



**UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE MEDICINA**

Caroline Baldini Prudencio

**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**

*The relation between pelvic floor electromyography and relaxin levels during
pregnancy and post-partum of women with gestational hyperglycaemic and
pregnancy specific urinary incontinence*

Tese apresentada à Faculdade de Medicina,
Universidade Estadual Paulista “Júlio de Mesquita
Filho”, Campus de Botucatu, como parte dos
requisitos para obtenção do título de Doutora em
Tocoginecologia.

Orientadora: Profa. Dra. Angélica Mércia Pascon Barbosa

Botucatu

2021

Caroline Baldini Prudencio

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

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de Botucatu, como parte dos requisitos para obtenção do título de Doutora em Tocoginecologia.

Thesis presented to the Botucatu Medical School, São Paulo State University “Júlio de Mesquita Filho”, as part of the requirements for obtaining the title of Doctor in Tocogynecology.

Orientadora: Profa. Dra. Angélica Mércia Pascon Barbosa

Botucatu

2021

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉCN. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Prudencio, Caroline Baldini.

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 / Caroline Baldini Prudencio. - Botucatu, 2021

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina de Botucatu

Orientador: Angélica Mércia Pascon Barbosa

Capes: 40101150

1. Diabetes gestacional. 2. Gravidez. 3. Distúrbios puerperais. 4. Relaxina. 5. Assoalho pélvico. 6. Hiperglicemia. 6. Incontinência urinária.

Palavras-chave: Assoalho pélvico; Diabetes gestacional; Gestação; Pós-parto; Relaxina.

Caroline Baldini Prudencio

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 específica da gestação

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de Botucatu, como parte dos requisitos para obtenção do título de Doutora em Tocoginecologia

Orientadora: Prof^a. Dra. Angélica Mércia Pascon Barbosa

Banca Examinadora

Prof^a. Dra. Angélica Mércia Pascon Barbosa
Faculdade de Medicina de Botucatu – UNESP/Botucatu

Prof^a. Dra. Fatima Fani Fitz
Escola Paulista de Medicina (UNIFESP/EPM)

Prof^a. Dra. Patricia Driusso
Universidade Federal de São Carlos (UFSCAR)

Prof^a. Dra. Flávia Ignácio Antônio
Faculty of Health Sciences- University of Ottawa

Prof. Dr. Luiz Gustavo Oliveira Brito
Faculdade de Ciências Médicas – UNICAMP

Botucatu, de 27 de agosto de 2021

Επίγραφε

"Sou o que quero ser, porque possuo apenas uma vida e nela
só tenho uma chance de fazer o que quero.
Tenho felicidade o bastante para fazê-la doce
dificuldades para fazê-la forte,
Tristeza para fazê-la humana e
esperança suficiente para fazê-la feliz.
As pessoas mais felizes não tem as melhores coisas
elas sabem fazer o melhor das oportunidades que aparecem em seus caminhos"
"Que ninguém se engane: só se consegue a simplicidade através de muito trabalho."

Clarice Lispector

Dedicatória

Esta tese é dedicada a **todas boas energias** que me guiaram até aqui, uma grande conquista como esta é resultado de tudo aquilo que plantamos no nosso dia a dia e das boas ações que proporcionamos ao próximo. Durante todo esse processo, lembrei das palavras do meu pai e pedi para que meus anjos da guarda estivessem sempre a minha frente para me guiar, ao meu lado para me acompanhar, atrás de mim para me guardar e acima de mim para me iluminar. Então, agora agradeço por **Deus e meus anjos da guarda** não me desampararem em nenhum momento e reservarem sempre o melhor. Gostaria também de homenagear a **Nossa Senhora Desatadora dos Nós**, por abrir os melhores caminhos quando precisei.

Aos **meus pais, Bete e Francisco**, os meus maiores incentivadores e melhores amigos. Os quais eu posso contar sempre e para qualquer coisa. Sei o quanto esta conquista é importante para nós. Apesar de saber que vocês estão orgulhosos pela conquista deste título acadêmico, o tempo passa e a maturidade vem e, portanto, hoje eu que tenho muito orgulho de tudo que vocês puderam proporcionar para nossa família. Meu amor por vocês não tem limites, vocês são incríveis na minha vida. Amo vocês imensamente.

À **minha irmã Mariana**, nossa conexão é tão intensa que parece que vem de outras vidas e tomara que sim, porque quero te encontrar em muitas outras. Obrigada por ser suporte, compreensão, puxão de orelha e acima de tudo minha companheira. Você é um ser humano, mulher, irmã, filha, neta, profissional, pesquisadora, nutricionista (e tudo mais que você possa ser) fenomenal. Muito obrigada pela leveza e pela sua intensidade, pelas suas mudanças de opinião na última hora (até isso eu amo em você) e pelos seus conselhos para minha vida pessoal e acadêmica (que foram tão importantes). Vamos combinar assim, você me segue e eu te sigo por todas

nossas vidas? Te amo.

Aos **meus avós**, quem me conhece sabe o quanto importante a família é para mim e o quanto acredito que o que conquistamos são uma sucessão de ações feitas por nossos antepassados. Sou neta e bisneta de imigrantes e fico imaginando o quão feliz eles ficariam em saber que sou a primeira doutora (com doutorado) da família. Obrigada por vocês trabalharem duro para proporcionar condições para que chegássemos aqui, dedico então esta tese a vocês. Obrigada por terem me guiado, vocês são meus **verdadeiros anjos da guarda**.

Em especial, gostaria de homenagear minha **vó Lucia**, a qual perdi fisicamente no começo do meu doutorado e que faz uma falta tão imensa na minha vida. Como eu gostaria de comemorar este momento com a senhora, com aquele chazinho e crostolis que só a senhora sabia fazer. A senhora deixou muitas saudades, mas nunca será esquecida. Quero agradecer também a **tia Marli**, por se preocupar tanto comigo e transmitir tanto amor durante minha vida, você é uma mulher muito especial e talentosa “tica”, amo você, obrigada por tudo.

Agradecimientos

Não poderia deixar de agradecer minha orientadora e acima de tudo grande amiga **Profa. Angélica Barbosa**. São tantos anos de convivência que é difícil não enxergar como uma mãe postiça que a academia me presenteou. Foram tantos momentos, muitos desafios e experiências partilhadas que é quase impossível imaginar trabalhar com alguém diferente da senhora. Sua maestria em ensinar, guiar e acolher é algo que ficará marcado na minha vida pessoal e profissional. A senhora me ensinou que com empatia, determinação e foco conseguimos driblar os percalços que encontramos quando mergulhamos neste mar de emoções que é academia. Muito obrigada por isto. Além destas qualidades que julgo raro nas pessoas, mas que na pós-graduação é quase nula em muitos orientadores, a senhora também consegue ser uma pesquisadora admirável. Me ensinou muito, oportunizou aprendizados que hoje me diferenciam como profissional e me incentivou imensamente em muitos sentidos. Muito obrigada não só pelo que faz por mim, mas também a todos a sua volta, sem distinção. A senhora é ímpar neste mundo. Aproveito também para estender meus agradecimentos à **família Barbosa e Pascon** obrigada pelos momentos que pudemos partilhar durante estes anos.

À **professora Emérita Marilza Rudge**, líder do grupo Diamater, que foi minha orientadora no mestrado e quando ingressei no doutorado. Mulher de grande determinação e rigor científico. Obrigada professora por auxiliar e apoiar as etapas da minha trajetória acadêmica. Tenho a senhora como grande inspiração. Obrigada por abrir os meus olhos para os “trens” que a vida estava deixando passar bem na minha frente e a senhora deu o empurrão para que eu pudesse entrar nele e vivenciar momentos que ficarão marcados para sempre.

À **Professora Dra. Cristiane R. Pedroni**, obrigada pela parceria de sempre. Seu auxílio no meu desenvolvimento em relação à eletromiografia foi de suma

importância na minha trajetória. Espero que nossa parceria permaneça por muitos anos. Obrigada por toda ajuda e disponibilidade.

Ao **Carlos Sartorão**, obrigada pela parceria desde o mestrado, pela troca acadêmica e pela oportunidade de ajuda na coleta de dados. Estendo o agradecimento à sua esposa Celyse e às funcionárias da Euroclínica, Deise, Leticia e Adriana por facilitarem a execução da pesquisa.

À **Fabiane A. Pinheiro**, minha amiga querida, companheira de trajetória acadêmica. Você é inspiração. Obrigada pela sintonia e amizade. Obrigada pela acolhida em tantos momentos e por me fazer sentir em casa, que saudades que eu tenho de estar mais pertinho. Agradeço aos seus filhos, **Thomaz e Tiago**, por me receberem sempre tão bem. A mãe de vocês é fabulosa.

À **Sthefanie K. Nunes**, amiga obrigada pela parceria neste desafio que é desenvolver um trabalho com eletromiografia. Por muitas vezes sem você perceber, lhe tive como espelho para não enlouquecer, você me fez enxergar que as coisas podem ser mais simples de acordo como você as encara.

À **Bruna Bologna, Raissa Escandiussi e Henrique Bassin**, obrigada amigos por trazerem leveza durante meu doutorado. Vocês são incríveis e quero sempre por perto.

Ao **meu grande amor Guilherme Nava**, como é bom partilhar minha vida com você. Você é meu melhor amigo, grande incentivador e àquele que sonha grande comigo. Obrigada por ser como você é, meu companheiro de tantos anos. Obrigada também por contribuir tanto para esta tese, tenho a sorte de ter alguém com tantas qualidades ao lado e ainda por cima um pesquisador e fisioterapeuta super competente. Suas observações e contribuições foram de suma importância para que esta tese fosse concluída. Obrigada pelas horas no carro me esperando durante

alguma disciplina, por transformar nosso intercambio para Australia muito especial, obrigada por aguentar meu mau humor quando foi preciso focar, obrigada por estar em coletas, cursos, reuniões e tantas outras tarefas extras que, por amor e companheirismo, você partilhou comigo. Você é o melhor *room mate*! O mundo é nosso, que venham as próximas aventuras juntos!

A **todos professores que participaram de toda minha formação até aqui**, a educação transforma vidas e abre janelas inimagináveis, toda minha admiração pela contribuição que dão a sociedade.

Agradeço a minha banca de qualificação, composta pelas **professoras Natalia Martinho e Cristiane Pedroni**, pela disponibilidade, atenção e sensibilidade durante a condução da minha arguição. Além das valiosas contribuições que ajudaram a moldar esta tese. **E em antecipação a minha banca de defesa**, tenho certeza que contribuirão para finalização do meu processo de aprendizagem nesta etapa.

À **Regina Moretto do “Laboratório de Métodos Imunoenzimáticos”**, você foi essencial para a realização das análises de ELISA desta tese, minha gratidão pela disponibilidade e profissionalismo. Ao **Hallur, Sofia, Rafael e Maiara**, pela disponibilidade, amizade e profissionalismo.

To **Professor Paul Hodges** and all his researcher partners, **Wolly, Rachel, Lori, Manuela**, thank you to hosted me on the lab and for the enriching academic environment, the time that I had spent in UQ was very important to my academic growth. Agradeço também as pesquisadoras brasileiras **Karine e Nathália**, pela amizade e por serem inspiração para mim, meninas vocês são incríveis até do outro lado do mundo. Além de **todos amigos** que pude fazer na passagem pela Austrália.

À toda **equipe administrativa, de limpeza e todos os funcionários** do Programa de Pós-Graduação da **Faculdade de Medicina de Botucatu**, ao

Departamento de Tocoginecologia e da **UNESP- Campus Marília**, em especial à Solange Sako pela ajuda de sempre.

Aos **funcionários das Unidades Básicas de Saúde e do Centro Especializado em Reabilitação, nosso querido CEES**, por facilitarem a execução da pesquisa e pela amizade.

A **todos meus companheiros do grupo de pesquisa Diamater**, obrigada pelas trocas científicas valiosas e pelos momentos de descontração também.

Agradeço também a dança (a dança, Carol? Você escreveu certo?), se tem uma dica que eu posso dar a você que está aqui lendo meus agradecimentos é que a vida é bem mais que a academia/pós-graduação/trabalho. Entenda que é necessário parar e fazer coisas que você ama! No meu caso é a dança, que me desconecta e me traz para o meu verdadeiro eu! Esta nota é para você leitor e para mim também. Quando me esqueço da minha verdadeira essência, a dança me faz lembrar quem eu realmente sou.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001. Agradeço, então à **CAPES** pelo financiamento pessoal e **FAPESP** a FAPESP pelo financiamento de projeto (**temático n 2016/01743-5**).

E enfim, e mais importante, a **todas as mulheres que participaram** desta pesquisa meu muito obrigada. Meu sonho é que a ciência trabalhe sempre em prol da melhoria e qualidade de vida de pessoas e que os resultados cheguem a todas, sem distinção. Acho muito lindo o fato de sermos um grupo majoritariamente feminino, entendendo, pensando, criando e desenvolvendo soluções para melhorar a vida de outras mulheres. Em nome do DIAMATER, nosso muito obrigada.

