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Original Article

Lower RXRA and TFRC expression despite higher mineral and vitamin intake in diabetic pregnant women with pregnancy-specific urinary incontinence

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SUMMARY

Background: Gestational diabetes mellitus (GDM) and pregnancy-specific urinary incontinence (PSUI) pose significant health challenges for pregnant women, but their metabolic and molecular underpinnings remain poorly understood.
Methods: In this cross-sectional study, 1,105 participants from the DIAMATER cohort were categorized based on GDM and PSUI status. Dietary intake of iron, magnesium, zinc, and vitamins A and D was assessed through dietary recalls, while serum levels, gene expression (MTF1, RXRA, TFRC, TRPM6), and protein expression were analyzed using standard techniques. Oxidative stress markers were also measured.

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Results: GDM-PSUI participants exhibited lower RXRA and TFRC gene expression and decreased TFRC protein levels despite higher intake of magnesium, zinc, and vitamin D compared to GDM controls without PSUI. Serum mineral levels and oxidative stress markers did not differ significantly between groups.
Conclusions: Lower RXRA and TFRC expression in GDM-PSUI women, despite increased mineral and vitamin intake, suggests potential molecular targets for interventions aimed at improving management strategies in this population.

Introduction

Gestational diabetes mellitus (GDM) is identified by impaired pancreatic β-cell functions and a comparatively insufficient secretion of insulin, resulting in hyperglycemia during pregnancy [1]. Given that GDM is considered a possible precursor to chronic Type 2 Diabetes Mellitus (T2DM), this poses a substantial challenge to global health and medical resource [2,3].

Literature shows that nutrients and the expression of their receptors can be altered as a cause or consequence of GDM [4–7], and urinary incontinence (UI) [8–10]. This highlights the pressing need of determining the influence of nutrients on the functionality of β-cell in the pancreas and to prevent any potential damage to β-cells during pregnancy [11]. Although the findings are inconclusive, recent research has investigated the correlation between diet and GDM. Particularly interesting is the fact that dietary modifications prior to and during pregnancy may influence the risk of GDM [12–16]. However, the existing body of evidence regarding the connection between diet and GDM is still limited in the primary global regions [17–19].

During the 20th century, Nutrigenomics emerged with the fundamental concept of gene-nutrient interaction in relation to the human post-genome [20,21]. Nutrigenomics investigates the relationship between bioactive compounds and nutrients present in food and the functioning of the genome, which may modulate gene expression and/or affect genome stability [22,23]. Dietary nutrients can have an indirect or direct effect on gene expression [24–26]. Indirectly influencing cell-signaling pathways [24,27] or directly modulating transcriptional changes by interacting with nuclear receptors [24].

Due to their higher fiber and micronutrient contents, fruits and vegetable-rich diets are associated with reduced risk of GDM [28]. Vitamin A, for instance, regulates the development and function of pancreatic beta cells in the underlying endoderm [29–31]. A correlation between glucose metabolism and vitamin A and its receptors has been identified in various studies [29,31,32]. The mechanism by which vitamin A exerts its effects involves interactions with retinoic acid (RXA) and retinoid acid X (RXR) receptors, including three subtypes of RXR: alpha, beta, and gamma [33]. The retinoid X alpha receptor (RXRA) is a nuclear receptor that functions in the metabolism of glucose, fatty acids, and cholesterol [34,35]. Glucose metabolism is disrupted and gene and protein expression and compromised due RXRA gene polymorphisms [34–36]. Furthermore, RXRA is essential for vitamin D signaling [37], which deficiency is linked to T2DM [38]. Regarding some dietary minerals literature describe a relationship between maternal iron stores and disturbances in glucose metabolism leading to the development of T2DM. In fact, during pregnancy, maternal iron nutritional requirements increase as gestation advances and become approximately 10 times higher by late pregnancy [39]. The transferrin receptor (TRFC) is a gene that encodes a cell surface receptor necessary for cellular iron uptake via endocytosis [40,41]. The TRFC expression is regulated by intracellular iron concentrations, i.e iron

deficiency increases TRFC expression; conversely, elevated intracellular iron concentrations inhibit iron incorporation in cells and decrease TRFC expression [42]. Magnesium is an additional mineral, as well as deficiency has been linked to glucose dysregulation in pregnant women [43]. This mineral acts on insulin regulation and is transported intracellularly by a protein containing an ion channel domain and a protein kinase domain encoded by the transient receptor potential cation channel subfamily M member 6 (TRPM6) gene [44]. An association between zinc, pancreatic function, and diabetes has also been suggested for years [45,46]. Zinc has the function of regulating glucose and maintaining glucose absorption at physiological levels [46]. During pregnancy, zinc deficiency is related to embryonic death and can also result in iron accumulation in various tissues, such as the liver and kidney [47,48]. Metal regulatory transcription factor 1 (MTF1) is responsible for activating genes related to zinc homeostasis and is reversibly activated in response to changes in free zinc concentrations [47,49].

In this study, we hypothesized that specific dietary nutrient intake would correlate with gene and protein expression patterns, influencing the development of pregnancy-specific urinary incontinence (PSUI) in women with GDM. To this end, the purpose of this study is to investigate the relationship between serum levels of iron, magnesium, zinc, vitamin A, and vitamin D and the expression of MTF1, RXRA, TFRC, and TRPM6 genes and proteins, as well as oxidative stress biomarkers in women with GDM and PSUI.

Materials and methods

Recruitment of pregnant patients

The study population included women who attended the Perinatal Diabetes Research Center (PDRC) at the University Clinical Hospital of Botucatu Medical School (UNESP), Brazil, and participated in the DIAMATER study, a prospective observational cohort approved by the Research Ethics Committee of Botucatu Medical School, the host institution (CAAE: 82225617.0.0000.5411). The full study protocol of the original trial is published elsewhere [50]. This study included four groups from the Diamater cohort =(2016–2023), comprising a total of 1,105 participants. After informed consent was provided, plasma samples were collected from all patients. Groups were classified according to GDM diagnosis and PSUI into 4 groups namely, non-GDM continent (non-GDM-C), non-GDM incontinent (non-GDM-PSUI) and GDM continent (GDM-C) as control groups and GDM incontinent (GDM-PSUI) as study group. The full methodology for the analysis presented in the original essay has been published elsewhere [51].

Dietary intake analysis

The amount of iron (mg), magnesium (mg), zinc (mg), vitamin A (mcg) and vitamin D (mcg) intake were performed using a 24-hour and 48-hour dietary recall, in which all information on the pregnant woman's food intake were noted. The recall showed all foods and beverages ingested, mealtimes, ways of preparing food, amounts in household measures, portions or grams of foods/beverages ingested, and brands of foods. Discrepant values of nutrients were disregarded. After dietary assessment, all nutrients were quantified using a nutritional software (Nutrilife version 9.12).

Micronutrients biochemical analysis

A total of 15 mL blood was collected from the pregnant women for quantification of retinol (mg/L) by high-pressure liquid chromatography method efficiency, magnesium (mg/dL) by colorimetric method, serum zinc (μg/dL) by atomic absorption spectrometry method, iron (μg/dL) by the colorimetric method and vitamin D (ng/mL) by ARCHITECT i2000SR analyzer (Abbott, Brazil); between 24 to 40 gestational weeks. Blood samples were centrifuged at 3,200 rpm for 12 min and serum aliquots were stored protect from light in a freezer at -80°C for later analysis at the Clinical Hospital of the Faculty of Medicine of Botucatu (FMB) and by Alvaro Laboratory (Barueri, Sao Paulo – Brazil) [52,53].

Gene expression by real-time PCR (qPCR)

The following genes were analyzed: MFT1, involved in the regulation of metal metabolism, mainly zinc; RXRA, involved in vitamin A and vitamin D signaling; TFRC, involved in iron transport; and TRPM6, related to magnesium transport. Total RNA was isolated and purified from whole blood by Trizol Reagent (Invitrogen®, Carlsbad, California, US) method, according to the manufacturer's instructions. The High Capacity cDNA kit (Applied Biosystems) was used for the synthesis of complementary DNA (cDNA) from 1000 ng of total RNA. The mRNA levels of the genes in Table 1 were determined by qPCR (QuantStudio® 3 Real-Time PCR System, Applied Biosystems, USA) in reactions containing PowerUp™ SYBR™ Green Master Mix 2 × (5 μL; Applied Biosystems, Foster City, CA), 0.4 μM of each assay, 50 ng cDNA and nuclease-free water. All analyses were performed in triplicate. Relative gene expression was determined by the 2-ΔΔCt method [54] using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), folylpolyglutamate synthase (FPGS), and TNF receptor-associated protein 1 (TRAP1) genes as endogenous controls.

