UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

Sarah Maria Barneze Costa

NÍVEIS DE NUTRIENTES E DA EXPRESSÃO DE SEUS RECEPTORES EM MULHERES COM DIABETES MELLITUS GESTACIONAL E INCONTINÊNCIA URINÁRIA ESPECÍFICA DA GESTAÇÃO

Nutrient levels and the expression of their receptors in women with gestational diabetes mellitus and pregnancy specific urinary incontinence

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Doutora em Tocoginecologia.

Orientadora: Prof.ª Emérita Marilza Vieira Cunha Rudge

Coorientadora: Prof.ª Dra. Angélica Mércia Pascon Barbosa

Coorientador: Prof. Dr. Raghavendra Hallur Lakshmana Shetty

Botucatu
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Orientador: Marilza Vieira Cunha Rudge
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Coorientador: Raghavendra Hallur Lakshmana Shetty
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1. Diabetes Gestacional. 2. Expressão gênica. 3. Incontinência urinária. 4. Minerais. 5. Vitaminas.

Palavras-chave: Diabetes Gestacional; Expressão Gênica; Incontinência Urinária; Minerais; Vitaminas.

Impacto esperado na sociedade desta tese de doutorado produzida no Programa de Pós-graduação em Tocoginecologia da UNESP

O conhecimento das possíveis alterações promovidas pelo Diabetes *Mellitus* Gestacional (DMG) nos níveis de macronutrientes e micronutrientes, bem como na expressão de seus receptores específicos, possui um potencial científico, técnico, social, inovador, econômico, educacional e cultural significativo. O estudo abrirá novas perspectivas para o diagnóstico, tratamento e prevenção da Incontinência Específica da Gestação (IU-EG), além de promover avanços na medicina personalizada e melhorar a saúde materna e fetal.

Do ponto de vista científico, entender essas alterações permitirá identificar biomarcadores para a IU-EG, possibilitando um diagnóstico mais preciso e eficiente. Isso impulsionará avanços na pesquisa científica relacionada à gravidez e às doenças metabólicas, abrindo caminho para novas descobertas e terapias.

Em termos técnicos, o conhecimento dessas alterações proporcionará a base para o desenvolvimento de métodos de triagem mais sofisticados e precisos, permitindo uma detecção precoce da IU-EG. Isso terá um impacto direto na prática clínica, possibilitando medidas terapêuticas e preventivas adequadas, promovendo a saúde materna e fetal.

No aspecto social, o entendimento dessas alterações permitirá fornecer orientações dietéticas e recomendações de suplementação nutricional personalizadas para mulheres com risco de desenvolver IU-EG. Isso contribuirá para a promoção de uma gestação saudável e redução dos impactos negativos na saúde das mães e bebês.

Além disso, do ponto de vista inovador, a descoberta de biomarcadores e a implementação de medidas terapêuticas e preventivas baseadas na alimentação adequada e suplementação nutricional abrirão portas para novas abordagens no campo da medicina personalizada,

onde o tratamento é adaptado às necessidades individuais.

No âmbito econômico, a identificação desses biomarcadores e a adoção de medidas preventivas terão um impacto positivo nos sistemas de saúde, reduzindo os custos associados ao tratamento de complicações da IU-EG e melhorando a eficiência dos cuidados pré-natais.

Do ponto de vista educacional e cultural, o avanço nesse campo de pesquisa ampliará o conhecimento científico sobre a gravidez e as doenças metabólicas associadas. Isso será compartilhado através de publicações, conferências e outros meios de divulgação, contribuindo para a educação médica e a conscientização pública sobre a importância da alimentação adequada durante a gravidez.

Expected Impact on Society of this doctoral thesis produced in the Postgraduate Program in Obstetrics and Gynecology at UNESP

The knowledge of the potential alterations caused by Gestational Diabetes Mellitus (GDM) in macronutrient and micronutrient levels, as well as in the expression of their specific receptors, holds significant scientific, technical, social, innovative, economic, educational, and cultural potential. This study will open new perspectives for the diagnosis, treatment, and prevention of Gestational-Specific Incontinence (PSUI) while promoting advances in personalized medicine and improving maternal and fetal health.

From a scientific standpoint, understanding these alterations will enable the identification of biomarkers for GSI, allowing for a more precise and efficient diagnosis. This will drive advancements in scientific research related to pregnancy and metabolic disorders, paving the way for discoveries and therapies.

In technical terms, knowledge of these alterations will provide the foundation for the development of more sophisticated and accurate screening methods, enabling early detection of GSI. This will have a direct impact on clinical practice, facilitating appropriate therapeutic and preventive measures that promote maternal and fetal health.

On the social front, understanding these alterations will enable the provision of personalized dietary guidelines and recommendations for nutritional supplementation to women at risk of developing GSI. This will contribute to the promotion of a healthy pregnancy and reduce negative impacts on the health of mothers and babies.

Moreover, from an innovative perspective, the discovery of biomarkers and the implementation of therapeutic and preventive measures based on appropriate nutrition and nutritional supplementation will open doors to new approaches in personalized medicine, where treatment is tailored to individual needs.

From an economic standpoint, the identification of these biomarkers and the adoption of preventive measures will have a positive impact on healthcare systems by reducing costs associated with the treatment of GSI complications and improving the efficiency of prenatal care.

From an educational and cultural standpoint, advancements in this research field will expand scientific knowledge about pregnancy and associated metabolic disorders. This knowledge will be shared through publications, conferences, and other means of dissemination, contributing to medical education and public awareness of the importance of proper nutrition during pregnancy.

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Epígrafe

“Nada verdadeiramente valioso surge da ambição ou de um mero senso de dever, mas de amor e devoção para com os homens e para com as coisas objetivas.”

Albert Einstein

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Lista de Abreviaturas

Português e Inglês

Lista de abreviaturas em português

CEP	Comitê de Ética em Pesquisa
CIDPN	Centro de Investigação do Diabete Perinatal
DM	Diabetes *Mellitus*
DMG	Diabetes *Mellitus* Gestacional
Dra.	Doutora
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
FMB	Faculdade de Medicina de Botucatu
HCFMB	Hospital das Clínicas da Faculdade de Medicina de Botucatu
IU-EG	Incontinência Urinária Específica da Gestação
Prof.	Professor
Prof.ª	Professora
UNESP	Universidade Estadual Paulista – “Julio de Mesquita Filho”
UNIPEX	Unidade de Pesquisa Experimental

Lista de abreviações em Inglês

%	Percentage
ºC	Celsius
µl	Microliter
AI	Adequate Intake
BMI	Body Mass Index
cm	Centimeter
cm²	Centimeter per Square
dL	Deciliter
DM	Diabetes Mellitus
DNA	DeoxyriboNucleic acid
DNPH	2,4-Dinitrophenylhydrazine
DRI	Dietary Reference Intakes
Et al.	“And Others”
FPGS	Folylpolyglutamate Synthase
g	Grams
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GDM	Gestational Diabetes Mellitus
HIV	Human Immunodeficiency Virus
Kg	Kilogram
L	Liters
m	Meter
m²	Square Meter
MDA	Malondialdehyde
mcg	Micrograms
ml	Milliliter
mg	Milligram
mm	Millimeter
MTF1	Metal Regulatory Transcription Factor 1
n	The population sizes
nm	Nanometer
OGTT	glucose tolerance test

PSUI	pregnancy-specific urinary incontinence
p	P-value
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
RXRA	Retinoid X Receptor Alpha
TBA	Trichloroacetic acid
TFRC	Transferrin Receptor
TRAP1	TNF Receptor Associated Protein 1
TRPM6	Transient Receptor Potential Cation Channel Subfamily M Member 6
ug	Microgram
UI	International Units

Sumário

Seção 1
Trajetória Acadêmica

Desde criança, fui interessada por alimentação saudável e elaborações de receitas culinárias. Além disso, sempre tive uma rotina equilibrada com a prática de exercícios físicos diários e fiz *ballet* por anos.

Quando cheguei ao terceiro colegial, optei por fazer Nutrição após um teste vocacional na escola, e iniciei meus estudos. Escolhi a nutrição como profissão com o intuito de ajudar as pessoas a se sentirem melhores e serem mais saudáveis, podendo prevenir futuras doenças.

Na época da faculdade, a disciplina que mais me chamava à atenção era a de nutrição materno-infantil, a que mais gostava e me identificava. A apostila de nutrição materno-infantil era a mais utilizada e lida.

Depois de me formar em 2012, comecei a clinicar em minha cidade, Jaú, e fazer especialização em nutrição clínica e aprimoramento hospitalar. Atendia gestantes e crianças, e trabalhei, temporariamente, na maternidade Santa Isabel em Bauru. Mas, uma coisa não saia da minha mente, desde a saída da faculdade, a vontade de fazer Mestrado, o desejo era grande.

Após dois anos clinicando em Jaú, conheci uma Prof.ª da especialização, Dra. Maria Fernanda Biagioni, que me falou da oportunidade de fazer Mestrado em Botucatu com o grupo de pesquisa de doenças endocrinológicas comandado pela Prof.ª Titular Célia Regina Nogueira.

Em 2015, entrei no Mestrado em Fisiopatologia em Clínica Médica pela FMB-UNESP com bolsa FAPESP (processo: 14/15529-0) e fiz sanduiche na Universidade de Lisboa, em Portugal. O estágio internacional foi na Universidade de Lisboa pelo projeto: “RNA regulomics the role of non coding RNA in human health and disease”. Também, na época do mestrado fui membro da comissão permanente de pesquisa por 1 ano da FMB-UNESP.

Depois de concluir meu mestrado em 2017, decidi me aperfeiçoar na pesquisa clínica e conheci o grupo de pesquisa Diamater através da Dra. Juliana Floriano. Me interessei pelos projetos do grupo voltados para a população de gestantes. No grupo Diamater, estavam precisando de alguém para realizar análise de expressão proteica, e como tinha experiência nessa prática, integrei no grupo de pesquisa Diamater como bolsista PROPe no Apoio a Equipamentos Multiusuários da UNESP (Projeto Vigentes de Grande Porte) sob responsabilidade da Prof.ª Emérita Marilza Vieira Cunha Rudge.

Em 2019, comecei o Doutorado em Tocoginecologia com a Prof.ª Emérita

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Seção 2
Contextualização

Conhecimento das possíveis alterações promovidas pelo Diabetes *Mellitus* Gestacional em nível de nutrientes e da expressão de seus receptores para fornecer informações para a identificação de biomarcadores para a Incontinência Urinária Específica da Gestação

O Diabetes *Mellitus* Gestacional (DMG) mesmo tratado de acordo com padrões internacionais da *American Diabetes Association* (ADA), e institucionalizado no Centro de Investigação do Diabete Perinatal (CIDPN- projeto InfraIV da Fapesp), está associado com a maior ocorrência de Incontinência Urinária (IU) 2 anos pós-parto cesárea.

O tratamento com dieta isolada ou associada à insulina foi insuficiente para evitar o efeito deletério da hiperglicemia gestacional controlada sobre a IU – específica da gestação (IU-EG) que foi fator de risco para IU 2 anos pós-parto e disfunção muscular do assoalho pélvico. Barbosa et al., 2011 [1], a procura de marcadores dessa miopatia caracteriza o projeto temático Diamater intitulado "Coorte da tríade gestacional: hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica na predição de incontinência e disfunção muscular e pesquisa translacional com biodevice para regeneração muscular em ratas" (Processo FAPESP: 2016/01743-5).

O Modelo Conceitual Integrado do Diamater estabelecido (Figura 1) evidencia essa relação entre DMG e IU-EG, miopatia hiperglicêmcia e IU 12 meses pós-cesárea.

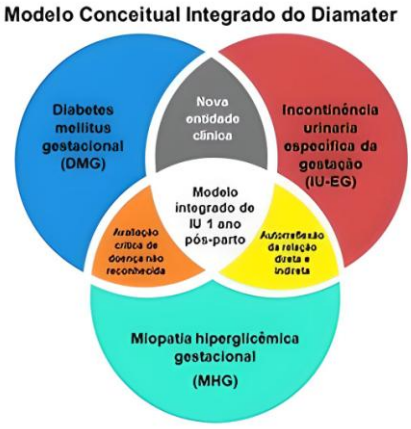

Figura 1. Modelo Conceitual Integral do Diamater entre DMG, UI-EG e miopatia hiperglicêmica na prevalência de UI em longo prazo.

A presente tese de Doutorado se insere como variável moderadora que precisa ser analisada em profundidade para avaliar o impacto da variável preditora (DMG e IU-EG) sobre o desfecho (IU 12 meses pós-parto cesária). Nessa tese de Doutorado, a variável mediadora (Miopatia hiperglicêmica) não foi analisada (Figura 2).

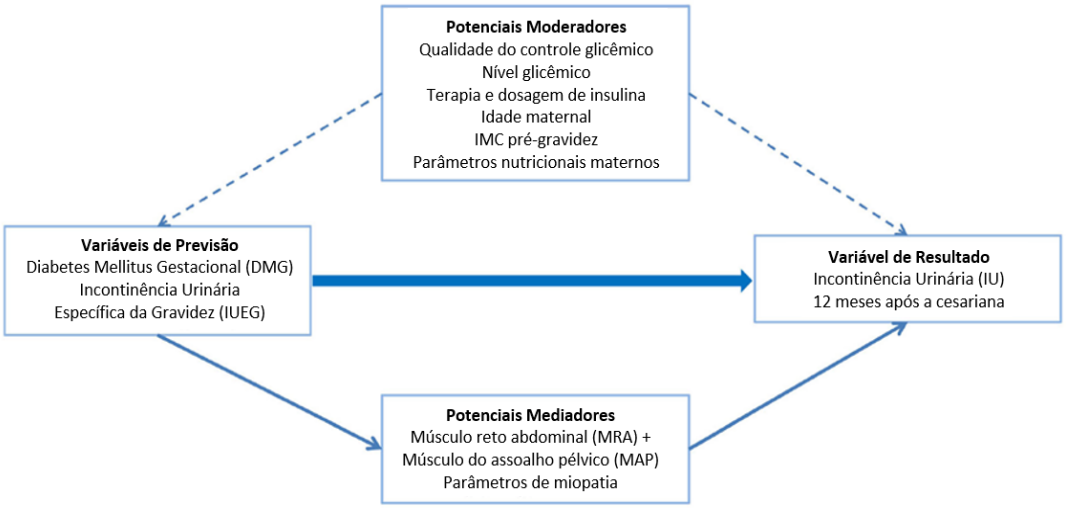

Figura 2. Mediadores e moderadores entre a variável preditora e o desfecho do DIAMATER (Projeto Temático).

Durante o período gestacional, a mulher passa por diversas adaptações endócrinas e metabólicas desde modificações na sensibilidade a insulina até alterações nos níveis circulantes do colesterol [2,3]. Com esses eventos, ocorre a produção de hormônios como é o caso do lactogênio placentário conforme o crescimento placentário. A placenta é o órgão separador da circulação materna e fetal, e a secreção de hormônios é associada ao efeito anti-insulina proporcionado por hormônios, o que contribui para a progressão da resistência insulínica, tendo o tecido muscular da gestante maior impacto com a resistência insulínica do que o tecido gorduroso [4]. Além disso, ainda no primeiro trimestre da gravidez, a resistência à insulina encontra-se em níveis normais, porém, conforme a evolução da gestação, essa condição tende a aumentar e levar ao quadro possilvelmente diabetogênico associado a outros fatores maternos como a idade avançada e a obesidade [5].

O DMG é caracterizado pela hiperglicemia e qualquer nível de intolerância a glicose diagnosticado durante a gravidez pelo rastreamento entre 24-28 semanas

gestacionais [5,6]. As mulheres que apresentaram disfunções metabólicas subclínicas tais como sensibilidade a insulina e/ou produção pancreática não adequada previamente à gravidez, possuem mais chances de desenvolver o DMG, bem como as gestantes obesas. Ao contrário, aquelas gestantes com peso normal tem menor probabilidade de desenvolver o DMG [2,3]. O DMG é a desordem metabólica mais comum na gestação. A prevalência mundial de DMG é de 14%, resultante em 20 milhões de nascimentos por ano [7]. As possíveis causas do DMG não são bem estabelecidas, porém o *status* nutricional, sedentarismo, predisposição genética e obesidade são as mais investigadas [8]. As alterações nos níveis do lactogênio placentário e fator de crescimento semelhante à insulina tipo 1 são relacionadas com o desenvolvimento do DMG [8,9]. Além disso, o DMG promove efeitos deletérios perinatais e tardios para o recém-nascido e para a gestante [9]. Os recém-nascidos de mães com DMG tem maiores chances de desenvolver macrossomia, distócia de ombro, hipoglicemia neonatal, síndrome do desconforto respiratório e obesidade na vida adulta [2,3,9]. Na gestante, há maior probabilidade de desenvolver diabetes *mellitus* do tipo 2 e complicações como a miopatia diabética [8,9]. Também, a obesidade foi determinada como risco para o desenvolvimento da IU devido à pressão excessiva intra-abdominal e pélvica da gordura [10-12].

Desta forma, nosso grupo de pesquisa “Diabetes e Gravidez - Clínico e Experimental” (CNPq - Faculdade de Medicina de Botucatu – UNESP) vem estudando, desde 2006, a relação da IU associado com o DMG [13-14], entretanto esse quadro clínico junto à ingestão de macronutrientes e micronutrientes dietéticos, medidas antropométricas e dosagens bioquímicas, bem como a expressão de receptores relacionados à nutrientes ainda não esta elucidada nesse contexto.

O adequado estado nutricional materno é essencial para o desenvolvimento fetal e a ingestão de nutrientes em quantidades adequadas, segundo a “*Dietary Reference Intakes”* [15], proporciona a mulher uma gestação normal, pois alterações metabólicas como a hiperglicemia levam a modificações na absorção, metabolização, excreção e biodisponibilidade de nutrientes, bem como na expressão de seus receptores. As quantidades de nutrientes em gestantes com DMG e IU-EG são pouco conhecidas, no momento apenas alguns estudos [16-20] descreveram essas condições clínicas separadamente, mas não associadas, e também não na condição de IU-EG. Além disso, o diagnóstico das deficiências de

micronutrientes deve ser melhorado, já que os estudos são limitados em relação às várias idades gestacionais e paridade.

Os micronutrientes fazem parte da composição de novas células, fatores de transcrição, enzimas e retardam o estresse oxidativo na gestação, porém o consumo inadequado de micronutrientes pode comprometer esses processos metabólicos. A carência de nutrientes leva a diminuição da fertilidade materna, alterações no peso do recém-nascido e resistência insulínica na vida adulta do bebê. Além disso, hábitos alimentares errôneos, falha na absorção intestinal, tabagismo e adaptações maternas gestacionais (cardiovasculares, gastrointestinais e renais) afetam ainda mais as deficiências nutricionais [19-21]. Gestantes fumantes têm as concentrações de vitamina A, C, e E diminuídas na corrente sanguínea comparadas àquelas gestantes não fumantes [19].

Durante a gestação, a hiperglicemia provoca modificações mitocondriais placentárias, desequilíbrio na homeostase do ferro e aumento das espécies reativas de oxigênio (ROS) [22]. A vitamina A, C e E são potentes antioxidantes e auxiliam na reversão do estresse oxidativo, visto ser amplamente documentado na literatura. A Organização Mundial da Saúde evidenciou que 50% das gestantes tem deficiência de vitamina A durante a gestação [19]. Também, durante esse período, há um maior fluxo sanguíneo materno, sendo frequente a carência de vitamina C, além dessa condição ser rotineira. De acordo com Mangir *et al*. [23], a vitamina C atua como cofator na produção da enzima prolil hidroxilase, responsável pela formação da reticulação de fibrilas de colágeno do tecido conjuntivo do assoalho pélvico. A falha no processo de síntese e degradação do colágeno, devido à carência da vitamina C, leva ao desenvolvimento da IU [19]. Ainda de acordo com Monaco *et al*. [24], altos níveis de vitaminas antioxidantes auxiliam na redução dos danos ocasionados pela miopatia diabética.

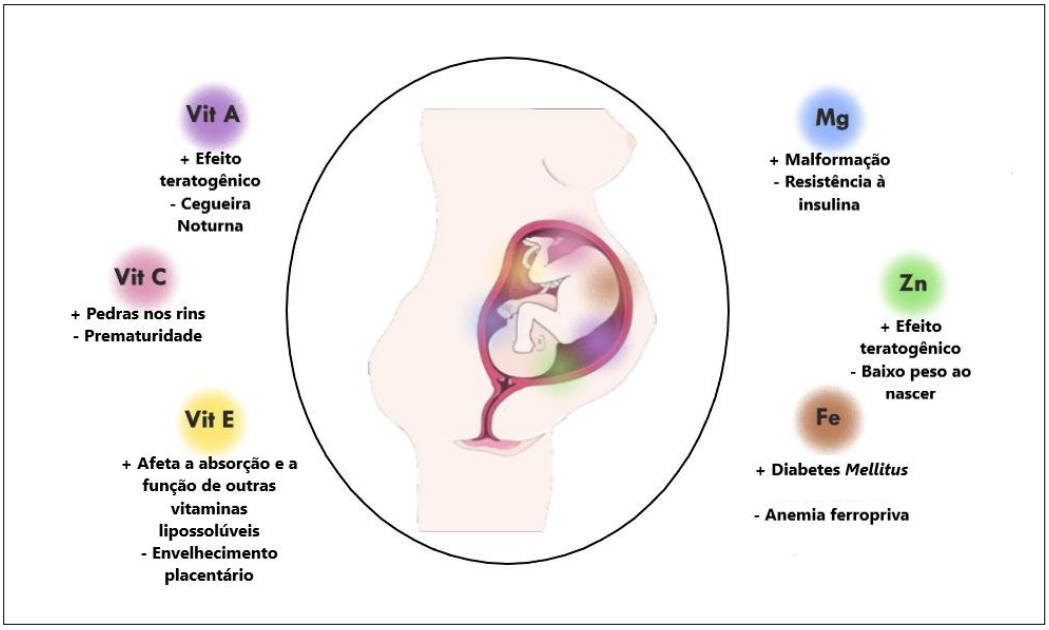

Figura 3. Consequências do excesso e da deficiência de micronutrientes (vitamina A, vitamina C, vitamina E, magnésio, zinco e ferro). Sinal Positivo (+): excesso do micronutriente; Sinal negativo (-): deficiência do micronutriente.