Lista de Figuras

Contextualização

Figura 1	Modelo conceitual integrado da tríade de investigação do <i>Diamater Study Group</i>	25
Figura 2	Comparação das análises musculares das primeiras evidências da ação miopática do diabetes nos grupos experimentais.....	27
Figura 3	Comparação de análises da matriz extracelular, morfológicas, imuno-histoquímicas entre os diferentes tipos de modelos de indução do diabetes em estudos experimentais.....	29
Figura 4	Estruturação dos achados e módulos do <i>Diamater Study Group</i>	31
Figura 5	Comparação dos achados morfológicos do músculo reto abdominal em ratas prenhes hiperglicêmicas e gestantes com diabetes mellitus gestacional.....	32

Artigo 1

Figura 1	Glazer Protocol plots showing full signal and intravaginal probe image (A), protocol segment tasks (B) and contraction time window with all performed tasks (C).....	87
Figura 2	Example EMG recording of the Flick task from a representative subject, illustrating the EMG variables used in the analyses.....	88
Figura 3	GDM women's screening, diagnosis, enrollment, follow-up analysis and reasons for signal exclusion from analysis.....	89
Figura 4	Group average and SD of the RMS EMG during the 1-sec Flick and 10-sec Hold PFM contraction tasks from Glazer protocol. Before averaging, the EMG patterns from each subject was expressed as percentage of the peak recorded during the 1-sec Flick contractions. Positive contrasts indicate that GDM < non-GDM.....	93

Artigo 2

Figura 1	Flow chart indicating the distributions of study participants according to glycaemic and continence status.....	111
Figura 2	Median \pm 25th - 75th percentiles of relaxin-2 serum concentration according to glycaemic and continence status during pregnancy...	113

Lista de Tabelas

Artigo 1

Table 1	Average participant characteristics for non-GDM and GDM groups along time-points.....	90
Table 2	Group mean \pm standard deviation (across subjects) of the parameters extracted from the EMG signals at each task of the Glazer protocol. Results are presented from the 2-way generalized linear model (GLM) using factors Group (non-GDM, GDM) and Time-Point (T1: 24–30 weeks of gestation, T2: 36–38 weeks of gestation, T3: 18-24 months postpartum) as factors, with repeated measures on Time-Point.....	91

Artigo 2

Table 1	Baseline characteristics of study participants according to glycaemic and continence status.....	112
Table 2	Urinary incontinence questionnaires and pelvic floor function according to groups.....	114
Table 3	Relaxin-2 serum concentrations regarding pelvic floor strength according to different groups stratifications.....	115

Lista de Abreviações
Português e Inglês

LISTA DE ABREVIATURAS EM PORTUGUÊS

CAPES	Coordenação De Aperfeiçoamento De Pessoal De Nível Superior
DMAP	Disfunção Muscular Do Assoalho Pélvico
DMG	<i>Diabetes Mellitus</i> Gestacional
DMG-C	Diabética gestacional continente
DMG-IUEG	Diabética gestacional incontinente
Dr.	Doutor
Dra.	Doutora
EMG	Eletromiografia
FAPESP	Fundação De Amparo À Pesquisa Do Estado De São Paulo
FI	Fator De Impacto
FMB	Faculdade De Medicina De Botucatu
FMUSP	Faculdade De Medicina Da Universidade De São Paulo
GAGs	Glicosaminoglicanos
HC	Hospital Das Clínicas
IU	Incontinência Urinária
IU-EG	Incontinência Urinária Específica Da Gestação
MAP	Músculos Do Assoalho Pélvico
MEC	Matriz Extracelular
mg/dL	Miligramas Por Decilitro
MRA	Músculo Reto Abdominal
Não-DMG-C	Não-diabética gestacional continente
Não-DMG-IUEG	Não-diabética gestacional incontinente
Print	Programa Institucional De Internacionalização
SG	Semana Gestacional
TCC	Trabalho De Conclusão De Curso
TCLE	Termo De Consentimento Livre E Esclarecido
UNESP	Universidade Estadual Paulista
US	Ultrassonografia
US-3D	Ultrassonografia Tridimensional

LISTA DE ABREVIATURAS EM INGLÊS

%/sec	Percentage Per Second
10-sec	Ten Seconds
1-sec	One Second
1st	First
2nd	Second
3D-US	Three-Dimension Ultrasonography
3rd	Third
60-sec	Sixty Seconds
75g- OGTT	75g Oral Glycemic Tolerance Test
A/D	Analog to Digital
BMI	Body Mass Index
CMRR	Common Mode Rejection Ratio
CV	Coefficient of Variation
dB	Decibels
ELISA	Enzyme linked immune-sorbent assay
EMG	Electromyography
GDM	Gestational Diabetes Mellitus
GDM-C	Diabetic continent
GDM-PSUI	Diabetic incontinent
Hz	Hertz
ICIQ-SF	International Consultation on Incontinence Questionnaire - Short Form
ICS	International Continence Society
ISI	Incontinence Severity Index
IUGA	International Urogynecological Association
Kg	Kilogram
M	Meters
mg/dL	Miligrams Per Deciliter
mm	Millimeters
MOS	Modified oxford scale
MVC	Maximal Voluntary Contraction
Non-GDM	Non-Gestational Diabetes Group

Non-GDM-C	Non-diabetic continent
Non-GDM-PSUI	Non-diabetic incontinent
PDRC	Perinatal Diabetes Research Center
PFD	Pelvic Floor Muscle Dysfunction
PFM	Pelvic Floor Muscles
pg/mL	Picograms/deciliter
PSUI	Pregnancy-Specific Urinary Incontinence
RMS	Root Mean Square
SD	Standard Deviation
Sec	Seconds
SENIAM	Surface Electromyography for The Non-Invasive Assessment Of The Muscles
SNR	Signal-To-Noise Ratio
SST	Serum Separation Transport Tube
UI	Urinary Incontinence



Sumário

Seção 1	Contextualização	
	<i>Timeline</i> do Grupo de Pesquisa: <i>Diamater</i> Study Group.....	24
	Trajatória Acadêmica e Perspectivas Acadêmicas Futuras.....	41
	Referências.....	42
Seção 2	Resumos Expandidos	
	Resumo da Tese.....	48
	<i>Thesis Abstract</i>	56
Seção 3	Artigos	
	Artigo 1: Gestational diabetes is associated with alteration on pelvic floor muscles activation pattern during pregnancy and postpartum: Prospective Cohort using electromyography assessment	62
	Abstract.....	64
	Introduction.....	65
	Methods.....	66
	Results.....	72
	Discussion.....	75
	Conclusion.....	81
	References.....	83
	Tables and Figures.....	87
	Artigo 2: Relaxin-2, gestational diabetes, urinary incontinence, and pelvic floor muscle function: a cross-sectional study	94
	Abstract.....	96
	Introduction.....	97
	Methods.....	98
	Results.....	102
	Discussion.....	103
	Conclusion.....	106
	References.....	108
	Tables and Figures.....	111
Seção 4	Diamater Study Group	116
Seção 5	Anexos	
	Artigos Publicados Durante a Tese.....	119
	Seleção pública para função docente.....	128
	Cover Letter – Article 1.....	129
	Parecer consubstanciado do comitê de ética em pesquisa.....	130
Seção 6	Apêndices	
	Termo de Consentimento Livre e Esclarecido (TCLE).....	135
	Ficha de Avaliação.....	136



Seção 1
Contextualização

Timeline do Diamater Study Group

O *Diamater Study Group* é liderado por 4 mulheres pesquisadoras, que com a aprovação de projeto Temático FAPESP (Processo nº 2016/01743-5) tornaram possível a consolidação e expansão da linha de pesquisa proveniente da tese de doutorado da professora Dra. Angélica Barbosa (2006) “Prevalência e fator de risco para incontinência urinária (IU) e disfunção do assoalho pélvico dois anos após Diabetes Mellito gestacional”. Esta linha de pesquisa deriva-se do Grupo de Pesquisa Acadêmico “Diabetes e Gravidez - Clínico e Experimental” que é liderado pela professora Dra. Emérita Marilza Rudge desde a década de 80 e está vinculado ao Programa de Pós-Graduação em Tocoginecologia da Faculdade de Medicina de Botucatu - FMB/UNESP.

A principal característica do *Diamater Study Group* é o modelo de abordagem translacional, no qual a partir de achados clínicos, há exploração da temática em modelos experimentais para que possam ser replicados e confirmados clinicamente. Décadas de pesquisa relacionadas as técnicas de indução experimental do diabetes, propiciaram que etapas importantes fossem adaptadas para o início da investigação pré-clínica acerca da associação da disfunção muscular do assoalho pélvico (DMAP), incontinência urinária específica da gestação (IU-EG) e *diabetes mellitus* gestacional (DMG) (figura 1). O principal desafio inicial, foi o estabelecimento do tempo de exposição e intensidade necessários para mimetizar o ambiente hiperglicêmico semelhante ao DMG no tecido muscular. Esta seção foi idealizada para demonstrar a *timeline* de publicações do grupo e oferecer arcabouço cronológico e introdução a justificativa da presente tese.

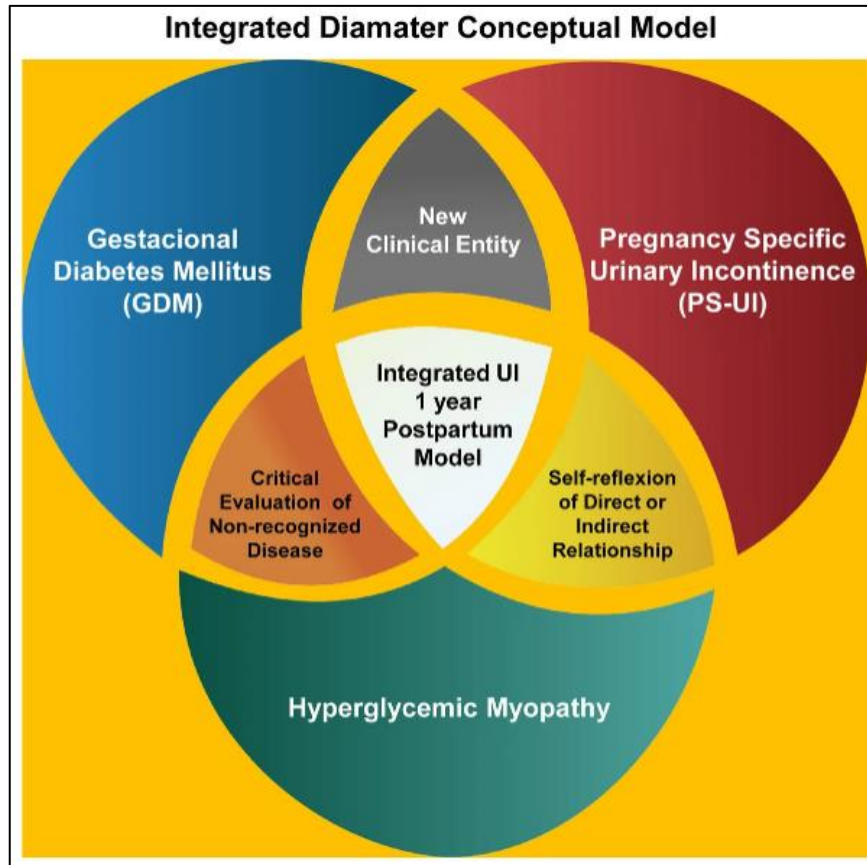


Figura 1. Modelo conceitual integrado da tríade de investigação do *Diamater Study Group*. Fonte: Rudge et al., 2020 (1).

O estudo clínico proveniente da dissertação de mestrado (2004) de **Barbosa, AM et al., publicado em 2013**, “*Prevalence of urinary incontinence and pelvic floor muscle dysfunction in primiparae two years after cesarean section: cross-sectional study*” embora não com enfoque no DMG, foi precursor para as próximas investigações. Neste estudo, o principal achado foi que a IU-EG foi determinante para IU pós-parto (OR: 8.675; IC 95%: 3,027-24,324). Além disso, a cesárea eletiva foi fator protetor para a IU pós-parto (2).

Os resultados do **estudo clínico** da tese de doutorado (2006) de **Barbosa, AM et al.**, foram publicados no artigo, “*Urinary incontinence and vaginal squeeze pressure two years post-cesarean delivery in primiparous women with previous gestational*

diabetes mellitus”, e identificou que, quando comparado com não-diabéticas, as mulheres com DMG apresentaram prevalência maior de IU-EG (50,8% vs. 31,6%) e que esta prevalência permaneceu elevada após 2 anos de parto cesárea (44.8% vs. 18.4%). A função muscular avaliada pela perineometria dois anos após o parto em mulheres com DMG prévio, foi significativamente diminuída em relação as não-diabética, sendo que, o aumento do ganho materno e peso do recém-nascido foram fator de risco para esta diminuição. Além disso, idade materna, IU-EG e força muscular diminuída (OR 20,416; IC 95%: 3,548; 117,479) foi apontado como fator de risco para IU mesmo após 2 anos depois de parto cesariana. O DMG em modelo de análise logística multivariada foi fator de risco para IU-EG (OR 2,26; IC 95%: 1,116-4,579) (3).

Aprofundando o conhecimento obtido dos achados de Barbosa, a dissertação (2010) e tese (2014) de **Marini G.** e dissertação de **Piculo F.** (2013) deram início a **etapa translacional** do grupo de pesquisa direcionando as pesquisas da área clínica para a **pré-clínica** (“*bedside to bench*”). O primeiro artigo oriundo da pesquisa experimental foi **publicado** por **Marini G et al.**, e foi composto por 4 grupos de estudo: ratas virgens não-diabéticas, ratas prenhes não-diabéticas, ratas virgens diabéticas e ratas prenhes diabéticas. Esta composição de grupos foi importante para estabelecer o nível de interação entre diabete, prenhez e dano muscular, controlando assim o risco de possíveis vieses de interpretação. Análises morfológicas e de imuno-histoquímica foram realizadas para diferenciar fibras *slow* and *fast*. É possível observar nos resultados (figura 2) que houve deterioração progressiva da musculatura esquelética periuretral das ratas, sendo que a presença da prenhez ou da hiperglicemia não concomitante com a prenhez promove alterações negativas. No entanto, quando se associa prenhez e hiperglicemia os efeitos são muito mais intensos, caracterizados

por atrofia, adelgaçamento, desorganização e rompimento associado à perda de localização anatômica normal e alteração na proporção das fibras rápidas e lentas (4).

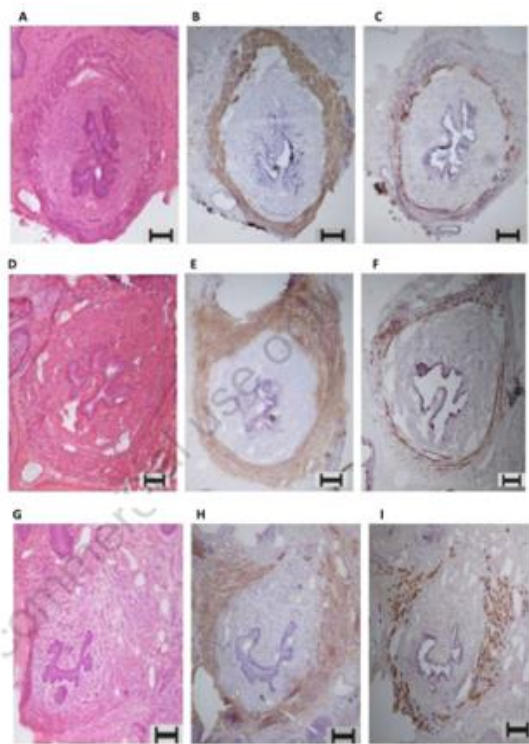


Figure 2. Microphotographs of transverse section of the urethra in virgin group (A, B, C), pregnant group (D, E, F), diabetic virgin group (G, H, I). H&E stained (H&E); immunohistochemical staining to visualize fast (FAST) and slow (SLOW) myosin heavy chain (MHCf, MHCs) in the striated muscle fibers. Scale bar, 100 μ m.