Protein expression by Western blot

The protein synthesis of MTF1, RXRA, TRPM6, and TFRC was analyzed by Western blot. Proteins were lysed with Ripa buffer (Radioimmunoprecipitation assay: 150 mM sodium chloride, 1% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris, pH 8.0), and the homogenate was centrifuged at 4°C for 20 min at 12000 rpm. The quantification was performed according to the BRADFORD method, following the standardized protocol, and samples containing 25 ng of protein were used. Electrophoresis was performed on polyacrylamide gel at a concentration of 12%, according to the molecular weight of the analyzed proteins. Then, the proteins were transferred to a nitrocellulose membrane using a transfer buffer. Non-specific binding sites of the primary antibody to the membrane were blocked by incubation with a blocking solution (0.5% skimmed milk powder diluted in basal solution: 1 M Tris-HCl pH 8.0, 2.5 M NaCl and Tween 20) at room temperature under constant stirring. Then, the membrane was incubated with the primary antibody under constant agitation at a temperature of 2°C–8°C overnight. The primary antibodies used were mouse monoclonal anti-MTF1 (Product#MAS-26739; 1:2000; Invitrogen, Waltham, US), rabbit monoclonal anti-RXRA (Product#MAS-35265; 1:1000; Invitrogen, Waltham, US), rabbit polyclonal anti-TRPM6 (Product#OST00108W; 1:1000; Invitrogen, Waltham, US) and mouse monoclonal anti-TRFC (Product#13–6800; 1:1000; Invitrogen, Waltham, US). After incubation with the primary antibody, the membrane was incubated with the secondary antibody, conjugated to peroxidase (anti-mouse or anti-rabbit; Invitrogen, Waltham, US) used in the titration of 1:1000 and 1:10000, respectively.

Table 1
Expression Primers Sequences and PCR cycle conditions

Gene	F and F sequences (5′-3′)	Tm (°C)	%GC	λ
RXRA	CTCAATGGCGTCCTCAAGGT	60.04	55.00	111
	CACTCCATAGTGCTTGCCTGA	60.07	52.38	
TRPM6	ATCCCTCCTTGGGGTGTCAT	60.25	55.00	67
	GGTACAGGCACACCACATCT	59.68	55.00	
MTF1	CGAGTGCACACGAAGGAGAA	60.32	55.00	91
	TCTGATGTGCTTTCAGCCTGT	59.93	47.62	
TFRC	GATCGTGTCATGAGAGTGGAGT	59.57	50.00	83
	CCCCAGAAGACATGTCGGAAA	60.00	52.38	
GAPDH	TCACCATCTTCCAGGAGCGA	60.61	55.00	50
	AGCATCGCCCCACTTGATTT	60.32	50.00	
FPGS	TCTCTTGGCATCGACCACAC	60.04	55.00	93
	GGCAGGGACACCTTGCTTAAA	60.82	52.38	
TRAP1	TCCACCTGAAATCCGACTGC	60.04	55.00	63
	TCGTTACCACATCTCGCACC	60.11	55.00	

F: Forward Primer; R: Reverse Primer; Tm: Melting Temperature; %GC: Percentage of Guanine-Cytosine Content; λ: Amplicon Length.

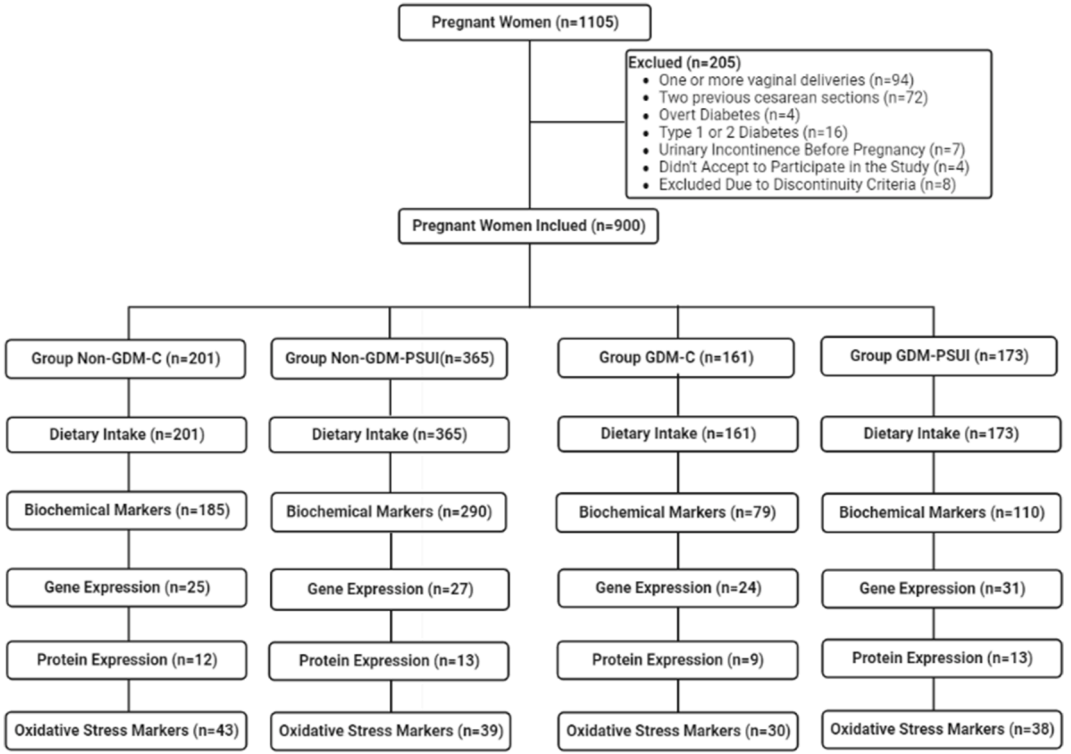

Figure 1. Flow diagram illustrating the screening and selection of the study population for analyses of dietary intake, biochemical markers of micronutrients, gene expression profiles, protein expression, and oxidative stress markers. This figure represents the categorization of 1,105 pregnant participants into four groups based on GDM and PSUI diagnoses. List of Abbreviations: Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus and incontinent; n: represents the number of participants in each group.