Portanto, o objetivo da presente tese de doutorado foi descrever as alterações no estado nutricional maternas, níveis de macronutrientes e micronutrientes dietéticos, exames bioquímicos, marcadores de estresse oxidativo e a análise da expressão gênica e proteica de receptores específicos em gestantes com DMG e IU-EG.

Assim, o conhecimento das possíveis alterações promovidas pelo DMG ao nível de macronutrientes e micronutrientes, bem como da expressão de seus receptores específicos, podem fornecer informações para a identificação de biomarcadores para a IU-EG, além de subsidiar a prática clínica com medidas terapêuticas e/ou preventivas por meio da alimentação adequada e/o suplementação nutricional, ampliando as áreas de estudos nessas doenças.

Os resultados obtidos na tese estão apresentados em 2 artigos de acordo o respectivo objetivo:

Artigo 1 - *Role of Dietary Food Intake Patterns, Antropometric Measures and Multiple Biochemical Markers in the Development of Pregnancy Specific Urinary Incontinence in Gestational Diabetes Mellitus.*

Artigo 2 - *Transferrin Receptor Gene and Protein Expression are Reduced in Diabetic Pregnant Women with Specific Urinary Incontinence of Pregnancy.*

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Seção 3
Resumos Expandidos

Resumo da Tese

Costa, S. M. B. Níveis de Nutrientes e da Expressão de Seus Receptores em Mulheres com Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação. 2023. Defesa para obtenção de titulo (Doutorado) – Faculdade de Medicina de Botucatu (FMB), Universidade Estadual Paulista (UNESP), Brasil.

Introdução: Durante o período gestacional, o corpo da gestante sofre diversas adaptações endócrinas e metabólicas, desde alterações na sensibilidade à insulina até alterações nos níveis circulantes do hormônio lactogênio placentário e colesterol, à medida que a placenta está em desenvolvimento. O Diabetes *Mellitus* Gestacional (DMG) é caracterizado por qualquer grau de intolerância a glicose, hiperglicemia e diagnosticado pela primeira vez durante a gestação. Nosso grupo de pesquisa “Diabetes e Gravidez - Clínico e Experimental” (CNPq - Faculdade de Medicina de Botucatu – UNESP) vem estudando, desde 2006, a relação da Incontinência Urinária (IU) associado com o DMG, entretanto esse quadro clínico junto ao estado nutricional materno, papel dos macronutrientes e micronutrientes dietéticos, exames bioquímicos e da expressão de seus receptores em gestantes com DMG e Incontinência Urinária Específica da Gestação (IU-EG) ainda não esta elucidado. O estado nutricional materno adequado é essencial para o desenvolvimento fetal e a ingestão de vitaminas e minerais em quantidades adequadas, de acordo com as DRIs, proporciona à mulher uma gestação saudável, uma vez que as alterações metabólicas como a hiperglicemia levam a alterações na absorção, metabolismo, excreção e biodisponibilidade de nutrientes. A identificação precoce de deficiências nutricionais deve ser aprimorada, pois os estudos são limitados em relação às várias idades gestacionais e paridade. A falta de micronutrientes leva à redução da fertilidade materna, alterações no peso corporal do recém-nascido e resistência insulínica na vida adulta do recém-nascido como descrito na literatura. Além disso, hábitos alimentares errôneos, falha na absorção intestinal, tabagismo e adaptações maternas gestacionais (cardiovasculares, gastrointestinais e renais) afetam ainda mais as deficiências nutricionais maternas. A deficiência de vitamina A é um problema de saúde pública durante o terceiro trimestre de gestação, e seu excesso está associado a defeitos congênitos. Além disso, durante o período gestacional, 30,8% das gestantes no Brasil apresentam deficiência de vitamina C. As quantidades de nutrientes em gestantes com DMG e

IU-EG são pouco conhecidas, no momento apenas alguns estudos descreveram essa condição clínica. Além disso, o diagnóstico das deficiências de nutrientes deve ser melhorado, já que os estudos são limitados.

Justificativa: Desta forma, o impacto das adaptações hemodinâmicas, cardiovasculares, renais e gastrointestinais em relação aos níveis de nutrientes deve ser considerado durante a gestação. Idealmente, os estudos que investigam o efeito dos micronutrientes no DMG devem medir a ingestão alimentar e os níveis bioquímicos de micronutrientes. Assim, o conhecimento das possíveis alterações promovidas pelo DMG ao nível de nutrientes e da expressão de seus receptores específicos podem fornecer informações para a identificação de biomarcadores para a IU-EG, além de subsidiar a prática clínica com medidas terapêuticas e/ou preventivas por meio da alimentação adequada e/o suplementação nutricional, ampliando os estudos na área.

Objetivos: Artigo 1) Avaliamos os padrões de ingestão de alimentos da dieta maternos, medidas antropométricas e múltiplos marcadores bioquímicos em mulheres com DMG e IU-EG, e exploramos se o ambiente anterior ao DMG afeta o desenvolvimento da IU-EG em uma coorte de mulheres grávidas com DMG e IU-EG.

Artigo 2) Descrever as alterações da expressão gênica e expressão proteica dos receptores/canal (MTF1, RXRA, TRPM6 e TFRC) relacionados a nutrientes, e marcadores de estresse oxidativo (carbonilação de proteínas e peroxidação lipídica) em gestantes com DMG e IU-EG.

Métodos: Artigo 1) Este foi um estudo transversal do estudo de coorte “Diamater” realizado em 900 mulheres gestantes. O desenho do estudo incluiu três grupos controle, ou seja, não DMG continente (NG-C), não DMG incontinente (NG-IU) e DMG continente (DMG-C), e um grupo DMG incontinente (DMG-IU) como grupo estudo. As informações dietéticas maternas foram coletadas por um recordatório alimentar de 24-48 horas, uma ferramenta de avaliação da dieta na qual os participantes foram solicitados a recordar todos os alimentos e bebidas que consumiram nas últimas 24 horas e 48 horas (inquérito alimentar). As medidas antropométricas incluíram as circunferências do braço, pulso, cintura, quadril e muscular do braço, e dobra cutânea do tríceps. Às 24 semanas de gestação, com uma amostra de punção venosa em jejum, amostras de sangue atuais para marcadores bioquímicos de hormônios, vitaminas e minerais foram analisadas. O

DMG foi diagnosticado por um teste oral de tolerância à glicose a 75g em 24 semanas de gestação, e o “International Consultation on Incontinence Questionnaire - Short Form” (ICIQ-SF) para IU. Urogenital Distress Inventory-Short Form e Incontinence Severity Index (ISI-short form) foram aplicados. Questionários baseados em sintomas de acordo com a International Continence Society foram usados para detectar se o vazamento involuntário de urina começou durante a gravidez para caracterizar IU-EG. Os grupos foram comparados em termos de variáveis numéricas usando ANOVA para amostras independentes seguidas de comparações múltiplas com pós-teste de Bonferroni. Para variáveis categóricas, os grupos foram comparados por meio dos testes qui-quadrado ou exato de Fisher. A análise foi realizada com o software SPSS 21.

Artigo 2) Análise da expressão gênica pelo PCR em tempo real (qPCR) dos genes FPGS, MTF1, RXRA, TRPM6, TRAP1 e TFRC e análise da expressão proteica pelo Western Blot dos genes MTF1, RXRA, TRPM6 e TFRC. Os marcadores de estresse oxidativo foram feitos por carbonilação de proteínas e pelos níveis de malondialdeído, utilizando o soro das pacientes gestantes.

Resultados: Artigo 1) Das 900 mulheres gestantes com dados completos, 334 desenvolveram DMG (critérios IADPSG, OGTT 24-28 semanas). As gestantes do grupo DMG-IU apresentaram maior peso pré-gestacional, ganho de peso durante a gravidez, IMC durante a gravidez, circunferência do braço, circunferência da cintura, circunferência muscular do braço e dobra cutânea triciptal do que os grupos NG-C e NG-IU, caracterizando um ambiente materno obesogênico. Em relação à ingestão de alimentos dietéticos, foi observado um aumento significativo de aminoácidos aromáticos, aminoácidos ramificados, cromo, fibra alimentar, magnésio e zinco no grupo DMG-IU em comparação com o grupo NG-C. Houve uma diminuição significativa de cálcio, gorduras poliinsaturadas e gorduras saturadas no grupo DMG-IU em comparação com o grupo NG-IU. A vitamina C sérica foi reduzida no grupo DMG-IU em comparação com o grupo NG-IU. Os níveis de insulina foram maiores no grupo DMG-IU em comparação com o grupo NG-IU. Nos níveis hormonais maternos, o hormônio lactogênio placentário foi menor no grupo DMG-IU em comparação com o grupo NG-C.

Artigo 2) Um aumento significativo de magnésio, vitamina D e zinco dietético foi observado no DMG-IU em comparação com o grupo controle NG-C. As expressões dos genes RXRA e TRFC foram diminuídas no grupo DMG-IU em comparação com

o grupo DMG-C. Não foram encontradas diferenças significativas na expressão gênica de FPGS, MTF1, TRAP1 e TRPM6 em relação ao grupo do estudo e aos demais grupos controle. Além disso, a expressão proteica de TRFC foi diminuída no grupo DMG-IU em comparação com o grupo DMG-C. Não foram encontradas diferenças significativas na expressão proteica de MTF1, RXRA e TRPM6 e os marcadores de estresse oxidativo em relação ao grupo estudo em comparação aos grupos controle.

Conclusão: Artigo 1) Este estudo enfatiza a necessidade de uma estratégia abrangente para mulheres DMG com IU-EG em termos de desvio na adaptação materna tendendo à obesidade e deficiências maternas de micronutrientes. Adaptações maternas que tendiam para a obesidade, com ingestão de alimentos dietéticos demonstram uma deficiência materna múltipla de micronutrientes, e aumento significativo de aminoácidos aromáticos, aminoácidos ramificados, cromo, fibra dietética, magnésio e zinco, e diminuição de cálcio, gorduras poliinsaturadas e saturadas gorduras. Houve também níveis hiperinsulinêmicos e níveis mais baixos de hormônio lactogênio associados a baixos níveis de vitamina C. Este estudo também ressalta a necessidade de focar em fatores modificáveis pré-gravidez como uma oportunidade para a prevenção do DMG, aumentando a ingestão de frutas e vegetais folhosos verde-escuros para benefício em mulheres com DMG e IU-EG.

Artigo 2: Compreender os processos celulares que regulam a homeostase do ferro, como o TRFC, é essencial para buscar novos tratamentos para doenças como o DMG. Além disso, o achado quanto à expressão do TRFC em gestantes com DMG e IU-EG pode ser utilizado como ferramenta para intervenção nutricional e/ou farmacológica nessa população de gestantes. Assim, a prática da nutrigenômica auxiliará no tratamento de doenças não transmissíveis como o DMG, sendo o futuro da nutrição individualizada para melhor atender os pacientes em suas necessidades e utilizar a terapêutica adequada.

Palavras-chave: Diabetes Gestacional; Incontinência Urinária; Minerais; Vitaminas; Expressão Gênica; Estresse Oxidativo**.**

Thesis Abstract

Costa, S. M. B. Nutrient Levels and Their Receptor Expression in Women with Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence. 2023. Doctorate - Botucatu Medical School (FMB), São Paulo State University (UNESP), Brazil.

Introduction: During the gestational period, the pregnant body undergoes various endocrine and metabolic adaptations, from changes in insulin sensitivity to changes in the circulating levels of placental lactogenic hormone and cholesterol, as the placenta develops. Gestational Diabetes Mellitus (GDM) is characterized by any degree of intolerance to glucose, and hyperglycemia and is diagnosed for the first time during pregnancy. Our research group "Diabetes and Pregnancy - Clinical and Experimental" (CNPq – Botucatu Medical School - UNESP) has been studying, since 2006, the relationship of Urinary Incontinence (UI) associated with GDM, meanwhile this clinical together with state maternal nutrition, the role of dietary macronutrients and micronutrients, biochemical tests and the expression of their receptors in pregnant women with GDM and Specific Urinary Incontinence of Gestation (PSUI) is still not elucidated. Adequate maternal nutritional status is essential for fetal development and the intake of vitamins and minerals in adequate amounts, according to the DRIs, provides women with a healthy pregnancy, since metabolic changes such as hyperglycemia lead to changes in absorption, metabolism, excretion and bioavailability of nutrients. Early identification of nutritional deficiencies should be improved, as studies are limited to different gestational ages and parity. The lack of micronutrients leads to reduced maternal fertility, changes in the newborn's body weight, and insulin resistance in the newborn's adult life, as described in the literature. In addition, erroneous eating habits, failure in intestinal absorption, smoking, and maternal gestational adaptations (cardiovascular, gastrointestinal, and renal) further affect maternal nutritional deficiencies. Vitamin A deficiency is a public health problem during the third trimester of pregnancy, and its excess is associated with birth defects. In addition, during the gestational period, 30.8% of pregnant women in Brazil have vitamin C deficiency. The amounts of nutrients in pregnant women with GDM and PSUI are little known, at the moment only a few studies have described this clinical condition. Furthermore, the diagnosis of nutrient deficiencies should be improved, as studies are limited.

Justification: Thus, the impact of hemodynamic, cardiovascular, renal, and

gastrointestinal adaptations about nutrient levels should be considered during pregnancy. Ideally, studies investigating the effect of micronutrients on GDM should measure dietary intake and biochemical levels of micronutrients. Thus, knowledge of the possible alterations promoted by GDM in terms of nutrients and the expression of its specific receptors can provide information for the identification of biomarkers for PSUI, in addition to subsidizing clinical practice with therapeutic and/or preventive measures through adequate food and/or nutritional supplementation, expanding studies in the area.

Aims: 1) We assessed maternal dietary food intake patterns, anthropometric measures, and multiple biochemical markers in GDM women with PSUI and we explored whether antedating GDM environment affects the PSUI development in a cohort of pregnant women with GDM and PSUI.

2) Describe changes in gene expression and protein expression of receptors/channels (MTF1, RXRA, TRPM6, and TFRC) related to nutrients, and markers of oxidative stress (protein carbonylation and lipid peroxidation) in pregnant women with GDM and PSUI.

Methods: 1) This was a cross-sectional study of the “Diamater” cohort study conducted on 900 pregnant women. The study design included three control groups, i.e. non-GDM continent (non-GDM-C), non-GDM incontinent (non-GDM-PSUI) and GDM continent (GDM-C), and a GDM incontinent group (GDM-PSUI) as group study. Anthropometric measurements included arm, wrist, waist, hip, and arm muscle circumferences, and triceps skinfolds. At 24 weeks of gestation, with a fasting venipuncture sample, current blood samples for biochemical markers of hormones, vitamins, and minerals were analyzed. GDM was diagnosed by an oral glucose tolerance test at 75g at 24 weeks of gestation, and the “International Consultation on Incontinence Questionnaire - Short Form” (ICIQ-SF) for UI. Urogenital Distress Inventory-Short Form and Incontinence Severity Index (ISI-short form) were applied. Symptom-based questionnaires according to International Continence Society were used to detect if the involuntary leakage of urine started during pregnancy to characterize PSUI. The groups were compared in terms of numerical variables using ANOVA for independent samples followed by multiple comparisons with Bonferroni correction. For categorical variables, the groups were compared using Chi-square or Fisher's exact tests. Analysis was performed with SPSS 21 software.

2) Analysis of gene expression by real-time PCR (q-PCR) of FPGS, MTF1, RXRA, TRPM6, TRAP1, and TFRC genes and analysis of protein expression by the Western Blot of genes MTF1, RXRA, TRPM6, and TFRC. Oxidative stress markers were made by protein carbonylation and malondialdehyde levels using patients' serum.

Results: 1) Of the 900 pregnat women with complete data, 334 developed GDM (IADPSG criteria, OGTT 24-28 weeks). Pregnant women in the GDM-PSUI group had higher pre-gestational weight, weight gain during pregnancy, BMI during pregnancy, arm circumference, waist circumference, arm muscle circumference, and triceps skinfold than the non-GDM-C and non-GDM-PSUI groups, characterizing an obesogenic maternal environment. Regarding dietary food intake, a significant increase in aromatic amino acids, branched amino acids, chromium, dietary fiber, magnesium, and zinc was observed in GDM-PSUI group compared to the non-GDM-C group. There was a significant decrease in calcium, polyunsaturated fats, and saturated fats in the GDM-PSUI group compared to the non-GDM-PSUI group. Serum vitamin C was reduced in the GDM-PSUI group compared to the non-GDM-PSUI group. The insulin levels were higher in GDM-PSUI group compared to the non-GDM-PSUI group. In maternal hormone levels, the placental lactogen hormone was lower in the GDM-PSUI group compared to the non-GDM-C group.

2) A significant increase in magnesium, vitamin D and zinc dietary was observed in the GDM-PSUI compared to the non-GDM-C control group. RXRA and TRFC gene expressions were decreased in the GDM-PSUI group compared to the GDM-C group. No significant differences were found in the gene expression of FPGS, MTF1, TRAP1 and TRPM6 in relation to the study group and the other control groups. Furthermore, TRFC protein expression was decreased in the GDM-PSUI group compared to the GDM-C group. No significant differences were found in the protein expression of MTF1, RXRA, and TRPM6, and oxidative stress markers in relation to the study group compared to the control groups.

Conclusion: 1) This study emphasizes the necessity for a comprehensive strategy for GDM women with PSUI in terms of deviation in maternal adaptation trending toward to obesity and maternal micronutrientes deficiencies. In this cohort of women at GDM diagnosis, the maternal environment in GDM women with PSUI demonstrated deviation in maternal adaptations which were trending toward obesity, with dietary food intake demonstrate a multiple maternal micronutrient deficiencies

significant increase in aromatic amino acids, branched amino acids, chromium, dietary fiber, magnesium, and zinc and decrease in decrease in calcium, polyunsaturated fats, and saturated fats. There were also hyperinsulinemic levels and lower levels of lactogen hormone associated with low levels of vitamin C. This study also underscores the need to focus on modifiable factors pre-pregnancy as an opportunity for GDM prevention, increasing dietary intake of fruits and dark green leafy vegetables as beneficial in women with GDM and PSUI.

2) To understand the cellular processes that regulate iron homeostasis, such as TRFC, is essential to seek new treatments for diseases such as GDM. In addition, the finding regarding the expression of TRFC in pregnant women with GDM-PSUI can be used as a tool for nutritional and/or pharmacological intervention in this population of pregnant women. Thus, the practice of nutrigenomics will help in the treatment of non-communicable diseases such as GDM, being the future of individualized nutrition to better serve patients in their needs and use the appropriate therapy.

Keywords: Gestational Diabetes; Urinary incontinence; Minerals; Vitamins; Gene expression; Oxidative stress.

Seção 4
Artigos

Artigo 1

Artigo Original redigido de acordo com as normas de publicação da *Nutrition* (Elsevier).

Role of Dietary Food Intake Patterns, Antropometric Measures and Multiple Biochemical Markers in the development of Pregnancy Specific Urinary Incontinence in Gestational Diabetes Mellitus.

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Abstract

Background: In the vast majority of cases, Pregnancy Specific Urinary Incontinence (PSUI) antedate Gestational Diabetes Mellitus (GDM) diagnosis. It is uncertain whether as gestation progresses, there is deviation in metabolic and nutritional maternal adaptations to pregnancy in GDM with PSUI. We hypothesize that the metabolic and nutritional mechanism predisposing to GDM may be important to provide effective treatment to PSUI. Understanding the dominant mechanism predisposing to GDM may be important to provide effective treatment in order to improve perinatal outcomes.

Aims: We assessed maternal dietary food intake patterns, anthropometric measures, and multiple biochemical markers in GDM women with PSUI and we explored whether antedating GDM environment affects the PSUI development in a cohort of pregnant women with GDM and PSUI.

Methods: This was a cross-sectional survey of the Diamater cohort study carried out in 900 pregnant women. The study design included three control groups namely non-GDM-C, non-GDM-PSUI, and GDM-C, and a GDM-PSUI as study group. Maternal dietary information was collected by a 24-48 hour dietary recall chart, a dietary assessment tool in which participants were asked to recall all food and drink they have consumed in the last 24 hours. Anthropometric measurements included arm, wrist, waist, hip, and arm muscle circumferences, and triceps skinfolds. At 24 weeks of gestation, with a fasting venipuncture sample, current blood samples for biochemical markers of hormones, vitamins, and minerals were analyzed. GDM was identified by a 75 g oral glucose tolerance test at 24 weeks of gestation. The “International Consultation on Incontinence Questionnaire - Short Form” (ICIQ-SF) for UI. Urogenital Distress Inventory-Short Form and Incontinence Severity Index (ISI-short form) were applied. Symptom-based questionnaires according to International Continence Society were used to detect if the involuntary leakage of urine started during pregnancy to characterize PSUI. The groups were compared in terms of numerical variables using ANOVA for independent samples followed by multiple comparisons with Bonferroni correction. For categorical variables, the groups were compared using Chi-square or Fisher's exact tests. Analysis was performed with SPSS 21 software.

