–4 meals/day	20 (23.5)
≥ 5 meals	65 (76.5)
<i>Main cereal consumed</i>	
Rice/refined	71 (83.5)
Wheat/millet	14 (16.5)
<i>Mean fruit and vegetable intake</i>	
Median (range) in g	200 (50–700)

*Significant at 5% level

Baseline characteristics

Table 1 represents the demographic, anthropometric and clinical characteristics of these pregnant women with diabetes. The mean age of the group was 29.9 ± 4.5 years with 66% of them being either overweight or obese with a mean HbA1c level of $6.3 \pm 1.4\%$. More than half of them had a parental history of diabetes while primary hypothyroidism was the most common associated comorbidity

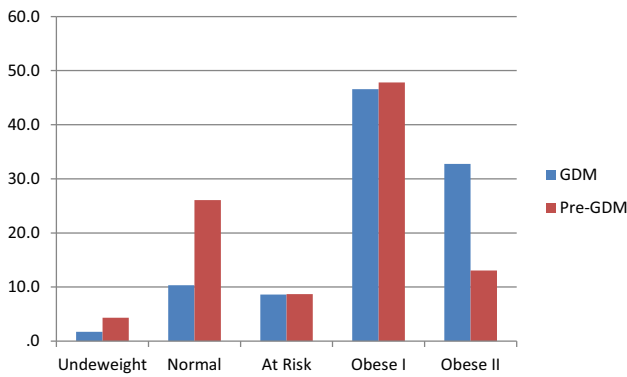


Fig. 1 Distribution of women with gestational diabetes mellitus ($n = 59$) and pre-gestational diabetes mellitus ($n = 26$) based on their body mass index classified according to Asia Pacific guidelines

(21%). Insulin was the commonest form of therapy, while 25% of them required a combination of insulin and metformin. The mean birth weight was 2.71 ± 0.64 kg. Considering their diet pattern, refined cereals like rice and refined flours were prevalent in more than 84% of the population while the mean intake of fruits and vegetables was 200 g/day (range 50–700 g/day).

Comparing the GDM and pre-GDM groups, women with pre-GDM were significantly older (31.7 ± 4.7 years; 29.1 ± 4.2 years; $p = 0.013$) with higher mean HbA1C levels (6.96 ± 1.8 ; $5.96.0 \pm .0.97$; $p = 0.004$). Though the mean BMI was similar, the GDM group had higher

number of women with BMI > 30 kg/m² than the pre-GDM group (32% vs 13%, $p = 0.03$)(Fig. 1). Other characteristics like family history of diabetes, hypertension and the presence of chronic medical disorders were similar ($p = 0.15$). There was no difference in foetal and maternal outcomes including birth weight, prematurity, maternal hypertension and stillbirths ($p = 0.21$) between the two groups. All the pre-GDM women (100%) required insulin when compared to 37% in the GDM women ($p = 0.02$).

Analysis of Nutritional Intake

Table 2 represents the actual nutrient intake of pregnant women with diabetes.

The mean calories were 1538.4 ± 318.7 cal/day which was below the RDA for an Indian pregnant woman with the pre-GDM consuming less calories than the GDM women (1417.2 ± 263.5 vs 1591.8 ± 328.12 cal/day, $p = 0.02$). Overall, there was a deficit in intake of all macro- and micronutrients with the exception of vitamin C.