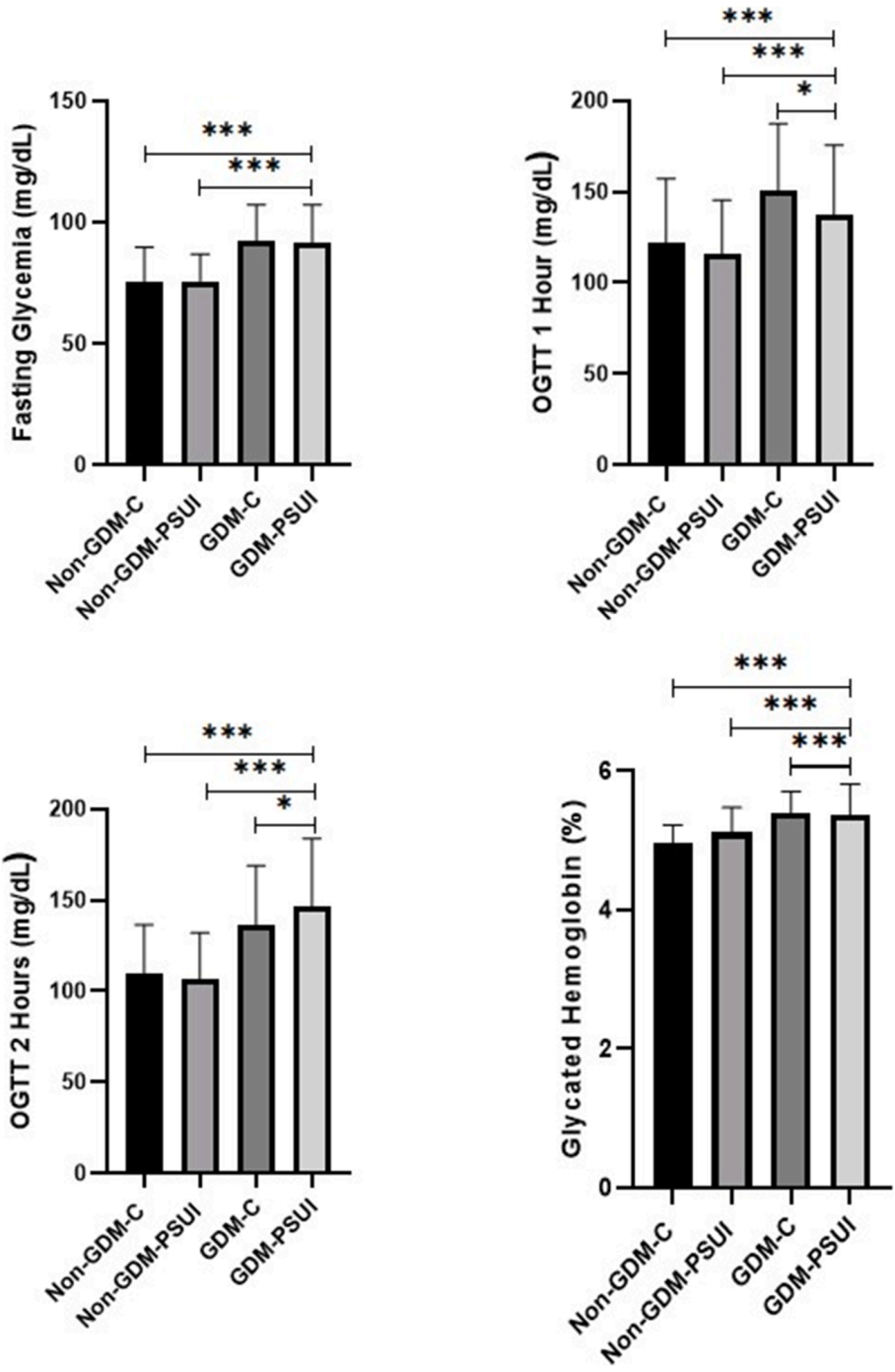

Figure 2. Comparison of fasting blood glucose, glucose levels one and two hours after the OGTT, and glycated hemoglobin across study groups. Data are presented as means ± standard deviation. Significant differences in glucose and glycated hemoglobin levels were observed between the groups, indicating the effect of gestational diabetes and stress urinary incontinence on metabolic parameters. Statistical analysis was performed using one-way ANOVA followed by Bonferroni-corrected multiple

Finally, immunodetection was performed using the chemiluminescence method according to the manufacturer's instructions (Enhancer Chemi-Luminescence, Amersham Biosciences, NJ-USA). Quantitative analyses of protein bands were performed using Carestream Molecular Imaging 5.0 software (Carestream Health, Rochester, NY, USA).

Oxidative stress biomarkers

Protein carbonylation level

Protein carbonylation was performed using serum samples to verify the irreversible protein damage caused by reactive oxygen species. These samples were measured using the non-specific DNPH method (2,4-dinitrophenylhydrazine as the derivatizing agent), and the amounts were expressed in mmol of DNPH per mg of protein [55,56].

Malondialdehyde (MDA) level

A total of 250 μL serum from pregnant patients was used at both times and 750 μL 10% trichloroacetic acid (TBA) for protein precipitation. Afterward, the samples were centrifuged (3000 rpm; for 5 min; Eppendorf Centrifuge 5804-R, Hamburg, Germany), and the supernatant was removed; 0.67% thiobarbituric acid was added in the ratio of 1:1, and later the samples were heated for 15 min at a temperature of 100ºC. Reading at 535 nm was performed on a Spectra Max 190 microplate (Molecular Devices, Sunnyvale, CA, USA). The MDA concentration was found by the molar extinction coefficient (1.56×10^5 M^{-1} $centimeter^{-1}$) [57].

Statistical analysis

In the statistical analysis, the sample size for the present study was determined based on a power analysis, as detailed in reference [50]. The study group was compared to three control groups related to numerical variables using the ANOVA test for independent samples followed by multiple comparisons with Bonferroni correction. Correlation analysis was performed using Pearson's correlation coefficient. The analysis was performed using the SPSS 21 software. The $P<0.05$ value was considered statistically significant.

Results

For the present prospective, population-based cohort study (Diamater Study), a screen survey study of 1,105 pregnant women was selected (Figure 1), 205 were excluded, turning the analysis of 900 pregnant women. Figure 2 shows the variables of fasting blood glucose, glucose 1 hour after the Oral glucose tolerance test of 75 g (OGTT), glucose 2 hours after the OGTT, and glycated hemoglobin. The GDM-PSUI group exhibited a significant increase in fasting blood glucose, OGTT 1h, OGTT 2h, and glycated hemoglobin when compared to the non-GDM groups. Figure 3 shows the dietary intake amounts and serum level of iron, magnesium, zinc, vitamin A, and vitamin D. GDM-PSUI had a significant increase in dietary intake of magnesium and zinc, and a significant decrease in vitamin D compared to the non-GDM-C group. There were no significant difference of dietary food intake of iron and vitamin A, and serum levels of iron, magnesium, zinc and vitamin D between study group and control groups.

Figure 4 shows FPGS, MTF1, RXRA, TRAP1, TRFC, and TRPM6 gene expression in GDM-PSUI compared with the three control groups. RXRA and TRFC expressions were decreased in the GDM-

comparisons. Pearson's correlation coefficient was used for correlation analysis. A *P*-value < 0.05 was considered statistically significant. *: $P<0.05$; ***: $P<0.0001$. List of Abbreviations: Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus and incontinent group; OGTT-75g: Oral glucose tolerance test of 75 g; *P*-value: the probability of observing a test statistic value greater than or equal to the one found; mg/dL: milligrams per deciliter.

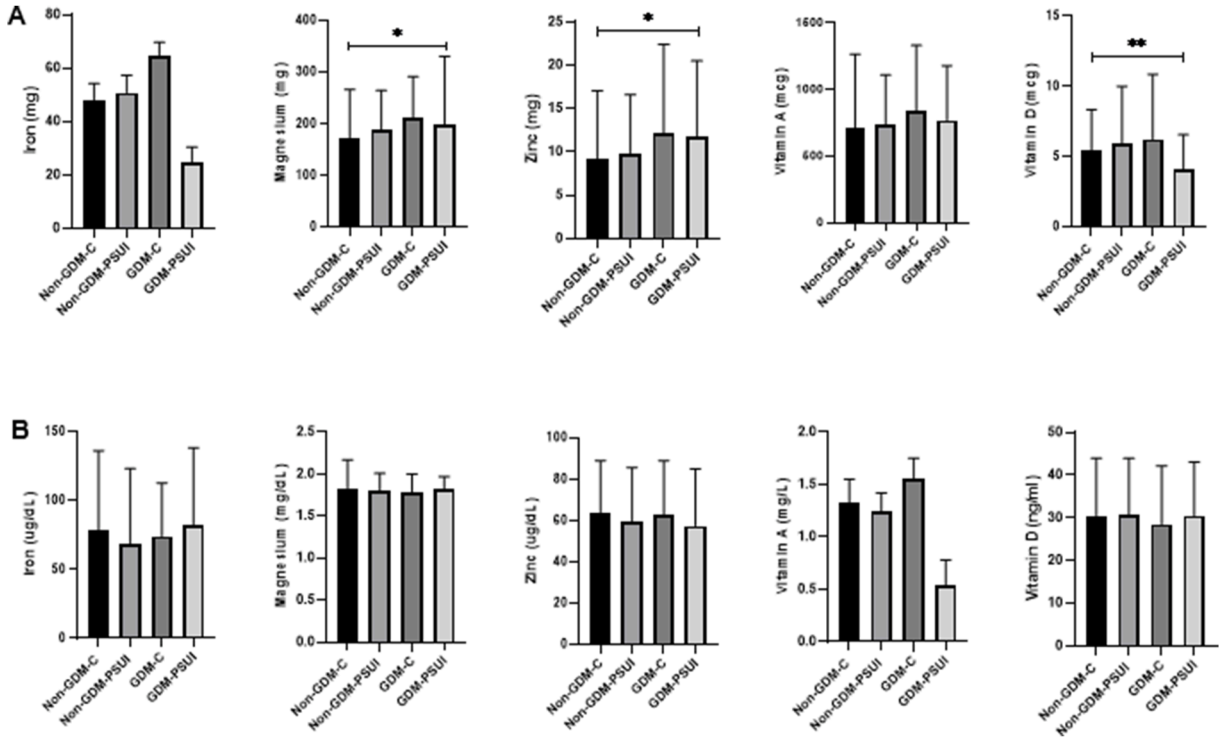

Figure 3. Comparison of dietary intake and serum micronutrient levels between GDM-PSUI and control groups. This figure illustrates the differences in dietary intake and serum levels of selected micronutrients—iron, magnesium, zinc, vitamin A, and vitamin D—between the GDM-PSUI group (GDM with PSUI) and three control groups. Significant increases in dietary intake of magnesium and zinc and a significant decrease in dietary intake of vitamin D were observed in the GDM-PSUI group compared to the non-GDM-C group, suggesting nutritional disparities associated with GDM-PSUI. Data are presented as means ± standard deviation. (**A**) Dietary intake levels of iron, magnesium, zinc, vitamin A, and vitamin D. (**B**) Serum levels of iron, magnesium, zinc, vitamin A, and vitamin D. Statistical analysis was performed using one-way ANOVA followed by Bonferroni-corrected multiple comparisons, with significance set at $P < 0.05$. *$P < 0.05$; **$P < 0.001$. List of Abbreviations: Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus and incontinent group; *P*-value: the probability of observing a test statistic value greater than or equal to the one found; mg: milligram; ug: microgram; ng/mL: nanogram per milliliter; mcg: microgram; mg/dL: milligrams per deciliter; ug/dL: micrograms per deciliter.