Results: Of the 900 pregnat women with complete data, 334 developed GDM

(IADPSG criteria, OGTT 24-28 weeks). Pregnant women in the GDM-PSUI group had higher pre-gestational weight, weight gain during pregnancy, BMI during pregnancy, arm circumference, waist circumference, arm muscle circumference, and triceps skinfold than the non-GDM-C and non-GDM-PSUI groups, characterizing an obesogenic maternal environment. Regarding dietary food intake, a significant increase in aromatic amino acids, branched amino acids, chromium, dietary fiber, magnesium, and zinc was observed in GDM-PSUI group compared to the non-GDM-C group. There was a significant decrease in calcium, polyunsaturated fats, and saturated fats in the GDM-PSUI group compared to the non-GDM-PSUI group. Serum vitamin C was reduced in the GDM-PSUI group compared to the non-GDM-PSUI group. The insulin levels were higher in GDM-PSUI group compared to the non-GDM-PSUI group. In maternal hormone levels, the placental lactogen hormone was lower in the GDM-PSUI group compared to the non-GDM-C group.

Conclusion: This study emphasizes the necessity for a comprehensive strategy for GDM women with PSUI in terms of deviation in maternal adaptation trending toward to obesity and maternal micronutrients deficiencies. In this cohort of women at GDM diagnosis, the maternal environment in GDM women with PSUI demonstrated deviation in maternal adaptations which were trending toward obesity, with dietary food intake demonstrate a multiple maternal micronutrient deficiencies significant increase in aromatic amino acids, branched amino acids, chromium, dietary fiber, magnesium, and zinc and decrease in decrease in calcium, polyunsaturated fats, and saturated fats. There were also hyperinsulinemic levels and lower levels of lactogen hormone associated with low levels of vitamin C. This study also underscores the need to focus on modifiable factors pre-pregnancy as an opportunity for GDM prevention, increasing dietary intake of fruits and dark green leafy vegetables as beneficial in women with GDM and PSUI.

Keywords: Gestational diabetes; Maternal nutrition; Macronutrients; Micronutrients; Anthropometry. Pregnancy Specific urinary Incontinence

Highlights

- The most concerned and modifiable factor, dietary food intake plays a significant role in human health.
- However, less is known about the role of maternal metabolic and nutritional adaptation to pregnancy just before the Gestational Diabetes Mellitus (GDM) diagnosis on the etiology of Pregnancy Specific Urinary Incontinence (PSUI).
- There was a significant decrease in calcium, polyunsaturated fats, and saturated fats in the GDM-PSUI group compared to the non-GDM-PSUI group.
- Serum vitamin C was reduced in the GDM-PSUI group compared to the non-GDMPSUI group.
- Understanding the dominant mechanism predisposing to GDM may be important to provide effective treatment in order to improve perinatal outcomes.

1. Introduction

Gestational diabetes mellitus (GDM) is characterized by a transitory form of diabetes induced by insulin resistance and pancreatic β-cell dysfunction during pregnancy. Untreated, GDM is related to an increased risk for adverse pregnancy outcomes (McIntyre et al., 2019). Pregnancy Specific-Urinary Incontinence (PSUI), a Urinary Incontinence started for the first time in pregnancy, may be considered a short-term consequence for mothers with GDM (Barbosa, Adriano Dias, et al., 2011; Chuang et al., 2012; Kim et al., 2008). However, less is known about the role of maternal metabolic and nutritional adaptation to pregnancy just before the GDM diagnosis on the etiology of PSUI. The analyses included anthropometric parameters at the beginning and during pregnancy, laboratory values of glycated hemoglobin, fructosamine, birth weight measures and the presence of neonatal complications. This temporality between the onset of PSUI at an early gestational age and the later development of GDM at around 24 weeks may be related to maternal nutritional and metabolic adaptation. such as anthropometric parameters during pregnancy, dietary intakes and multiple laboratory markers. Understanding these maternal adaptations related to PSUI in GDM women allow us to introduce modifiable lifestyle factors to prevent or delay the development of PSUI in early stage of GDM women´s life (Ellerbrock et al., 2022).

PSUI in GDM womenis a risk fator for long-Urinary incontinence (UI) (Barbosa, Adriano Dias, et al., 2011; Sangsawang, 2014). PSUI, a symptom undervalued by classical obstetric area remains rather controversial. However, the direct association between GDM diagnosis and PSUI needs to be deeply analysed as the associations has not been confirmed in early gestation. Few studies have reported women with GDM to have a high prevalence of UI due to higher body mass index, obesity, and macrosomia in their infants (Melville et al., 2005; Saydah et al., 2005). Although the exact mechanism remains unclear, excess weight gain during pregnancy may exert pressure on the PFM, which increases pressure on the bladder and influences urethral mobility, leading to UI (Brown et al., 2003). Nutrition therapy, defined as the use of nutrients and whole foods in the management of the disease remains a cornerstone of GDM management. Although the evidence base on optimal diets and foods as well as maternal blood levels of nutrients remains inconclusive and warrants more studies (Basu et al., 2021).

PSUI was described by the first time in 2002 (Hvidman et al., 2002). Since the first detection of (Barbosa, Adriano Dias, et al., 2011) our research group detected the GDM induced Myopathy (GDiM) as the underline etiological factor (Barbosa, Dias, et al., 2011; Pinheiro et al., 2022; Prudencio et al., 2022; Sartorão Filho et al., 2020; Vesentini et al., 2020). Maternal metabolic adaptation to pregnancy could be involved with PSUI development in GDM women (Powe et al., 2016). PSUI may be considered as one short consequences of GDM. According to the available evidence, the pathophysiology of short term consequences of GDM is a complex and still not a fully understood issue. However, it appears that an increase in insulin resistance and an inability of beta cells to respond with sufficient insulin secretion are crucial factors for development of GDM in predisposed pregnant women (Powe et al., 2016). As insulin resistance physiologically increases in the second half of pregnancy, GDM is most likely to be detected during this period. Gestational diabetes, characterized by FPG ≥ 5.1 mmol/L in early pregnancy, is associated with higher body weight and BMI at the beginning of pregnancy (Krystynik et al., 2023).

BMI has been the focal assessment of GDM risk in weight heterogeneous women, with relatively little attention to more direct measures of adiposity such as skinfold thicknesses, and limb and waist circumferences which may better reflect pathological distributions of adipose tissue (Hartz et al., 1983). In women with obesity these measures when evaluated early in pregnancy, may add increased granularity to maternal GDM risk assessment which BMI doesn't provide. Gestational weight gain (GWG) is associated with the development of GDM amongst weight heterogeneous women, with one meta-analysis quoting an unadjusted odds ratio (OR) of 1.40 (95% CI 1.21–1.61) for the association between excessive GWG and GDM, with no evidence of interaction with maternal pre-pregnancy BMI (Brunner et al., 2015). Despite this evidence, interventions designed to improve lifestyle or reduce GWG in women with obesity, have not translated to a reduction in GDM (Poston et al., 2015; Simmons et al., 2017; Syngelaki et al., 2016).

PSUI is a short-term consequence for mothers with GDM. Findings from prior studies have demonstrated that Gestational Diabetic induced Myopathy (GDiM) seems to be the underline etiological factor (Barbosa, Adriano Dias, et al., 2011; Pinheiro et al., 2022; Prudencio et al., 2022; Sartorão Filho et al., 2020; Vesentini et al., 2020). Although,for the most cases, PSUI antedate the own GDM diagnosis. For

many years it was intensivily debates whether the poor outcomes associated with GDM were due to maternal hyperglycemia per se or other risk factors. However, it may be difficult to capture the overall effects of diet on health by studying the relationship of single nutrient with diseases. Incorrect eating habits, failure in intestinal absorption, smoking, and maternal adaptations to pregnancy, affect maternal nutritional deficiencies during pregnancy (Cikot et al., 2001; Simpson et al., 2010). Studies (Maged et al., 2016; Morisset et al., 2014) have associated either the dietary intake of GDM women or the dietary intake of non-pregnant women with UI (Dallosso et al., 2004; Maserejian et al., 2010, 2011). Meanwhile, these two associated clinical conditions, GDM and PSUI, need to be investigated. Nevertheless, there remains insufficient studies concerning whether nutritional maternal intake patterns,biochemical maternal markers and maternal anthropometric measurements influences the PSUI development in GDM women.

For medical researchers, preventing the pancreatic β-cell damage reducing the GDM incidence are an incumbent responsability. This highly necessitates the identification of the effects of nutrients on β-cell functionality and the prevention of pancreatic β-cell impairment (Gong et al., 2022). Collectively, it is imperative to identify the modifiable factors for GDM prevention, among which nutritional strategies play an essential role. Maternal dietary intake in adequate (Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, 2005; Kominiarek & Rajan, 2016; Ma et al., 2021; Trumbo et al., 2001) amounts provides women with a healthy pregnancy and adequate safe fetal development, since metabolic changes, such as hyperglycemia, lead to changes in the absorption, metabolism, excretion, and bioavailability of nutrients (Cikot et al., 2001; Mcardle et al., 1999; J. L. Simpson et al., 2010b).

Nutrients are part of the composition of new cells, transcription factors, and enzymes and delay oxidative stress during pregnancy (Gombart et al., 2020; Manford et al., 2021; J. L. Simpson et al., 2010b; Toboła-Wróbel et al., 2020). During pregnancy, hyperglycemia causes placental mitochondrial changes, imbalance in iron homeostasis, cell apoptosis, and increased reactive oxygen species (ROS) (Gombart et al., 2020; Pereira & Martel, 2014; Zhuang et al., 2014). Some studies (Dallosso et al., 2004; J. H. Lee & Lee, 2017; Maserejian et al., 2010, 2011) have shown nutrient imbalances related to female urinary tract symptoms or UI. According to (Maserejian et al., 2010, 2011), higher consumption of saturated fats, as opposed

to polyunsaturated fats, was associated with UI, with the type of fat being related to the development and progression of UI. Regarding vitamins, Vitamin A deficiency is a problem during the third trimester of pregnancy (Biesalski & Nohr, 2003; Radhika, 2002; Semba & Bloem, 2002), and 30.8% of Brazilian pregnant women have vitamin C deficiency (Dror & Allen, 2012; Rumbold et al., 2015; Siega-Riz et al., 2003). Vitamin C is a collagen stimulator, important for the connective tissue, acting as a cofactor in the production of the enzyme prolyl hydroxylase, responsible for the formation of the cross-linking of collagen fibrils in the connective tissue of the pelvic floor (Mangir et al., 2016). In vitamin C deficiency, there is a failure in the process of synthesis and degradation of collagen, with defective production and/or decrease in collagen being associated with the development of UI (Mangir et al., 2016; Weber et al., 2004). In the study by (Lee & Lee, 2017) dietary amounts of vitamin C and vitamin E were similar in 661 incontinent women and 1322 continent women. According to (Monaco et al., 2017), high levels of antioxidant vitamins assist in reducing the damage caused by diabetic myopathy.

In a meta-analysis of 18 randomized controlled trials on dietary modifications in GDM, low-glycemic-index diets or manipulation of dietary fats and proteins revealed a decrease in maternal hyperglycemia and risks of macrosomia. However, owing to large variations in dietary exposure and participant characteristics in the reported studies, challenges remain in reaching a consensus on the best dietary practices to decrease risks of GDM (Yamamoto et al., 2018). GDM has been identified as the commonest medical pregnancy complication. It has a growing problem around the world as the obesity epidemic continues and is one of the major obstacles in achieving improved maternal health (Simmons, 2019).

To better understand the maternal environment antedating the real hyperglycemic medium (after the GDM diagnoses) related to the emergence of PSUI illnesses, this project was delineate. Our hypothesis is that before maternal hyperglycemia environment have been identified (GDM diagnosis), other maternal nutritional characteristics may be found. We hypothesize that GDM women with inadequate maternal environment have higher risk of developing PSUI.

Thus, the main aim of present study was to investigate maternal dietary nutrient patterns during pregnancy and to address the anthropometric measures and serum concentration of vitamins, minerals, and hormones in GDM-PSUI women assessed in the early second and third trimester.

2. Patients and method

2.1. Capture of pregnant patients in the study

The cross-sectional study took place between June 2020 and October 2021. For the present cross-sectional study, pregnant women were representatively selected from the FAPESP Thematic Project (process: 2016/01743-5) entitled “Cohort of the gestational triad: hyperglycemia, urinary incontinence and clinical, molecular and omic profile of hyperglycemic myopathy in the prediction of incontinence and muscle dysfunction and translational research with biodevice for muscle regeneration in female rats”. The pregnant women were divided into four groups: non-GDM continent (non-GDM-C), non-GDM incontinent (non-GDM-PSUI), GDM continent (GDM-C) and GDM incontinent (GDM-PSUI). The study design included three control groups namely non-GDM-C, non-GDM-PSUI, and GDM-C. All pregnant women were evaluated at one stage of pregnancy, monitored in Perinatal Diabetes Research Center, Clinical Hospital of Botucatu Medical School (FMB) after signing the Informed Consent Form. The study was approved by the Research Ethics Committee of FMB, São Paulo, Brazil (CAAE: 82225617.0.0000.5411). The collection of biological material from the pregnant women was carried out at the Perinatal Diabetes Research Center of the Clinical Hospital of FMB. As inclusion criteria, all primiparous or second pregnant women with previous elective cesarean section, aged over 18 years, without collagen-related diseases, without malignant neoplasms, without active infection by the hepatitis A virus, hepatitis B or hepatitis C and HIV, without diseases degenerative muscles, miscarriages, with or without a diagnosis of GDM in the current pregnancy were considered eligible. For the diagnosis of GDM, the following protocol was performed: with less than 20 weeks, the pregnant woman was submitted to fasting glycemia. After the test, if the result was between 92 and 125 mg/dL, the pregnant woman was diagnosed with GDM. On the other hand, if the result of the fasting glycemia test is <92 mg/dL, between 24-28 weeks of gestation, the pregnant woman was submitted to the 75g oral glucose tolerance test, and the diagnosis of GDM was established when at least one of the following values is altered: fasting blood glucose between 92 and 125 mg/dL, 1-hour blood glucose after overload greater than or equal to 180 mg/dL and 2-hour blood glucose after overload greater than or equal to 153-199 mg/dL. The GDM diagnosis protocol was performed as recommended by the Brazilian Ministry of Health (OPAS - Organização Pan-Americana da Saúde, 2019) and the “International Association of

Diabetes in Pregnancy Study Group" (International Association of Diabetes and Pregnancy Study Groups Consensus Panel et al., 2010). The diagnosis of PSUI was performed using the "International Consultation on Incontinence Questionnaire Short Form" questionnaire (Tamanini et al., 2004). Exclusion criteria for the study were pregnant women with two or more previous cesareans, diabetes mellitus before pregnancy, and twin pregnancy. As discontinuity criteria, participants who progressed to premature birth and/or intrauterine fetal death were excluded.

2.2. Dietary intake of pregnant women

The intake amount of aromatic amino acids (mg), branched amino acids (mg), ascorbic acid (mg), beta-carotene (mcg), caffeine (mg), calcium (mg), carbohydrates (g), total cholesterol (mg), chromium (mcg), iron (mg), heme iron (mg), nonheme iron (mg), total dietary fiber (g), insoluble fiber (g), soluble fiber (g), monounsaturated fats (g), fats polyunsaturated (g), saturated fat (g), total fat (g), glycemic index (g), lycopene (mg), magnesium (mg), omega-3 (g), omega-6 (g), total protein (g), retinol (mcg), selenium (mcg), tocopherol (mg) and zinc (mg) was performed using a 24-hour and 48-hour dietary recall, in which all information on the pregnant woman's food intake in the last 24 hours and 48 hours 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 the nutrition software (Nutrilife version 9.12).

2.3. Anthropometric measurements of pregnant women

Prepregnancy height and weight notes were identified in clinical records and were used for the calculation of prepregnancy BMI (Nutrition During Pregnancy, 1990). The current weight and height of the pregnant women were measured using a W300 electronic platform scale (Welmy) according to a weighing protocol established for adults (Brasil. Ministério da Saude., 2011), and thus, with these measurements, the current BMI of the pregnant women between 24-40 gestational weeks. In addition, measurements of the mid-arm circumference (cm), wrist circumference (cm), waist circumference (cm), hip circumference (cm), arm muscle circumference (cm), and triceps skinfold (mm) were performed of pregnant women according to the techniques described in the following articles (Boucher et al., 2022;

Mousapour et al., 2021; Ren et al., 2019; Widen & Gallagher, 2014). Furthermore, maternal weight gain (Nutrition During Pregnancy, 1990; Stephansson et al., 2001) was recorded at the time of collection.

2.4. Biochemical markers of pregnant women in the study

15 ml of blood were collected from the pregnant women for placental lactogen (mg/L) by the enzyme immunoassay method, insulin (pU/mL) by the electrochemiluminescence method, vitamin A (mg/L), vitamin C (mg/L) and vitamin E by high-performance liquid chromatography method, magnesium (mg/dL) by colorimetric method, serum zinc (ug/dL) by spectrometry method of atomic absorption and iron (ug/dL) by the colorimetric method between 24-40 gestational weeks. Blood samples were centrifuged at 3,200 rpm for 12 minutes and serum aliquots were stored in a freezer at -80°C for later analysis at the clinical analysis laboratory at the Clinical Hospital of FMB and for some time private laboratory (Funk & Clinical and Laboratory Standards Institute., 2008; LIPPI et al., 2006).

2.5. Assessment of the general characteristics of the pregnant women

An interview was carried out with the pregnant women and an evaluation form was filled out with the participant's personal data such as name, identity document, address, age, gestational age, parity, marital status, education, ethnicity, profession, religion, personal contact, frequency and time of physical activity per week/month, amount of alcohol consumed during the week/month, use or not of medication/drugs, planning the pregnancy or not, follow-up with a nutritionist during pregnancy or not, constipation or not, and history family member of diabetes mellitus or not for further analysis of the differences in these variables between the groups.

2.6. Statistical analysis

The study groups were compared in relation to numerical variables using the ANOVA test for independent samples followed by multiple comparisons with Bonferroni correction. For categorical variables, the groups were compared using the Chi-square or Fisher's Exact tests. The analysis was performed using the SPSS 21 software. The $p<0.05$ value was considered statistically significant.

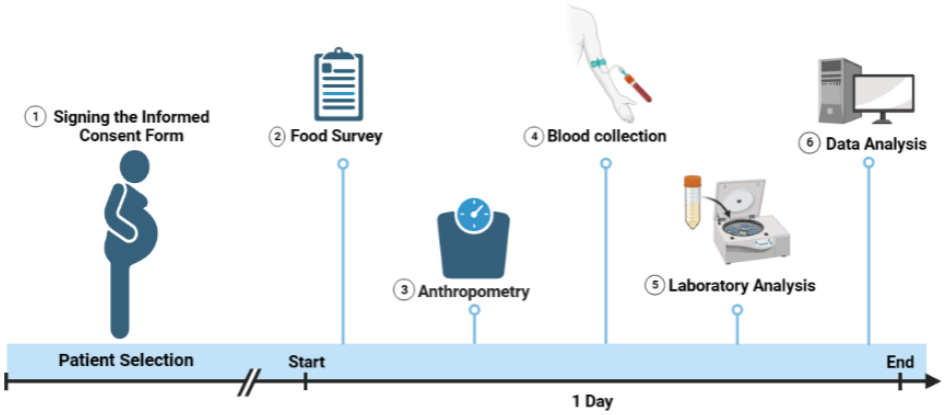

Figure 1. Study design.

3. Results

For present prospective, population-based cohort study (Diamater Study), a screeny survey study 1105 pregnant women were selected, of which 205 patients were excluded (94 patients had one or more vaginal deliveries; 72 patients had two previous cesarean sections; 4 patients had Overt Diabetes; 16 patients had type 1 or 2 diabetes; 7 patients had urinary incontinence before pregnancy, 4 patients didn't accept to participate in the research and 8 patients were excluded due to discontinuity criteria. For this analysis, 900 patients were included.

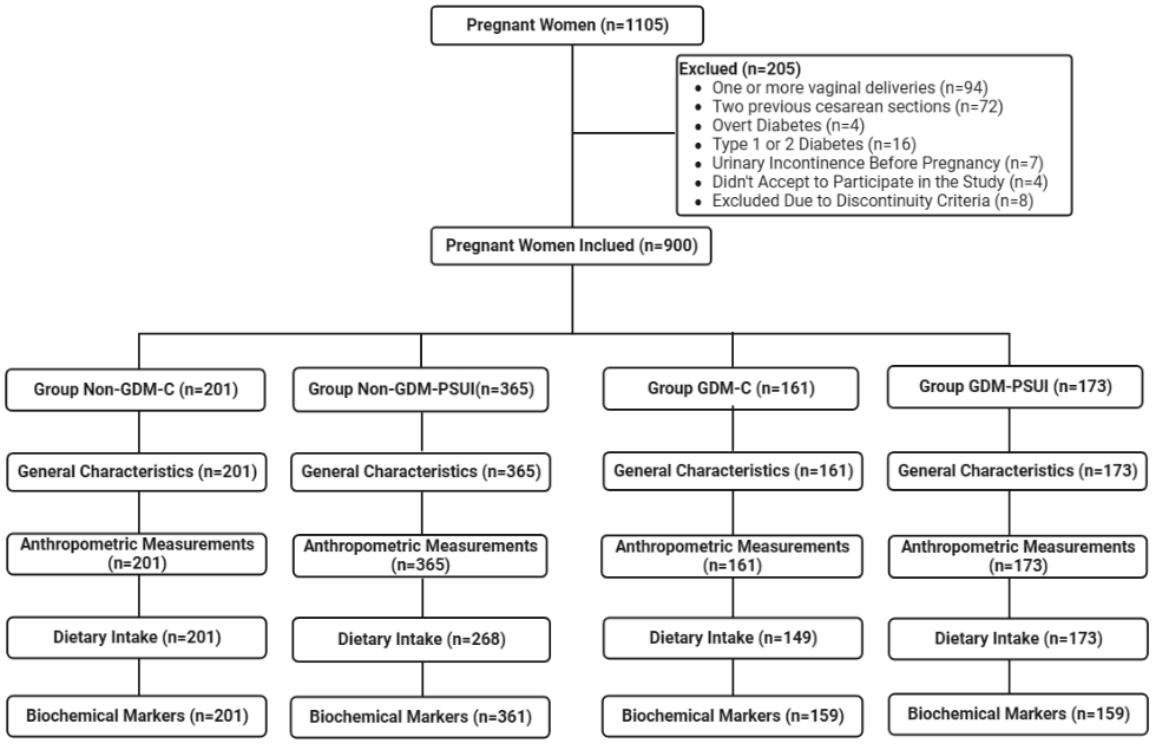

Fig. 2. Flow diagram of the study. A total of 1105 pregnant patients were screened.