Table 1. Semi-quantitative analysis of slow and fast fibers according the presence of each type of fiber throughout circumference of the layer; thickness of the muscle fiber layer; the degree to which the layers maintained a normal anatomic localization; fast index; slow index; and fast:slow index in each group.

Groups	Virgin	Pregnant	Diabetic virgin	Diabetic pregnant
Fast throughout circumference/ thickness/ normal anatomic localization	++++/++++/++++	++++/++++/++	+++/++++/+	+++/+++/+
Slow throughout circumference/ thickness/ normal anatomic localization	++++/+/++++	++++/+/++	+++/+++/+	+++/+++/+
Fast index	64	32	12	9
Slow index	16	8	9	6
Fast:Slow index	4:1	4:1	1.5:1	1.5:1

Figura 2. Primeira evidência da ação miopática do diabetes, a partir da observação das análises musculares de diferentes grupos experimentais. *Fonte: Marini et al., 2011 (4).*

Colaborando para **refinar o modelo experimental do DMG**, o artigo de **Piculo F. et al. em 2014**, atentou-se ao fato de que no artigo anterior publicado pelo grupo, a hiperglicemia mimetizada no modelo experimental foi compatível com taxas glicêmicas do Diabetes Mellitus Tipo 1 descontrolado e, portanto, não refletiu os valores glicêmicos de mulheres com DMG. A partir de protocolo de indução do diabetes moderado, o qual reproduziu de forma mais fidedigna os valores glicêmicos encontrados no DMG, foram realizadas análises musculares acrescidas de análise da

matriz extracelular (MEC), devido sua importância no mecanismo de continência urinária. Os achados deste estudo apresentam que as fibras uretrais são finas, desorganizadas e atroficas, com aumento do colágeno na área do músculo estriado, aumento dos vasos sanguíneos, diminuição da área do urotélio, acúmulo de mitocôndrias subsarcolemais e intermiofibrilares, aumento das gotículas lipídicas e mais grânulos de glicogênio. Das descobertas mais significativas a colocação de fibras rápidas e lentas e diminuição constante na proporção de fibras rápidas em relação as lentas, se destacam. Na MEC, foram encontradas fibrose/deposição de fibra de colágeno associada à atrofia muscular (5).

Entre 2011 e 2014, como **estratégia de comunicação científica** nacional foram publicados artigos em língua portuguesa para divulgar *briefing* dos achados relacionados ao risco aumentado de IU-EG e IU pós-parto relacionado ao DMG e aos achados experimentais preliminares caracterizando a miopatia diabética gestacional (6–8).

Avanços relacionados à metodologia e interpretação dos resultados da fase pré-clínica foram estabelecidos com **parcerias internacionais**, dentre elas, a Universidade *Case Western Reserve*. **Em 2016 Marini et al.** publicaram resultados do estudo sobre os efeitos na musculatura estriada periuretral de ratas, em relação a **interação intensidade hiperglicêmica versus tempo de exposição**. Dois grupos foram estudados em comparação ao controle (não-diabético): diabetes grave *short-term* (120 and 300mg/dl) e diabetes moderado *long-term* (>300 mg/d). Os resultados expressos na tabela 2 demonstram que em ambos os grupos de estudo houve deterioração da composição muscular. Especificamente o diabetes moderado *long-term* ocasionou aumento da fibrose uretral e maior número de alterações ultra

estruturais. Em contraste, diabetes grave de *short-term* está associado à atrofia muscular (figura 3) (9).

Control	Long-term mild diabetes	Short-term severe diabetes
Pathological analysis (Fibrosis)		
Minimal	Minimal	Minimal
Minimal to moderate	Minimal to moderate	Minimal to moderate
	Moderate	Moderate
	Severe	
Morphometric analysis		
Well-organized tissue	↑↑↑ Total collagen area ↓ Striated muscle area	↓ Total collagen area ↓↓ Striated muscle area ↑↑ Blood vessels
Immunohistochemical		
Fast myosin heavy chain isoform predominantly	Loss of specific localization for each fiber type	Loss of specific localization for each fiber type
Fast fibers present throughout the outer circular layer	Greater quantity of slow fibers in the inner layer	Greater quantity of slow fibers in the inner layer
Slow fibers present throughout the inner circular layer	Significantly decreased of fast fibers	Significantly decreased of fast fibers
Ultrastructural analysis		
Well-organized myofibrils	↑↑ Interstitial collagen	Centrally myonuclei presence
Collagen normally distributed	↑↑ Lipid droplets ↓ Mitochondria ↓ Glycogen granules	

Figura 3. Comparação de análises da matriz extracelular, morfológicas e imunohistoquímicas entre os diferentes tipos de modelos de indução do diabetes em estudos experimentais. *Fonte: Marini et al., 2016 (9).*

Em 2017 foi publicado **artigo dedicado à composição da MEC** devido sua importância no suporte do sistema geniturinário e na transmissão de força, manutenção e reparação da fibra muscular. **Marini et al.** analisaram a distribuição e quantificaram os principais componentes estruturais da MEC, como colágenos tipo I e III, a relação do colágeno tipo I/III e glicosaminoglicanos (GAGs), na musculatura periuretral em **ratas prenhes hiperglicêmicas** (10). Os achados deste estudo demonstram que, em ratas prenhes hiperglicêmicas, o músculo estriado uretral apresenta-se diminuído e envolvido com mais tecido conjuntivo, evidenciado pela mudança na organização das fibrilas de colágeno, aumento na proporção relativa do colágeno tipo I/III e diminuição nos níveis de GAGs totais.

Estas últimas publicações citadas foram fruto de dissertação e doutorado de componentes do *Diamater Study Group*. Voltando **na linha do tempo em 2015**, devido aos achados parciais dos estudos anteriores iniciou-se a **investigação do impacto da hiperglicemia no músculo reto abdominal (MRA)** de ratas prenhas, sendo que os resultados provenientes deste estudo foram tema do **mestrado de Vesentini, G**. A investigação desta musculatura justificou-se pela ação sinérgica que estes músculos desempenham no processo de continência e pela viabilidade de, posteriormente, justificar a realização de biópsia do MRA de gestantes, por meio da coleta de retalho no momento da cesárea.

Os **resultados de seu mestrado** foram **publicados** no artigo **“Morphological changes in rat rectus abdominis muscle induced by diabetes and pregnancy”**, este estudo incluiu 6 grupos de ratas: virgem, prenhe não diabético, diabetes moderado *long-term*, diabetes moderado *long-term* prenhe, diabetes grave de *short-term*, diabetes grave de *short-term* prenhe, e, portanto, foi possível isolar a ação da gestação e diabete sobre os músculos, assim como a relação entre intensidade e exposição. Em relação aos resultados, as ratas prenhes com diabetes moderado *long-term* e ratas grávidas diabetes grave de *short-term* apresentaram fibras rápidas diminuídas e fibras lentas aumentadas, áreas interrompidas do sarcômero, mitocôndrias intermiofibrilares e figuras de mielina no MRA. O diabetes e a gravidez induzem a transformação intramuscular e a reorganização do MRA por meio da troca de tipo de fibra ajustando sua arquitetura de acordo com a intensidade e a duração hiperglicêmica durante a gravidez (11).

Seguindo a linha do tempo, **em 2016 após a consolidação** dos dados provenientes dos estudos de Barbosa AMP., Piculo F., Marini G e Vesentini G., foi

aprovado o projeto temático FAPESP (2016/01743-5) que possibilitou a **translação dos estudos experimentais para a clínica** ("bench to bedside"). Iniciou-se então, a investigação clínica da miopatia diabética gestacional por meio de análises morfológicas, moleculares, hormonais e/ou funcionais, potenciais marcadores para explicação da tríade DMAP, IU-EG e DMG, sendo que o **Study Protocol publicado** explica detalhadamente a metodologia empregada. Esta **fase clínica é um dos braços** que compõe o projeto temático que ainda conta com fase pré-clínica relacionada à primeira proposta de intervenção. Há ainda mais dois módulos em andamento concomitantes: **módulo pré-clínico** que propõe tratamento da miopatia diabética gestacional por meio do biodevice para regeneração muscular induzida por células tronco mesenquimais, e o **módulo de revisão sistemática** (figura 4).

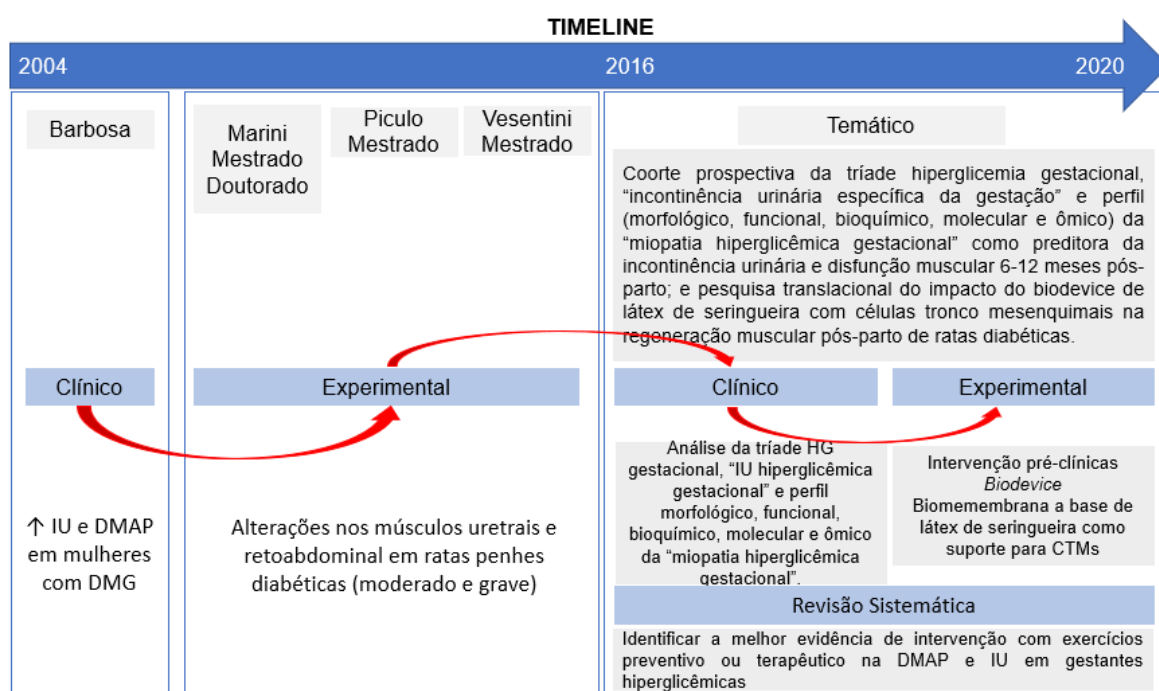


Figura 4. Estruturação dos achados e módulos do *Diamater Study Group*. Fonte: Projeto temático Diamater.

Desde então, mestrandos, doutorandos e pós-doutorandos desenvolvem pesquisas nos módulos do temático. Aprofundando o conhecimento sobre a miopatia diabética gestacional, o doutorado de Vesentini demonstrou os primeiros resultados relacionados a miopatia diabética gestacional no MRA de gestantes com DMG. Os retalhos musculares foram coletados durante o parto cesariana. Os resultados publicados demonstraram que, em análise comparativa entre o modelo animal e gestantes, a hiperglicemia foi capaz de produzir efeitos deletérios estruturais no MRA em ambos. O DMG modifica a quantidade dos tipos de fibras do MRA e diminui a área de fibra rápida. Embora o hiperglicêmico modelo animal e DMG tenham mostrado mudanças semelhantes no número de fibras rápidas, área de fibras rápidas e número de fibras lentas, a área de colágeno em DMG mostrou diminuição. Tomados em conjunto, nossos resultados demonstram que o MRA é vulnerável a mudanças na arquitetura histológica devido ao DMG em humanos. As alterações no padrão das fibras musculares do MRA poderiam influenciar sua funcionalidade tanto em ratos com diabetes moderado quanto mulheres com DMG. A figura 5 demonstra alterações morfológicas comparando os achados experimentais e clínicos (12).

Morphological changes in the RAM of pregnant rats and women with diabetes.		
	MHP (rats) (Vesentini et al., 2018)	GDM (women)
Collagen area	Ns	↓
Fiber type area	↓ FAST	↓ FAST
	↑ SLOW	↓ SLOW
Fiber type number	↓ FAST	↓ FAST
	↑ SLOW	↑ SLOW

Figura 5. Comparação dos achados morfológicos do músculo reto abdominal em ratas prenhes hiperglicêmicas e gestantes com diabetes mellitus gestacional.

Fonte: Vesentini et al., 2020 (12). MHP: mild hyperglycemic pregnant; GDM: gestational diabetes mellitus; Ns: not significant.

Os dois artigos subsequentes são provenientes dos achados das teses de Vesentini, G (2015-2019) e Piculo, F (2014-2017), nos quais foram incluídas análises estratificadas por grupos de acordo com presença de hiperglicemia e de IU-EG. No artigo *“Deleterious effects of gestational diabetes mellitus on the characteristics of the rectus abdominis muscle associated with pregnancy-specific urinary incontinence”*, o grupo não-DMG com IU-EG, apresentou diminuição da expressão, área de colágeno e da área de fibra lenta e rápida. O grupo DMG sem IU-EG aumentou o número de fibras lentas e os núcleos localizados centralmente e diminuiu o número e a área das fibras rápidas, além da área das fibras lentas em relação aos grupos não-DMG. As gestantes com DMG e IU-EG mostraram aumento de núcleos localizados centralmente, diminuição da área e conteúdo de colágeno, número e área de fibra rápida e expressão e área de fibra lenta. Além de alterações ultra estruturais nas fibras musculares, como numerosas estruturas mieloides, desorganização do sarcômero e mitocôndrias morfologicamente anormais (13).

O artigo *“Pregnancy-specific urinary incontinence in women with gestational hyperglycaemia worsens the occurrence and severity of urinary incontinence and quality of life over the first year postpartum”* avaliou mulheres em 5 momentos: 24-28, 34-38 semanas gestacionais (SG), 24-48 horas, 6 semanas e 6-12 meses pós-parto. Todas as participantes do estudo apresentavam IU-EG no momento da inclusão (24-28 SG) e os 2 grupos estudados foram diferenciados pela presença ou ausência do DMG. Sendo assim, os resultados demonstraram que a prevalência geral de IU-EG foi de 54,1%, sendo que a prevalência entre as não-diabéticas foi de 43,3% e 56,7%

nas mulheres com DMG. Durante os dois momentos da gestação a IU-EG é mais severa ($p=.000$) e tem impacto maior no grupo hiperglicêmico, devido ao maior volume ($p<.002$), maior frequência ($p<.001$) e maior impacto na qualidade de vida ($p<.001$). Esta característica no grupo DMG persiste até 1 ano após o parto (14).