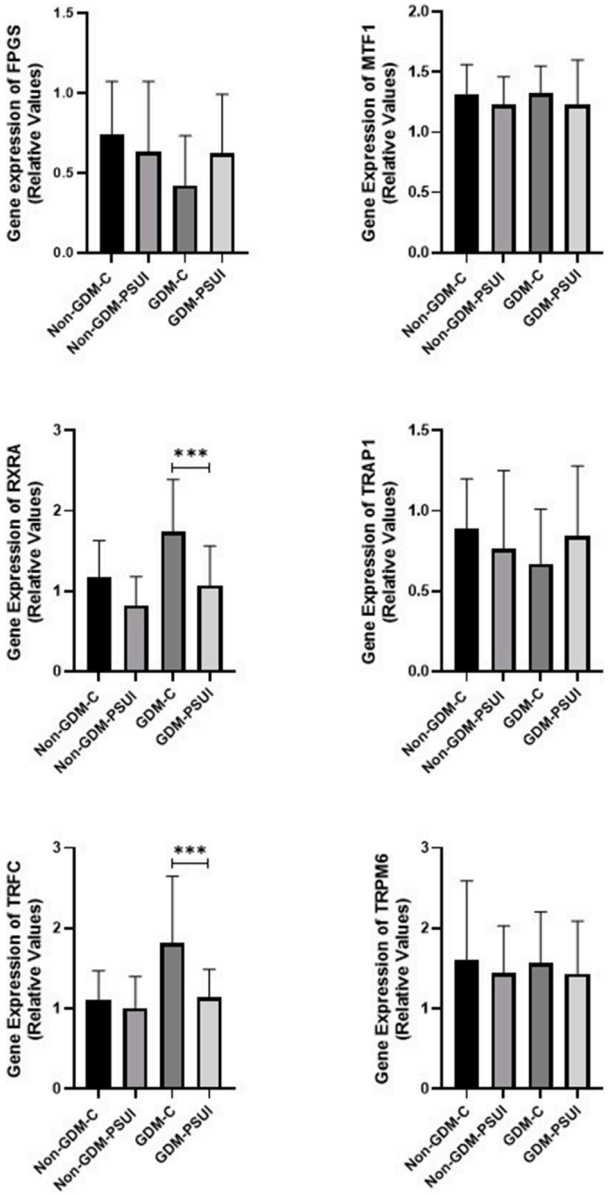

Figure 4. Comparison of gene expression levels in the GDM-PSUI group and three control groups. Data are presented as means ± standard deviation. Gene expression analysis includes key genes involved in nutrient metabolism and cellular stress responses: FPGS, MTF1, RXRA, TRAP1, TRFC, and TRPM6. Notably, the GDM-PSUI group exhibited a significant reduction in RXRA and TRFC expression levels compared to the GDM-C group, suggesting a possible link to metabolic or inflammatory pathways involved in GDM-PSUI. Statistical analysis was performed using one-way ANOVA followed by multiple comparisons. A *P*-value < 0.05 was considered statistically significant. ***$P<0.0001$. List of Abbreviations: Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus and incontinent group; *P*-value: the probability of observing a test statistic value greater than or equal to the one found; FPGS: folylpolyglutamate synthase; MTF1: metal regulatory transcription factor 1; RXRA: retinoid X receptor alpha; TRAP1: TNF receptor associated protein 1; TRFC: transferrin receptor; TRPM6: transient receptor potential cation channel subfamily M member 6.

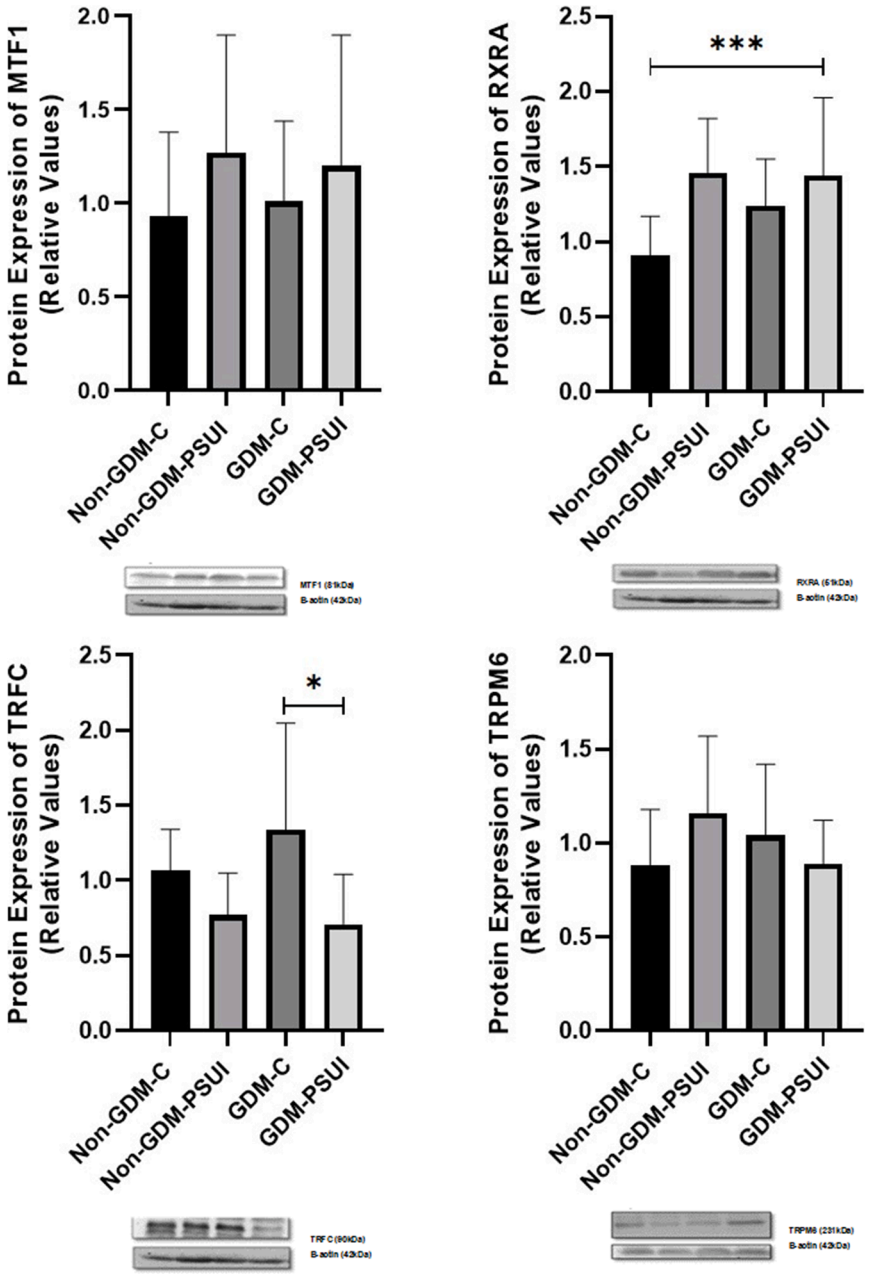

Figure 5. Comparison of protein expression levels in the GDM-PSUI group and three control groups. Data are presented as means ± standard deviation. Protein expression analysis includes key proteins involved in nutrient regulation and cellular signaling: MTF1, RXRA, TRFC, and TRPM6. Significant differences in protein expression between groups emphasize the impact of GDM and PSUI on metabolic pathways and cellular regulation. Notably, TRFC protein expression was significantly reduced in the GDM-PSUI group relative to the GDM-C group, indicating potential disruptions in cellular iron transport linked to GDM-PSUI. Statistical analysis was conducted using one-way ANOVA followed by Bonferroni-corrected multiple comparisons. A P-value < 0.05 was considered statistically significant; *$P < 0.05$ and ***$P < 0.0001$ indicate levels of significance. List of abbreviations: Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus and incontinent group;

PSUI group relatively to the GDM-C group. No significant differences were found FPGS, MTF1, TRAP1, and TRPM6 expression in relation to the study group and the other control groups.

MTF1, RXRA, TRFC, and TRPM6 protein expression in GDM-PSUI and in the three control groups are showed in Figure 5 (values are expressed as means and standard deviations). The TRFC protein expression was decreased in the GDM-PSUI group relatively to the GDM-C group. No significant differences were found for MTF1, RXRA, and TRPM6.