Table 1 shows the characteristics of pregnant women by group. The groups of non-GDM-PSUI and GDM-C pregnant women planned the pregnancy more previously than the GDM-PSUI group. Pregnant women in the GDM-PSUI group had more follow-up with a nutritionist during pregnancy than the non-GDM groups. The GDM-PSUI group had a greater history of DM in the family than the non-GDM-C group. The GDM-PSUI group practiced more physical activity than the non-GDM-PSUI group. Regarding smoking, pregnant women in the GDM-PSUI group smoke less than pregnant women in the non-GDM-PSUI group. There were no significant differences between the study group and the control groups regarding partner, complete higher education, work, had constipation, alcoholic and drug user.

Table 1. Characteristics of pregnant women by groups (percentage values).

Group	Non-GDM-C (n=201)	Non-GDM-PSUI (n=365)	GDM-C (n=161)	GDM-PSUI (n=173)	
Variable	%	%	%	%	*P-Value*
Partner	86.00	90.99	87.42	88.76	*0.314*
Complete Higher Education	10.95	17.26	18.87	16.18	*0.155*
Work	96.02	96.44	96.23	96.53	*0.994*
Pregnancy Planning	26.37	37.53	33.96	28.00	*<0,05*
Follow-up by a Nutritionist	19.68	18.27	48.98	39.16	*<0.05*
Had Constipation	25.37	26.30	17.61	27.17	*0.144*
Has History of DM	51.74	60.82	59.75	67.05	*<005*
Practice Physical Activity	17.91	12.33	23.90	25.43	*<0.05*
Smoker	5.97	10.41	7.55	4.05	*<0.05*
Alcoholic	1.99	1.37	0.63	1.73	*0.728*
Drug User	1.49	0.00	0.63	0.58	*0.056*

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-UI: gestational diabetes mellitus and incontinent group; p-value: probability of observing a test statistic value greater than or equal to the one found. For categorical variables, groups were compared by Chi-square or Fisher's Exact tests.

Figure 3 shows the GDM-PSUI group compared 3 control groups (values expressed as mean and standard deviation). Pregnant women in the GDM-PSUI group were older, in pre-pregnancy weight, weight during pregnancy, BMI during pregnancy, arm circumference, waist circumference, arm muscle circumference, and triceps skinfold than the non-GDM-C groups and the group non-GDM-PSUI. In addition, gestational height was greater in the GDM-PSUI group than in the non-GDM-C group. Gestational age was lower in the GDM-PSUI group than in the GDM-C group.

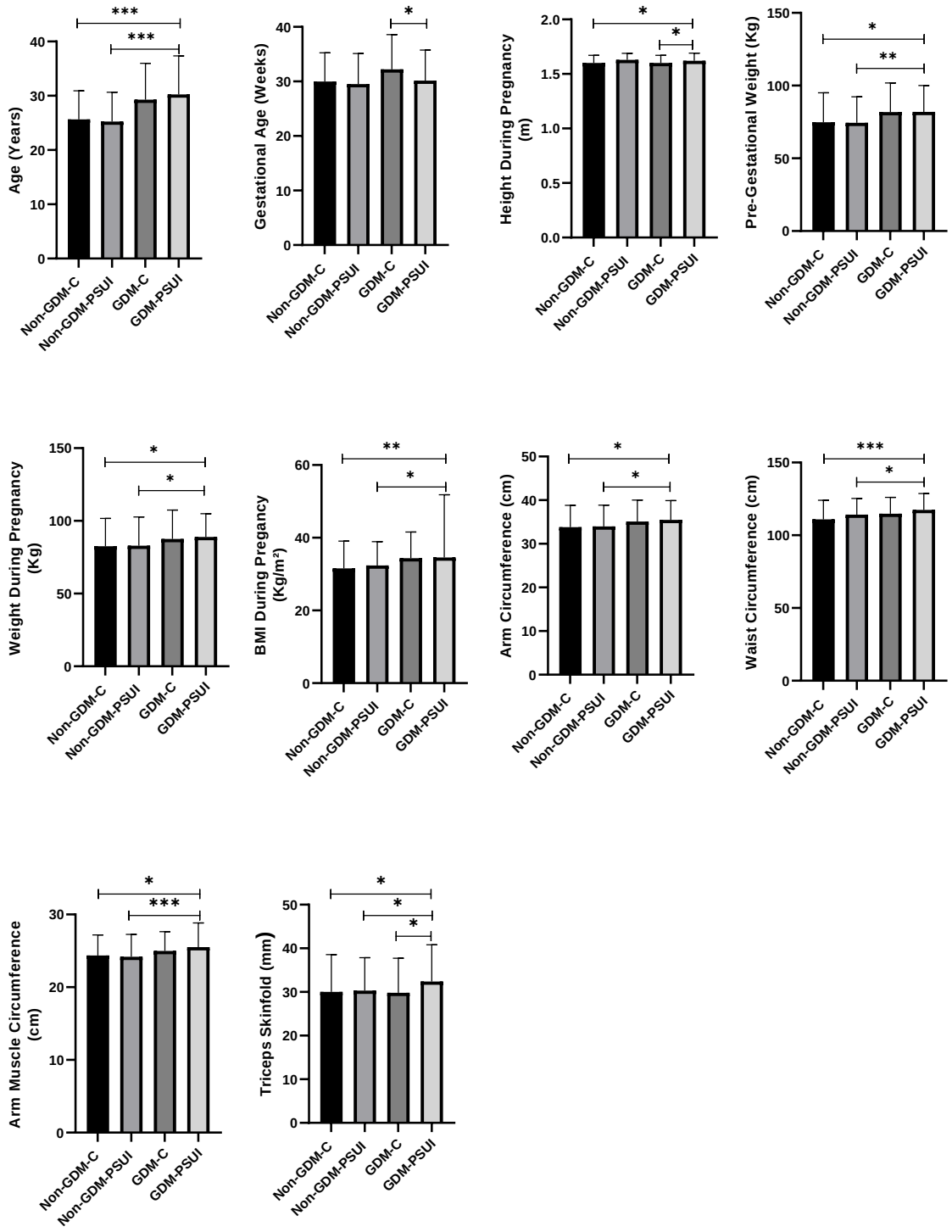

Fig. 3. Anthropometric measurements of GDM-PSUI group compared with 3 control groups (values expressed as mean and standard deviation). n: number of pregnant women; SD: standard deviation; non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: gestational diabetes mellitus and incontinent group; p-value: the probability of observing a test statistic value greater than or equal to the one found; *: p<0.05; **: p<0.001; ***: p<0.0001; m: meters; Kg: kilogram; BMI: Body Mass Index; cm: centimeters; mm: millimeter.

Figure 4 shows the dietary intake of nutrients in GDM-PSUI compared with 3 control groups (values expressed as mean and standard deviation). A significant increase in aromatic amino acids, branched amino acids, chromium, dietary fiber, glycemic index, dietary magnesium, and zinc was observed in the GDM-PSUI compared to the non-GDM-C control group. Aromatic amino acids and chromium were also increased in the GDM-PSUI group compared to the non-GDM-PSUI group. On the other hand, there was a significant decrease in calcium, polyunsaturated fats, and saturated fats in the GDM-PSUI group compared to the non-GDM-PSUI group. Furthermore, the GDM-PSUI group had lower levels of polyunsaturated fats and selenium than the GDM-C and non-GDM-C groups, in addition to having lower total dietary cholesterol than the GDM-C group.

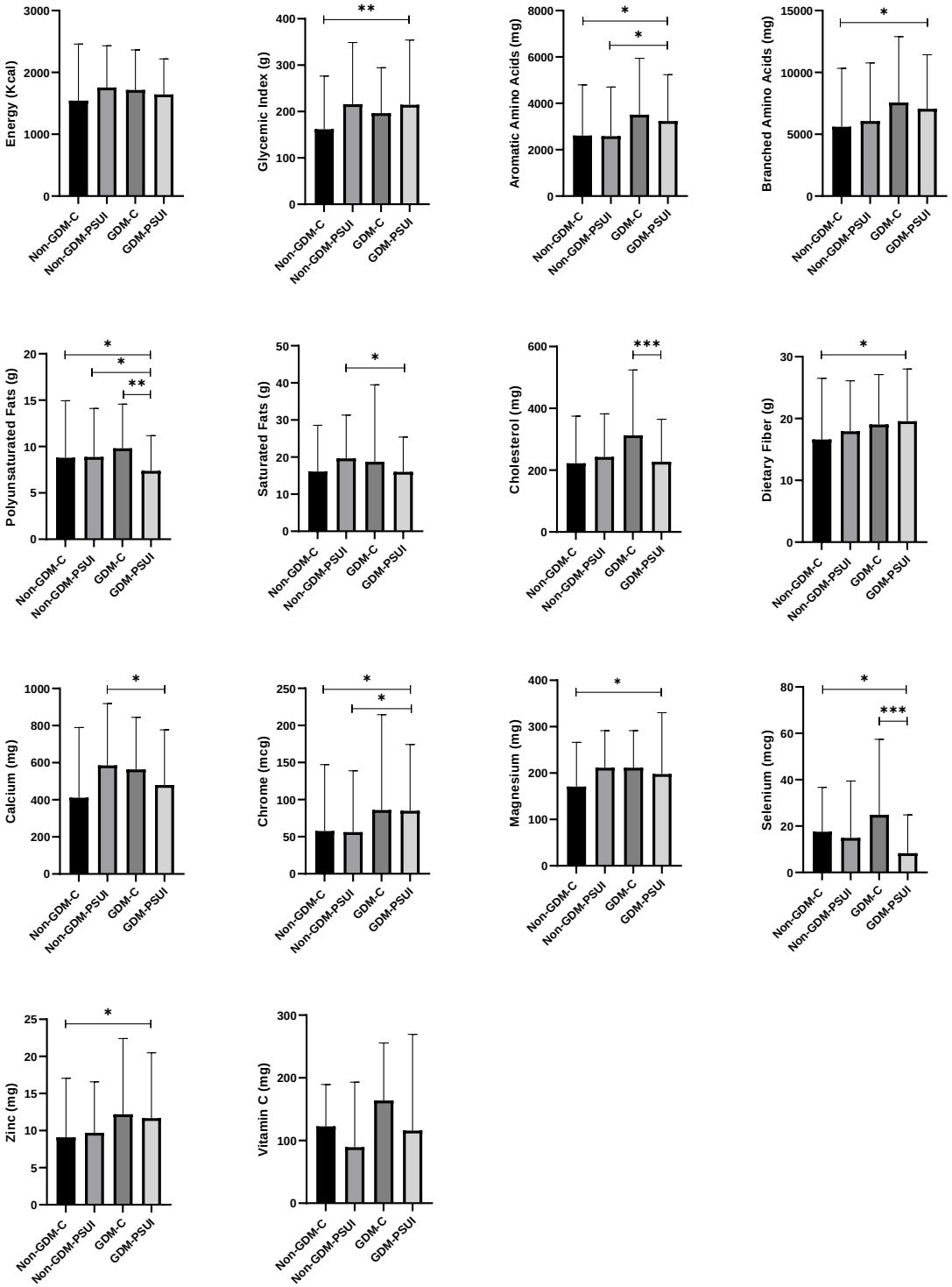

Fig. 4. Dietary intake of nutrients in GDM-PSUI compared with 3 control groups (values expressed as mean and standard deviation). n: number of pregnant women; SD: standard deviation; non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-UI: gestational diabetes mellitus and incontinent group; p-value: probability of observing a test statistic value greater than or equal to the one found; *: p<0.05; **: p<0.001; ***: p<0.0001; mg: milligram; ml: milliliters; mcg: microgram; g: grams.

Figure 5 shows the biochemical markers in GDM-PSUI compared with 3 control groups (values expressed as mean and standard deviation). There was a significant increase in fasting blood glucose, GTT 1 hour, and GTT 2 hours in the GDM-PSUI group compared to the non-GDM groups. HOMA-IR and HOMA-β increased in GDM-PSUI group compared to non-GDM-C group. In maternal hormone levels, the placental lactogen hormone decreased in the GDM-PSUI group compared to the non-GDM-C group. The hormone insulin showed a significant increase in the GDM-PSUI group compared to the non-GDM-PSUI group. Regarding the amounts of maternal vitamins, vitamin C was reduced in the GDM-PSUI group compared to the non-GDM-PSUI group.

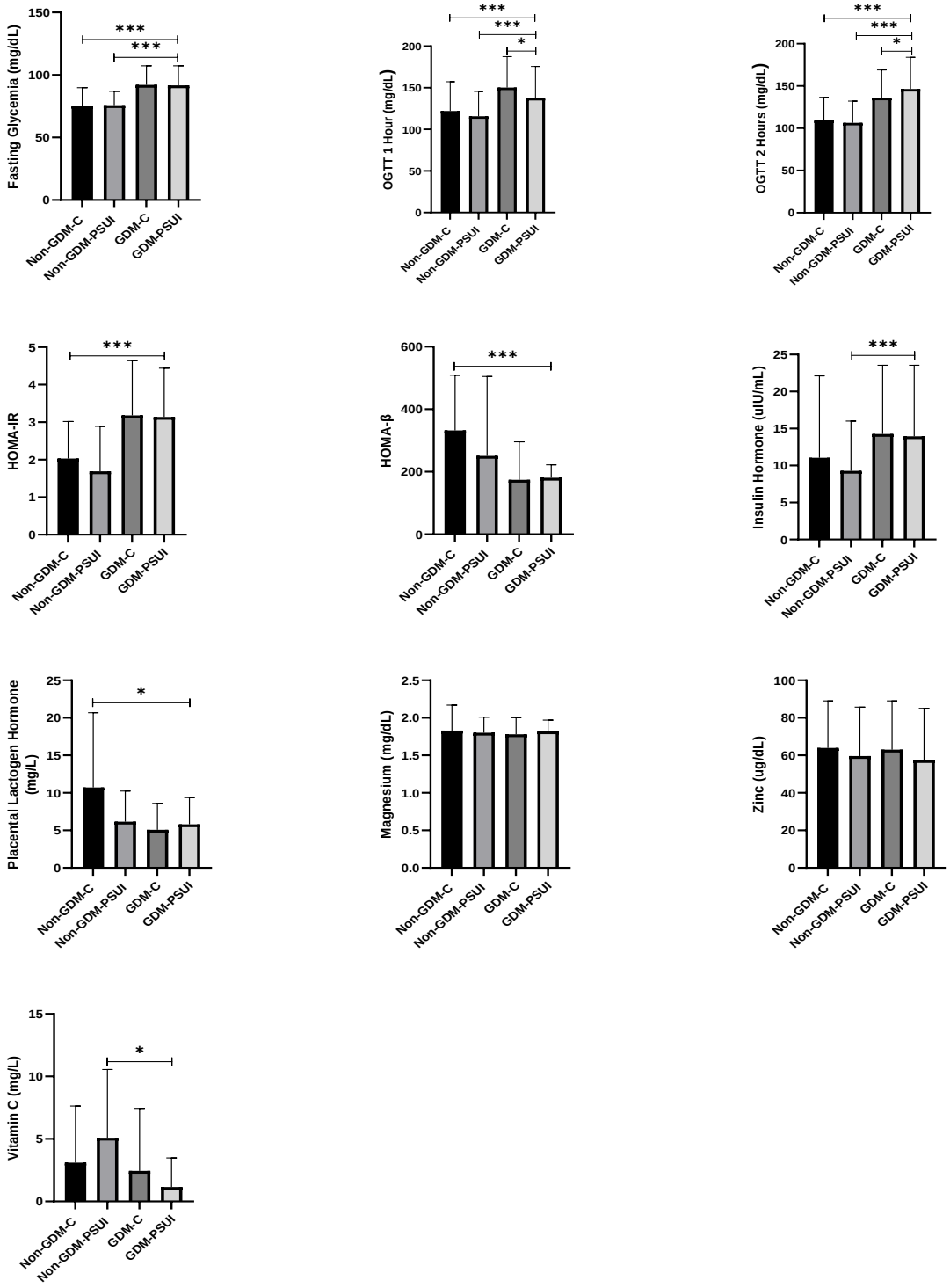

Fig. 5. Biochemical markers in GDM-PSUI compared with 3 control groups (values expressed as mean and standard deviation). n: number of pregnant women; SD: standard deviation; non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-UI: 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; *: p<0.05; **: p<0.001; ***: p<0.0001; mg/dl: milligrams per deciliter; mg/L: milligram per liter; uIU/mL: international units per milliliter; ug/dL: microgram per deciliter; mg/dL: milligram per deciliter.

4. Discussion

The results provide valuable insights into the characteristics and differences between pregnant women with gestational diabetes mellitus (GDM) and pelvic floor symptom-related urinary incontinence (PSUI) to those without these conditions. The findings indicate key differences in demographic, anthropometric, dietary, and biochemical markers between the GDM-PSUI group and the control groups. Therefore, the study findings contribute to the understanding of actors that may be associated with GDM-PSUI.

Comparison of demographic characteristics of GDM-PSUI expectant mothers to non-GDM-PSUI expectant mothers revealed an increased likelihood of association between hereditary diabetes and PSUI with the pregnancy condition (Table 1). This finding conforms to findings by Dyck et al. (2002) and Damm et al. (2016) who reported family history of diabetes as a risk factor for GDM. Moreover, active collaboration with nutritionists and commitment to physical exercise were higher among GDM-PSUI mothers than non-GDM groups, results that aligns to findings reported by Wang et al. (2015) that indicated both dietary and exercise intervention were more recommended for GDM mothers. In addition, family planning was more common among GDM-PSUI expectant mothers. This finding could be explained by higher levels of awareness of the risks associated with GDM and the role of proper nutrition and physical activity in effectively managing the condition during the pregnancy (Avery & Walker, 2016). Therefore, the results reveal that GDM-PSUI group were more likely to adopt a more proactive approach in addressing their health during pregnancy. This could attributed to their understanding and knowledge of the possible complications related to GDM and the importance of preventive measures.

Anthropometric measurements results showed a higher pre-pregnancy and pregnancy weight among GDM-PSUI mothers than GDM-C and the non-GDM-PSUI groups. Furthermore, the results also demonstrated higher pre-pregnancy weight that GDM-PSUI mothers had higher BMI during pregnancy. In addition, arm circumference, waist circumference, arm muscle circumference, and triceps skinfold were higher among GDM than non-GDM-C groups and the non-GDM-PSUI group (Figure 3). These findings align with Torloni et al. (2009) and Kim et al. (2012) found that elevated maternal weight and BMI were linked to a heightened risk of GDM. Furthermore, the GDM-PSUI group displayed a greater gestational height than the non-GDM-C group. This could be linked to the increased insulin resistance observed

in the GDM-PSUI group and reported by Buchanan and Xiang (2005) and Catalano et al. (2012). This result suggests that the anthropometric differences in the GDM-PSUI group could contribute to the development or exacerbation of GDM and its associated complications. Therefore, further investigation into preventive measures and management strategies is needed. Pregnant women in the GDM-PSUI group were older which is consistent with previous research that has linked increased maternal age to a higher risk of developing GDM (Catalano et al., 2012).

The comparison of dietary nutrient intake in the GDM-PSUI group with the control groups revealed several noteworthy differences (Figure 4). Significantly, the GDM-PSUI group exhibited higher consumption of aromatic amino acids, branched amino acids, chromium, dietary fiber, glycemic index, dietary magnesium, and zinc in comparison to the non-GDM-C group. A higher intake of dietary fiber has been associated with improved glycemic control and reduced risk of GDM (Zhang et al., 2006). Similarly, chromium supplementation has been found to enhance insulin sensitivity in pregnant women with gestational diabetes (Jamilian et al., 2015). Magnesium is also known to play a vital role in glucose metabolism, and its deficiency has been linked to insulin resistance and GDM (Asemi et al., 2015). Furthermore, branched amino acids and aromatic amino acids have been reported to influence insulin resistance and glucose homeostasis (Newgard et al., 2009). A higher glycemic index diet has been associated with an increased risk of GDM (Bao et al., 2013), and increased zinc intake during pregnancy has been found to be beneficial for glucose metabolism (Scholl & Chen, 2002).

The increased intake of these nutrients might be associated with the increased prevalence of nutritionist follow-up observed in the GDM-PSUI group (Hillier et al., 2007). Intriguingly, the GDM-PSUI group displayed lower intakes of calcium, polyunsaturated fats, saturated fats, and selenium compared to the control groups (Scholl & Chen, 2002). These findings imply that while the GDM-PSUI group may demonstrate a better overall nutrient consumption, there remains room for enhancement in their dietary choices to ensure the best possible maternal and fetal health outcomes (Hillier et al., 2007; Poston, 2016).

Polyunsaturated fats, particularly omega-3 fatty acids, have been reported to have beneficial effects on insulin sensitivity and glucose metabolism (Lorente-Cebrián et al., 2013). A higher intake of polyunsaturated fats has been associated with a reduced risk of GDM (Zhang et al., 2014). Lower consumption of these fats in

the GDM-PSUI group may be indicative of a suboptimal dietary pattern that could contribute to the development of GDM. On the other hand, saturated fats have been associated with impaired glucose metabolism and insulin resistance (Vessby et al., 2001). However, a lower intake of saturated fats in the GDM-PSUI group compared to the non-GDM-PSUI group suggests that the GDM-PSUI group may have been attempting to follow a healthier dietary pattern in response to their condition.