Macronutrients

Analysing the individual macronutrient intake compared to the RDA for Indian woman, the mean carbohydrate intake of 228.3 ± 50.2 g/day in this population was inadequate for pregnancy. There was a deficit of 95 and 130 g/day in

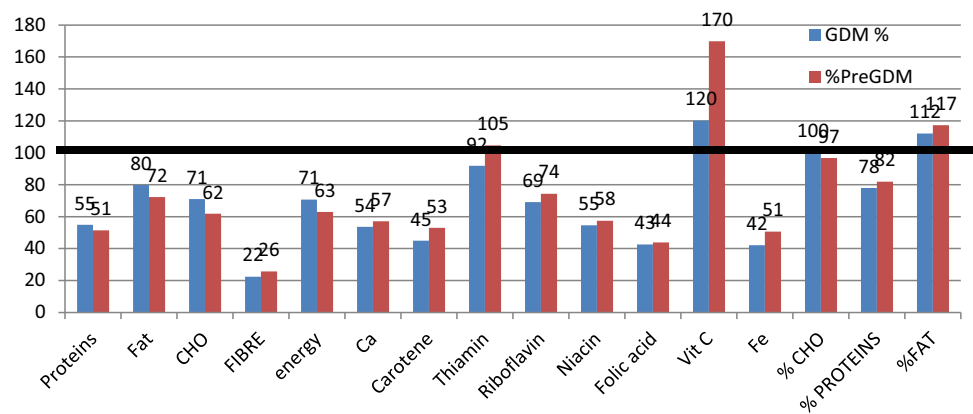
Table 2 Adequacy of nutrient intake of pregnant women with gestational diabetes mellitus ($n = 59$) and pre-gestational diabetes mellitus ($n = 26$)

Nutrients	RDA*	Pregnant women with diabetes ($n = 85$)	GDM ($n = 59$) Mean \pm SD	Pre-GDM ($n = 26$) Mean \pm SD	p value
Energy kcal	2250	1538.4 ± 318.7	1591.8 ± 328.12	1417.2 ± 263.5	0.019*
Proteins gm	85	45.7 ± 11.3	46.6 ± 12.1	43.7 ± 9.2	0.274
Fat gm	63	48.8 ± 13.7	50.3 ± 14.6	45.5 ± 11.1	0.141
Carbohydrates gm	335	228.3 ± 50.2	237.7 ± 51.9	207.0 ± 39.3	0.009*
% Carbohydrates	60	59.5 ± 5.4	60.1 ± 5.8	58.0 ± 4.3	0.102
% Proteins	15	11.9 ± 1.7	11.7 ± 1.9	12.3 ± 1.4	0.158
% Fats	25	28.4 ± 4.7	28.0 ± 4.9	29.3 ± 4.0	0.247
Fibre gm	40	9.4 ± 4.6	9.0 ± 4.7	10.3 ± 4.6	0.241
Calcium mg	1200	656.5 ± 280.7	643.9 ± 275.9	684.9 ± 294.9	0.539
Carotene mcg ^a	6400	2850 (221–11,125)	2622 (221–10,113)	3323(464–11,125)	0.358
Thiamine mg	1.2	1.1 ± 0.35	1.1 ± 0.34	1.3 ± 0.36	0.064
Riboflavin mg ^a	1.4	0.99 ± 0.5	0.97 ± 0.50	1.04 ± 0.52	0.551
Niacin mg	14	7.8 ± 2.9	7.6 ± 2.65	8.1 ± 3.69	0.556
Folic acid mg	500	214.9 ± 76.3	212.9 ± 77.6	219.4 ± 74.7	0.719
Vitamin C ^a mg	60	55.8 (22–290)	53.8 (36.5–273.9)	64.0 (12.3–289.5)	0.084
Iron mg ^a	35	10.7 (5.7–45)	10.7 (5.7–44.9)	10.7 (6.8–44.9)	0.239

*Nutrient requirements and recommended dietary allowances (A report of the expert group of the Indian Council of Medical Research 2009 NIN, Hyderabad)

^aReported in median (range)

Fig. 2 Comparison of nutrient intake of pregnant women with gestational diabetes mellitus ($n = 59$) and pre-gestational diabetes mellitus ($n = 26$) as percentages of recommended daily allowance (RDA)



the intake of GDM and pre-GDM women, respectively, and this was statistically significant ($p = 0.009$). The mean protein intake was inadequate in both GDM and pre-GDM groups (46.6 ± 12.1 vs 43.7 ± 9.2 g/day, $p = 0.27$), though not significantly different. The mean fat intake also showed a similar finding in the GDM and pre-GDM women (50.3 ± 14.6 vs 45.5 ± 11.1 g/day, $p = 0.14$). In the pre-GDM group, calories from fat was positively associated with BMI ($r = 0.268$, $p = 0.040$).