Figure 6 presents the Pearson correlation coefficients among the analyzed variables. In the negative control group (non-GDM-C), a negative correlation was observed between serum vitamin A levels (Y) and RXRA protein expression (Figure 6A). Positive correlations were detected between TRFC (Y) and RXRA gene expression (X), and also between RXRA gene (X) and RXRA protein expression (Y) in the GDM-C Group (Figure 6C and D) Additionally, a positive correlation was found between the TRFC (Y) AND RXRA (X) protein expression (Y) in the non-GDM-PSUI Group (Figure 6B). Figure 6E highlighted that the GDM-PSUI Group presented a positive correlation between TRFC (Y) and RXRA (X) gene expression and also a negative correlation between serum iron (Y) and protein expression of RXRA (X). This means that as RXRA protein expression increases, iron levels tend to decrease significantly.

Figure 7 shows the two oxidative stress biomarkers measured in GDM-PSUI and in control groups (values are expressed as means and standard deviations). None statistically significant difference was detected.

Discussion

In this study, we aimed to explore the correlations between dietary intake and the expression of specific genes and proteins in women with GDM and PSUI. Our results revealed significant elevations in fasting blood glucose and glycated hemoglobin in the GDM-PSUI group compared to non-GDM counterparts, along with increased magnesium and zinc intake but reduced vitamin D levels. However, these dietary changes did not translate into corresponding alterations in serum micronutrient levels, indicating a complex interplay that warrants further investigation. Thus, while our hypothesis is partially supported through observed correlations, the lack of direct associations between dietary intake and biomarker levels suggests the need for additional research to clarify these relationships.

GDM is a transient condition by definition, neverthess data indicate that women with GDM and PSUI remain at an elevated risk for long-term UI[58–60]. Therefore, in order to enhance the quality of life for women diagnosed with GDM, it is imperative to understand the correlation between GDM and PSUI. It is noteworthy that in previous studies, we have established maternal Hyperglycemic Myopathy as the fundamental mechanism underlying PSUI in GDM women [61–65].

Pregnant women frequently experience nutritional deficiencies, and following a diet rich in macronutrients and micronutrients during pregnancy and childbirth may promote optimal fetal development and protect mother and fetus from potential metabolic complications [28]. This study detailed the combined effects of pregnancy dietary food intake and serum micronutrient levels on the expression of MTF1, RXRA, TFRC, and TRPM6 genes and proteins, as well as oxidative stress biomarkers in GDM women with PSUI. According to the results women with GDM and PSUI exhibited higher magnesium and zinc intake and lower vitamin D dietary intake compared to non-GDM continent women. However, this variation had no impact on the serum concentrations of equivalent plasmatic micronutrients. Despite the GDM and PSUI group having a higher magnesium intake, pregnant women across all four groups failing to meet the recommended levels (290 mg–300 mg) as outlined by [66]. As reported in a study lipid profiles and glucose regulation in pregnant women with GDM were improved after six weeks of magnesium supplementation (250mg/day) and vitamin E (400IU/day) improved [67]. Moreover, according to a recent meta-analysis women with GDM achieved substantial gains glycemic control by supplementing their diets with magnesium, zinc, selenium, calcium, vitamin D, and E (either individually or in combination) [68]. This observed improvement can be attributable to a

P-value: the probability of observing a test statistic value greater than or equal to the one found; MTF1: metal regulatory transcription factor 1; RXRA: retinoid X receptor alpha; TRFC: transferrin receptor; TRPM6: transient receptor potential cation channel subfamily M member 6.

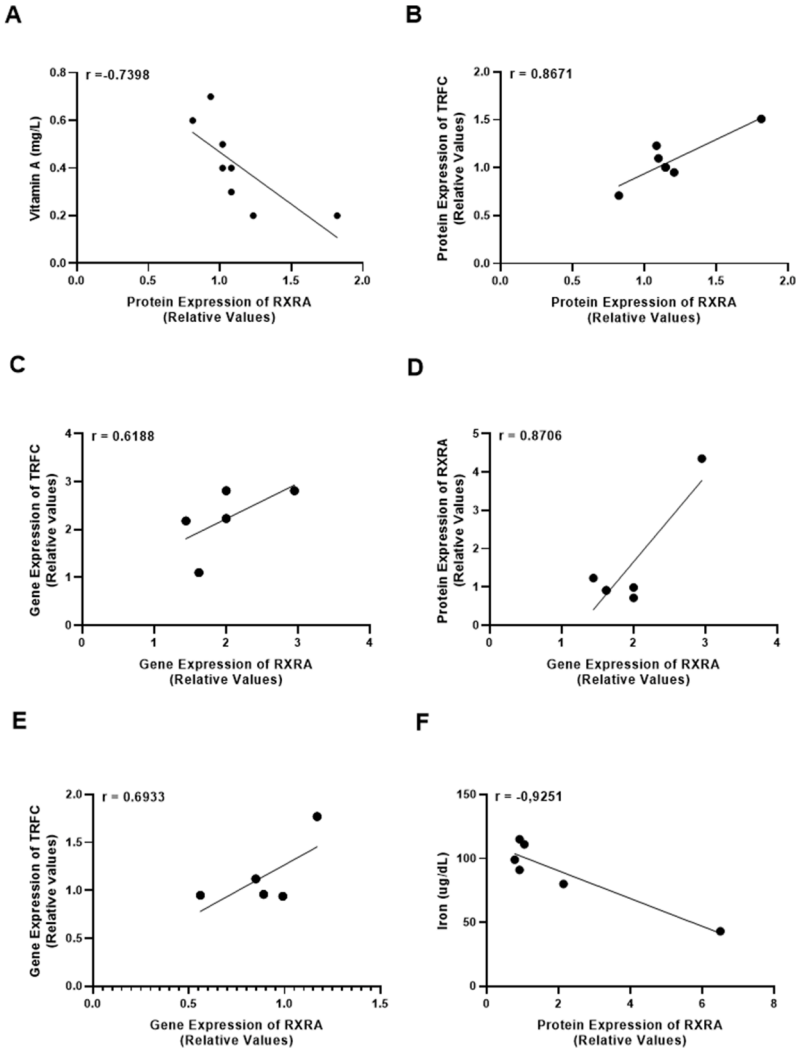

Figure 6. Pearson correlation analysis among selected variables in different study groups, highlighting associations between nutrient levels, protein expression, and gene expression. Correlation coefficients and *P*-values indicate the strength and significance of relationships, suggesting potential interactions relevant to GDM and PSUI status. (**A**) Correlation between serum vitamin A (Y-axis) and RXRA protein expression (X-axis) in the non-gestational diabetes mellitus and continent group (non-GDM-C; $P = 0.0359$; $^*P<0.05$). (**B**) Correlation between TRFC protein expression (Y-axis) and RXRA protein expression (X-axis) in the non-gestational diabetes mellitus and incontinent group (non-GDM-PSUI; $P = 0.0115$; $^*P<0.05$). (**C**) Correlation between TRFC gene expression (Y-axis) and RXRA gene expression (X-axis) in the gestational diabetes mellitus and continent group (GDM-C; $P = 0.0139$; $^*P<0.05$). (**D**) Correlation between RXRA protein expression (Y-axis) and RXRA gene expression (X-axis) in the GDM-C group ($P = 0.0240$; $^*P<0.05$). (**E**) Correlation between TRFC gene expression (Y-axis) and RXRA gene expression (X-axis) in the gestational diabetes mellitus and postpartum stress urinary incontinence group (GDM-PSUI; $P = 0.0042$; $^*P<0.05$). (**F**) Correlation between serum iron (Y-axis) and RXRA protein expression (X-axis) in the GDM-PSUI group ($P = 0.0082$; $^*P<0.05$). List of abbreviations: Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus and incontinent group; *P*-value: probability of observing a test statistic as extreme as, or more extreme than, the observed value under the null hypothesis; r: Pearson correlation coefficient; RXRA: retinoid X receptor alpha; TRFC: transferrin receptor.

reduction in MDA levels, oxidative stress, inflammation and overall antioxidant capacity. Given the link between oxidative stress and endothelial dysfunction, these findings suggest potential improvements in endothelial performance among the participants in this cohort supplementation with 4,000 IU