The biochemical markers in the GDM-PSUI group displayed several remarkable differences when compared to the control groups (Figure 5). Pregnant women in the GDM-PSUI group exhibited significantly elevated fasting blood glucose, GTT 1 hour, and GTT 2 hours in comparison to the non-GDM groups. These observations align with the diagnostic criteria for GDM, which are grounded in heightened blood glucose levels during pregnancy (International Association of Diabetes and Pregnancy Study Groups Consensus Panel et al., 2010). Elevated fasting blood glucose and glucose levels during oral glucose tolerance tests (OGTT) are hallmarks of GDM, as these measures indicate impaired glucose metabolism and insulin resistance (Metzger et al., 2010). The higher levels of these markers in the GDM-PSUI group confirm the presence of GDM in this population, as reported in previous studies (Catalano et al., 2012).

Moreover, higher glucose levels during pregnancy, as observed in the GDM-PSUI group, have been associated with adverse maternal and fetal outcomes, such as increased risk of macrosomia, preterm birth, and cesarean delivery (HAPO Study Cooperative Research Group, 2008). These findings emphasize the importance of early detection and appropriate management of GDM to improve pregnancy outcomes and reduce the risk of long-term complications for both the mother and the baby.

Moreover, the GDM-PSUI group demonstrated increased HOMA-IR and HOMA-β levels relative to the non-GDM-C group, signifying enhanced insulin resistance and beta-cell dysfunction within this group. This concurs with previous research that has associated GDM with changes in insulin sensitivity and secretion (Buchanan et al., 2012; Catalano et al., 2012). The biochemical marker findings underscore the importance of monitoring and managing glucose levels and insulin resistance during pregnancy to minimize potential adverse outcomes for both mother and child.

In the PSUI group, there was a significant decrease in placental lactogen

hormone levels compared to the non-GDM-C group. The placental lactogen hormone has been reported to play a crucial role in the regulation of maternal glucose metabolism and insulin resistance throughout pregnancy (Napso et al., 2018). As such, the diminished levels of this hormone in the GDM-PSUI group could potentially contribute to the emergence of GDM and associated metabolic alterations. Furthermore, the GDM-PSUI group exhibited elevated insulin levels relative to the non-GDM-PSUI group, a finding that aligns with the increased insulin resistance observed in women diagnosed with GDM (Catalano et al., 2012; Retnakaran et al., 2006). These results highlight the complex interplay between hormones, insulin resistance, and glucose metabolism in pregnant women, which ultimately affects the risk of developing GDM and other pregnancy complications (Barbour et al., 2007).

Pertaining to maternal vitamin levels, the GDM-PSUI group exhibited reduced levels of vitamin C in comparison to the non-GDM-PSUI group (Figure 5). Vitamin C is a vital micronutrient with antioxidant properties, and its deficiency has been linked to a heightened risk of GDM (Rumbold et al., 2005). The diminished levels of vitamin C observed in the GDM-PSUI group could be indicative of suboptimal dietary intake or heightened oxidative stress, either of which might potentially contribute to the emergence of GDM and related complications (Asemi et al., 2016; Zhang et al., 2016). These findings emphasize the importance of adequate vitamin C intake during pregnancy to maintain optimal maternal and fetal health and mitigate the risk of developing GDM (Casanueva & Viteri, 2003).

5. Conclusion

This study sheds light on the distinct demographic, anthropometric, dietary, and biochemical characteristics of pregnant women with both gestational diabetes mellitus (GDM) and pelvic floor symptom-related urinary incontinence (PSUI) compared to those without these conditions. Notably, the GDM-PSUI group exhibited increased risk factors such as older age, higher pre-pregnancy weight, elevated BMI, greater anthropometric measurements, and a family history of diabetes. The GDM-PSUI group was also more likely to actively engage with nutritionists and participate in physical exercise, reflecting a proactive approach to managing their health during pregnancy.

Dietary differences were observed between the GDM-PSUI and control groups, with the GDM-PSUI group consuming higher amounts of nutrients known to

influence glucose metabolism and insulin resistance. However, there remains room for improvement in their dietary choices, particularly regarding polyunsaturated fats and vitamin C intake. Biochemical markers revealed enhanced insulin resistance, beta-cell dysfunction, and altered hormonal levels in the GDM-PSUI group, highlighting the complex interplay between hormones, insulin resistance, and glucose metabolism during pregnancy.

Author Contributions

SMBC and HRCN performed data processing and statistical analysis. SMBC, DRAR, RHLS, JFF, FCBA, MABLC, LS, PV, AMPB, and MVCR designed the study, wrote the manuscript and critically reviewed the article.

Declaration of competing interest

The authors declare that they have no competing interests.

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Artigo 2

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Transferrin Receptor Gene and Protein Expression are Reduced in Diabetic Pregnant Women with Specific Urinary Incontinence of Pregnancy

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Abstract

Aim: This study aims to identify dietary intake food and the serum concentration of iron, magnesium, zinc, vitamin A, and vitamin D during pregnancy and to evaluate the gene and protein expression of the MTF1, RXRA, TFRC and TRPM6 genes, as well as markers of oxidative stress in women with GDM and PSUI.

Methods: A cross-sectional study was carried out in which pregnant women were selected for dietary food intake and serum concentration minerals and vitamins, gene expression (real-time PCR) and protein expression (Western Blot) of the MTF1, RXRA, TFRC, and TRPM6 genes, as well as the measurement of markers of oxidative stress, carbonylation of proteins and malondialdehyde through the serum of pregnant patients. The study design included three control groups namely non-GDM-C, non-GDM-PSUI, and GDM-C, and a DMG-PSUI study group. The groups were compared in terms of numerical variables using ANOVA for independent samples followed by multiple comparisons with Bonferroni correction. Analysis was performed with SPSS 21 software.

Results: For present prospective, population-based cohort study (Diamater Study), a screen survey study 1105 pregnant women were selected. A significant increase in magnesium, vitamin D and zinc dietary was observed in the GDM-PSUI compared to the non-GDM-C control group. RXRA and TRFC gene expressions were decreased in the GDM-PSUI group compared to the GDM-C group. No significant differences were found in the gene expression of FPGS, MTF1, TRAP1 and TRPM6 in relation to the study group and the other control groups. Furthermore, TRFC protein expression was decreased in the DMG-PSUI group compared to the GDM-C group. No significant differences were found in the protein expression of MTF1, RXRA, and TRPM6, and oxidative stress markers in relation to the study group compared to the control groups.

Conclusions: Understanding the cellular processes that regulate iron homeostasis, such as TRFC, is essential to seek new treatments for GDM. In addition, the finding regarding the expression of TRFC in pregnant women with GDM-PSUI can be used as a tool for nutritional and/or pharmacological intervention in this population of pregnant women.

Keywords: Gestational Diabetes; Maternal Nutrition; Oxidative Stress Injury; Protein Expression.

Highlights

- This study investigated the dietary food intake, serum concentrations of minerals and vitamins, gene expression, protein expression, and oxidative stress markers in pregnant women with gestational diabetes mellitus (GDM) and pregnancy-specific urinary incontinence (PSUI).
- The results showed that pregnant women with GDM and PSUI had a significant increase in dietary intake of magnesium, zinc, and vitamin D compared to non-GDM control groups. However, there were no significant differences in the intake of iron and vitamin A.
- The gene expression analysis revealed a decreased expression of retinoid X receptor alpha (RXRA) and transferring receptor (TFRC) genes in the GDM-PSUI group compared to the GDM control group. However, there were no significant differences in the gene expression of other genes related to glucose metabolism and iron homeostasis.
- The protein expression analysis showed a decreased expression of transferrin receptor (TRFC) protein in the GDM-PSUI group compared to the GDM control group. No significant differences were found in the protein expression of other metal regulatory transcription factors.

1. Introduction

In the 20th century, the practice of nutrigenomics began in the context of the human post-genome, having the gene-nutrient interaction as its basic concept (Neeha and Kinth, 2013; Marcum, 2020). Nutrigenomics investigates the interaction between nutrients and bioactive compounds in food with the functioning of the genome, resulting in the modulation of gene expression and/or affecting the genome stability (Bull and Fenech, 2008; Hendriks, 2013; Fenech, 2020). Moreover, polymorphisms in genes that regulate the uptake and metabolism of micronutrients alter the levels of micronutrients in plasma, leading to genome instability, tissue dysfunction, and increased risk for chronic degenerative diseases (Bull and Fenech, 2008; Fenech, 2008).

Nutrients and the expression of their receptors are altered as a cause or consequence of gestational diabetes mellitus (GDM) (Bo et al., 2005; Morisset et al., 2014; Maged et al., 2016; Parast and Paknahad, 2017), urinary incontinence (UI) (Dallosso et al., 2004; Maserejian et al., 2010; Maserejian et al., 2011) and/or pregnancy-specific Urinary Incontinence (PSUI); research is needed in this last clinical condition.

Nutrients from the diet influence the gene expressions of an individual in two ways, directly or indirectly (Cousins, 1999; Fenech, 2002; Bull and Fenech, 2008). Nutrients can interact directly with the nuclear receptors, promoting transcriptional changes (Cousins, 1999), or they can indirectly influence cell-signaling pathways (Cousins, 1999; Rist et al., 2006). Knowledge of nutrigenomics at the molecular, tissue, and body level can reduce the risks associated with chronic non-communicable diseases such as obesity and diabetes mellitus (Fenech, 2003; Hsu and Tain, 2019).

Diets rich in fruits and vegetables are linked to a lower risk of GDM due to their higher fiber and micronutrient contents (Plows et al., 2019), including vitamin A, which regulates the development and function of pancreatic beta cells in the underlying endoderm (Trasino et al., 2015; Trasino and Gudas, 2015). Several studies have found a correlation between levels of vitamin A and its receptors with glucose metabolism (Trasino et al., 2015; Trasino and Gudas, 2015; Zhou et al., 2021). Vitamin A activity is mediated by interactions with retinoic acid (RXA) and retinoid acid X (RXR) receptors, including three subtypes of RXR: alpha, beta, and gamma (Almeida and Conda-Sheridan, 2019). The retinoid X alpha receptor (RXRA)

is a nuclear receptor, is a member of the NR2B receptor family, and is involved in the metabolism of glucose, fatty acids, and cholesterol (Kölsch et al., 2009; Yu et al., 2021). Polymorphisms of the RXRA gene lead to changes in gene and protein expression and compromise glucose metabolism (Kölsch et al., 2009; Zhao et al., 2012; Yu et al., 2021). Furthermore, RXRA is essential for vitamin D signaling (Curtis et al., 2019). Low vitamin D levels are associated with type 2 diabetes (Afzal et al., 2013). Vitamin D improves pelvic floor skeletal muscle strength (Bischoff-Ferrari et al., 2004); however, the association between vitamin D status and UI is uncertain. Vitamin D supplementation in 11,646 elderly women wasn't associated wih a reduction in the prevalence of UI (Markland et al., 2022).

The role of other minerals, such as iron and the gene expression of transferrin receptor (TRFC) in the pathophysiological mechanisms of GDM and PSUI are still incomplete. According to several studies (Montonen et al., 2012; Zhao et al., 2012; Zhang and Rawal, 2017) there is a relationship between maternal iron stores and disturbances in glucose metabolisms leading to the development of T2DM. During pregnancy, maternal iron nutritional requirements increase to meet the requirements of the fetus, placenta, and red cells (Næss-Andresen et al., 2019). Due to changes in hemodilution, the nutritional recommendations for iron for pregnant women increase to 27 mg iron/day, and it is common for pregnant women to present anemia (Kominiarek and Rajan, 2016; Zhang and Rawal, 2017). When there is a functional iron deficiency, the body's iron levels are reduced, and iron stores are depleted (Skikne et al., 1990). TRFC is present in plasma membranes and is responsible for iron uptake via endocytosis (Gammella et al., 2017; Gammella et al., 2021). TRFC expression is modulated by intracellular iron concentrations; when intracellular iron is high, TRFC expression decreases and limits iron absorption in cells; on the other hand, iron deficiency increases TRFC expression (Macedo and Sousa, 2008). In addition, a significant association can be observed between higher maternal iron stores and increased risk of impaired glucose metabolism (Zhang and Rawal, 2017).

Magnesium deficiency is also associated with glucose dysregulation in pregnant women (Lee et al., 2022). According to Goker Tasdemir et al. (2015), magnesium acts on insulin regulation and is transported intracellularly by transient receptor potential cation channel subfamily M member 6 (TRPM6). TRPM6 is a member of the transient receptor potential channel superfamily and plays a role in regulating whole-body magnesium homeostasis and epithelial magnesium transport,

maintaining the integrity of cell membranes, and mediating active magnesium absorption in the intestine and kidney (Cuffe et al., 2015; Lee et al., 2022). A defect in TRPM6 is marked by the occurrence of diseases related to magnesium deficiency, such as glucose dysregulation and hypertension (Lee et al., 2022), in addition, it is not known how TRPM6 expression changes during adulthood (Cuffe et al., 2015).

An association between zinc, pancreatic function, and diabetes has been suggested for years (Chausmer, 1998; Fan et al., 2021). Zinc has the function of regulating glucose and maintaining glucose absorption at physiological levels (Fan et al., 2021). 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 (Lindert et al., 2009; Troadec et al., 2010). In pregnant women, zinc supplementation decreases vaginal bleeding and increases the risk of childbirth-related complications (Mcardle et al., 1999). 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 (Lindert et al., 2009; Hennigar et al., 2021).

Healthy eating patterns and food supplementation, in therapeutic dosages, are strategies for maintaining genome health in individuals with a specific genetic background (Bull and Fenech, 2008). Micronutrients may act as cofactors (magnesium for several DNA polymerases) or as an integral part of the DNA repair genes (zinc as component of hOGG1), Therefore, alterations of the physiologic levels of such micronutrients may alter enzyme activity, contributing to genome instability, tissue dysfunction, and the development of genetic-related diseases (Petrović et al., 2016; Chasapis et al., 2020; Fenech, 2020; Rajakumari et al., 2023).

This study investigated dietary intake food and the concentrations of iron, magnesium, zinc, vitamin A, and vitamin D in serum during pregnancy and to evaluate the gene and protein expression of the MTF1, RXRA, TFRC, and TRPM6 genes, as well as markers of oxidative stress in women with GDM and PSUI. Our hypothesis is that nutrients and their receptor expressions are altered as a cause or consequence of the development of GDM and PSUI.

2. Patients and methods

2.1. Recruitment of pregnant patients

This study was approved by the Research Ethics Committee of FMB, São Paulo, Brazil (CAAE: 82225617.0.0000.5411). This cross-sectional study was carried out between June 2020 and October 2021. For the present cross-sectional study, pregnant women were representatively selected from the FAPESP Thematic Project (process: 2016/01743-5) entitled “Cohort of the gestational triad: hyperglycemia, urinary incontinence and clinical, molecular, and omic profile of hyperglycemic myopathy in the prediction of incontinence and muscle dysfunction and translational research with biodevice for muscle regeneration in female rats”. The pregnant women were divided into four groups: non-GDM continent (non-GDM-C), non-GDM incontinent (non-GDM-PSUI), GDM continent (GDM-C), and GDM incontinent (GDM-PSUI). The study design included three control groups, namely, non-GDM-C, non-GDM-PSUI, and GDM-C. All pregnant women were evaluated at one stage of pregnancy, monitored in Perinatal Diabetes Research Center, Clinical Hospital of Botucatu Medical School (FMB) after signing the Informed Consent Form. The collection of biological material from the pregnant women was carried out at the Perinatal Diabetes Research Center of the Clinical Hospital of the FMB. As inclusion criteria, all primiparous or second pregnant women with a previous elective cesarean section, aged over 18 years, without collagen-related diseases, without malignant neoplasms, without active infection by the hepatitis A virus, hepatitis B, or hepatitis C and HIV, without diseases degenerative muscles, miscarriages, with or without a diagnosis of GDM in the current pregnancy were considered eligible. For the diagnosis of GDM, the following protocol was performed: before 20 weeks of gestation, the pregnant woman was submitted to a fasting glycemia evaluation. After the test, for results between 92 and 125 mg/dL, the pregnant woman was diagnosed with GDM. On the other hand, if the result of the fasting glycemia test is <92 mg/dL, between 24–28 weeks of gestation, the pregnant woman was administered the 75 g oral glucose tolerance test, and the diagnosis of GDM was established when at least one of the following values was altered: fasting blood glucose between 92 and 125 mg/dL, 1 h blood glucose after overload greater than or equal to 180 mg/dL and 2 h blood glucose after overload greater than or equal to 153–199 mg/dL. The GDM diagnosis protocol was performed as recommended by the Brazilian Ministry of Health (Organização Pan-Americana da Saúde, 2019) and the International

Association of Diabetes in Pregnancy Study Group (International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010). The diagnosis of PSUI was performed using the International Consultation on Incontinence Questionnaire Short Form questionnaire (Tamanini et al., 2004). Exclusion criteria for the study were pregnant women with two or more previous cesareans, diabetes mellitus before pregnancy, and twin pregnancy. As exclusion criteria, participants who progressed to premature birth and/or intrauterine fetal death were excluded.

2.2. Dietary intake analysis

The intake amount of iron (mg), magnesium (mg), zinc (mg), vitamin A (mcg), and vitamin D (mcg) was performed using a 24-hour and 48-hour dietary recall, in which all information on the pregnant woman's food intake in the last 24 and 48 h was 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 nutrition software (Nutrilife version 9.12).

2.3. 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 FMB and by Alvaro Laboratory (Barueri, Sao Paulo – Brazil) (LIPPI et al., 2006; Funk and Clinical and Laboratory Standards Institute., 2008).

2.4. Analysis of gene expression by real-time PCR (qPCR)

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 (Livak and Schmittgen, 2001) using the GAPDH, FPGS, and TRAP1 genes as endogenous controls.

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	50
GAPDH	TCACCATCTTCCAGGAGCGA	60.61	55.00	
	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	

2.5. Analysis of 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 to 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. 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).

2.6. Oxidative stress markers analysis

2.6.1. Protein carbonylation levels

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 derivatizing agent) and the amounts were given in mmol DNPH/mg protein (Suzuki et al., 2010; Mesquita et al., 2014).

2.6.2. Malondialdehyde (MDA) levels

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} $centimeter^{-1}$) (Samarghandian et al., 2016).

2.7. Statistical analysis

The study groups were compared in relation to numerical variables using the ANOVA test for independent samples, followed by multiple comparisons with Bonferroni correction. The analysis was performed using the SPSS 21 software; $p<0.05$ was considered statistically significant.

3. Results

For the present prospective, a population-based cohort study (Diamater Study), a screen survey study of 1105 pregnant women was selected (Figure 1). Figure 2 shows the dietary intake amounts and serum level of iron, magnesium, zinc, vitamin A, and vitamin D. A significant increase in dietary intake of magnesium, zinc, and vitamin D was observed in the GDM-PSUI compared to the non-GDM-C. There were no significant differences between the study group and the control groups regarding dietary intake of iron and vitamin A, and serum levels of iron, magnesium, zinc, and vitamin D.

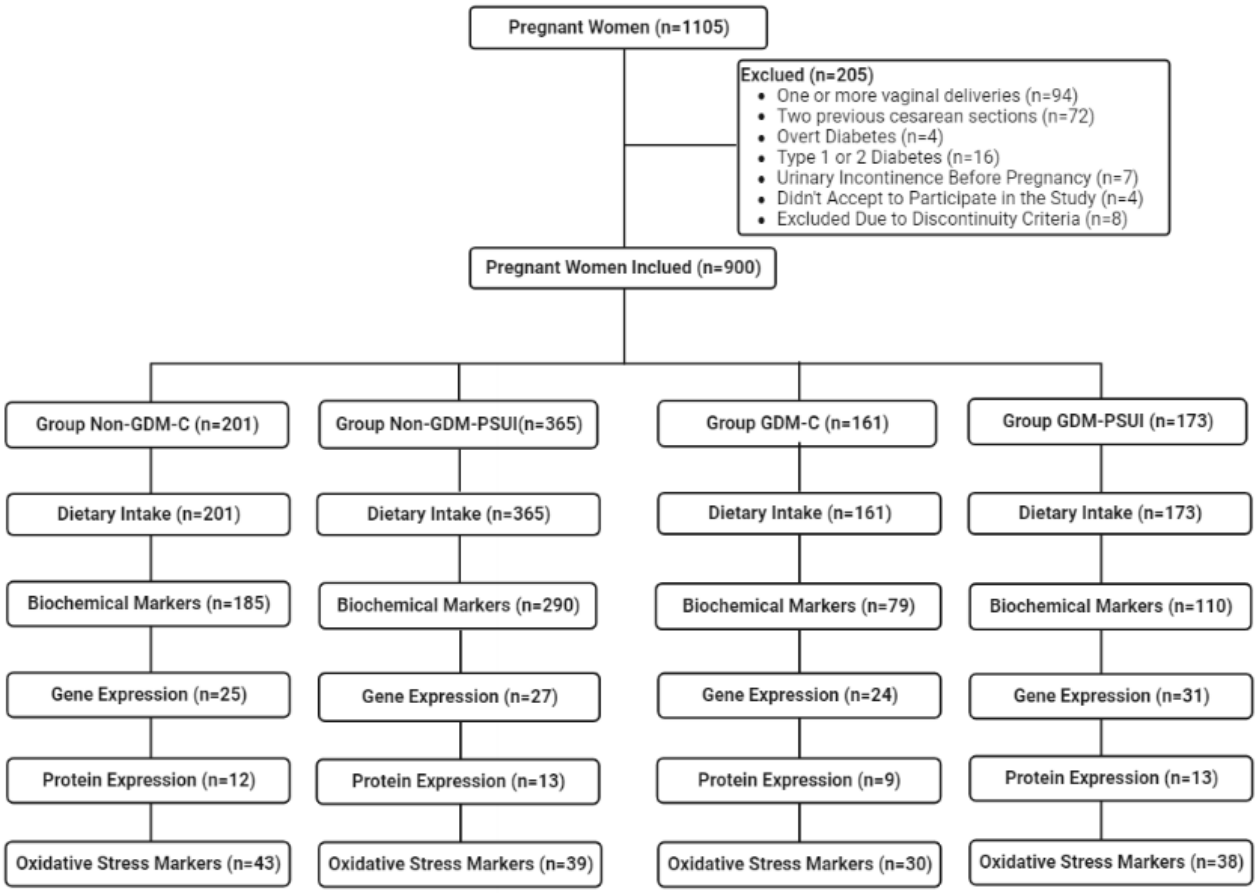

Fig. 1. Flow diagram of the study. A total of 1105 pregnant patients were screened.