Micronutrients

Analysis of the micronutrient intake revealed that the overall mean intake of micronutrients like calcium (656.5 ± 280.7 mg), carotene (median = 2850 mcg), thiamine (1.1 ± 0.35 mg), riboflavin (0.99 ± 0.5 mg), niacin (7.8 ± 2.9 mg), folic acid (214.9 ± 76.3 mg) and iron (median = 10.7 mg) was inadequate in this group of pregnant women. Statistical analysis showed both the GDM and pre-GDM women to be similarly deficient in these micronutrients. Only the daily vitamin C intake was adequate in both the groups (median = 55.6 mg).

Dietary Habits

Considering the dietary habits between the two groups, majority of pre-GDM mothers (80%) consumed meals more frequently (> 5 meals per day; 3 meals and 2 or more snacks daily) and a higher percentage of them (30%) consumed high-fibre cereals compared to GDM women ($p = 0.03$). 69% of pre-GDM mothers consumed refined cereals as against 90% of the GDM women (90%) ($p = 0.04$). The daily intake of pulses, vegetable and fruit consumption was below the recommended allowance in both groups.

Adequacy of Nutritional Intake Compared to the RDA

Figure 2 illustrates the intake of the GDM and pre-GDM mothers as percentages of their RDA. There was a 29 and

37% deficit in energy intake amongst GDM and pre-GDM women when compared to the RDA. With regard to protein intake, only half (50–55% of the RDA) of the daily requirement was met in both the groups of women. The fat and carbohydrate met approximately 60–80% of the RDA in women with GDM and pre-GDM. The intake of fibre was less than 30%, and folic acid intake was less than 50% of the RDA in both groups of women with GDM consuming less than 50% of the requirement of carotene and iron.

Factors Affecting Nutritional Intake

Our study looked at the influence of gravidity status on the pattern of nutritional intake. In our study of 85 women with diabetes in pregnancy, 32(38%) were primigravida and 53(62%) were multigravida. Amongst these two groups, the individual macronutrient intake compared to the RDA for Indian woman was inadequate for pregnancy. The mean protein intake was similar in both groups (42.8 ± 16.2 vs 44.5 ± 10.7 g/day, $p = 0.34$) while mean fat intake was significantly lower in the multigravida than the primigravida group (44.8 ± 11.3 vs 52.2 ± 14.1 g/day, $p = 0.04$). In the primigravida group, carbohydrate intake was significantly higher (238 ± 46.6 g/day) than the multigravida (211 ± 40.5 g/day, $p = 0.02$), though the deficit in carbohydrate intake was similar for both primigravida and multigravida (105 vs 112 g/day, $p = 0.19$). The micronutrient intake was similarly deficient in both the groups, with the exception of calcium which was slightly better in the multigravida group (686 ± 226.6 g/day) than the primigravida (617 ± 212.5 g/day, $p = 0.05$). Considering the dietary habit, the proper pattern of meals/snacks was being followed by a higher number of multigravida (78%) as opposed to the primigravida (56%, $p = 0.03$), while there was no significant difference between the percentages taking high-fibre cereals (32 vs 28%, $p = 0.34$). The intake of pulses, vegetables and fruits was inadequate in both.

In our study population of 85 pregnant women, 61 women (72%) were taking a nonvegetarian diet while 24 women ($n = 28\%$) were taking vegetarian diet. The intake of micronutrient and macronutrient intake was inadequate compared to the RDA in both these groups, though the vegetarian group was more deficient in protein ($p = 0.02$) and iron ($p = 0.04$), while having similar deficiencies in fat and carbohydrate intake ($p = 0.22$).