Na **sequência cronológica, em 2017** os primeiros resultados de dissertações com objetivo de investigar o comportamento funcional dos MAP de gestantes foram descritos e se encontram na sequência. **Sartorão Filho, C. et al.** publicaram os **resultados de coorte prospectiva** em 2 momentos da gestação (segundo e terceiro trimestres), que caracterizaram a **biometria dos MAP** por meio da ultrassonografia (US) tridimensional (3D) (**US-3D**). Os resultados demonstraram que em gestantes com DMG houve aumento na área hiatal, diminuição do diâmetro póstero-anterior e redução da espessura do músculo elevador do ânus (15). Complementando os achados de Sartorão, advindos de avaliação durante o repouso dos MAP, **Pinheiro F.** apresentou em sua **dissertação** a proposta metodológica de avaliar a contração e distensão (valsalva) dos MAP e além de índice relativos ao repouso chamados de **índice de contratilidade** e **distensibilidade** e o índice de **mobilidade** que é relativo à diferença de medida entre contração e distensão. O grupo DMG quando comparado ao não-DMG apresenta diminuição no índice de contratilidade, índice de distensibilidade e índice de mobilidade ($p=.000$; $p=.000$ e $P=.000$, respectivamente), o que significa que ao tentar contrair os MAP o grupo DGM tem menor capacidade de fechamento da área hiatal e que ao realizar a distensão por meio da valsalva apresentam dificuldade no aumento dos diâmetros, assim como redução na excursão total dos MAP (mobilidade), demonstrando que os MAP apresentam maior rigidez ao final da gestação e menor capacidade funcional.

Prudencio CB et al. publicaram o artigo *“Negative impact of gestational*

diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study.”, por meio da **eletromiografia dos MAP**. Este estudo demonstra que houve diminuição progressiva do recrutamento muscular entre o segundo e terceiro trimestre relacionados as contrações sustentadas por 10 segundos e que o nível de atividade basal dos MAP também diminuiu entre momentos no grupo com DMG (16). Paralelamente, **em 2018, Kenickel, SN** utilizou o questionário “**Inventário da resposta sexual na gestação**” (17,18) desenvolvido e validado sob orientação da professora Marilza Rudge e Angélica Barbosa em 2009 para avaliar o impacto do DMG na função sexual de gestantes. As participantes com DMG e não-DMG se apresentavam pareadas em relação a **função sexual antes da gestação. Durante a gestação** o grupo DMG apresentou pontuação composta PSRI menor que 50, indicando risco de diminuição da função sexual quando comparadas com o controle. Após o diagnóstico de DMG e o tratamento proposto, as diferenças foram significativas ($p < 0,0001$) em relação a frequência, excitação, orgasmo, satisfação e escore de dispareunia (19). Este estudo clínico demonstrou que, além da IU-EG e IU pós-parto, outras disfunções relacionadas com a DMAP podem acontecer e impactar a vida deste grupo de mulheres.

Outra pesquisa traz conhecimento acerca das repercussões sistêmicas para o binômio, como na **dissertação de Pascon T (2019)**, que demonstrou **impacto no desenvolvimento do esmalte dos dentes de crianças**, filhos de mães com histórico de DMG (20).

Neste momento da linha do tempo, no **eixo clínico do grupo** relacionado com marcadores funcionais da miopatia diabética gestacional, encontra-se **esta tese**. A partir dos conhecimentos prévios experimentais e clínicos, que indicam o comprometimento dos MAP durante a gestação complicada pelo DMG, os resultados

que serão apresentados direcionarão o entendimento do processo de comprometimento funcional dos MAP no segundo e terceiro trimestre gestacional até 18-24 meses pós-parto. É amplamente difundido que o treino muscular do assoalho pélvico é efetivo para tratamento e prevenção da IU-EG. No entanto, faltam estudos básicos de fisiopatologia que empreguem métodos objetivos que possam delinear o comportamento dos MAP ao longo da gestação e seu processo de recuperação no pós-parto, uma vez que sua função tem ligação com a presença ou ausência de disfunções, como a IU (3,21,22). Na literatura há lacuna no conhecimento da relação entre gestação sem comorbidades e a função dos MAP ao longo da gestação, bem como no seguimento após o parto. Nos grupos com DMG o assunto é inédito considerando a análise dos MAP por EMG ao longo da gestação e no seguimento após o parto.

Além disso, outros marcadores bioquímicos podem estar envolvidos no processo. A relaxina é um hormônio da superfamília da insulina e está relacionado à manutenção da gestação e com as principais adaptações gravídicas, atuando sobre diversos sistemas, mais especificamente sobre o sistema cardiovascular, tecido conectivo e muscular. (23) A literatura demonstra que gestantes com diagnóstico de diabetes clínico e DMG apresentaram níveis de relaxina aumentados em relação a gestantes não-diabéticas independente do trimestre (24,25). O mecanismo deste aumento em sua concentração ainda não foi esclarecido e parece estar relacionado com ajustes da própria gestação complicada pelo diabete (26). Embora a temáticas gestação hiperglicêmica, IU e relaxina serem tratadas na literatura, os estudos apresentam os resultados separadamente e não há estudos que inter-relacionem os três fatores.

Com o propósito de preencher parte destas lacunas do conhecimento esta

Tese apresenta 2 artigos com análises inéditas. No artigo 1, estão apresentados resultados de estudo coorte da gestação até o parto, no qual foram analisados o recrutamento dos MAP durante protocolo padronizado de EMG. O artigo 2 demonstra a interação entre relaxina, função dos MAP, IU-EG e DMG, uma vez que embora seja amplamente difundido que este hormônio tem relação com ocorrência de IU-EG, justificado pela sua ação negativa sobre a função dos MAP, não foram encontrados estudos com metodologia consistente para avaliar o grau de interação entre as variáveis.

Referências

1. Rudge MVC, Souza FP, Abbade JF, Hallur RLS, Marcondes JPC, Piculo F, et al. Study protocol to investigate biomolecular muscle profile as predictors of long-term urinary incontinence in women with gestational diabetes mellitus. *BMC Pregnancy Childbirth* [Internet]. 2020 Dec 19;20(1):117. Available from: <https://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/s12884-020-2749-x>
2. Barbosa AMP, Marini G, Piculo F, Rudge CVC, Calderon IMP, Rudge MVC. Prevalence of urinary incontinence and pelvic floor muscle dysfunction in primiparae two years after cesarean section: cross-sectional study. *Sao Paulo Med J*. 2013;131(2):95–9.
3. Barbosa A, Dias A, Marini G, Calderon I. Urinary incontinence and vaginal squeeze pressure two years post-cesarean delivery in primiparous women with previous gestational diabetes mellitus. *Clinics*. 2011;66(8):1341–5.
4. Marini G, Pascon Barbosa AM, Damasceno DC, Michelin Matheus SM, De Aquino Castro R, Castello Girão MJB, et al. Morphological changes in the fast vs slow fiber profiles of the urethras of diabetic pregnant rats. *Urogynaecologia*. 2011 Jul;25(1).
5. Piculo F, Marini G, Barbosa AMP, Damasceno DC, Matheus SMM, Felisbino SL, et al. Urethral striated muscle and extracellular matrix morphological characteristics among mildly diabetic pregnant rats: translational approach. *Int Urogynecol J*. 2014 Mar;25(3):403–15.
6. Marini G, Piculo F, Barbosa AMP, Damasceno DC, Matheus SMM, Rudge MVC. Importância do modelo animal para testar hipóteses sobre a fisiopatologia do binômio diabetes e incontinência urinária feminina. *Sci Med (Porto Alegre)*. 2011;21(4):191–5.
7. Rudge MVC, Piculo F, Marini G, Damasceno DC, Calderon IMP, Barbosa AP. Pesquisa translacional em diabetes melito gestacional e hiperglicemia gestacional leve: conhecimento atual e nossa experiência. *Arq Bras Endocrinol Metabol* [Internet]. 2013 Oct;57(7):497–508. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004-27302013000700001&lng=pt&tlng=pt
8. Marini G, Rinaldi J de C, Damasceno DC, Felisbino SL, Rudge MVC. Alterações da matriz extracelular causadas pelo diabetes: o impacto sobre a continência urinária. *Rev Bras Ginecol e Obs* [Internet]. 2014 Jul;36(7):328–33. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-72032014000700328&lng=pt&nrm=iso&tlng=en
9. Marini G, Piculo F, Vesentini G, Barbosa AMP, Damasceno DC, Matheus SMM, et al. Effects of short-term severe and long-term mild STZ-induced diabetes in urethral tissue of female rats. *Neurourol Urodyn*. 2016 Mar;n/a-n/a.
10. Marini G, Piculo F, Vesentini G, Damasceno DC, Delella FK, Calderon IMP, et al. The influence of hyperglycemia on the remodeling of urethral connective tissue in pregnant rats. *Eur J Obstet Gynecol Reprod Biol*. 2018 Feb;221:81–8.
11. Vesentini G, Marini G, Piculo F, Damasceno DC, Matheus SMM, Felisbino SL, et al. Morphological changes in rat rectus abdominis muscle induced by diabetes and pregnancy. *Brazilian J Med Biol Res*. 2018 Mar;51(4).
12. Vesentini G, Barbosa AMP, Damasceno DC, Marini G, Piculo F, Matheus SMM,

- et al. Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women. Li L, editor. *PLoS One* [Internet]. 2020 Apr 3;15(4):e0231096. Available from: <https://dx.plos.org/10.1371/journal.pone.0231096>
13. Vesentini G, Barbosa AMP, Floriano JF, Felisbino SL, Costa SMB, Piculo F, et al. Deleterious effects of gestational diabetes mellitus on the characteristics of the rectus abdominis muscle associated with pregnancy-specific urinary incontinence. *Diabetes Res Clin Pract* [Internet]. 2020 Aug;166:108315. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0168822720305672>
 14. Piculo F, Marini G, Vesentini G, Morceli G, Damasceno DC, Sobrevia L, et al. Pregnancy-specific urinary incontinence in women with gestational hyperglycaemia worsens the occurrence and severity of urinary incontinence and quality of life over the first year post partum. *Eur J Obstet Gynecol Reprod Biol* [Internet]. 2020 Sep;252:336–43. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0301211520304036>
 15. Sartorão Filho CI, Pinheiro FA, Prudencio CB, Nunes SK, Takano L, Enriquez EMA, et al. Impact of gestational diabetes on pelvic floor: A prospective cohort study with three-dimensional ultrasound during two-time points in pregnancy. *Neurourol Urodyn* [Internet]. 2020 Aug 28;nau.24491. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/nau.24491>
 16. Prudencio CB, Rudge MVC, Pinheiro FA, Sartorão Filho CI, Nunes SK, Pedroni CR, et al. Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. Palatnik A, editor. *PLoS One* [Internet]. 2019 Nov 7;14(11):e0223261. Available from: <https://dx.plos.org/10.1371/journal.pone.0223261>
 17. Rudge C, Calderon I, Almeida A, Piculo F, Rudge M, Barbosa A. Score Establishment and Brazilian Portuguese version of the Pregnancy Sexual Response Inventory (PSRI). *Rev Bras Ginecol e Obs / RBGO Gynecol Obstet* [Internet]. 2018 Jun 6;40(06):322–31. Available from: <http://www.thieme-connect.de/DOI/DOI?10.1055/s-0038-1656536>
 18. Rudge CV, Calderon IM, Dias A, Lopes GP, Barbosa AP, Maestá I, et al. Design and validity of a questionnaire to assess sexuality in pregnant women. *Reprod Health* [Internet]. 2009 Dec 29;6(1):12. Available from: <https://reproductive-health-journal.biomedcentral.com/articles/10.1186/1742-4755-6-12>
 19. Nunes SK, Rudge CVC, Quiroz SCBV, Hallur RL, Prudencio CB, Pinheiro FA, et al. Impact of Gestational Diabetes Mellitus on Sexual Function: A Case–Control Study. *J Women’s Heal* [Internet]. 2020 Sep 1;29(9):1216–25. Available from: <https://www.liebertpub.com/doi/10.1089/jwh.2019.7794>
 20. Pascon T, Barbosa AMP, Cordeiro RCL, Bussaneli DG, Prudencio CB, Nunes SK, et al. Prenatal exposure to gestational diabetes mellitus increases developmental defects in the enamel of offspring. *PLoS One*. 2019;14(2).
 21. Van Geelen H, Ostergard D, Sand P. A review of the impact of pregnancy and childbirth on pelvic floor function as assessed by objective measurement techniques. Vol. 29, *International Urogynecology Journal*. Springer London; 2018. p. 327–38.
 22. Barbosa AMP. Prevalência e fator de risco para incontinência urinária e disfunção do assoalho pélvico dois anos após Diabete Melito gestacional. *Rev Bras Ginecol e Obs*. 2007 Apr;29(4).
 23. Goldsmith LT, Weiss G. Relaxin in Human Pregnancy. *Ann N Y Acad Sci*

- [Internet]. 2009 Apr;1160(1):130–5. Available from:
<http://doi.wiley.com/10.1111/j.1749-6632.2008.03800.x>
24. Alonso Lopez Y, Dereke J, Landin-Olsson M, Strevens H, Nilsson C, Hillman M. Plasma levels of relaxin-2 are higher and correlated to C-peptide levels in early gestational diabetes mellitus. *Endocrine* [Internet]. 2017 Sep 29;57(3):545–7. Available from: <http://link.springer.com/10.1007/s12020-017-1354-x>
 25. Zaman I, Swaminathan R, Brackenridge A, Sankaralingam A, McGowan B. Assessment of relaxin levels in pregnant women with gestational diabetes mellitus. *Endocr Abstr*. 2014 Apr;
 26. Bani D, Pini A, Ka-Sheng Yue S. Relaxin, Insulin and Diabetes: An Intriguing Connection. *Curr Diabetes Rev*. 2012 Jul;8(5):329–35.