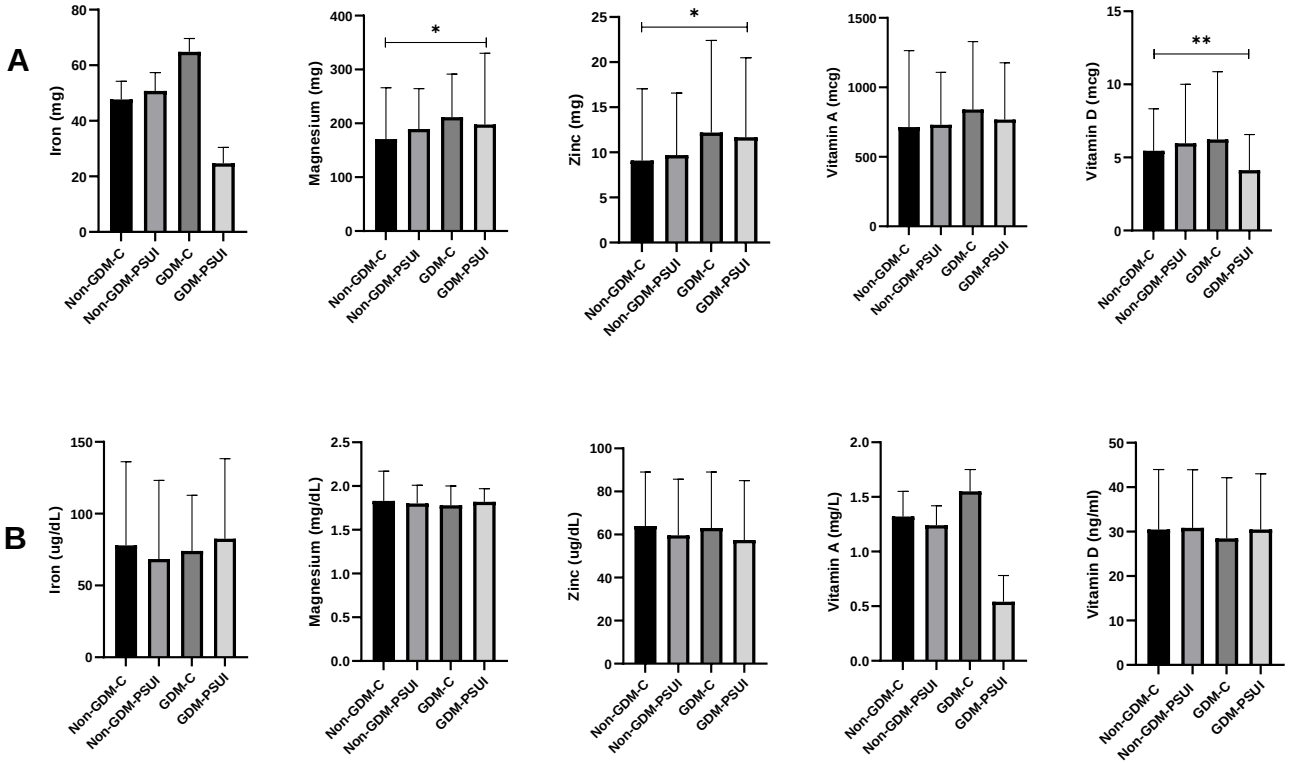

Fig. 2. Dietary intake and biochemical markers of nutrients in GDM-PSUI compared with three control groups (values expressed as means and standard deviations). (**A**) Dietary intake of iron, magnesium, zinc, vitamin A and vitamin D. Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-PSUI: gestational diabetes mellitus group; p-value: probability of observing a test statistic value greater than or equal to the one found; *p<0.05; **p<0.001; mg: milligram. (**B**) Biochemical markers of iron, magnesium, zinc, vitamin A and vitamin D. 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 group; ug/dL: microgram per deciliter; mg/dL: milligram per deciliter; ng/mL: nanogram per milliliter; mcg: microgram.

Figure 3 shows FPGS, MTF1, RXRA, TRAP1, TRFC, and TRPM6 gene expression in GDM-PSUI compared with three control groups. RXRA and TRFC gene expressions were decreased in the GDM-IU group relative to the GDM-C group. No significant differences were found in the gene expression of FPGS, MTF1, TRAP1, and TRPM6 in relation to the study group and the other control groups.

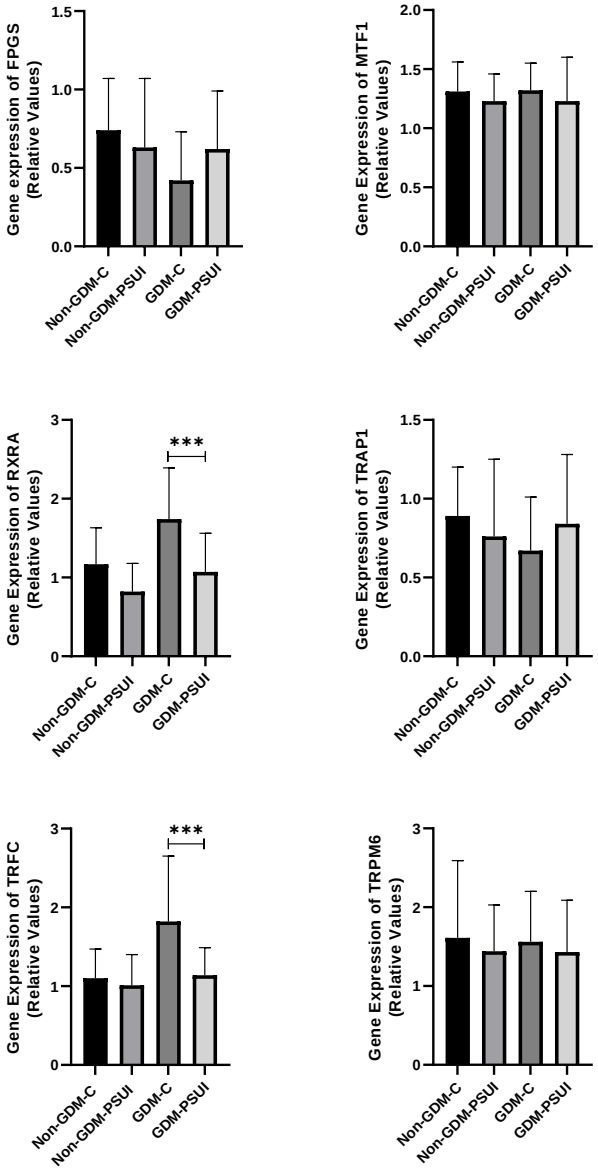

Fig. 3. Gene expression in GDM-PSUI compared with 3 control groups (values expressed as means and standard deviations). Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-UI: gestational diabetes mellitus and incontinent group ; p-value: probability of observing a test statistic value greater than or equal to the one found;

***p<0.0001; 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 4 shows the protein expression of MTF1, RXRA, TRFC, and TRPM6 in GDM-PSUI compared with three groups (values expressed as means and standard deviations). TRFC protein expression was decreased in the GDM-PSUI group relative to the GDM-C group. No significant differences were found in the protein expression of MTF1, RXRA, and TRPM6 to the study group and the other control groups.

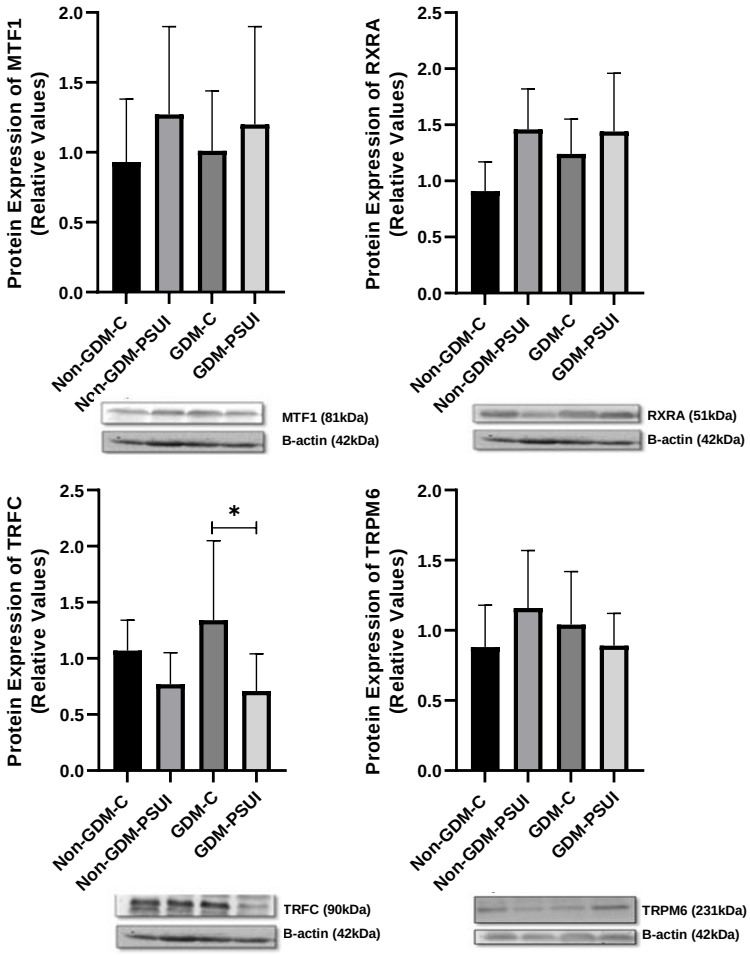

Fig. 4. Protein expression in GDM-PSUI compared with three control groups (values expressed as means and standard deviations). Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-UI: gestational diabetes mellitus and incontinent group; p-value: probability of observing a test statistic value greater than or equal to the one found; *p<0.05; 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 5 shows oxidative stress markers in GDM-PSUI relative to three groups (values expressed as means and standard deviations). There were no significant differences between the groups regarding the evaluated oxidative stress markers.

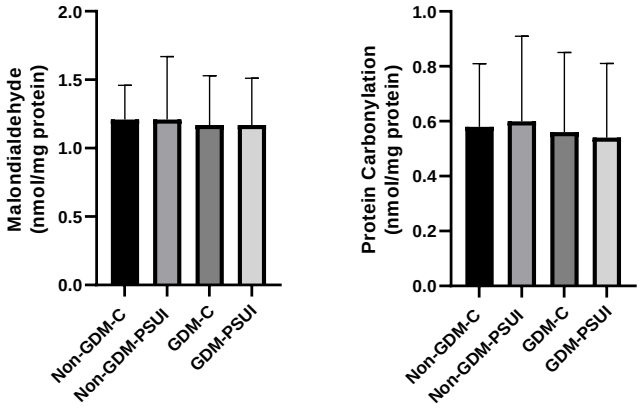

Fig. 5. Oxidative stress markers in GDM-PSUI compared with three control groups (values expressed as means and standard deviations). Non-GDM-C: non-gestational diabetes mellitus and continent group; non-GDM-PSUI: non-gestational diabetes mellitus and incontinent group; GDM-C: gestational diabetes mellitus and continent group; GDM-UI: gestational diabetes mellitus and incontinent group; nmol/mg protein: nanomole per milligram.

4. Discussion

Pregnancy is a of important physiological changes in a woman's body, reflected in the homeostasis of maternal glucose and micronutrients (Hernandez and Brand-Miller, 2018). Nutritional deficiencies are common in pregnant women, and a diet full of macronutrients and micronutrients ensures optimal nutritional support for the development of the fetus and prevents possible maternal and fetal metabolic complications throughout pregnancy and childbirth (Plows et al., 2019).

Thus, in this study, the expression of genes related to micronutrient uptake was verified on whole blood of pregnant patients with or without GDM and PSUI. The results showed a decreased gene expression of RXRA in GDM women with urinary incontinence relative to continent GDM women. The

expression of the RXRA nuclear receptor changes with vitamin A status, and vitamin A activity depends on RXR activation (Yu et al., 2021b). Vitamin A deficiency is linked to a decrease in the expression of RXR and an increase in the expression of PPAR, which alters the lipid metabolism in the heart of rats, according to a study by (Vega et al., 2009). Godfrey et al. (2011) found greater methylation of the RXRA gene was noted in the umbilical cord blood of neonates in the group of pregnant women who ingested smaller amounts of carbohydrates in early pregnancy. According to Ren and Zhu (2022), in the presence of thyroid hormones (TH), ligand-bound thyroid hormone receptors (THRs) couple to thyroid hormone response elements (TREs) as a heterodimer with the retinoid X receptor (RXR). This ligand-THR/RXR complex then recruits coactivators, which are proteins that facilitate gene transcription. This complex and coordinated interaction allows THs to exert their regulatory effect on various physiological processes such as development, metabolism, and homeostasis. Mukwaya et al. (2018) demonstrated that the inflammatory process inhibits the expression of RXR; this suggests that inflammation can interfere with the function of the RXR and GDM for being an inflammatory process, possibly affecting the biological processes in which the RXR is involved.

A decreased gene and protein expression of TRFC was observed in GDM incontinent women compared to GDM continent women. TRFC is regulated according to the amounts of intracellular iron (Wallander et al., 2006). According to Macedo and Sousa (2008), when TRFC expression is increased, this is evidence of iron deficiency in cells. Furthermore, Yang et al. (2016) found that maternal TRFC levels in serum were elevated in women with GDM compared to non-pregnant women, which was not observed in our study about the GDM-PSUI group. According to Khan et al. (2005) in human vascular endothelial cells, after exposure to high levels of glucose, TRFC mRNA decreased, along with increasing oxidative stress. TRFC concentrations are useful for detecting iron deficiency and assessing the degree of iron supply in GDM (Skikne et al., 1990; Yang et al., 2016). The role of iron in the development of GDM is uncertain, but high iron stores are linked to disturbances in glucose metabolism (Yang et al., 2016) and increased risk of type 2 diabetes (Montonen et al., 2012). Although the best-known function of TRFC is the regulation of iron metabolism, it also plays an important role in

muscle physiology. Specific deletion of TRFC in muscle in animal models resulted in a number of negative effects on muscle tissue such as retardation of muscle growth. The absence of TRFC impairs iron uptake by muscle cells, negatively affecting protein synthesis and proper muscle development (Ding et al., 2021).

No significant difference was seen with respect to oxidative stress between the groups of pregnant women regarding MDA and protein carbonylation. Diabetes is a condition of glucotoxicity that leads to post-translational modifications, such as protein carbonylation (Zheng et al., 2016). Biochemically, modified proteins are the causes of tissue damage (Cederberg et al., 2001) however, this condition was not observed in our study. In the study by (Gelisgen et al., 2011) with pregnant women evaluated between 24–28 gestational weeks, an increase in plasma protein oxidation was observed in healthy pregnant women. A significant increase in MDA and vitamin E was also seen in pregnant women with GDM at 24–28 weeks of gestation, relative to healthy pregnant women (Ma et al., 2021). According to Surapaneni and Vishnu Priya (2008), oxidative stress during pregnancy is associated with the risk of anomalies, and when antioxidant vitamins are low and lipid peroxidation is increased, it is believed that GDM is a cause of the pathogenesis of lipid peroxidation, which was not noticed in the present study.

For oxidative stress, there was no significant difference between the groups of pregnant women regarding MDA and protein carbonylation. Diabetes is a condition of glucotoxicity that leads to post-translational modifications, such as protein carbonylation (Zheng et al., 2016). Biochemically, modified proteins are the cause of tissue damage (Cederberg et al., 2001); however, this condition was not observed in our study. Gelisgen et al. (2011), who evaluated pregnant women evaluated between 24–28 gestational weeks, an increase in plasma protein oxidation was observed in healthy pregnant women. A significant increase in MDA and vitamin E was also seen in pregnant women with GDM at 24–28 weeks of gestation relative to healthy pregnant women (Ma et al., 2021). According to Surapaneni and Vishnu Priya (2008), oxidative stress during pregnancy is associated with a risk of anomalies, and when antioxidant vitamins are low and lipid peroxidation is increased, GDM may be a cause of

the pathogenesis of lipid peroxidation, although this was not observed in this study.

5. Conclusion

To understand the cellular processes that regulate iron homeostasis, such as TRFC, is essential to seek new treatments for diseases such as GDM. In addition, the finding regarding the expression of TRFC in pregnant women with GDM-PSUI can be used as a tool for nutritional and/or pharmacological intervention in this population of pregnant women. Thus, the practice of nutrigenomics will help in the treatment of non-communicable diseases such as GDM, being the future of individualized nutrition to better serve patients in their needs and use the appropriate therapy.

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Author Contributions

SMBC and CRC carried out the protein carbonylation and lipid peroxidation - MDA experiments. SMBC and HRCN performed data processing and statistical analysis. SMBC, CRC, RHLS, HRCN, AMPB, and MVCR outlined the work, wrote the manuscript, and critically reviewed the article.

Declaration of competing interest

The authors declare that they have no competing interests.

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Seção 5
Perspectivas Acadêmicas e Científicas

Perspectivas em curto prazo

A publicação dos dois artigos da tese de Doutorado e a continuação das publicações na área de nutrição e medicina, a fim de contribuir para a pesquisa científica do Brasil. A divulgação da pesquisa a outros pesquisadores com o intuito de propagação da ciência e do conhecimento.

Perspectivas em médio prazo

Dar continuidade a pesquisa clínica iniciada na enfermaria da maternidade do HC por meio de um projeto de pós-doutorado para orientar as gestantes seja por meio da alimentação e/ou suplementação alimentar.

Perspectivas em longo prazo

Lecionar na disciplina de nutrição materno-infantil ou outra disciplina relacionada à área da saúde, e o atendimento a pacientes gestantes e não gestantes em consultório, bem como a construção de guidelines para a população de gestantes.

Seção 6

Diamater Study Group

Pesquisadores Nacionais

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Rita de Cássia Athanázio
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Dra. Juliana Ferreira Floriano
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Ms. Raissa Escandiusi Avramidis
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Seção 7
Anexos

Anexo 1 - Termo de Consentimento Livre e Esclarecido da Pesquisa Clínica

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE
(TERMINOLOGIA OBRIGATÓRIA EM ATENDIMENTO A RESOLUÇÃO 466/12-CNS-MS)

A sra. está sendo convidada a participar de uma pesquisa intitulada **"Estudo de coorte prospectivo: nova tríade gestacional (hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica) na predição de incontinência. Pesquisa translacional com *biodevice* para regeneração muscular em ratas com diabetes"** que pretende estudar a influência da hiperglicemia gestacional na incontinência específica da gestação e na função dos músculos do assoalho pélvico (músculos que envolvem a vagina e o ânus) , além de estudar composição dos músculos do abdome e do sangue para detectar o processo patológico desta musculatura durante a gestação e 6-12 meses pós-parto. Este projeto contempla ainda subprojetos

( ) Subprojeto 1: Relação dos achados eletromiográficos dos músculos do assoalho pélvico com os níveis de relaxina ao longo da gestação e após o parto de mulheres com hiperglicemia gestacional e incontinência urinária específica da gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Caroline Baldini Prudencio/ Nível: Doutorado);

( ) Subprojeto 2: Ultrassonografia Tridimensional do assoalho pélvico de mulheres com hiperglicemia gestacional 16-18 meses após a gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Carlos Izaias Sartorão Filho/ Nível: Doutorado);

( ) Subprojeto 3: Coorte prospectiva da tríade hiperglicemia gestacional, incontinência urinária específica da gestação e disfunção muscular do assoalho pélvico, avaliada pela ultrassonografia funcional, como preditora da incontinência urinária e disfunção muscular 6-18 meses após o parto (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Fabiane Affonso Pinheiro/ Nível: Doutorado).

( ) Subprojeto 4: Níveis de micronutrientes e da expressão de seus receptores em mulheres com Diabetes *Mellitus* Gestacional e Incontinência Urinária Específica da Gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Sarah Maria Barneze Costa/ Nível: Doutorado).

A sra. foi selecionada a participar dessa pesquisa por apresentar características que se adequam a este estudo.

( ) Assinalar: 1) Esta etapa consta de algumas perguntas sobre sua vida pessoal, histórico de doenças e hábitos. Os questionários deverão ser respondidos pela sra antes da avaliação física e farão parte de um estudo com demais mulheres que também responderão aos mesmos questionários. A entrevista durará cerca de 15 minutos.

( ) Assinalar: 2) Nesta etapa será realizado o exame físico que consta de avaliação das medidas corporais e dos músculos vaginais pelo toque digital, pela sonda intravaginal e pela ultrassonografia. O exame físico durará cerca de 40 minutos. Todos os procedimentos são seguros, porém são considerados possíveis riscos do procedimento: desconforto e dor. O procedimento tem por finalidade ajudar a esclarecer como funcionam os músculos do assoalho pélvico de gestantes com diagnóstico de diabete melito gestacional e também sem diabete gestacional.