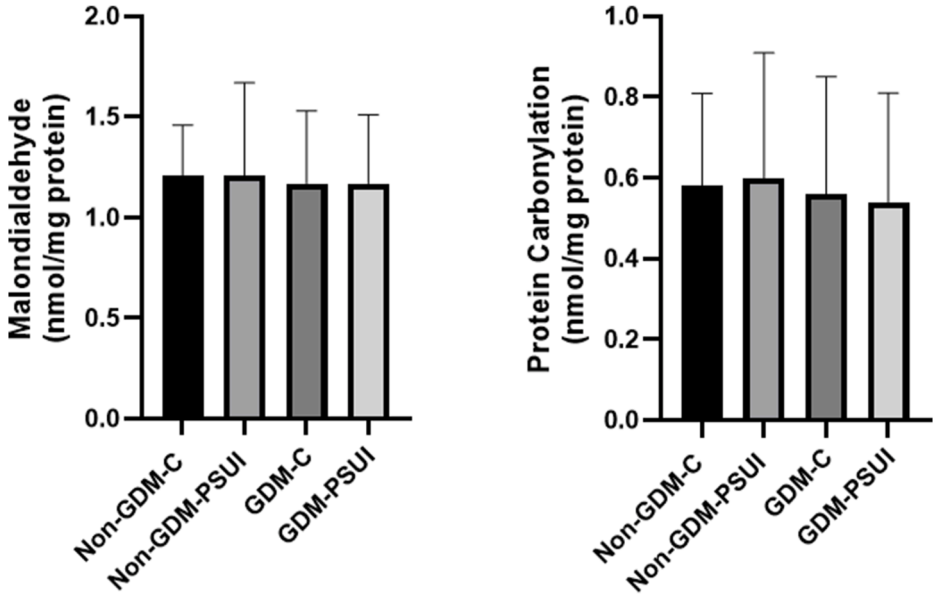

Figure 7. Oxidative stress biomarker levels in GDM-PSUI and control groups, demonstrating that no statistically significant differences were detected among the groups (values are expressed as means and standard deviations). This figure highlights that the presence of GDM and/or PSUI doesn't appear to significantly alter oxidative stress biomarker levels compared to controls. Statistical analysis was performed using ANOVA. List of Abbreviations: Non-GDM-C, non-gestational diabetes mellitus and continent group; Non-GDM-PSUI, non-gestational diabetes mellitus and incontinent group; GDM-C, gestational diabetes mellitus and continent group; GDM-PSUI, gestational diabetes mellitus and incontinent group; nmol/mg protein, nanomole per milligram.

vitamin D3 for six months in overweight, vitamin D-deficient GDM patients enhanced pancreatic beta cell function, as measured by the Quantitative Insulin Sensitivity Check Index [69].

In contrast to magnesium, the zinc intake of our study groups was in accordance with the daily recommendation of [66], which is 11 milligrams. Bo *et al.* [4] conducted a study examining the correlation between dietary zinc consumption and gestational hyperglycemia. The results indicated that a daily increase of 1 mg in zinc ingestion reduced the risk of GDM by 11% during the first 24–28 weeks of pregnancy. Conversely, Behboudi-Gandevani *et al.* [70] performed a study that investigated women between 14 and 20 weeks of gestation and revealed that dietary zinc intake falling below 50% of the recommended daily allowance did not correlate significantly with any adverse effects.

As mentioned before, we investigated the relationship between gene and protein expression and micronutrient uptake in blood cells of pregnant women with and without GDM and PSUI Initially, it was noted that there was no correlation between the elevated vitamin A levels and the RXRA protein expression in the healthy women (non-GDM-C). The results showed a decreased expression of RXRA in GDM women with urinary incontinence relative to continent GDM women. However, the levels of transcripts did not directly correlate with alterations in the corresponding protein levels. Perhaps, the protein biosynthesis was constrained by some limited nutrient uptake. Indeed, the explanatory power of genome-wide correlations between mRNA and protein expression levels is notoriously low, remaining around 40% across numerous studies [71]. Typically, alternative levels of regulation exist between the transcript and the protein product, which account for the discrepancy [72,73]. For example, recent research has shown that the activity of vitamin A depended on the activation of the RXRA nuclear receptor, which is affected by vitamin A status [35]. Furthermore, Mukwaya *et al.* [74] have demonstrated that the inflammatory process inhibits the expression of RXR. This finding implies that inflammation may disrupt the functionality of RXR and GDM, possibly affecting the biological processes in which the RXR is involved.

A concomitant decrease of TRFC gene and protein expression was observed in GDM incontinent women compared to GDM continent women. Literature shows that TRFC can be regulated by the amounts of intracellular iron [75]. According to Macedo and Sousa [42], when TRFC expression is increased, there is evidence of iron deficiency in cells. These results are consistent with our findings, as we also detected an increase in iron uptake and a reduction in TRFC expression in GDM and PSUI group. While the modulation of iron metabolism is the most widely recognized function of TRFC, it also exhibits an important impact on muscle physiology. In animal models, specific deletion of TRFC in muscle tissue resulted in numerous detrimental effects on muscle tissue, including muscle growth retardation. Iron uptake by muscle cells is impaired in the absence of TRFC, which has negative implications on protein synthesis and proper muscle development [76]. The role of iron in the development of GDM is uncertain, but high iron stores are linked to disturbances in glucose metabolism [77] and increased risk of type 2 diabetes [78].

In relation to oxidative stress, as assessed by MDA and protein carbonylation levels, there was no significant variation observed across the groups. Diabetes is a condition of glucotoxicity that leads to post-translational modifications, such as protein carbonylation [79]. A study involving health pregnant women from 24–28 gestational weeks, revealed an increase in plasma protein oxidation [80]. Moreover, at 24–28 weeks of gestation, pregnant women with GDM had also elevated levels of MDA and vitamin E compared to healthy pregnant women [81]. Biochemically, modified proteins are the causes of tissue damage [82]; however, our study did not detect this particular condition.

This study is limited by the lack of significant changes observed in serum micronutrient levels, despite differences in dietary intake. This observation suggests that variables such as nutrient bioavailability and the metabolic adaptations occurring during pregnancy may significantly influence the results, potentially masking the true effects of diet on health outcomes. Understanding these dynamics is crucial, as they underscore the complexity of nutrient metabolism in pregnant women.

Conclusion

This study presented new insight into the potential impact of dietary choices and intake in on pregnant women. Understanding homeostasis of minerals and vitamins via cellular processes appears to be crucial for the development of new therapies for disorders such as GDM. Our result mainly concerning the expression of TRFC in pregnant women with GDM-PSUI, might be used as a model for nutritional and/or pharmacological intervention in the population of pregnant women. It is our belief that individualized nutrition protocols, such as those offered by the field of nutrigenomics, could potentially contribute to treatment strategies in future.

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to privacy and ethical restrictions involving patient data but are available from the corresponding author on reasonable request.

Credit author statement

- **Conceptualization**: SMBC, JPCM, DCHF, DRAR, AMPB, MCVR
- **Methodology**: SMBC, MNF, ICD, SLF
- **Investigation**: SMBC, CRC
- **Data Curation**: SMBC, RLSH
- **Formal Analysis**: SMBC, RLSH
- **Writing - Original Draft**: SMBC, JPCM
- **Writing - Review & Editing**: SMBC, DCHF, DRAR, AMPB, MCVR
- **Supervision**: MCVR

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marilza Vieira Cunha Rudge reports financial support was provided by São Paulo State University Faculty of Medicine. Marilza Vieira Cunha Rudge reports a relationship with State of Sao Paulo Research Foundation that includes: board membership, funding grants, and non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Diamater Study Group

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References

[1] Memon B, Abdelalim EM. Stem Cell Therapy for Diabetes: Beta Cells versus Pancreatic Progenitors. Cells 2020 Jan 23;9(2):283.
[2] Ásbjörnsdóttir B, Ronneby H, Vestgaard M, Ringholm L, Nichum VL, Jensen DM, et al. Lower daily carbohydrate consumption than recommended by the Institute of Medicine is common among women with type 2 diabetes in early pregnancy in Denmark. Diabetes Res Clin Pract 2019 Jun;152:88–95.
[3] Johnson ST, Lynch B, Vallance J, Davenport MH, Gardiner PA, Butalia S. Sedentary behavior, gestational diabetes mellitus, and type 2 diabetes risk: where do we stand? Endocrine 2016 Apr 28;52(1):5–10.
[4] Bo S, Lezo A, Menato G, Gallo ML, Bardelli C, Signorile A, et al. Gestational hyperglycemia, zinc, selenium, and antioxidant vitamins. Nutrition 2005 Feb;21(2):186–91.
[5] Morisset AS, Côté JA, Michaud A, Robitaille J, Dubé MC, Veillette J, et al. Dietary intakes in the nutritional management of gestational diabetes mellitus. Can J Diet Pract Res 2014;75(2):64–71.
[6] Maged AM, Torky H, Fouad MA, GadAllah SH, Waked NM, Gayed AS, et al. Role of antioxidants in gestational diabetes mellitus and relation to fetal outcome: a randomized controlled trial. J Maternal-Fetal Neonatal Med 2016;29(24):4049–54.
[7] Parast VM, Paknahad Z. Antioxidant Status and Risk of Gestational Diabetes Mellitus: a Case-Control Study. Clin Nutr Res 2017;6(2):81.
[8] Dallosso H, Matthews R, McGrother C, Donaldson M. Diet as a risk factor for the development of stress urinary incontinence: a longitudinal study in women. Eur J Clin Nutr 2004 Jun 1;58(6):920–6.
[9] Maserejian NN, Mcvary KT, Giovannucci EL, Mckinlay JB. Dietary Macronutrient Intake and Lower Urinary Tract Symptoms in Women. Ann Epidemiol 2011 Jun;21(6):421–9.