We also assessed the maternal and foetal outcomes in our study population of 85 women and their association with macro- and micronutrient deficiencies. In our study group, 24% ($n = 20$) had caesarean sections, 76% ($n = 65$) had spontaneous vaginal delivery, 1.1% ($n = 1$) had pre-eclampsia, 1 ($n = 1.1\%$) had placenta previa, 9.4% ($n = 8$) had anaemia and 2.3% ($n = 2$) women had oligo/polyhydramnios. Preterm birth was seen in 8.2% ($n = 7$), stillbirths/intrauterine death in 1.1% ($n = 1$), macrosomia (birth weight > 3.5 kg) in 11.9% ($n = 11$), low birth weight (birth weight < 2.5 kg) in 8.2% ($n = 7$) and neonatal hypoglycaemia in 2.3% ($n = 2$) of the study subjects.

Carbohydrate, protein or fat deficiencies were not significantly associated with the maternal and foetal outcomes in our study. Amongst the micronutrients, deficiencies in iron and folic acid were associated with maternal anaemia ($p = 0.02$) and neonatal low birth weight ($p = 0.03$), while none of the other micronutrients had significant associations with any of the maternal and foetal outcomes.

Discussion

Our study throws light on the nutritional profile of South Indian pregnant women with diabetes. The mean HbA1C of the subjects was above 6.3%, and it was significantly higher in the women with pre-GDM. A study of a multi-ethnic population of pregnant women has reported that women with a HbA1C $\geq 5.9\%$ ($n = 48$) had a threefold increased risk of macrosomia (95% CI: 1.127–8.603, $p = 0.028$) and pre-eclampsia (95% CI: 1.086–11.532, $p = 0.036$) independent of GDM later on pregnancy [9].

Several Indian and global studies have shown a strong association between family history and the prevalence of GDM [10]. As expected, our study also identified a family history of diabetes in both groups of women with GDM and pre-GDM. Most of the subjects in our study developed GDM in the second trimester of pregnancy.

Overall, only a small percentage of the population were in the normal weight category. It was interesting to note that there were significantly more normal weight individuals in the pre-GDM group when compared to GDM women. This could be due to the fact that women with pre-GDM have previously been sensitized to the principles of

healthy eating during their routine medical care and also due to the nature of their disease.

Medical nutrition therapy is the most influential and cost-effective strategy in the treatment of diabetes mellitus in pregnancy [11]. This should include the provision of adequate calories and nutrients to attain appropriate weight gain, meet the increased energy needs of pregnancy and should be consistent with the maternal blood glucose goals that have been established [12]. According to the recommended dietary allowance for Indians, a woman requires an additional 350 cal/day during the second and third trimester of pregnancy. Thus, a sedentary Indian pregnant mother requires 2250 cal/day. Energy-related studies agree that caloric restrictions are necessary for the overweight or obese mother [13]. The protein requirement is an additional 23 g/day during the gestation period. An Indian woman thus requires 70–80 g/day. There is an increased need of micronutrients to meet the energy demands of pregnancy.

In our study, the overall nutrient intake of the subjects in the baseline state was suboptimal. There was a gross deficit in the intake of calories, protein, fat and carbohydrates in both groups of women. The meal composition was inappropriate with a high quantity of calories from fat and an inadequate contribution from proteins. Similar findings were reported in a South Indian study on patients with type 1 diabetes mellitus patients [14].

A Korean study found that the GDM group had an undesirable macronutrient composition and obtained 56.6% of their calories by carbohydrate intake, which exceeded the recommended levels (125.8%) [15].

The conventional approach to nutrition therapy in GDM has focused on a diet high in complex carbohydrates as this can blunt the postprandial sugars and prevent worsening of insulin resistance and macrosomia [11]. Our study found that the intake of fibre in the diet was less than one-third of the requirement. Complex carbohydrates (wheat, millets) were higher in the pre-GDM group. There is an urgent need to educate Asian Indian society about the benefits of reverting to our traditional coarse grain diet as staple food. Promoting kitchen gardens and encouraging the use of seasonally grown crops can enhance the intake of fibre-rich fruits and vegetables across all sections of society.