Trajetória acadêmica

A minha história com a pesquisa iniciou-se na graduação em fisioterapia na UNESP de Marília (2009-2012), onde participei de projetos de extensão e de pesquisa em diversas áreas como cardiologia, respiratória e no 3º ano da graduação resolvi explorar a área de saúde da mulher. Durante a execução do meu trabalho de conclusão de curso (TCC) que resultou, posteriormente, no artigo intitulado “*Evolution of female urinary continence after physical therapy and associated factors*”, percebi o quanto problemas uroginecológicos afetam a vida de mulheres e a partir de então o tema saúde da mulher se tornou de grande interesse.

Quando entrei na graduação já tinha a vontade de seguir carreira acadêmica e de pesquisa, mas ao chegar ao 4º ano notei que precisava encontrar uma linha de pesquisa que me despertasse interesse, uma vez que a atuação da fisioterapia é bastante ampla. Decidi então, ampliar meus conhecimentos cursando a especialização em fisioterapia na saúde da mulher no Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC/FMUSP), no qual tive contato com gestantes de alto risco no maior complexo hospitalar da América Latina. Neste tempo, me aproximei muito da área de uroginecologia e percebi que a maior parte das mulheres relatavam que os sintomas haviam aparecido a partir da gestação e que a grande maioria apresentava ou apresentou comorbidades durante a gestação. Neste momento conversei com a professora Angélica que me orientou sobre sua linha de pesquisa, a qual tinha semelhanças com o que gostaria de estudar.

Iniciei o mestrado no PPG-GOM em 2014 e concluí em 2016, e a dissertação resultou na publicação do artigo “*Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study*”. Durante o

período do mestrado tive oportunidade de acompanhar e colaborar na elaboração do Projeto Temático Diamater, tendo sido uma experiência de grande privilégio e crescimento profissional participar de todos os trâmites envolvidos na submissão. Além disso, durante este período foi oportunizado a conexão com diversos pesquisadores nacionais e internacionais, possibilitando a ampliação da rede de colaboradores. Durante todo este período ampliei minha formação científica extracurricular, oportunizado pelas professoras Marilza Rudge (orientadora) e Angélica Barbosa (orientadora).

Após o mestrado, tive oportunidade de permanecer no *Diamater Study Group* para o seguimento da minha pesquisa, durante a gestação e incluindo análises hormonais e acompanhamento após o parto. Ingressei no doutorado em 2017 e, desde o início, os esforços para ampliar a amostra do mestrado no pós-parto foi bastante desafiador. Com o grupo mais consolidado, reuniões semanais de discussão de artigos e mensais para assuntos gerais foram conduzidas durante os anos do doutorado, fato que proporcionou discussões de estratégias de condução da pesquisa e de coletas, discussão de resultados parciais e introdução a temáticas variadas por meio do convite das professoras para que pesquisadores externos nos fornecessem treinamento. Em momentos específicos fui responsável por ministrar temas durante as disciplinas do Diamater, fato que me proporcionou experiência e possibilidade de treinamento a nível de ensino para pós-graduação.

Outra grande oportunidade durante o doutorado foi a execução do doutorado sanduíche financiado por meio do edital da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) do Programa Institucional de Internacionalização (CAPES-Print). Tive oportunidade vivenciar, durante seis meses, a experiência de estar no *“The University of Queensland’s Centre for Clinical Research Excellence in*

Spinal Pain, Injury and Health”, liderado pelo professor Paul Hodges, que é um dos principais pesquisadores na área de fisioterapia a nível mundial. Durante meu intercâmbio tive chance de analisar dados parciais de eletromiografia e aprofundar meus conhecimentos em relação a coleta e análise de dados. Além disso, fui apresentada ao pesquisador Sauro Salomoni, o qual é engenheiro biomédico e nos auxilia no desenvolvimento de rotinas de Matlab. Além disso, tive contato com o sistema *Covidence* quando participei como segunda revisora da revisão sistemática liderada pela aluna de doutorado Rachel Worman, “*What is the evidence for: Overactivity, hyperactivity (what is threshold), increased tone, increased stiffness (passive and active) of PFM in pelvic dysfunction*”. Em paralelo participei de coletas de dados da pesquisadora Lori Forner, com temáticas relacionadas a área de US3D dos MAP e prolapso genital de mulheres que praticam exercício físico com carga elevada. Também participei de coletas da pesquisadora Manuela Besomi que envolviam o estudo do efeito da angulação do probe nas imagens captadas do músculo tensor da fáscia lata por meio do equipamento de elastografia *shear-wave*.

Agradeço imensamente o incentivo das professoras Marilza e Angélica para que fosse possível cada etapa do meu doutorado, em especial a do meu doutorado sanduíche, pois me proporcionou crescimento nos mais variados aspectos da minha vida. É preciso ressaltar também a importância do programa de bolsas da CAPES/DS (demanda social), pois foi por meio dela que minha dedicação integral a pesquisa foi viabilizada. Acredito que muito ainda temos para progredir na área acadêmica em relação a valorização das partes envolvidas no “fazer ciência” que em grande parte é conduzida por alunos de pós-graduação/pesquisadores e deixo aqui meu testemunho do quão importante é o incentivo financeiro para viabilizar ciência de qualidade.

Para finalizar, durante estes 7 anos de dedicação a pesquisa pude vivenciar as atividades que compõe a universidade pública. Participei do **ensino**, incentivada pela professora Dra. Angélica em sua disciplina de graduação na UNESP/Marília, ministrando aulas e participando da formação prática dos alunos no estágio supervisionado. Neste último ano, também tive oportunidade de ser contratada em uma universidade particular para disciplina de anatomia e estágio supervisionado em saúde da mulher. Além disso, a nível de **extensão** participei do projeto RENOVE de auxílio da Professora Angélica, auxiliando na execução e análises. Em nível de **pesquisa**, participei como ouvinte de 20 congressos nacionais e internacionais, 16 cursos de formação complementar, proferi 15 palestras autorais, organizei 2 congressos, orientei de forma oficial 2 trabalhos de conclusão de curso (especialização e residência), coorientei de forma voluntária/informal 26 TCC nível graduação, participei de 16 bancas de TCC nível graduação, fui autora ou coautora de 36 resumos de trabalhos apresentados em congresso, 14 trabalhos publicados em anais de congresso, 1 prêmio de melhor trabalho em congresso como coautora, 10 artigos publicados em revistas de alto impacto como autora ou coautora (ANEXO 1) e 10 artigos em processo de submissão como autora ou coautora, 1 capítulo de livro na qualidade coautora intitulado *“recursos físicos terapêuticos para tratamento da incontinência urinária de urgência em mulheres”* em processo de publicação, 1 convite de autoria de capítulo de livro na área de eletromiografia do assoalho pélvico. Com as considerações dos membros da banca, finalização dos 2 artigos que compõe esta tese e obtendo a aprovação na defesa, a trajetória continuará.

E agora o que fazer? Quais as perspectivas futuras após a aprovação da tese? Onde quero estar? Toda esta trajetória consolidou e construiu o começo da minha história com a docência e pesquisa. Tenho intenção de continuar me aperfeiçoando e

colaborar para publicações futuras junto ao *Diamater Study Group*, continuar como colaboradora de pesquisa com grupos da UNESP Marília e UNIFESP. Além disso, tenho interesse em desenvolver pós-doutorado para dar sequência aos achados construídos a partir dos resultados dos meus trabalhos e dos trabalhos do grupo de pesquisa Diamater. Em paralelo, tenho intenção me preparar para editais externos para ampliar minha experiência internacional e minha rede de colaboração. Além de continuar dando aulas na faculdade particular que estou contratada e em paralelo prestar editais de seleção pública para docentes (ANEXO 2).

Seção 2

Resumos Expandidos

Exame Geral de Qualificação

Resumo Tese

Prudencio, C. B. **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.** 2021.138f Exame Geral de Qualificação (Doutorado) – Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, Brasil.

Introdução: A gestação é o evento no qual os músculos do assoalho pélvico (MAP) recebem importante sobrecarga. A ocorrência de alterações funcionais dos MAP durante e/ou após a gestação dependerá de diferentes fatores, sendo que dentre os principais estão o ganho de peso materno, aumento do volume uterino, alterações no alinhamento postural, comorbidades associadas à gestação e fatores hormonais. Adicionalmente, o peso do recém-nascido soma-se aos fatores de risco para desenvolvimento ou agravamento de disfunções no pós-parto. O *Diabetes Mellitus Gestacional* (DMG) é alteração patológica específica da gestação e que é rastreada pela primeira vez no decorrer do período gravídico. Preconiza-se pela *American Diabetes Association* (ADA) que entre a 24^a e a 28^a semana gestacional seja realizado o exame de glicemia de jejum e teste de tolerância oral a glicose. No âmbito das alterações musculares a ação da hiperglicemia gestacional (HG) foi documentada por estudos experimentais no tecido periuretral de ratas que demonstraram impacto similar quando induzido diabetes severo e moderado retratando alterações morfológicas nas fibras musculares uretrais, caracterizado por adelgaçamento, desorganização, além de atrofia muscular. No tecido musculoesquelético de gestantes estudos morfológicos demonstraram no reto abdominal alterações similares ao experimental. Além disso, outras estruturas também são acometidas pela hiperglicemia, uma vez que estudos clínicos e experimentais demonstraram alterações na composição da matriz extracelular, fibrose, alteração na ultraestrutura

como acúmulo de mitocôndrias, aumento de gotas de lipídios e acúmulo de grânulos de glicogênio no grupo diabético moderado prenhe. Todas estas modificações podem influenciar negativamente a funcionalidade dos MAP e ocasionar disfunções musculares do assoalho pélvico (DMAP), como por exemplo, a incontinência urinária (IU). Somado a este panorama existem as alterações hormonais gestacionais que também podem influenciar na capacidade funcional dos MAP. O hormônio relaxina, faz parte da superfamília da insulina e está relacionado à manutenção da gestação e com as principais adaptações gravídicas, principalmente, sobre o sistema cardiovascular e tecido conjuntivo. No tecido conjuntivo, a relaxina altera a densidade e quantidade de colágeno na matriz extracelular, além de aumentar o colágeno solúvel e diminuir o insolúvel, proporcionando a remodelação de um tecido mais firme para mais elástico. Há ainda a ação de enzimas degradadoras de colágeno que conferem características anti-fibrótica a este hormônio, processo que prepara a pelve para o processo do parto que resulta em frouxidão dos ligamentos, fâscias e sínfise púbica, com conseqüente interferência no sistema de suporte pélvico. Além disso, a relaxina aumenta a atividade de metaloproteinases no trato urinário o que pode diminuir a capacidade recuperação dos MAP após o parto. Isto se deve, provavelmente, porque os MAP dependem desses componentes para que o suporte ideal ocorra e garanta que sua função seja executada de maneira eficaz. A relação de dependência entre a integridade do tecido conjuntivo e a ação muscular em conjunto com a teoria de ação hormonal durante gestação ainda não está clara na literatura e traz dados conflitantes em relação ao aumento ou diminuição da relaxina e a presença de DMAP na gestação. A literatura demonstra que gestantes com diagnóstico de diabetes clínico e DMG podem apresentar níveis de relaxina diminuídos ou aumentados em relação a gestantes não-diabéticas independente do trimestre. Fato que pode ser explicado pela

relaxina aumentar a ligação de insulina aos receptores de insulina nos adipócitos. Porém, o mecanismo deste aumento em sua concentração ainda não foi esclarecido e parece estar relacionado com ajustes da própria gestação complicada pelo diabetes. Embora as temáticas gestação hiperglicêmica, IU e relaxina serem tratadas na literatura, os estudos apresentam os resultados separadamente e não há estudos que inter-relacionem os três fatores. Com objetivo de entender esta dinâmica, a presente tese é estruturada em 2 artigos que trarão maior conhecimento sobre a temática. Para tanto, dosagens séricas do hormônio relaxina foram realizadas analisadas. A incontinência urinária específica da gestação (IU-EG) e IU pós-parto foram avaliadas por instrumentos validados que mensuram a severidade e impacto da IU. A função dos MAP foi avaliada pela eletromiografia, exame recomendado pela *International Continence Society (ICS)* e *International Urogynecological Association (IUGA)*, para registrar a atividade extracelular bioelétrica gerada pelas fibras musculares e, desta forma, inferir sobre o comportamento neuromuscular. **Justificativa:** O grupo de pesquisa *Diamater Study Group* empenhou-se em estudar nos últimos anos o efeito do DMG sobre os MAP. Barbosa et al. (2011) em seu estudo confirmou associação entre a DMG e a presença de baixa pressão de contração dos MAP dois anos após cesárea. Os estudos translacionais clínicos e experimentais identificaram alterações nas características das fibras musculares e matriz extracelular (MEC). Estes resultados corroboram com os resultados clínicos de que a IU-EG em gestação com DMG está associada à DMAP. Os temas relaxina, DMG e função dos MAP são abordados de modo individual, porém observa-se que eles têm pontos que convergem e que precisam ser investigados em associação. Não foram encontrados na literatura trabalhos que abordem o impacto do aumento nos níveis do hormônio relaxina sobre a função dos MAP em gestantes com DMG, indicando importante lacuna no

conhecimento sobre os fatores que interferem no desempenho dos MAP durante a gestação. Os resultados da presente tese poderão auxiliar no manejo e detecção de possíveis fatores de risco para DMAP e desta maneira planejar e propor condutas tanto de caráter minimizador, curativo ou preventivo. **Artigo 1 Objetivos: 1) Comparar o padrão de ativação dos MAP entre mulheres com GDM e não-GDM entre 24-30 semanas de gestação até 18-24 meses pós-parto durante um teste clínico padrão.**