[10] Maserejian NN, Giovannucci EL, McVary KT, McKinlay JB. Intakes of Vitamins and Minerals in Relation to Urinary Incontinence, Voiding, and Storage Symptoms in Women: A Cross-Sectional Analysis from the Boston Area Community Health Survey. Eur Urol 2011 Jun;59(6):1039–47.
[11] Gong R, Liu Y, Luo G, Yang L. Dietary Magnesium Intake Affects the Vitamin D Effects on HOMA-β and Risk of Pancreatic β-Cell Dysfunction: A Cross-Sectional Study. Front Nutr 2022;9.
[12] Blumer I, Hadar E, Hadden DR, Jovanovič L, Mestman JH, Murad MH, et al. Diabetes and Pregnancy: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2013 Nov 1;98(11):4227–49.
[13] Dianna J. Magliano EJBIDA 10th edition scientific committee. IDF DIABETES ATLAS. 10th edition 2021.
[14] Donazar-Ezcurra M, López-del Burgo C, Bes-Rastrollo M. Primary prevention of gestational diabetes mellitus through nutritional factors: a systematic review. BMC Pregnancy Childbirth 2017 Dec 13;17(1):30.
[15] Orozco Marilin Camacho R, Mercado N. Silvia Gorbán de Lapertosa (Argentina), María Amelia Linari (Argentina). Revista de la Sociedad Argentina de Diabetes 2016;50:117–28.
[16] Raghavan R, Dreibelbis C, Kingshipp BL, Wong YP, Abrams B, Gernand AD, et al. Dietary patterns before and during pregnancy and maternal outcomes: a systematic review. Am J Clin Nutr 2019 Mar;109:705S–28S.
[17] Jacobs DR, Tapsell LC. Food, Not Nutrients, Is the Fundamental Unit in Nutrition. Nutr Rev 2008 Jun 28;65(10):439–50.
[18] Mijatovic-Vukas J, Capling L, Cheng S, Stamatakis E, Louie J, Cheung N, et al. Associations of Diet and Physical Activity with Risk for Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis. Nutrients 2018 May 30;10(6):698.
[19] Mousa A, Naqash A, Lim S. Macronutrient and Micronutrient Intake during Pregnancy: An Overview of Recent Evidence. Nutrients 2019 Feb 20;11(2):443.
[20] Neeha VS, Kinth P. Nutrigenomics research: a review. J Food Sci Technol 2013 Jun 19;50(3):415–28.
[21] Marcum JA. Nutrigenetics/Nutrigenomics, Personalized Nutrition, and Precision Healthcare. Curr Nutr Rep 2020 Dec 23;9(4):338–45.
[22] Bull C, Fenech M. Genome-health nutrigenomics and nutrigenetics: nutritional requirements or 'nutriomes' for chromosomal stability and telomere maintenance at the individual level. Proc Nutr Soc 2008 May 15;67(2):146–56.
[23] Hendriks HFJ. Use of nutrigenomics endpoints in dietary interventions. Proc Nutr Soc 2013 Aug 28;72(3):348–51.
[24] Cousins RJ. Nutritional regulation of gene expression. Am J Med 1999 Jan;106(1):20–3.
[25] Fenech M. Micronutrients and genomic stability: a new paradigm for recommended dietary allowances (RDAs). Food Chem Toxicol 2002 Aug;40(8):1113–7.
[26] Fenech M. Genome health nutrigenomics and nutrigenetics – diagnosis and nutritional treatment of genome damage on an individual basis. Food Chem Toxicol 2008 Apr;46(4):1365–70.
[27] Rist MJ, Wenzel U, Daniel H. Nutrition and food science go genomic. Trends Biotechnol 2006 Apr;24(4):172–8.
[28] Plows JF, Reynolds CM, Vickers MH, Baker PN, Stanley JL. Nutritional Supplementation for the Prevention and/or Treatment of Gestational Diabetes Mellitus. Curr Diab Rep 2019 Sep 1;19(9):73.
[29] Trasino SE, Benoit YD, Gudas LJ. Vitamin A Deficiency Causes Hyperglycemia and Loss of Pancreatic β-Cell Mass. J Biol Chem 2015 Jan;290(3):1456–73.
[30] Trasino SE, Tang XH, Jessurun J, Gudas LJ. Retinoic acid receptor β2 agonists restore glycaemic control in diabetes and reduce steatosis. Diabetes Obes Metab 2016;18:142–51.
[31] Trasino SE, Gudas LJ. Vitamin A: a missing link in diabetes? Diabetes Manag 2015 Sep;5(5):359–67.
[32] Zhou Y, Wang H, Zhou J, Qiu S, Cai T, Li H, et al. Vitamin A and Its Multi-Effects on Pancreas: Recent Advances and Prospects. Front Endocrinol (Lausanne) 2021 Feb 18;12.
[33] Almeida NR, Conda-Sheridan M. A review of the molecular design and biological activities of RXR agonists. Med Res Rev 2019 Jul 3;39(4):1372–97.
[34] Kölsch H, Lütjohann D, Jessen F, Popp J, Hentschel F, Kelemen P, et al. RXRA gene variations influence Alzheimer's disease risk and cholesterol metabolism. J Cell Mol Med 2009 Mar 24;13(3):589–98.
[35] yuan Yu X, Song L ping, Zheng H ting, Wei S dan, Wen X lan, Huang B, et al. Association between functional genetic variants in retinoid X receptor -α/γ and the risk of gestational diabetes mellitus in a southern Chinese population. Biosci Rep 2021 Oct 29;41(10).
[36] Zhao S, Li R, Li Y, Chen W, Zhang Y, Chen G. Roles of vitamin A status and retinoids in glucose and fatty acid metabolism. Biochem Cell Biol 2012 Apr;90(2):142–52.
[37] Curtis EM, Krstic N, Cook E, D'Angelo S, Crozier SR, Moon RJ, et al. Gestational Vitamin D Supplementation Leads to Reduced Perinatal RXRA DNA Methylation: Results From the MAVIDOS Trial. J Bone Mineral Res 2019;34(2).
[38] Afzal S, Bojesen SE, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: A prospective cohort study and metaanalysis. Clin Chem 2013;59(2).
[39] Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990 May 1;75(9):1870–6.
[40] Gammella E, Buratti P, Cairo G, Recalcati S. The transferrin receptor: the cellular iron gate. Metallomics 2017;9(10):1367–75.
[41] Gammella E, Lomoriello IS, Conte A, Freddi S, Alberghini A, Poli M, et al. Unconventional endocytosis and trafficking of transferrin receptor induced by iron. Mol Biol Cell 2021 Jan 15;32(2):98–108.
[42] Macedo M, Sousa M. Transferrin and the Transferrin Receptor: Of Magic Bullets and Other Concerns. Inflamm Allergy-Drug Targets 2008 Mar 1;7(1):41–52.
[43] Lee CC, Yang PK, Chen LC, Cheong ML, Tsai YL, Tsai MS. Associations between gene expression of magnesium transporters and glucose metabolism in pregnancy. J Formos Med Assoc 2022 Jul;121(7):1231–7.
[44] Goker Tasdemir U, Tasdemir N, Kilic S, Abali R, Celik C, Gulerman HC. Alterations of ionized and total magnesium levels in pregnant women with gestational diabetes mellitus. Gynecol Obstet Invest 2015;79(1):19–24.
[45] Chausmer AB. Zinc, Insulin and Diabetes. J Am Coll Nutr 1998 Apr;17(2):109–15.
[46] Fan J, Zhang T, Yu Y, Zhang B. Is serum zinc status related to gestational diabetes mellitus? A meta-analysis. Matern Child Nutr 2021 Oct 4;17(4).