An adequate intake of micronutrients is necessary to prevent maternal complications and reduce other adverse pregnancy outcomes—SGA births, low birth weight, stillbirths, perinatal and neonatal mortality. Although all our patients were on standard calcium, iron and folate supplementation, there was a significant deficiency of minerals, vitamins and fibre in their diets. Only the intake of vitamin C was adequate in our study subjects.

A Korean study has found that the majority of GDM subjects do not meet the recommended intake levels for most micronutrients [15]. Studies have shown that

supplementation with multiple micronutrients in pregnancy can reduce SGA births [16]. A Mumbai Indian study has indicated that by increasing the intake of leafy green vegetables, fruit and/or milk in low-income families (with poor intake of micronutrient-rich foods) a protective effect against the evolution of GDM occurs [17]. Our study shows association of iron and folate deficiencies with maternal anaemia and low birth weight in the newborn. Further, studies have shown that maternal anaemia can significantly impact maternal and foetal outcomes including prematurity, birth weight and gestational age [18].

However, owing to the smaller sample size, our study may not have identified possible associations of other micronutrients with maternal and foetal outcomes and this can be actively looked at in future larger Indian studies. Further, there could have been changes in their dietary intake following nutritional counselling and hence follow-up data on their nutritional intake would have made these associations more robust.

Between the two groups of GDM and pre-GDM, the pre-GDM women had a marginally better nutrient intake. Though we compared data at the first antenatal visit to the GDM clinic in both the groups, the fact that the pre-GDM women could have been exposed to general diabetes education and nutritional counselling prior to coming to our clinic during the earlier part of their diabetes care cannot be fully excluded. They consumed a lower carbohydrate percentage with a higher protein proportion, with a better intake of fibre, calcium, carotene, thiamine, riboflavin, niacin, folic acid, vitamin C and iron in their diet. The consumption of fibre-rich foods like vegetables, fruits, millets and wheat-based products was higher amongst them. They had three meals with small snacks in between for better glycaemic control.

Similarly, the findings of better nutritional intake and dietary habits in multigravida as opposed to primigravida women in our study probably reflect better understanding and knowledge due to repeated counsellings and education during previous childbirths in the multigravida group.

To our knowledge, this is the first study from a tertiary centre in Southern India that looked at the dietary pattern of pregnant women with diabetes mellitus. It throws light on the dismal nutritional intake of women with GDM and pre-GDM. The imbalanced macronutrient composition of each meal, inadequate micronutrients in the diet and the inappropriate meal pattern call for urgent nutritional counselling. Increasing the proteins and micronutrients in the diet is vital to achieving a better dietary composition which will meet the increased requirements of pregnancy.

While our study utilized the 24-h dietary recall and records of food diary to obtain the nutritional history, more objective measures of nutrient intake in future studies may add valuable insights into the nutritional pattern of women

with GDM and pre-GDM. Moreover, follow-up nutritional data on GDM women after the first nutritional counselling and need for a larger study population from different regional and ethnic backgrounds are potential limitations in our study that if overcome can help in understanding the influence of macro- and micronutrients on maternal and foetal outcomes in a better way.

Conclusion

The gestational period should be viewed as a window of opportunity to modify dietary patterns and introduce healthy lifestyle practices for the woman and her family. Adequate intake of micro- and macronutrients will not only facilitate glycaemic control during pregnancy, but also influences the metabolic health of the mother and the child.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The study protocol conformed to the ethical guidelines of the Declaration of Helsinki (2013), and approval was obtained from the Institutional Review Board of Christian Medical College, Vellore, India (IRB Min. No. 10045 dated 4/4/2016).

Informed Consent Informed consent was obtained from all the participants.

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