( ) Assinalar: 3) Esta etapa é para coleta de sangue que será realizada após o exame físico, em que será coletado no máximo 10 mL de sangue para análise de componentes sanguíneos como hormônios, enzimas, vitaminas e minerais. Neste procedimento pode ocorrer dor, desconforto e hematoma no local da coleta do sangue. Caso seja necessário repetir a dosagem sanguínea de alguma análise descrita acima, utilizaremos parte ou a totalidade de no máximo 2 mL de soro do sangue que ficará armazenado em alíquotas no Biorrepositório. Este material ficará armazenado por no máximo 10 anos e caso não seja utilizado será descartado seguindo as normas específicas deste procedimento.

( ) Assinalar: 4) Esta etapa ocorrerá durante a cirurgia cesariana, momento que será coletado no máximo 3,0 cm de músculo de cada lado do músculo reto abdominal, este procedimento não oferece riscos adicionais aos já considerados ao realizar a cirurgia cesariana. Serão realizadas análises no músculo coletado. Caso seja necessário repetir alguma análise, utilizaremos parte ou a totalidade de no máximo 0,5 cm do músculo que ficará armazenado no Biorrepositório. Este material ficará armazenado por no máximo 10 anos e caso não seja utilizado será descartado seguindo as normas específicas deste procedimento.

( ) Assinalar: 5) No pós-parto a Sra será acompanhada e reavaliada em 6 momentos diferentes, sendo eles: entre 24 e 48 horas após o parto, 6 semanas após o parto, e também 6, 12, 18 e 24 meses após o parto. No momento 24 e 28 horas após o parto serão realizadas as etapas 1 e 3 somente, nos outros momentos serão realizadas todas as etapas exceto a 4.

Caso sejam utilizadas sobras de amostras de sangue ou músculo para outras pesquisas será formalizado um novo projeto de pesquisa. Solicito também seu consentimento para consultar seu prontuário médico para coletar outras informações lá contidas como suas medidas, exames de sangue, dados das gestações e do pré-natal referentes a consultas feitas anteriormente pela Senhora.

Seu benefício em participar da pesquisa será ser avaliada durante toda gestação sobre a evolução da musculatura de seus músculos vaginais e abdominal e se detectado alguma alteração você será orientada e encaminhada para o tratamento necessário. Além disso, receberá orientações de como manter sua saúde perineal para evitar possíveis perdas de urina. O deslocamento para a coleta de dados será no mesmo dia que você for realizar sua consulta médica ou outros exames de rotina relacionados à gestação, portanto, não trará ônus adicional.

Fique ciente de que sua participação neste estudo é voluntária e que mesmo após ter dado seu consentimento para participar da pesquisa, você poderá retira-lo a qualquer momento, sem qualquer prejuízo na continuidade do seu tratamento. Este Termo de Consentimento Livre e Esclarecido será elaborado em 2 vias de igual teor, o qual 01 via será entregue ao Senhor (a) devidamente rubricada, e a outra via será arquivada e mantida pelos pesquisadores por um período de 5 anos após o término da pesquisa. Qualquer dúvida adicional você poderá entrar em contrato com o Comitê de Ética em Pesquisa através dos telefones (14) 3880-1608 ou 3880-1609 que funciona de 2ª a 6ª feira das 8.00 às 11.30 e das 14.00 às 17horas, na Chácara Butignolli s/nº em Rubião Júnior – Botucatu - São Paulo. A principal investigadora é a Dra Marilza Rudge que pode ser encontrada no Distrito Rubião Jr - s/n, SP **Telefone:**(14) 3815-1711 ou por e-mail: marilzarudge@gmail.com.

Após terem sido sanadas todas minhas dúvidas a respeito deste estudo, CONCORDO EM PARTICIPAR de forma voluntária, estando ciente que todos os meus dados estarão resguardados através do sigilo que os pesquisadores se comprometeram. Estou ciente que os resultados desse estudo poderão ser publicados em revistas científicas, sem no entanto, que minha identidade seja revelada.

Eu: _____ RG:_____
Tel:(___) _____ Cel:(___) _____ End:_____
Concordo voluntariamente em participar dos procedimentos de avaliação e intervenção, os quais fui devidamente esclarecida.

Data:___/___/_____ _____
Assinatura da Participante

Pesquisador Responsável pela Coleta:
Nome: _____ Telefone: _____

Pesquisadora Responsável: _____

Anexo 2 – Aprovação do CEP

UNESP -FACULDADE DE MEDICINA DE BOTUCATU

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Projeto Mãe: Estudo de coorte prospectivo: nova tríade gestacional (hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica) na predição de incontinência. Pesquisa translacional com biodevice para regeneração muscular em ratas com diabetes

Subprojeto 1: Relação dos achados eletromiográficos dos músculos do assoalho pélvico com os níveis de relaxina ao longo da gestação e após o parto de mulheres com hiperglicemia gestacional e incontinência urinária específica da gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Caroline Baldini Prudencio/ Nível: Doutorado)

Subprojeto 2: Ultrassonografia Tridimensional do assoalho pélvico de mulheres com hiperglicemia gestacional 16-18 meses após a gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Carlos Izaias Sartorão Filho/ Nível: Doutorado);

Subprojeto 3: Coorte prospectiva da tríade hiperglicemia gestacional, incontinência urinária específica da gestação e disfunção muscular do assoalho pélvico, avaliada pela ultrassonografia funcional, como preditora da incontinência urinária e disfunção muscular 6-18 meses após o parto (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Fabiane Affonso Pinheiro/ Nível: Doutorado)

Subprojeto 4: Níveis de micronutrientes e da expressão de seus receptores em mulheres com diabetes mellitus gestacional e incontinência urinária específica da gestação(Orientador: Marilza Vieira Cunha Rudge/ Orientando: Sarah Maria Barneze Costa/ Nível: Doutorado).

Pesquisador: Marilza Vieira Cunha Rudge

Área Temática:

Versão: 4

CAAE: 82225617.0.0000.5411

Instituição Proponente: Departamento de Ginecologia e Obstetrícia

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 3.342.965

Endereço: Chácara Butignoli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

Apresentação do Projeto:

As informações descritas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas dos documentos e arquivo - Informações Básicas da Pesquisa.

No estudo aprovado (Projeto Mãe), intitulado: Estudo de coorte prospectivo: nova tríade gestacional (hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica) na predição de incontinência. Pesquisa translacional com biodevice para regeneração muscular em ratas com diabetes, constam: 3 subprojetos aprovados anteriormente:

Subprojeto 1: Relação dos achados eletromiográficos dos músculos do assoalho pélvico com os níveis de relaxina ao longo da gestação e após o parto de mulheres com hiperglicemia gestacional e incontinência urinária específica da gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Caroline Baldini Prudencio/ Nível: Doutorado)

Subprojeto 2: Ultrassonografia Tridimensional do assoalho pélvico de mulheres com hiperglicemia gestacional 16- 18 meses após a gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Carlos Izaias Sartorão Filho/ Nível: Doutorado);

Subprojeto 3: Coorte prospectiva da tríade hiperglicemia gestacional, incontinência urinária específica da gestação e disfunção muscular do assoalho pélvico, avaliada pela ultrassonografia funcional, como preditora da incontinência urinária e disfunção muscular 6-18 meses após o parto (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Fabiane Affonso Pinheiro/ Nível: Doutorado) .

Trata-se de emenda para inclusão da Discente Sarah Barneze e do subprojeto 4: Níveis de micronutrientes e da expressão de seus receptores em mulheres com Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Sarah Maria Barneze Costa/ Nível: Doutorado), com objetivo de realização de doutorado (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Sarah Maria Barneze Costa/ Nível: Doutorado). Segundo os pesquisadores, os objetivos e metodologia deste subprojeto 4 estão contemplados no projeto maior, não sendo alterados substancialmente, apenas será incluído um questionário de frequência alimentar e os exames bioquímicos do lactogênio placentário, do fator de crescimento semelhante à insulina tipo 1, da vitamina B9, da vitamina C, da vitamina E, do zinco e do ferro.

No estudo clínico, as participantes serão selecionadas entre março 2016 e dezembro de 2019 no Centro de Investigação do Diabete Perinatal da FMB UNESP, no Ambulatório de Gestação de Risco do Hospital Materno Infantil da FAMEMA e no Hospital Regional de Assis. O total de participantes

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será de 160 considerando as quatro coortes que serão constituídas. Será realizada análise do perfil bioquímico, obtido pelas análises sanguíneas dos níveis de CCL7, Relaxina, Ca, PTH, calcitonina, vitamina D, glicose e insulina; nos MAPs, serão realizados a Avaliação Funcional do Assoalho pélvico (AFA), Eletromiografia (EMG) e Ultrassonografia (US), nos MRA serão realizados EMG e US, no momento da cesárea a contratilidade ex vivo obtida pelo Miógrafo, e os perfis morfológico (ultraestrutura das fibras musculares e da matriz extracelular), molecular (Expressão Gênica e Proteica das fibras musculares e da matriz extracelular), organóide, Ômico (transcriptômica, proteômica e metabolômica). Para o estudo experimental, serão utilizadas ratas Wistar prenhes, divididas em grupos (n=20 cada) não diabéticas e diabéticas, que serão submetidas à instalação do biodevice de biomembrana de látex revestida de células-tronco mesenquimais, implantados na musculatura estriada uretral e MRA. O estudo de Revisão Sistemática seguirá as normas e será registado na Colaboração Cochrane.
Tamanho da Amostra no Brasil: 160 participantes.

Objetivo da Pesquisa:

Analisar a influência da associação entre HGG (DMG e HGL), "IUEG" no perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico) dos MRA e MAPs. Hipótese nula correspondente: Não há associação entre HG gestacional, "IUEG" com o perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico).Objetivo 2: Analisar a tríade HGG, "IUEG" e perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico) do MRA e do MAPs, com incidência de IU 6-12 meses PP e perfil morfológico, funcional e bioquímico, dentro das quatro coortes constituídas pela combinação entre HGG e "IUEG".Hipótese nula correspondente: O risco de IU 6-12 meses PP e o perfil morfológico, bioquímico e funcional do MRA e MAPs não sofrem efeito da tríade HGG, "IUEG" e perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico) do MRA e do MAPs em mulheres com HGG.Objetivo 3: Biodevice Avaliar o efeito do biodevice de látex de seringueira com CTMs na regeneração do músculo estriado uretral e MRA no PP de ratas com diabete moderado, no perfil morfológico, microvascular, molecular e ômico. Hipótese nula correspondente: Não há efeito estatisticamente significativo do uso do biodevice com CTMs sobre a miopatia diabética. Objetivo 4: Revisão Sistemática: Determinar a melhor evidência científica, por meio da revisão sistemática, sobre efetividade dos exercícios sobre a DMAP e "IUEG" em gestantes HGs.

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UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

Avaliação dos Riscos e Benefícios:

EMG, US, AFA, dinamometro: Desconforto e dor leves na região vaginal. Coleta sangue: dor, desconforto, hematoma local (solução, compressão e gelo). Retalho de músculo: este procedimento não oferece riscos adicionais aos já considerados ao realizar a cirurgia cesariana Benefícios: A- Que o conjunto das análises morfológicas, funcionais, bioquímicas, moleculares e o mapeamento ômico caracterizem a "MHG" relacionada à "IUEG" em gestantes HGs em dois estádios de gravidade da miopatia diferenciados pelo tipo de distúrbio hiperglicêmico (HGL e DMG). B - Que a tríade caracterizada por HGG, "IUEG" e perfil (morfológico, funcional, bioquímico, molecular e ômico) da "MHG" do MRA e MAPs seja preditora de IU 6-12 meses PP. C - Que o conjunto das análises morfológicas, funcionais, bioquímicas, moleculares e o mapeamento ômico definam marcadores úteis. no diagnóstico e em futuras terapias. D - Que a revisão sistemática evidencie a primeira linha de intervenção preventiva ou reabilitadora para a DMAP e "IUEG" em gestantes HGs. E - Que a "IUEG" em gestantes HGs seja incluída de modo inovador como intercorrência clínica na gestação diabética e com HGL e represente nova ferramenta na definição do impacto materno de longo prazo do DMG e HGL. F - Espera-se que a "IUEG" em gestantes HGs seja considerada problema de saúde pública pela imbricação de duas patologias com elevada prevalência (IU e DMG), que tem impacto no custo em saúde e que uma (HGG-DMG ou HGL), por sua ação sobre os músculos (miopatia), se confirme como a base fisiopatológica da ocorrência da outra (IU). G - Que seja confirmada a potencialidade da utilização do biodevice de biomembrana de látex como suporte para CTMs, para regeneração da musculatura estriada uretral e no MRA PP cesárea de ratas com diabete moderado para futuros estudos nas fases pré-clínica e clínica. Esta abordagem translacional proposta trará esta pesquisa para a fronteira do conhecimento na área de Saúde da Mulher, uma vez que estão sendo aplicadas metodologias de análise que se complementam na abordagem de problemática de interesse médico/social.

Comentários e Considerações sobre a Pesquisa:

Trata-se de emenda para inclusão da Discente Sarah Barneze e do subprojeto 4: Níveis de micronutrientes e da expressão de seus receptores em mulheres com Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Sarah Maria Barneze Costa/ Nível: Doutorado), com objetivo de realização de doutorado (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Sarah Maria Barneze Costa/ Nível: Doutorado). Segundo os pesquisadores, os objetivos e metodologia deste subprojeto 4 estão contemplados no projeto maior, não sendo alterados substancialmente, apenas será incluído um questionário de frequência alimentar e os exames bioquímicos do lactogênio placentário, do fator

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de crescimento semelhante à insulina tipo 1, da vitamina B9, da vitamina C, da vitamina E, do zinco e do ferro.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados os documentos necessários. Novo TCLE foi apresentado com a inclusão do subprojeto 4. A folha de rosto consta o subprojeto 4.

Recomendações:

Apresentar relatório final de atividades após conclusão da pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Após análise em REUNIÃO ORDINÁRIA, o Colegiado deliberou APROVADA a emenda apresentada.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado em reunião EXTRAORDINÁRIA do Comitê de Ética em Pesquisa da FMB/UNESP, realizada em 21 de MAIO de 2019, o documento enviado na forma de "EMENDA", encontra-se APROVADO, sem necessidade de envio à CONEP.

Atenciosamente,

Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu – UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1255054_E1.pdf	11/04/2019 13:42:25		Aceito
Outros	Carta_resposta_comite_c.pdf	11/04/2019 13:41:35	Marilza Vieira Cunha Rudge	Aceito
Folha de Rosto	FolhaDeRostoAssinada_Sub_Proje_4_Sarah.pdf	29/01/2019 11:51:24	Sarah Maria Barneze Costa	Aceito
Outros	TermoDeAnuenciaInstitucional_Sub_Proj_4_Sarah.pdf	29/01/2019 11:44:05	Sarah Maria Barneze Costa	Aceito
Projeto Detalhado / Brochura Investigador	Oficio_ao_comite_sub_proj_4_Sarah.pdf	29/01/2019 11:41:10	Sarah Maria Barneze Costa	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_tematico_com_subprojeto_4_de_Sarah_Barneze.pdf	29/01/2019 11:35:52	Sarah Maria Barneze Costa	Aceito
Projeto Detalhado / Brochura	Projeto_doutorado_Sarah_Barneze_sub_projeto_4.pdf	29/01/2019 11:32:45	Sarah Maria Barneze Costa	Aceito

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Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

Continuação do Parecer: 3.342.965

Investigador	Projeto_doutorado_Sarah_Barneze_sub projeto_4.pdf	29/01/2019 11:32:45	Sarah Maria Barneze Costa	Aceito
Outros	EAP_Marilza_Rudge.pdf	18/01/2018 17:27:08	Marilza Vieira Cunha Rudge	Aceito
Outros	Fluxograma_Tematico_CEP.pptx	26/11/2017 19:18:14	Marilza Vieira Cunha Rudge	Aceito
Outros	HRA.pdf	26/11/2017 19:09:17	Marilza Vieira Cunha Rudge	Aceito
Outros	termodeautorizacao_HRA.pdf	26/11/2017 19:01:53	Marilza Vieira Cunha Rudge	Aceito
Outros	coparticipacao_assis_municipio.jpeg	26/11/2017 18:53:44	Marilza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_3.doc	05/10/2017 23:41:43	Marilza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_2.docx	05/10/2017 23:41:04	Marilza Vieira Cunha Rudge	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_mae.docx	05/10/2017 23:39:04	Marilza Vieira Cunha Rudge	Aceito
Outros	Projeto_mae_Subprojetos.docx	05/10/2017 23:37:51	Marilza Vieira Cunha Rudge	Aceito
Orçamento	orcamento_tematico.pdf	05/10/2017 23:36:42	Marilza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_1.doc	05/10/2017 22:54:11	Marilza Vieira Cunha Rudge	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_tematico.docx	05/10/2017 22:48:47	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_FFC.pdf	21/09/2017 17:02:46	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Famema.jpeg	21/09/2017 17:02:11	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Araraquara.pdf	21/09/2017 17:00:09	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Bauru.pdf	21/09/2017 16:59:28	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_RioPreto.pdf	21/09/2017 16:58:52	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_RioClaro.pdf	21/09/2017 16:57:58	Marilza Vieira Cunha Rudge	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:

Endereço: Chácara Butignolli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
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Anexo 3 - Termo de Ciência e Anuência da Responsável pela Seção Técnica de Laboratório de Análises Clínicas do HCFMB

FACULDADE DE MEDICINA DE BOTUCATU – UNESP

TERMO DE CIÊNCIA E ANUÊNCIA
(Preenchido pela Chefia Imediata do Serviço)

Eu, Maria Salete Sartori, responsável pela Seção Técnica de Laboratório de Análises Clínicas declaro que estou ciente da realização dos exames laboratoriais do projeto de pesquisa intitulado: Níveis de micronutrientes e da expressão de seus receptores em mulheres com Diabetes *Mellitus* Gestacional e Incontinência Urinária Específica da Gestação a ser desenvolvido pela Pesquisadora Sarah Barneze sob orientação da Professora Emérita Marilza Vieira Cunha Rudge no período de seu doutorado (01 de março de 2019 a 01 de dezembro de 2022).

Após a aprovação do projeto de pesquisa pelo Comitê de Ética (CEP), a sua condução nos Serviços do HCFMB, deverá ser planejada entre Pesquisador Principal e Responsável pelo Serviço para não interferir na operacionalização e/ou atividades cotidianas dos mesmos. O HCFMB não arcará com despesa decorrente das atividades relacionadas à pesquisa desenvolvida.

Botucatu, 09 de Janeiro de 2018.

Assinatura e Carimbo do Responsável pelo Serviço

Superintendência do Hospital das Clínicas- HCFMB
Distrito de Rubião Júnior, s/n | CEP 18618-970
Botucatu | São Paulo | Brasil
Tel. (14) 3811-6215 | 3811-6218 | 3811-6100 | Fax 3882-5387
www.hcfmb.unesp.br |

Depto. de Gestão de Atividades Acadêmicas-DGAA
Núcleo de Pesquisa
Tel. (14) 3811-6426
pesquisadgaa@hcfmb.unesp.br

SÃO PAULO

Anexo 4 – Termo de Ciência e Anuência da Responsável pelo Serviço da Enfermaria de Obstetrícia

HOSPITAL DAS CLÍNICAS
FACULDADE DE MEDICINA DE BOTUCATU – UNESP

TERMO DE CIÊNCIA E ANUÊNCIA
(Preenchido pela Chefia Imediata do Serviço)

Eu, Claudia Garcia Magalhães, responsável pelo Serviço da Enfermaria de Obstetrícia, declaro que estou ciente e de acordo com o projeto de pesquisa intitulado: Níveis de micronutrientes e da expressão de seus receptores em mulheres com Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação, a ser desenvolvido pela Pesquisadora Sarah Barneze sob orientação da Profa. Emérita Marilza Vieira Cunha Rudge nesta unidade no período de 01 de fevereiro de 2019 a 30 de dezembro de 2022.

Após a aprovação do projeto de pesquisa pelo Comitê de Ética (CEP), a sua condução nos Serviços do HCFMB, deverá ser planejada entre Pesquisador Principal e Responsável pelo Serviço para não interferir na operacionalização e/ou atividades cotidianas dos mesmos. O HCFMB não arcará com despesa decorrente das atividades relacionadas à pesquisa desenvolvida.

Botucatu, 26 de Novembro de 2018.

Assinatura e Carimbo do Responsável pelo Serviço

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Núcleo de Pesquisa
Tel. (14) 3811-6426
pesquisadgaa@hcfmb.unesp.br

Anexo 5 – Termo de Anuência Institucional

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

ESCRITÓRIO DE APOIO À PESQUISA

Documento de Anuência Institucional

Em tendo cumprido as análises solicitadas pelo Fluxo Institucional de Projetos de Pesquisa da Faculdade de Medicina de Botucatu e Hospital das Clínicas da Faculdade de Medicina de Botucatu, informamos que a proposta de pesquisa "Níveis de micronutrientes e da expressão de seus receptores em mulheres com Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação", protocolo 421/2018, que tem como autor principal "Sarah Maria Barneze Costa" sob orientação de "Marilza Vieira Cunha Rudge" está liberada para ser submetida ao Órgão de Ética da Instituição.