Métodos: 1) Trata-se de estudo de coorte prospectivo que avaliou as participantes em três momentos distintos: de 24-30 semanas de gestação (1º momento), 36-38 semanas de gestação (2º momento) e 18-24 meses após o parto (3º momento). Os principais critérios de inclusão foram: gestantes que no momento da avaliação estivessem com 24-30 semanas gestacionais, gravidez única sem IU prévia. O critério para composição dos 2 grupos de estudo foram as diretrizes propostas pela ADA, utilizando o teste oral de tolerância glicêmica de 75g (75g-TOTG), participantes que obtiveram níveis em jejum ≥ 92 mg / dL ou 1 hora ≥ 180 mg / dL ou 2 horas ≥ 153 mg / dL foram alocadas no grupo DMG. As participantes que apresentaram valores abaixo compuseram o grupo não-DMG. O recrutamento ocorreu no Centro de Pesquisa em Diabetes Perinatal da Faculdade de Medicina de Botucatu/UNESP/Brasil, entre 2015 e 2018. As participantes que aceitaram participar foram avaliadas com 24-30 semanas de gestação por questionário com dados pessoais, clínicos e histórico obstétrico e o índice de massa corporal (IMC) foi aferido. Na sequência, foi explicado sobre o exame funcional dos MAP e as participantes foram posicionadas na maca em decúbito dorsal e foi realizada a palpação digital dos MAP para confirmar que as participantes conseguiam entender o comando verbal de contração e relaxamento. Em seguida, foi realizado o preparo do exame eletromiográfico, por meio da introdução de sonda vaginal com dois eletrodos opostos de aço inoxidável (85 x 25 mm) posicionados em

ambas as paredes vaginais. O protocolo Glazer foi utilizado para padronizar as contrações: 60 segundos de repouso (pré-linha de base); contração fásica de 1 segundo (contração rápida) repetida por 5 vezes (5x), com 10 segundos de descanso entre as contrações; 10 segundos de contração tônica por 5x, com 10 segundos de descanso; e uma contração lenta sustentada por 60 segundos seguido de 60 segundos de repouso (pós-linha de base). Após a avaliação no 1º momento as participantes foram agendadas para a reavaliação no 2º momento e 3º momento, e os mesmos procedimentos foram realizados em cada etapa. Em relação a análise dos dados, as medidas foram realizadas no domínio do tempo por meio das medidas de *root mean square* (RMS), as quais predizem sobre a amplitude do sinal durante as diferentes tarefas do protocolo, através das variáveis média, pico e variabilidade do desvio padrão e de seu coeficiente de variação. Além disso, o comportamento do sinal em relação ao tempo entre o início da contração e o pico e do pico até o final da contração foram expressos em segundos. A taxa de ativação neural durante o início e o fim da contração e, também, antes e depois do pico foram avaliados. Com objetivo de visualizar o comportamento da contração durante todo o protocolo, foi realizado análise de wf-wavelets para comparar o formato das curvas entre os grupos em cada tarefa específica. **Resultados: 1)** Os dados demográficos e obstétricos foram homogêneos entre os grupos. O grupo GDM mostrou durante as contrações Flick de 1 seg menores amplitudes no pós-parto. Além disso, embora o grupo DMG atingiu o pico de amplitude de forma semelhante ao não-GDM, eles demoram mais para retornar aos níveis basais. Durante as contrações sustentadas de 10 segundos, o grupo DMG manteve níveis mais baixos de ativação dos MAP do que o grupo não-DMG ao final da gestação e no pós-parto quando comparado ao grupo não-DMG. **Conclusão: 1)** Os resultados sugerem que o DMG prejudica a controle da ativação

do MAP durante as contrações rápidas (1-seg) e lentas (10-seg), sendo que estas alterações aparecem no final da gestação e se estendem até 2 anos após o parto. O comportamento motor encontrado pode ter influência sobre as DMAP e deve ser foco de futuros estudos mecanísticos e clínicos envolvendo a temática. **Artigo 2 Objetivo:**

2) Investigar os níveis de relaxina em mulheres com e sem DMG de acordo com status da incontinência urinária e função muscular do assoalho pélvico. **Métodos: 2)** Trata-se de estudo transversal que avaliou 282 gestantes em qualquer momento gestacional a partir da 24ª semana gestacional. Os principais critérios de inclusão foram: gestantes entre 24 até 38 semanas de gestação, primigesta ou secundigesta com parto cesárea anterior, gravidez de feto única e sem histórico de IU prévio a atual gestação. As participantes foram divididas em 2 grupos principais, não-DMG e DGM, de acordo com a presença de diabetes avaliado pelo TOTG-75g. Além disso, de acordo com sub-análises os grupos foram subdivididos de acordo com o status da continência urinária durante a gestação, sendo assim formado 4 subgrupos: não-DGM-C (não-DMG continente), não-DGM-IUEG (não-DMG incontinente), DGM-C (DMG continente), DGM-IUEG (DMG incontinente). O protocolo de avaliação seguiu a mesma sequência de avaliação do estudo anterior, foi administrado questionário com dados pessoais, clínicos e obstétricos e adicionou-se questionários validados no Brasil para avaliação da IU, sendo eles, o *"International Consultation on Incontinence Questionnaire - Short Form" (ICIQ-SF)* e o *"Incontinence Severity Index" (ISI)*. Na sequência foram realizadas as mesmas instruções do artigo anterior em relação ao treinamento prévio da musculatura e foi performado a avaliação funcional do assoalho pélvico por meio da escala PERFECT, no qual foram coletados os dados de força, endurance e repetições de contrações rápidas (P,E,F). **Resultados 2)** As concentrações relaxina-2 foram significativamente menores em participantes com IUEG, tanto no grupo não-

DMG ($p=,023$) quanto no grupo DMG ($p=,027$), no entanto o grupo DMG-IUEG apresentou menores níveis de relaxina ($p=,001$). Além disso, a estratificação do grupo de acordo com a força do assoalho pélvico demonstrou que participantes com DMG e força 0-2 (MOS) tiveram menores níveis de relaxina que o grupo não-DMG ($p=,023$) e que participantes com DMG e força 3-5 (MOS) ($p=,029$). As concentrações de relaxina apresentaram-se ainda mais diminuídas no grupo DMG-IUEG quanto comparado a todos os outros 3 grupos ($p=,001$). **Conclusão 2)** Os níveis de relaxina estão relacionados com a presença de IUEG, no entanto a combinação do DMG e IUEG e força muscular do AP diminuída apresenta menores valores de relaxina quando comparado com outros grupos.

Palavras-Chaves: Assoalho pélvico; *diabetes mellitus* gestacional; músculo do assoalho pélvico; relaxina; eletromiografia.

Thesis Abstract

Prudencio, C. B. **The relation between pelvic floor electromyography and relaxin levels during pregnancy and post-partum of women with gestational hyperglycemic and pregnancy specific urinary incontinence.** 2021.138f. General Qualification Exam (PhD) – São Paulo State University (Unesp), Medical School, Botucatu, Brazil

Introduction: Pregnancy is the event in which the pelvic floor muscles (PFM) receive an important overload. The appearance of functional changes during and/or after pregnancy will depend on the pregnant woman's muscular response to these overloads. The most widespread factors that will influence PFM are maternal weight gain, uterine volume, changes in alignment, pathological changes associated with pregnancy and hormonal factors. The newborn's weight and episiotomy are added to the risk factors for the development or worsening of postpartum dysfunctions. Gestational Diabetes Mellitus (GDM) is a pathological alteration specific to pregnancy that is screened for the first time during the pregnancy period. It is recommended by the American Diabetes Association (ADA) that between the 24th and the 28th gestational week the blood glucose test and oral tolerance test be performed. In the context of muscle changes, the action of gestational hyperglycemia (GH) was documented by experimental studies that demonstrated the same behavior in severe and moderate GDM, depicting morphological changes in the urethral muscle fibers, characterized by thinning, disorganization, in addition to muscle atrophy. Other structures are also affected by hyperglycemia, clinical and experimental studies have shown changes in the composition of the extracellular matrix, fibrosis, changes in the ultrastructure such as accumulation of mitochondria, increase in drops of lipids and accumulation of glycogen granules in the moderate pregnant diabetic group. All of these changes can negatively influence the functioning of PFM and cause pelvic floor muscle dysfunctions (PFMD), such as urinary incontinence (UI). Added to this information are the hormonal changes in pregnancy that can contribute to a decrease in muscle function. The hormone relaxin is part of the insulin superfamily and is related to the maintenance of pregnancy and the main pregnancy adaptations, mainly on the cardiovascular system and connective tissue. In connective tissue, relaxin-2 alters the density and amount of collagen in the extracellular matrix, in addition to increasing soluble collagen and decreasing insoluble ones, this alteration remodels the tissue

more firmly into more elastic. There is also the action of collagen-degrading enzymes that give anti-fibrotic characteristics to this hormone, a process that prepares the pelvis for the delivery process that results in laxity of the ligaments, fascias and pubic symphysis, with consequent interference in the pelvic support system. In addition, relaxin increases the activity of metalloproteinases in the urinary tract, which can decrease the ability of PFM to recover after delivery. This is probably due to the fact that PFM depend on these components for the ideal support to occur and ensure that their function is performed effectively. This dependence relationship between the integrity of the connective tissue and the effective muscle action is evidenced through a study that demonstrated high levels of relaxin are significantly related to the presence of PFMD during pregnancy. The literature shows that pregnant women diagnosed with clinical diabetes and GDM had increased levels of relaxin in relation to non-diabetic pregnant women regardless of the trimester. A fact that can be explained by relaxin increasing the binding of insulin-to-insulin receptors in adipocytes, however the mechanism of this increase in its concentration has not yet been clarified and seems to be related to adjustments in the pregnancy itself complicates by diabetes. Despite the themes of hyperglycemic pregnancy, UI, relaxin being treated in the literature, studies present the results separately and there are no studies that interrelate the three factors, to understand their dynamics together. The present thesis is structured in 2 articles that will bring more knowledge about the theme. Therefore, serum dosages of the hormone relaxin will be performed on the participants. Pregnancy-specific urinary incontinence (PS – UI) and postpartum UI will be assessed by validated instruments that measure the severity and impact of UI. PFM function will be assessed by electromyography, an exam that is recommended by the International Continence Society and International Urogynecological Association, to record the bioelectric extracellular activity generated by muscle fibers and, thus, to infer about neuromuscular behavior. **Justification:** The Diamater Study Group of the Faculty of Medicine of Botucatu has endeavored to study in recent years the effect of GDM on pelvic floor (PF). Barbosa et al. (2011) in their study confirmed an association between GDM and the presence of low pressure of contraction of the PFM, two years after cesarean section. Translational clinical and experimental studies have identified changes in the characteristics of muscle fibers and extracellular matrix. These results corroborate the clinical results that specific urinary incontinence of pregnancy with

GDM is associated with PFMD. The themes relaxin, GH and PFM function are addressed individually, but it is observed that they have points that converge and that need to be investigated in association. No studies were found in the literature that address the impact of this increase in the levels of the hormone relaxin on the electromyographic parameters in the PFM in pregnant women with GDM and GH, indicating that an important gap needs to be answered to identify factors that interfere performance of PFM. The results may assist in the management and detection of possible risk factors for dysfunctions and in this way plan and propose conducts of a minimizing, curative or preventive character. **Article 1 Objectives:** 1) To analyze the progression of the neuromuscular pattern of the PF in women with a history of GDM during two gestational moments and 18-24 months after delivery. **Methods: 1)** The aim of was to compare PFM activation pattern between GDM and non-GDM women from 24-30 gestational weeks to 18-24 months postpartum during a standard clinical test during gestation and postpartum. The main inclusion criteria to the study were: pregnant women between 24-30 weeks of gestation; singleton pregnancy. The threshold to compose the 2 groups of study was the guidelines proposed by American Diabetes Association using the 75g oral glyceimic tolerance test (75g-OGTT), participants who had fasting ≥ 92 mg/dL or 1 hour ≥ 180 mg/dL or 2 hours ≥ 153 mg/dL were allocated to the GDM group. The participants who had lower values allocated the non-GDM group. The recruitment takes place on Perinatal Diabetes Research Center (PDRC) of Botucatu Medical School/UNESP/Brazil, between 2015 and 2018. The participants who accepted to participate were evaluate at 24-30 weeks of gestation by questionnaire with personal details, clinical and obstetric historic and body mass index (BMI) was measured. In sequence, they were explained about the PFM examination, positioned in a starcher in supine position and a digital palpation were performed to confirm that participants were able to understand the command. After, the electromyography exam preparation was performed using a vaginal probe sensor with two opposite stainless-steel electrodes (85 x 25 mm) positioned on both vaginal sidewalls. The glazer protocol was used to standardize the contractions: 60-seconds rest (pre-baseline), 1-sec phasic (flick) contraction repeated 5 times (5x), with 10-sec rest in between, 5 x 10-sec tonic contractions, with 10-sec rest in between, 60-sec endurance contraction and 1x 60-sec rest (post-Baseline). After the end of the first time-point the participant were scheduled to 36-38 weeks of gestation and 18-24

months postpartum and the same procedures were performed in each step. Data analyses focus on the amplitude measurements RMS related to average, peak and standard deviation variability was done. Analysis about the time between onset to peak and peak to offset was performed. In addition, rate of neural drive during the beginning and end of the contraction and before and after peak. The full RMS EMG waveforms from the Flick and Hold tasks were compared between groups using the technique of wavelet-functional ANOVA. **Results: 1)** Demographic and obstetric data showed homogeneity between groups. GDM group showed during 1-sec Flick contractions lower EMG amplitudes on post-partum. Additionally, although GDM group achieve peak PFM EMG amplitudes similarly to the non-GDM, they took longer to return to baseline levels. During 10-sec Hold contractions, the GDM group sustained lower levels of PFM activation than the non-GDM group at both 36-38 weeks of gestation and 18-24 months postpartum when compared to the non-GDM group. **Conclusion: 1)** The results suggest that GDM impaired PFM control mainly on 1 sec-flicks and 10-sec hold contraction, which appears to develop late-pregnancy and extends long-term postpartum. This motor behaviour may play a role on pelvic floor dysfunctions and should be focus of following mechanistic and clinical research. **Article 2 Objective: 2)** To investigate relaxin-2 concentration between DMG and non-DGM pregnant according to UI and pelvic function status. **Methods: 2)** This is a cross-sectional study which evaluate 282 pregnant from 24 weeks of gestation. The participants were divided into two groups, non-GDM and GDM, according to American diabetes Association's diabetes mellitus gestational threshold. In addition, according to sub analysis the both groups were subdivided according to the presence of pregnancy specific urinary incontinence (PSUI): non-GDM-C (non-GDM continent), non-GDM-PSUI (non-GDM incontinent), GDM-C (DMG continent), GDM-PSUI (GDM incontinent). All participants carried out questionnaires about clinical, obstetric and urinary continence status ("International Consultation on Incontinence Questionnaire - Short Form"- ICIQ-SF and the "Incontinence Severity Index" -ISI). Followed by pelvic floor muscle evaluation by the PERFECT scheme which strength, endurance and fast contraction were evaluated. **Results 2)** Serum relaxin-2 concentrations were significantly lower in pregnant with PSUI both in non-GDM and GDM pregnancies, but GDM showed the lowest concentration. In addition, the stratification of the groups according to PFM strength showed that pregnancy with GDM and MOS 0-2 had significantly lower levels than

non-GDM and GDM with MOS 3-5. Relaxin-2 level was intensely lower in GDM-PSUI pregnant with MOS 0-2 when compared with the other 3 groups. **Conclusion: 2)** The relaxin-2 concentration was related to the presence of PSUI, but the combination between GDM and PSUI and lower levels of pelvic floor strength leads to lower levels of relaxin-2 when compared to the other 3 groups.