[47] Lindert U, Cramer M, Meuli M, Georgiev O, Schaffner W. Metal-Responsive Transcription Factor 1 (MTF-1) Activity Is Regulated by a Nonconventional Nuclear Localization Signal and a Metal-Responsive Transactivation Domain. Mol Cell Biol 2009;29(23):6283–93.
[48] Troadec MB, Ward DM, Lo E, Kaplan J, De Domenico I. Induction of FPN1 transcription by MTF-1 reveals a role for ferroportin in transition metal efflux. Blood 2010 Nov 25;116(22):4657–64.
[49] Hennigar SR, Kelley AM, Anderson BJ, Armstrong NJ, McClung HL, Berryman CE, et al. Sensitivity and reliability of zinc transporter and metallothionein gene expression in peripheral blood mononuclear cells as indicators of zinc status: responses to *ex vivo* zinc exposure and habitual zinc intake in humans. Br J Nutr 2021 Feb 28;125(4):361–8.
[50] Rudge MVC, Souza FP, Abbade JF, Hallur RLS, Marcondes JPC, Piculo F, et al. Study protocol to investigate biomolecular muscle profile as predictors of long-term urinary incontinence in women with gestational diabetes mellitus. BMC Pregnancy Childbirth 2020 Dec 19;20(1):117.
[51] Costa SMB, Hallur RLS, Reyes DRA, Floriano JF, de Barros Leite Carvalhaes MA, de Carvalho Nunes HR, et al. Role of dietary food intake patterns, anthropometric measures, and multiple biochemical markers in the development of pregnancy-specific urinary incontinence in gestational diabetes mellitus. Nutrition 2024 Jan 1:117.
[52] Lippi G, Salvagno GL, Montagnana M, Franchini M, Guidi GC. Venous stasis and routine hematologic testing. Clin Lab Haematol 2006 Oct;28(5):332–7.
[53] Funk DM, Clinical and Laboratory Standards Institute. Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays: approved guideline. Clinical and Laboratory Standards Institute; 2008. p. 33.
[54] Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2-\Delta\Delta CT$ Method. Methods 2001 Dec;25(4):402–8 [cited 2020 Feb 4] Available from: https://linkinghub.elsevier.com/retrieve/pii/S1046202301912629.
[55] Suzuki YJ, Carini M, Butterfield DA. Protein Carbonylation. Antioxid Redox Signal 2010 Feb;12(3):323–5.
[56] Mesquita CS, Oliveira R, Bento F, Geraldo D, Rodrigues JV, Marcos JC. Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal Biochem 2014 Aug 1;458:69–71.
[57] Samarghandian S, Farkhondeh T, Samini F, Borji A. Protective Effects of Carvacrol against Oxidative Stress Induced by Chronic Stress in Rat's Brain, Liver, and Kidney. Biochem Res Int 2016;2016:1–7.
[58] Barbosa AMP, Dias A, Marini G, Calderon IMP, Witkin S, Rudge MVC. Urinary incontinence and vaginal squeeze pressure two years post-cesarean delivery in primiparous women with previous gestational diabetes mellitus. Clinics (Sao Paulo) 2011;66(8) [cited 2017 Jan 24] 1341–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21915481.
[59] Chuang CM, Lin IF, Horng HC, Hsiao YH, Shyu IL, Chou P. The impact of gestational diabetes mellitus on postpartum urinary incontinence: A longitudinal cohort study on singleton pregnancies. BJOG 2012;119(11).
[60] Kim C, McEwen LN, Sarma AV, Piette JD, Herman WH. Stress urinary incontinence in women with a history of gestational diabetes mellitus. J Womens Health 2008;17(5).
[61] Kron-Rodrigues MR, Rudge MVC, Lima SAM. Supplementation of Vitamin D in the Postdelivery Period of Women with Previous Gestational Diabetes Mellitus: Systematic Review and Meta-Analysis of Randomized Trials. Revista Brasileira de Ginecologia e Obstetrícia/RBGO Gynecol Obstet 2021 Sep 20;43(9):699–709.
[62] Prudencio CB, Rudge MVC, Pinheiro FA, Sartorão Filho CI, Nunes SK, Pedroni CR, et al. Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. PLoS One 2019 Nov 7;14(11):e0223261.
[63] Raimondo D, Youssef A, Mabrouk M, Del Forno S, Martelli V, Pilu G, et al. Pelvic floor muscle dysfunction on 3D/4D transperineal ultrasound in patients with deep infiltrating endometriosis: a pilot study. Ultrasound Obstet Gynecol 2017 Oct;50(4):527–32.
[64] Sartorão Filho CI, Barbosa AMP, Calderon I de MP, Rudge MVC. Assessment of Pelvic Floor Disorders due to the Gestational Diabetes Mellitus Using Three-Dimensional Ultrasonography: A Narrative Review. Revista Brasileira de Ginecologia e Obstetrícia/RBGO Gynecol Obstet 2022 Dec 29;44(12):1134–40.
[65] Vesentini G, Barbosa AMP, Damasceno DC, Marini G, Piculo F, Matheus SMM, et al. Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women. PLoS One 2020 Apr 3;15(4):e0231096.
[66] Kominiarek MA, Rajan P. Nutrition Recommendations in Pregnancy and Lactation. Med Clin North America 2016 Nov;100(6):1199–215.
[67] Maktabi M, Jamilian M, Amirani E, Chamani M, Asemi Z. The effects of magnesium and vitamin e co-supplementation on parameters of glucose homeostasis and lipid profiles in patients with gestational diabetes. Lipids Health Dis 2018;17(1).
[68] Li D, Cai Z, Pan Z, Yang Y, Zhang J. The effects of vitamin and mineral supplementation on women with gestational diabetes mellitus. BMC Endocr Disord 2021 Dec 24;21(1):106.
[69] Yeow TP, Lim SL, Hor CP, Khir AS, Wan Mohamud WN, Pacini G. Impact of Vitamin D Replacement on Markers of Glucose Metabolism and Cardio-Metabolic Risk in Women with Former Gestational Diabetes—A Double-Blind, Randomized Controlled Trial. PLoS One 2015 Jun 9;10(6):e0129017.
[70] Behboudi-Gandevani S, Safary K, Moghaddam-Banaem L, Lamyian M, Goshtasbi A, Alian-Moghaddam N. The relationship between maternal serum iron and zinc levels and their nutritional intakes in early pregnancy with gestational diabetes. Biol Trace Elem Res 2013;154(1):7–13.
[71] Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 2012;13(4).
[72] Maier T, Güell M, Serrano L. Correlation of mRNA and protein in complex biological samples. FEBS Lett 2009;583.
[73] Li X, Jie Q, Zhang H, Zhao Y, Lin Y, Du J, et al. Disturbed MEK/ERK signaling increases osteoclast activity via the Hedgehog-Gli pathway in postmenopausal osteoporosis, 122. Progress in Biophysics and Molecular Biology. Elsevier Ltd; 2016. p. 101–11.
[74] Mukwaya A, Lennikov A, Xeroudaki M, Mirabelli P, Lachota M, Jensen L, et al. Time-dependent LXR/RXR pathway modulation characterizes capillary remodeling in inflammatory corneal neovascularization. Angiogenesis 2018;21(2).

[75] Wallander ML, Leibold EA, Eisenstein RS. Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochim Biophys Acta (Bba) - Mol Cell Res 2006 Jul;1763(7):668–89.
[76] Ding H, Chen S, Pan X, Dai X, Pan G, Li Z, et al. Transferrin receptor 1 ablation in satellite cells impedes skeletal muscle regeneration through activation of ferroptosis. J Cachexia Sarcopenia Muscle 2021;12(3).
[77] Yang A, Zhao J, Lu M, Gu Y, Zhu Y, Chen D, et al. Expression of hepcidin and ferroportin in the placenta, and ferritin and transferrin receptor 1 levels in maternal and umbilical cord blood in pregnant women with and without gestational diabetes. Int J Environ Res Public Health 2016;13(8).
[78] Montonen J, Boeing H, Steffen A, Lehmann R, Fritsche A, Joost HG, et al. Body iron stores and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Diabetologia 2012 Oct 1;55(10):2613–21.
[79] Zheng H, Wu J, Jin Z, Yan LJ. Protein Modifications as Manifestations of Hyperglycemic Glucotoxicity in Diabetes and Its Complications. Biochem Insights 2016 Jan 23;9. BCI.S36141.
[80] Gelisgen R, Genc H, Kayali R, Oncul M, Benian A, Guralp O, et al. Protein oxidation markers in women with and without gestational diabetes mellitus: A possible relation with paraoxonase activity. Diabetes Res Clin Pract 2011 Dec;94(3):404–9.
[81] Ma H, Qiao Z, Li N, Zhao Y, Zhang S. The relationship between changes in vitamin A, vitamin E, and oxidative stress levels, and pregnancy outcomes in patients with gestational diabetes mellitus. Ann Palliat Med 2021 Jun;10(6):6630–6.
[82] Cederberg J, Basu S, Eriksson UJ. Increased rate of lipid peroxidation and protein carbonylation in experimental diabetic pregnancy. Diabetologia 2001 May 28;44(6):766–74.