Atenciosamente

Prof. Adjunto Pasqual Barretti
Diretor da Faculdade de Medicina

Botucatu, 29 de janeiro de 2019

Documento gerado pelo SIPE.
Unidade Responsável: EAP - Escritório de Apoio à Pesquisa
Funcionário responsável: Fernanda Chiuso Minicucci

Anexo 6 – Disciplinas cursadas

DISCIPLINA	CRÉDITOS	CARGA HORÁRIA	FREQUÊNCIA	SITUAÇÃO
"A Captação de Recursos Externos para Pesquisa"	2	30h	100%	Aprovada
"Seminários de Projetos de Teses"	3	45h	100%	Aprovada
"Seminars in Maternal and Offspring Health"	12	180h	100%	Aprovada
"Tópicos Especiais: Diabetes and Obesity in Pregnancy: From Patients to Molecular Mechanisms"	3	45h	100%	Aprovada
"Ação dos Alimentos, da Flora Bacteriana e da Absorção Intestinal como Reguladores Inflamatórios em Doenças Crônicas"	3	45h	100%	Aprovada
"Alterações Morfológico-funcionais e Bioquímicas Induzidas pela Obesidade"	3	45h	100%	Aprovada
"Seminários de Trabalhos Científicos"	2	30h	100%	Aprovada

DISCIPLINA	CRÉDITOS	CARGA HORÁRIA	FREQUÊNCIA	SITUAÇÃO
"Tópicos Especiais: Melhore a Qualidade das suas Sequencias"	3	45h	100%	Aprovada
"Alterações Funcionais e Metabólicas no Paciente Crítico"	2	30h	100%	Aprovada
"Saúde Baseada em Evidências"	3	45h	100%	Aprovada
"Apresentação e Avaliação dos Projetos Científicos"	2	30h	100%	Aprovada
"Tópicos Especiais: A Inteligência Artificial (AI) na estruturação e análise de dados para apoiar a geração de conhecimento no projeto temático DIAMATER"	2	30h	100%	Aprovada

Anexo 7 – Artigos publicados em periódicos

DIABETES RESEARCH AND CLINICAL PRACTICE 166 (2020) 108315

Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Deleterious effects of gestational diabetes mellitus on the characteristics of the rectus abdominis muscle associated with pregnancy-specific urinary incontinence

Giovana Vesentini [a,b], *Angélica M.P. Barbosa* [c], *Juliana F. Floriano* [a,b], *Sérgio L. Felisbino* [d], *Sarah M.B. Costa* [a,b], *Fernanda Piculo* [a,b], *Gabriela Marini* [a,b,e], *Sthefanie K. Nunes* [a,b], *David R.A. Reyes* [a,b], *João P.C. Marcondes* [a,b], *Raghavendra L.S. Hallur* [a,b], *Ariane L. Rozza* [d], *Cláudia G. Magalhães* [a,b], *Roberto Costa* [a,b], *Joelcio F. Abbade* [a,b], *José E. Corrente* [f], *Iracema M.P. Calderon* [a,b], *Selma M.M. Matheus* [g], *Marilza V.C. Rudge* [a,b,*], *The Diamater Study Group* [1]

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Keywords:
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Skeletal muscle

ABSTRACT

Aims: To evaluate the effects of gestational diabetes mellitus (GDM) on the structural characteristics of the rectus abdominis muscle (RAM) and its indirect effects on pregnancy-specific urinary incontinence (PSUI).

Methods: A total of 92 pregnant women were divided into four groups, according to their clinical conditions: non-GDM continent, non-GDM associated PSUI, GDM continent and GDM associated PSUI. The muscle morphometry (histochemistry and immunohistochemistry) for the fiber types and collagen fiber distribution, the ultrastructural analysis (transmission electron microscopy), the protein expression of fiber types and calcium signaling (Western blotting), and the content of types I and III collagen fiber (ELISA) in RAM collected at delivery were assessed.

Results: The GDM groups presented a significantly increased number of slow fibers and slow-twitch oxidative fiber expression; decreased fiber area, number of fast fibers, and area of collagen; an increase in central nuclei; ultrastructural alterations with focal lesion areas such as myeloid structures, sarcomere disorganization, and mitochondrial alteration. The

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BioMedical Engineering OnLine

RESEARCH Open Access

Viability of ex-vivo myography as a diagnostic tool for rectus abdominis muscle electrical activity collected at Cesarean section within a diamater cohort study

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Abstract

Background: *Ex-vivo* myography enables the assessment of muscle electrical activity response. This study explored the viability of determining the physiological responses in muscles without tendon, as rectus abdominis muscle (RAM), through *ex-vivo* myography to assess its potential as a diagnostic tool.

Results: All tested RAM samples (five different samples) show patterns of electrical activity. A positive response was observed in 100% of the programmed stimulation. RAM 3 showed greater weight (0.47 g), length (1.66 cm), and width (0.77 cm) compared to RAM 1, RAM 2, RAM 4 and RAM 5 with more sustained electrical activity over time, a higher percentage of fatigue was analyzed at half the time of the electrical activity. The order of electrical activity (Mn) was RAM 3 > RAM 5 > RAM 1 > RAM 4 > RAM 2. No electrical activity was recorded in the Sham group.

Conclusions: This study shows that it is feasible to assess the physiological responses of striated muscle without tendon as RAM, obtained at C-section, under *ex vivo* myography. These results could be recorded, properly analyzed, and demonstrated its potential as a diagnostic tool for rectus abdominis muscle electrical activity.

Keywords: Contractility, Rectus abdominis muscle, C-section, Myography, Skeletal muscle

Background

The Rectus Abdominis Muscle (RAM), a striated muscle without tendon, extending along the abdomen to the pubic symphysis, undergoes physiological adaptations during pregnancy [1–5]. As a striated muscle, the main focus of research has been on contractile muscle cells [6]. RAM has synergistic contractile activity with the pelvic floor

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Infraphysiological 17β-estradiol (E2) concentration compromises osteoblast differentiation through Src stimulation of cell proliferation and ECM remodeling stimulus

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ARTICLE INFO

Keywords:
Mesenchymal stem cells
Osteoblast
Hormone
Estrogen
Gene expression

ABSTRACT

It has been shown that 17β-estradiol (E2) helps to prevent bone loss. This study was undertaken to verify whether E2 action in human osteoblasts involves changes in the transcriptional profile of the TNF-α, IFN-γ, NF-κB, TRAIL, TGF-β, MMP2, MMP9, RECK, TIMP1, TIMP2, CDK2, CDK4, SRC, RUNX2, and SHH genes. Infraphysiological doses of E2 elevated mRNAs in all genes except for INF-γ, TRAIL, and TGF-β. Importantly, a significant increase in the CDKs −2 and −4 genes was found, which strongly suggests cell cycle progression, with a potential dependency of Src involvement, as well as a suppression of the osteoblast differentiation machinery, with ECM remodeling being involved. These data suggest that E2 plays an important role in bone formation and remodeling, and Src seems to play a pivotal role in driving cell proliferation and ECM remodeling. Taken together, these findings contribute to an understanding of the effects of infraphysiological E2 on modulating bone homeostasis, favoring bone resorption, and leading to osteoporosis.

1. Introduction

It is well known that the estrogen hormone, 17β-estradiol (E2) acts on osteoblasts through nuclear receptors and regulates the cytokines involved in bone remodeling (Kisakol et al., 2003). The activation of osteoclastogenesis via the receptor activator of the nuclear factor kappa-B (RANK)/receptor of the nuclear factor kappa-B ligand (RANKL) is well known (Menezes et al., 2006), but the role that the genes involved in osteoblastic differentiation play in responding to E2 remains little known (Nicks et al., 2009).

E2 deficiency in the body establishes an imbalance in the activity of bone cells, mainly in relation to the coupling of osteoclasts and osteoblasts, and this culminates in a disruption of the synchrony required for bone remodeling, resulting in increased bone loss and subsequent osteoporosis, mainly occurring in postmenopausal women (Nicks et al., 2009; Weitzmann and Pacifici, 2006). The biological issues of aging deserve attention.

It is known that osteoclastogenesis develops from monocyte cells responding to trophic molecules such as tumor necrosis factor (TNF-α) and RANKL, and an increase has been reported in the number of pre-osteoclasts in subjects with E2 deficiency (D'Amelio et al., 2008), possibly in scenarios with higher inflammation. Specifically, in the bone resorption area, increased osteoclast activity is associated with cytokines other than RANKL, such as interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 11 (IL-11), and macrophage colony stimulating factor (Kwan Tat et al., 2004; Wittrant et al., 2004). At normal levels, E2suppresses the cytokines that induce osteoclastogenesis (Rodan and Martin, 2000). The inflammation landscape revels a progressive resorption scenario in bone, and recent studies have revealed that hedgehog signaling is important in acute injury and inflammation. In bone, E2 acts through direct and/or indirect routes and regulates biological responses in terms of osteoblast performance (proliferation and apoptosis) to

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Available online 8 September 2020
0303-7207/

Anexo 8 – Cursos complementares

CERTIFICADO

CERTIFICAMOS QUE:

BRASPEN

Sarah Barneze

Participou do Webinar BRASPEN: **Webinar BRASPEN: Highlights do Congresso ASPEN 2021**, promovido pela Sociedade Brasileira de Nutrição Parenteral e Enteral (BRASPEN), com carga horária de 01 hora e 30 minutos.

São Paulo, 30 de março de 2021

Vale 05 pontos para prova de título BRASPEN.

Dra. Melina Gouveia Castro
Presidente da BRASPEN - Gestão 2020-2021

Dra. Clarissa Martins Zambelli
2ª Secretária BRASPEN - Gestão 2020-2021

WEBINAR BRASPEN
Vale 5 pontos para Prova de Título BRASPEN

braspen.org

CERTIFICADO

Nestlé Health Science e SBD certificam que

Sarah Maria Barneze Costa

concluiu e foi aprovado(a) na avaliação final do curso de

Manejo multidisciplinar do diabetes

Essa aprovação contará com uma carga horária de 3 horas.

09/04/2021

Domingos Augusto Malerbi
Presidente da Sociedade Brasileira de Diabetes

Monica Meale
Vice-Presidente
Nestlé Health Science

Apoio científico:

SOCIEDADE BRASILEIRA DE DIABETES

Avante
Nestlé HealthScience

The Physiological Society

Placental Adaptation in Gestational Diabetes and Obesity

25 May 2021 | 19:00 – 20:00 BST

To Whom It May Concern,

Certificate of Attendance

This is to certify that Sarah Maria Barneze Costa attended the 'Placental Adaptation in Gestational Diabetes and Obesity' webinar on **25 May 2021.**

Yours faithfully,
The Physiological Society

UNICAMP

PÓS GRADUAÇÃO CIÊNCIAS DA CIRURGIA FCM/UNICAMP

Unifal MG Universidade Federal de Alfenas

CIÊNCIAS DA REABILITAÇÃO

Programa de Pós-Graduação de Ciências da Reabilitação (UNIFAL-MG)
&
Programa de Pós-Graduação em Ciências da Cirurgia (FCM/Unicamp)

Certificamos que

Sarah Barneze

participou do ***Encontro Científico do grupo de UroFisioterapia***, realizado no dia 30 de novembro de 2018, na Universidade Estadual de Campinas – Unicamp, com carga-horária de 8 horas.

Cidade Universitária "Zeferino Vaz", 30 de novembro de 2018

Profa. Dra. Raquel Franco Leal
Coordenadora
Programa de Pós-Graduação em Ciências da Cirurgia
FCM/Unicamp

Prof. Dra. Simone Botelho
Coordenadora
Programa de Pós-Graduação de Ciências da Reabilitação
UNIFAL/MG

The Physiological Society

Obesity Inflammasome as a Risk for Developing Gestational Diabetes

27 April 2021 | 19:00 – 20:00 BST

To Whom It May Concern,

Certificate of Attendance

This is to certify that Sarah Maria Barneze Costa attended the 'Obesity Inflammasome as a Risk for Developing Gestational Diabetes' webinar on **27 April 2021.**

Yours faithfully,
The Physiological Society

Diabetes, Obesity and Pregnancy Outcomes

29 June 2021 | 19:00 – 20:00 BST

To Whom It May Concern,

Certificate of Attendance

This is to certify that Sarah Maria Barneze Costa attended the 'Diabetes, Obesity and Pregnancy Outcomes' webinar on **29 June 2021.**

Yours faithfully,
The Physiological Society

Anexo 9 – Participação em eventos

XXIII Congresso Paulista de Obstetrícia e Ginecologia

23 a 25 de agosto de 2018
Transamerica Expo Center • São Paulo, SP

SOGESP

CERTIFICADO

Certificamos que
QUIROZ, S.B.C.V.; PRUDENCIO, C.B.; HELIODORO, M.L.A.; COSTA, S.M.B.; PINHEIRO, F.A.; BARBOSA, A.M.P.
participaram do XXIII Congresso Paulista de Obstetrícia e Ginecologia 2018,
realizado do dia 23 a 25 de Agosto de 2018, no Transamerica Expo Center,
em São Paulo - SP, com o trabalho
O071 - "INCONTINENCIA URINÁRIA ESPECÍFICA DA GESTAÇÃO E DIABETES GESTACIONAL"
em forma de e-pôster.

Rossana Pulcineli Vieira Francisco — *Presidente do Congresso*
Manoel João Batista Castello Girão — *Diretor Científico*
Rosiane Mattar — *Coordenadora Científica de Obstetrícia*
Rogério Bonassi Machado — *Coordenador Científico de Ginecologia*

ICS 2018 PHILADELPHIA

Certificate of Acceptance

Presented to

Rudge M V C1, Vesentini G1, Piculo F2, Harada B S1, Leite A P S1, Fernandes J N1, Pinto C G3, Damasceno D C1, Matheus S M M3, Barneze S1, Barbosa A M P4, Marini G5

Had the following abstract accepted at the 48th International Continence Society Annual Meeting
28-31 August, Philadelphia, United States

Open Discussion ePoster, Abstract 559: Morphological analysis of the rectus abdominis muscle in pregnant women with hyperglycemia and urinary incontinence.

Laurence Stewart — ICS Scientific Chair
Roger R. Dmochowski — ICS 2018 Scientific Co-Chair
Lori Birder — ICS 2018 Scientific Co-Chair


FIGO WORLD CONGRESS OF GYNECOLOGY AND OBSTETRICS
XXII · RIO
CERTIFICATE
FIGO International Federation of Gynecology and Obstetrics
the Global Voice for Women's Health
We hereby certify that the paper entitled
IMPACT OF OBESITY AND GESTATIONAL DIABETES ON PREGNANCY-SPECIFIC URINARY INCONTINENCE
by the authors GIOVANA VESENTINI; FERNANDA PICULO; SARAH BARNEZE; STHEFANIE KENICKEL NUNES; SOFIA VEGA QUIROZ; GABRIELA MARINI; ANGÉLICA MÉRCIA PASCON BARBOSA; MARILZA VIEIRA CUNHA RUDGE
Was presented as an E-POSTER during the International Federation of Gynecology and Obstetrics (FIGO) XXII FIGO World Congress 2018 at RioCentro, Brazil, from October 14 to 19, 2018.
CN Purandare
FIGO President
Luis Cabero
Chair of Organizing Committee FIGO 2018
Johan Vos
FIGO Chief Executive
EMS

Anexo 10 - Estágio em Docência

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

CERTIFICADO

Certificamos que o(a) discente Sarah Maria Barneze Costa, CPF nº 38295726897, participou do Programa de Aperfeiçoamento e Apoio à Docência no Ensino Superior - PAADES, na disciplina Dietoterapia I de responsabilidade do Departamento de Ciências Humanas e Ciências da Nutrição e Alimentação do Câmpus de Botucatu, no periodo de 14/06/2021 a 15/10/2021 (CH= 56 h em atividades de apoio à docência e 4 h em atividades de docência).

Maria Valnice Boldrin
Pró-reitora de Pós Graduação
PROPG/UNESP

Celia Maria Giacheti
Pró-reitora Graduação
PROGRAD/UNESP

ANEXO 11 – Tabela da quantidade de cada nutriente ingerido pelas gestantes em inglês - Dietary intake of nutrients in GDM-PSUI compared with 3 control groups (values expressed as mean and standard deviation

Group	Non-GDM-C	Non-GDM-PSUI	GDM-C	GDM-PSUI		
Variable	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	***P-value***	**Differences Between Groups**
Carbohydrates (g)	203.39 ± 121.76	218.95 ± 88.11	213.90 ± 92.85	212.59 ± 79.46	*0.418*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Glycemic Index (g)	161.74 ± 114.78	215.73 ± 133.19	196.60 ± 97.81	214.49 ± 139.62	*0.000*	GDM-PSUI>Non-GDM-C**; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Proteins (g)	74.58 ± 53.22	86.13 ± 46.67	97.94 ± 52.09	83.28 ± 47.41	*0.000*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C; Non-GDM-C<GDM-C***
Aromatic Amino Acids (mg)	2608.88 ± 2188.66	2582.05 ± 2119.65	3510.05 ± 2430.56	3235.78 ± 1997.79	*0.000*	GDM-PSUI>Non-GDM-C*; GDM-PSUI>Non-GDM-PSUI*; GDM-PSUI=GDM-C
Branched Amino Acids (mg)	5604.88 ± 4726.32	6063.76 ± 4698.98	7564.04 ± 5329.83	7054.93 ± 4379.35	*0.000*	GDM-PSUI>Non-GDM-C*; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Total Fat (g)	48.85 ± 34.54	57.71 ± 29.19	52.94 ± 25.73	52.10 ± 32.08	*0.024*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Monounsaturated Fats (g)	13.34 ± 10.49	15.72 ± 9.74	14.86 ± 8.53	15.48 ± 11.16	*0.072*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Polyunsaturated Fats (g)	8.79 ± 6.15	8.87 ± 5.24	9.80 ± 4.77	7.38 ± 3.79	*0.000*	GDM-PSUI<Non-GDM-C*; GDM-PSUI<Non-GDM-PSUI*; GDM-PSUI<GDM-C**
Omega-3 (g)	1.25 ± 12.82	0.38 ± 0.55	0.41 ± 0,65	0.38 ± 0.62	*0.466*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Omega-6 (g)	6.38 ± 9.05	5.12 ± 6.36	5.30 ± 7.43	5.16 ± 6.64	*0.266*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Saturated Fat (g)	16.10 ± 12.48	19.64 ± 11.69	18.72 ± 20.76	16 ± 9.39	*0.012*	GDM-PSUI<Non-GDM-PSUI*; GDM-PSUI=Non-GDM-C; GDM-PSUI=GDM-C
Cholesterol (mg)	222.40 ± 152.46	242.71 ± 139.41	312.63 ± 211.54	227.11 ± 137.45	*0.000*	GDM-PSUI<GDM-C***; GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI
Dietary Fiber (g)	16.62 ± 9.88	17.95 ± 8.17	19.03 ± 8.07	19.56 ± 8.45	*0.006*	GDM-PSUI>Non-GDM-C*; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Insoluble Fiber (g)	8.80 ± 5.87	9.60 ± 5.14	9.88 ± 4.53	10.16 ± 4.73	*0.066*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Soluble Fiber (g)	4.90 ± 3.26	5.36 ± 2.80	5.51 ± 2.47	5.45 ± 2.21	*0.130*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Ascorbic acid (mg)	122.64 ± 829.42	89.37 ± 103.63	163.99 ± 649.54	115.84 ± 153.49	*0.634*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Retinol (mcg)	714.14 ± 1256.74	730.26 ± 825.79	841.24 ± 931.55	768.66 ± 1404.35	*0.729*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Beta Carotene (mcg)	1361.71 ± 2750.47	1403.97 ± 2924.03	2469.52 ± 4402.12	1720.88 ± 3744.80	*0.011*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Lycopene (mg)	5.00 ± 2.16	3.42 ± 12.14	6.76 ± 25.73	6.25 ± 23.29	*0.451*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Tocopherol (mg)	10.62 ± 8.96	10.32 ± 5.97	11.91 ± 6.39	10.06 ± 4.83	*0.070*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Calcium (mg)	410.90 ± 378.55	585.56 ± 333.02	563.50 ± 281.03	479.44 ± 297.51	*0.000*	GDM-PSUI<Non-GDM-PSUI*; GDM-PSUI=Non-GDM-C; GDM-PSUI=GDM-C
Chrome (mcg)	57.50 ± 89.64	56.18 ± 82.62	85.94 ± 128.54	84.84 ± 89.53	*0.001*	GDM-PSUI>Non-GDM-C*; GDM-PSUI>Non-GDM-PSUI*; GDM-PSUI=GDM-C
Iron (mg)	47.73 ± 257.88	50.79 ± 271.60	64.81 ± 321.36	24.70 ± 153.18	*0.559*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Heme Iron (mg)	1.90 ± 3.35	1.52 ± 3.34	1.84 ± 3.63	2.25 ± 3.92	*0.223*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Non-Heme Iron (mg)	0.69 ± 1.48	189.52 ± 75.06	0.35 ± 0.62	0.46 ± 0.61	*0.073*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Magnesium (mg)	170.83 ± 95.17	0.54 ± 1.62	211.45 ± 80.09	197.96 ± 132.10	*0.001*	GDM-PSUI>Non-GDM-C*; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C

Selenium (mcg)	17.59 ± 48.85	14.92 ± 24.50	24.82 ± 32.59	8.32 ± 16.54	*0.000*	GDM-PSUI<Non-GDM-C*; GDM-PSUI<GDM-C***; GDM-PSUI=Non-GDM-PSUI
Zinc (mg)	9.10 ± 7.95	9.69 ± 6.89	12.20 ± 10.20	11.68 ± 8.80	*0.001*	GDM-PSUI>Non-GDM-C*; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C
Caffeine (mg)	127.39 ± 553.24	96.92 ± 480.66	161.37 ± 823.85	67.94 ± 443.10	*0.490*	GDM-PSUI=Non-GDM-C; GDM-PSUI=Non-GDM-PSUI; GDM-PSUI=GDM-C

n: number of pregnant women; SD: standard deviation; 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-UI: gestational diabetes mellitus and incontinent group; p-value: probability of observing a test statistic value greater than or equal to the one found; *: p<0.05; **: p<0.001; ***: p<0.0001; mg: milligram; ml: milliliters; mcg: microgram; g: grams.