Keywords: Pelvic floor; gestational diabetes mellitus; pelvic floor muscle; relaxin; electromyography.

Seção 3
Artigos

Artigo 1

Seção redigida como Artigo Original de acordo com as normas de publicação da revista Plos One para a qual está em processo de submissão.

Qualis A1 MED III – FI: 3,124 <https://journals.plos.org/plosone/s/submission-guidelines>. Cover Letter (ANEXO 3)

Gestational diabetes is associated with alteration on pelvic floor muscles activation pattern during pregnancy and postpartum: Prospective Cohort using electromyography assessment

Caroline B. Prudencio^{1‡}, Sthefanie K. Nunes^{1‡}, Fabiane A. Pinheiro^{1*}, Carlos I. Sartorão Filho^{1*}, Guilherme T. A. Nava^{3*}, Sauro E. Salomoni⁴, Cristiane Rodrigues Pedroni^{2*}, Marilza V. C. Rudge^{1*}, Angélica M. P. Barbosa^{1,2*}, Diamater Study Group[#]

¹ São Paulo State University (Unesp), Medical School, Botucatu, Brazil, Department of Gynecology and Obstetrics, Botucatu Medical School, Universidade Estadual Paulista (UNESP), Brazil;

² São Paulo State University (Unesp), School of Philosophy and Sciences, Marília, Brazil,

³ Department of Physiotherapy and Occupational Therapy, Universidade Estadual Paulista (UNESP), Brazil.

⁴ The University of Queensland, NHMRC Centre for Clinical Research Excellence in Spinal Pain, Injury and Health, School of Health and Rehabilitation Sciences, Brisbane, Australia.

‡ CBP and SKN are first authors on this work.

● MVCR and AMPB are last authors on this work.

* These authors contributed equally to this work.

#Diamater Study Group - Rudge MVC, Barbosa, AMP, Caldeiron IMP, Souza FP, Berghmans B, de Bie R, Thabane L, Junginger B, Graeff CFO, Magalhães CG, Costa RA, Lima SAM, Kron-Rodrigues MR, Felisbino S, Barbosa W, Campos FJ, Bossolan G, Corrente JE, Nunes HRC Abbade J, Rossignoli PS, Pedroni CR, Atallah AN, Di Bella ZIKJ, Uchoa SMM, Hungaro MA, Mareco EA, Sakalem ME, Martinho N, Hallur LSR, Reyes DRA, Alves FCB, Marcondes JPC, Prudencio CB, Pinheiro FA, Sartorão CI, Quiroz SBCV, Pascon T, Nunes SK, Catinelli BB, Reis FVDS, Oliveira RG, Barneze S, Enriquez EMA, Takano L, Carr AM, Magyori ABM, Iamundo LF, Carvalho CNF, Jacomin M, Avramidis RE, Silva AJB, Orlandi MIG, Dangió TD, Bassin HCM, Melo JVF, Takemoto MLS, Menezes MD, Caldeirão TD, Santos NJ, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Garcia GA, Nava GTA, Pascon C, Bussaneli DG, Nogueira VKC, Rudge CVC, Piculo F, Prata GM.

* Corresponding Author: Angélica Mércia Pascon Barbosa; Botucatu Medical School, Univ Estadual Paulista (UNESP), Department of Gynecology and Obstetrics, Botucatu, Brazil. Email: angelicapascon@gmail.com.

Short Title: Gestational Diabetes and Pelvic Floor Electromyography

Financial Disclosure: The authors have no potential conflicts of interest related to this study. Funding received: Supported by Sao Paulo Research Foundation protocol number 2016/01743-5. Brazilian Federal Agency for Support and Evaluation of graduate Education/SD (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES/DS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ABSTRACT

Background and objective: Gestational diabetes mellitus (GDM) is a comorbidity which may cause acute and lifelong disorders to mother and child. Alterations in muscular and connective tissues have been associated with GDM in translation studies, characterizing gestational diabetic myopathy. Pregnancy-specific urinary incontinence and sexual disabilities, disorders that depend on the pelvic floor muscle (PFM) integrity, are also associated with GDM both during and after pregnancy. The aim of was to compare PFM activation pattern between GDM and non-GDM women from 24-30 gestational weeks to 18-24 months postpartum during a standard clinical test during gestation and postpartum. **Methods:** We conducted a prospective three-time points cohort study from gestation (24-30 weeks – T1, and 36-38 weeks – T2) to 18-24 months postpartum (T3). PFM electromyography was recorded in primigravida or primiparous women with one previous elective c-section with or without the diagnosis of GDM according to the American Diabetes Association criteria. A careful explanation of the muscle anatomy and functionality of the PFM was given to participants before EMG assessment. The outcome measures were PFM activation patterns assessed during pregnancy and postpartum, compared intra and between groups. PFM activation patterns were assessed by normalized electromyography signal at rest and during 1-second (sec) phasic, 10-sec hold and 60-sec sustained contractions. **Results:** Demographic and obstetric data showed homogeneity between groups. GDM group showed during 1-sec Flick contractions lower EMG amplitudes on post-partum. Additionally, although GDM group achieve peak PFM EMG amplitudes similarly to the non-GDM, they took longer to return to baseline levels. During 10-sec Hold contractions, the GDM group sustained lower levels of PFM activation than the non-GDM group at both 36-38 weeks of gestation and and 18-24 months postpartum when compared to the non-GDM group. **Conclusion:** The results suggest that GDM impaired PFM control mainly on 1 sec-flicks and 10-sec hold contraction, which appears to develop late-pregnancy and extends long-term postpartum. This motor behaviour may play a role on pelvic floor dysfunctions.

Key-words: Gestational diabetes mellitus, pelvic floor, electromyography and post-partum.

INTRODUCTION

Gestational diabetes mellitus (GDM) and gestational diabetic myopathy have been described as risk factors to pelvic floor muscle dysfunction (PFMD) during pregnancy and postpartum [1-10]. Compromised PFM integrity may predispose women to PFMD such as pregnancy specific-urinary incontinence (PS-UI) [2] and postpartum urinary incontinence, which have substantial social and economic burden, in addition to high public health costs [11]. More specifically, GDM has been associated with higher prevalence of both PS-UI and IU postpartum, with worsening of severity and quality of life during pregnancy and over first year postpartum compared to non-GDM women [1,2,5,12,13]. Taken together, current evidence indicates that PFM could be failing to perform contractions properly in women with GDM. A clinical triad composed by pelvic floor muscle (PFM) myopathy, PS-UI, GDM is focus of research. However, there is a lack of studies with longitudinal design assessing PFM function during and after pregnancy, especially in GDM group [14].

Experimental studies in moderate diabetic rat models have shown that the periurethral and rectus abdominis muscles present deterioration, such as atrophy, thinning, disorganization, and co-localization of fast and slow fibers. [7,8,10,15]. These data are consistent with those observed in rectus abdominis muscles tissue collected from pregnant women with GDM during C-section [6,16], which suggests that GDM is indeed capable of damaging the muscular tissue causing a myopathic process [6,16-18]. Establishing a rationale line by the morphological findings from urethral and rectus abdominis muscle of rats [7,8,10,15] and rectus abdominis on pregnant [6,16] the PFM are also potentially impacted by the myopathic process [6]. Due to the invasive nature of PFM biopsy, functional tests have been employed to evaluate the impact of GDM on its function. Studies have shown that GDM implies PFM function impairments when

compared to non-diabetic pregnant women: In a study using electromyography (EMG), the amplitude of PFM signals during rest and hold contraction was decreased from the second to the third trimester. When three-dimension ultrasonography (3D-US) was used, negative biometric changes, such as low increase of hiatal area, decrease in the anteroposterior diameter and a reduced levator ani muscle thickness, has also been observed between these two time points [17,18].

Although previous studies have demonstrated impairments in PFM function associated with GDM, current evidence is still inconclusive in relation to the time frame in which these impairments evolve, and whether women with GDM are capable to recover PFM function after delivery [19]. These are important clinical questions to understand the underlying pathophysiology of PFMD. Hence, the aim of this longitudinal study was to compare PFM activation pattern between GDM and non-GDM women from 24-30 gestational weeks to 18-24 months postpartum during a standard clinical test during gestation and postpartum.

METHODS

Study design, participants and group composition

This prospective cohort study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethical Committee of Botucatu Medical School of Sao Paulo State University (Protocol Number CAAE 82225617.0.0000.5411). The STROBE checklist was applied on the study. Written informed consent was obtained from all participants after careful explanation of all research procedures.

The inclusion criteria were: pregnant women between 24-30 weeks of gestation in the first assessment; singleton pregnancy; 18–40 years of age; ability to perform a palpable contraction of the PFM [20], had not received PFM training or any musculoskeletal PFM treatment previously or during pregnancy. The exclusion criteria were clinical diagnose of diabetes (type I or II, or overt diabetes in previous pregnancy), history of urinary incontinence (UI), having had more than two pregnancies, previous vaginal delivery, previous prolapse or incontinence surgery, failure to understanding or follow of the command to contract PFM, history of neurological diseases, visible genital prolapse, cervical isthmus incompetence, smoking, preterm birth, abortion, as well as participants who withdrew their consent during cohort.

The diagnosis guidelines proposed by American Diabetes Association were used to identify patients with GDM [21] using the 75g oral glyceimic tolerance test (75g-OGTT). The test was applied to all participants at 24 gestational weeks, and participants were assigned to the GDM group if they presented fasting glyceimic levels ≥ 92 mg/dL or 1 hour ≥ 180 mg/dL or 2 hours ≥ 153 mg/dL. Conversely, participants who had lower glyceimic levels were allocated to the non-GDM group.

Participants recruitment and assessment

Participants were evaluated at three time points: 24–30 weeks of gestation (T1), at 36–38 weeks of gestation (T2) and 18-24 months postpartum (T3). The same procedures were followed at each time point.

82 participants between 24-30 weeks of gestation who met the criteria were recruited from the Perinatal Diabetes Research Center (PDRC) of Botucatu Medical School/UNESP/Brazil, between 2017 and 2019. After giving their written consent, they were invited to answer a questionnaire with personal details, clinical and obstetric

historic and anthropometric measures were taken.

Afterwards, the PFM examination was explained and subsequently conducted by a single trained physiotherapist (CBP) with 4 years of experience in PFM evaluation. After emptying their bladder, participants were asked to lie down on the stretcher in supine position with their lower limbs flexed. Explanation about the anatomy and function of PFM was provided. To guarantee that participants understood the instructions, vaginal digital palpation was performed, and a PFM contraction was requested by giving the verbal instruction “squeeze the vaginal muscle and hold them as hard as possible, as if you were holding urine until I say to relax”. After we considered that an isolated PFM contraction was well-executed without co-contraction of the adductor, gluteus, hip movements, or expulsion movements [17,22,23], participants were asked to perform a short sequence of PFM contractions, in preparation to the Glazer protocol of clinical evaluation [24] that would be used for the PFM EMG assessment: 3 brief contractions of 1-sec (Flick) phasic contraction and 3 contractions sustained for 10-sec (Hold) sustained PFM contractions. Participants received strong verbal encouragement and during contractions, and digital palpation was used to confirm that they performed maximal voluntary contractions (MVCs) on every attempt. During the five minutes rest period before EMG recordings, additional instructions were given depending on the performance, any possible doubts were clarified addressed, and the instruction to contract the PFM as hard as they could before relaxing was re-inforced.

EMG recordings and experimental protocol

The EMG signals were recorded using a two-channel device (Miotool 200 Uro; Porto Alegre, Brazil) with a gain of 1000, 14-bit A/D converter, input impedance of 10^{10}

Ohm/2 pF, Common Mode Rejection Ratio (CMRR) at 126 dB. Signals sampled at 2000 Hz. PFM EMG was recorded using an intravaginal probe sensor (Fig 1A) with two opposite stainless-steel electrodes (85x25 mm) positioned on both sides of the vaginal sidewall, coupled to a differential sensor with ring connection. A water-soluble gel was applied before introducing the probe into the vaginal canal. The reference electrode was placed on the ulna's styloid process following the SENIAM recommendations [25].

The Glazer clinical protocol (Fig 1A) was used to standardize PFM activation. The protocol consists of the following sequence: (i) 60-sec rest (Baseline-pre), (ii) brief 1-sec phasic contractions (Flicks) repeated 5 times, followed by 10-sec rest interval, (iii) 10-sec sustained contractions (Hold) repeated 5 times, with 10-sec rest in between, (iv) 60-sec sustained endurance contraction (Endurance), and (v) 60-sec rest (Baseline-post) [24,26] (Fig 1B). The following verbal instructions were given to all participants to explain the execution of each task: (i) "Please, stay relaxed as quite as possible, until I say to you to contract the PFM" (Baseline-pre); (ii) "Please, squeeze your vaginal and anus muscles as harder as possible and relax as soon as instruct you" (Flick); (iii) "Please, squeeze and hold your vagina and anus as harder and as long as possible until 10 seconds" (Hold). They were encouraged to sustain the MVC during 10 seconds by the verbal instruction: "keep squeezing, keep going, keep going"; (iv) during the 60-sec endurance contraction, the same instruction used for the 10-sec Hold contraction was given, but the instruction to "keep squeezing, contract, keep going, contract as harder as possible, keep going" was continuously repeated during the 60-sec; (v) and in the last 60-sec rest period (baseline-post), participants were instructed to "Relax your vagina and anus as much as possible and stay relaxed for 60-sec".

Electromyographic signal processing

The EMG signals were processed offline using custom programs implemented in MATLAB (2014b, The MathWorks, Inc., Natick, MA, USA). First, the quality of the signals from each data collection was evaluated based on visual inspection and signal-to-noise ratio (SNR). Recordings with low SNR, where the EMG was not discernible from the background or containing excessive signal artifacts were removed from the analyses ($n = 13$). Because we detected significant contamination from the power line (60 Hz), an adaptive least mean squares (LMS) filter was implemented, using MATLAB function `dsp.LMSFilter`, in order to selectively remove contamination at 60 Hz and higher harmonics. The central frequency of the filter was adjusted in each case, depending on the presence of contamination in each harmonic.

The EMG profiles were obtained by applying the root mean square (RMS) the entire signal using a sliding window of 200 msec. Consistent with previous studies using the same protocol, the RMS EMG profiles were then normalized by the highest peak detected across the 5 repetitions of the Flick task [27]. Although the Glazer protocol defines fixed time windows for the execution of each task, we ensured the precise selection of time windows of each contraction task by using a single-threshold algorithm to automatically detect the EMG onset and offset of muscle activity [28], which were confirmed by visual inspection (see Figure 1). Rest periods (Baseline-pre and -post) were initially selected from the timing expected from the protocol, and were also visually inspected, with adjustments when necessary.

In order to characterize the muscle activation patterns of each subject, we extracted the following parameters from the normalized RMS EMG profiles of each task: average and peak amplitudes; standard deviation of the amplitude and coefficient of variation. For the Flick and Hold tasks, we also extracted the time from EMG onset to peak

amplitude and the time from peak amplitude to EMG offset. Using time windows of 200 msec, we also estimated the slopes (%/sec) of the RMS EMG after EMG onset (i.e. “increase rate of activity”) and before EMG offset (i.e. “decrease rate of activity”), as well as the slopes before and after the time of peak amplitude (Figure 2).

Finally, the full RMS EMG waveforms from the Flick and Hold tasks were compared between groups using the technique of wavelet-functional ANOVA (wfANOVA) [29,30]. As we were interested in both the phasic activation patterns and the rest amplitudes before and after each contraction, we selected time windows that included 3 seconds before and after each contraction. Using Subject as a random effect, all task repetitions from each subject were included in the wfANOVA model. For each task, the RMS EMG waveforms were transformed into the wavelet domain, allowing temporally localized features to be represented by a small number of orthogonal (independent) wavelet coefficients. These coefficients were then statistically tested between groups using a 1-way ANOVA at each Time-Point to evaluate if there were differences in PFM activation patterns between groups. Significant *between-group* contrasts were identified and transformed back from the wavelet domain into the time domain for visualization.

Sample size estimation

The sample size calculation was performed *a priori* using the G*Power. Calculations were performed considering a repeated measures design, power of 0.80, probability of error α 0.05, effect size of 0.25 calculated by the partial η^2 of 0.06. According to the study design, it was considered for the calculation two groups (GDM and non-GDM) and three measurements (i.e. 3 time points), an estimated correlation among repetition measures of 0.5 and non-sphericity correction of 1, the estimated

sample size required was at least 28 participants (14 in each group).

Statistical methods

The software IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA) was used to statistical analysis. The Chi-square test or Fisher's exact test was applied to compare the nominal data between groups. Mann–Whitney U test was applied to compare independent categories on table. The EMG parameters were tested using a 2-way generalized linear model (GLM), with Group (GDM, non-GDM) and Time Point (1, 2, 3) as factors, with repeated measures on the time point factor (i.e. within-subject). The hypothesis of sphericity was tested by the Mauchly test and when the sphericity was rejected the Greenhouse-Geisser correction was applied. When a significant main effect or interaction effect was found, pair-wise post-hoc tests were applied using Bonferroni correction. Furthermore, as mentioned previously, the full RMS EMG waveforms from the Flick and Hold tasks were compared between groups using the technique of wfANOVA. Differences were considered statistically significant if $p < 0.05$.

RESULTS

Flow of participants through the study

The flow chart in Figure 3 illustrates the number of women examined at each time point and the reasons for dropout. Amongst all initially included participants (n=82), 48 women were allocated in the non-GDM group and 34 in the GDM group. 62 participants remained on T2 (34 non-GDM and 28 GDM) and 46 returned to complete the T3 on the postpartum (26 non-GDM and 20 GDM). The reasons for dropout were

not related with DMG complications. Due to technical fail related to inappropriate signal noise ratio and/or not detectable EMGs burst made the inclusion of from 13 participants unfeasible. So, the EMG analysis were proceeded with participants who had all time-points completed and with good EMG signal quality (19 non-GDM and 14 GDM).

No significant group differences were found in participant characteristics during gestation or postpartum (Table 1). The glucose tolerance test values, as expected, showed marked group differences on fasting, 1 and 2 hours after OGTT.

Table 2 shows the average (and standard deviation) of the parameters extracted from the RMS EMG divided across groups and time points, as well as the results from the GLM. The variables evaluated during the 60-sec pre-baseline did not differ between or intragroup. During the 1-sec Flick contractions, there was interaction between time-points and groups on the average EMG amplitude [$F=(1.619,43.759)=4.568$; $p=.022$]. Post-hoc analyses revealed that the GDM group decreased the activation levels from T1 to T3 ($p=.040$). Additionally, during T1, the GDM group showed greater slope after onset (increase rate of EMG activity) compared to non-GDM [main effect of group, $F=(1,27)=4.504$; $p=.043$, post-hoc $p=.043$]. Moreover, main effects of time point revealed that, independent of group, women took more time to reach peak EMG during T1 compared to T3 [$F(2,54)=8.354$; $p<.001$, post-hoc $p<.001$]; the task duration was lower at T1 compared to T2 and T3 in both groups decreased from T1 to T2 [$F(2,54)=9.536$; $p<.001$, post-hoc T1 to T2 $p=.008$ and T1 to T3 $p<.001$]; and the rate of EMG increase after onset was lower during T1 compared to T2 [$F(2,54)=3.633$; $p<.033$, post-hoc $p=.041$]. Finally, interactions between Group and Time-Points revealed that the standard deviation of the EMG amplitude on non-GDM group increased from T1 to T2 [$F(2,54)=3.345$; $p<.043$, post-hoc $p<.031$] and the EMG slope before offset (decrease rate of EMG activity) was less

intense at T1 compared to T2 and T3 in non-GDM group [$F(2,54) = 4.812$; $p < .012$ post-hoc T1 to T2 $p = .005$ and T1 to T3 $p < .015$].

During the 10-sec hold task, there was interaction between Group and Time-Point on the time from peak to EMG offset [$F(2,62) = 5.068$; $p = .009$], indicating that the GDM group took less time to return to baseline after peak in T3 compared to T2 ($p = .023$). Main effects of Time-Point revealed that, independently of group, task duration was larger at T3 than both T1 and T2, [$F(1.580,46.735) = 3.895$; $p = .038$, post-hoc T1 to T3 $p = .023$ and T1 to T3 $p < .023$], and that the EMG slope before peak was greater at T3 compared to T1 [$F(2,62) = 3.335$; $p = .042$, post-hoc $p = .035$].

During the last PFM contraction task of the protocol, the 60-sec hold, there were no significant main effects of interactions with Group or Time-Point. During the 60-sec post-baseline rest period there was a significant effect of Group on the standard deviation of EMG amplitude [$F(1,10) = 5.319$; $p = .044$]. A main effect of Time-Point revealed that the peak amplitude was greater at T3 than T2 [$F(2,20) = 4.152$; $p = .031$, $p = .023$] independent of group.

Figure 4 shows the results of the wf-ANOVA analysis, with the average EMG patterns of each group and the significant Group contrasts during the Flick and Hold PFM contraction tasks at each time point. The significant contrasts indicate that, during the Flick contractions, the GDM group generally had smaller PFM EMG amplitude than non-GDM after ~1 second of contraction, suggesting shorter contractions. During the 10-sec Hold contractions, the non-GDM group activated the PFM at higher contraction intensities than the GDM group at both time points T2 and T3, although the timing of the contrasts differed between time points: At T2, the GDM group had lower initial peak amplitude during Hold, but similar amplitudes after ~2 seconds of contraction; at T3, the initial peaks from both groups had similar (normalized) amplitudes, after which the

levels of PFM activation decreased faster for the GDM group, remaining lower than the non-GDM group until near the end of the contraction.

DISCUSSION

This study assessed PFM EMG patterns from pregnancy to long-term post-partum (18-24 months) in women with and without GDM. Using a well-established protocol for pelvic floor assessment, we reproduced a similar sequence of PFM contractions requested in clinical consultations, commonly used to identify the motor strategy during brief and sustained PFM tasks. No significant group differences were found during the Baseline-pre and Endurance tasks, and only minor differences in during Baseline-post. During 1-sec Flick contractions, the EMG activation of all participants decreased on post-partum compared to the T1. Wavelet analysis showed that, although the GDM group achieve peak PFM EMG amplitudes similar to the non-GDM, they took longer to return to baseline levels. During 10-sec Hold contractions, the GDM group sustained lower levels of PFM activation than the non-GDM group at both T2 and T3.

Our study was based on evidence of changes in physiological and anatomical factors in the female PFM demonstrated by morphological studies in pregnant rats and humans [6]. Reduced ratio of fast to slow fibers and co-localization of fast and slow fibers has been observed in striated urethral muscle of diabetic pregnant rats compared with non-diabetics and non-pregnant [8,9]. More recently, similar findings were found in rectus abdominis muscles of pregnant women with GDM, who showed decreased cross-sectional area of both slow and fast muscle fibers, in addition to decreased number of fast fibers and increased number of slow fibers [6,31].

It is reasonable that morphologic and metabolic changes, in PFMs are likely to

contribute to UI [27,32,33]. Indeed, higher UI prevalence and severity have been associated with hyperglycemic disturbances not only during pregnancy [2,13,34], but also on pre-diabetic and clinical diabetes [35,36]. Three-dimension ultrasonography during rest showed that there is a decrement of the thickness of the levator ani muscle [18] during pregnancy which is consistent with previous morphological findings of a myopathic process on musculoskeletal tissue of GDM pregnant [6,16]. However, conclusive evidence to support this relationship have not yet been assessed due to the lack of studies assessing pelvic floor function by direct measures [37-39], particularly, on pregnancy until medium and long-term postpartum [14].

Autonomic neuropathic dysfunctions in the bladder, are associated with hyperglycemia. [40,41] Besides it, findings on external anal sphincter using electrophysiological methods showed that diabetic polyneuropathy caused by clinical diabetes mellitus (DM) affects the pudendal nerve by an increase in motor unit action potentials MUP mean duration, mean amplitude, mean phases, satellite rate, and in percentage of long duration MUPs and polyphasic potentials. [37,42]

Previous studies in pregnant women populations with GDM showed differences in PFM activation between GDM and non-GDM group at 24-30 and 36-38 weeks of gestation, particularly during rest and Hold contractions. [43] Although different EMG processing and normalization methods hinder direct comparison with the present study, our results complement previous findings by demonstrating that, as they approach the end of pregnancy, women with GDM show reduced ability to perform brief PFM contractions and to sustain long PFM contractions at the same level as their non-GDM counterparts.

Motor control studies have shown that the reduction in EMG amplitude secondary to muscle weakness could not fully explain the UI and they showed that the

pre activation of the PFM could have a great contribution on the continence mechanisms [27]. Other mechanisms should be addressed to explain better the motor strategy used by the GDM group along [44]. Thus, we decided to include besides the amplitude and peak quantification, analyses of contraction oscillation, temporal analyses related to onset, peak and offset and rate of recruitment during the begin and end of the contraction and the peak.

The findings from the 1-sec Flick contractions showed that the GDM group decreased their levels of PFM activation from T1 to T3, whereas the non-GDM group maintained similar levels of activation along time-points. We believe that the significant increase in amplitude standard deviation which clinically implies about amplitude variability during the same task non-GDM group could contribute to allow non-GDM group to maintain the level of activation. The implications of low or high variability still controversially in literature, but there is evidence that a higher variability may represent an adaptive mechanism to maintain the task performance. [45]

The impairments in PFM function observed in women with GDM have been attributed to physiological and anatomical changes to the musculoskeletal system, namely reduced cross-sectional area and reduced number of fast fiber type, in addition to impairments in ionic channels, as well as fat infiltration and proliferation of connective tissue in the PFM. [46] Nevertheless, we cannot exclude other confounding factors, including the volitional component (i.e. choose not to activate) and technical aspects inherent to EMG acquisition which could affect both groups. [47]

Both groups achieved peak quicker on T3 compared to T1 on flicks task. As this characteristic was the same on the groups and no differences were found between T1 and T2, the pregnancy itself may have implication on it. A quicker response of the pelvic floor is important mainly when intra-abdominal pressure is higher to promote

continence. Other studies should consider explore the latency of PFM onset to peak in comparing it with other structures involved on the modulation of intra-abdominal pressure. [48]

Although our protocol had a standard task duration, we observed that both groups decreased duration in the flicks contractions on T2 and T3 compared to T1 to T2 in both groups and achieved peak on T3 quicker than T1. We believe that this is probably a result of a learning effect: as participant got familiar with the tasks, both groups were able to reach peak amplitude more quickly than before, increasing the rate of EMG activity (slope after onset). Additionally, we expected on T1 that the groups may have the same recruitment characteristics, but the GDM group activates PFM around 60% more per second compared to non-GDM.

Concerning the deactivation on the end of the task (slope before offset) the non-GDM decrease rate of EMG activity intensely from T1 to T2 and T1 to T3. The GDM group used the same strategy to relax pelvic floor muscle along time-points. When comparing the full RMS EMG waveforms between groups, we found that, at all three time points, the GDM group took longer T1 to return from peak amplitude to baseline levels, as revealed by smaller EMG amplitude compared to non-GDM after peak EMG. This observation wave characteristic is corroborated by a recent study applying the same protocol to continent and incontinent women that found that the incontinent group took more time to relax after Flick contractions. [49]

On the 10-sec Hold tasks, whereas traditional amplitude measurements were not able to identify major differences between groups or time-points, the analyses in the wavelet domain found reduced EMG amplitude in the GDM group compared to non-GDM at time points T2 and T3, which means that when events along the task are taking into consideration different motor control patterns are found between groups

along the task duration in each time-point. During T1 the motor pattern was mostly similar between groups. It could be explained by the fact that this is the screening period to GDM, so it is the point that glycemia starts to get higher and maybe there is no drastic influence on muscle yet. Also, the discrete but significant differences on T2 could be explained by the fact that the cross-section area of slow fibers are decreased on GDM group [6,16]. Although the capacity of the morphological recovery on post-partum is unknown, our findings suggest that PFM control continues to be impaired post-partum in the GDM group. Additionally, the GDM group take more time to return from peak to offset from T2 and T3. Although the task duration statistically increased from T1 to T2 and T3, it was less than 1 second and may not be relevant clinically.

During post-baseline resting there were differences related to the peak from T2 to T3 in both groups and the group GDM on T2 oscillated less during the final resting. Although significant these two characteristics without an additional change on average amplitude, clinically, does not provide a valuable reflection about the task in general.

EMG is a valuable but challenging method to evaluate PFM function, hence interpretation of the present results should be made with caution to avoid mistaken conclusions. [50] Although the findings from the present study may be partially explained by morpho-pathological processes involved in GDM, there are several concerns to consider: First, the test-retest reliability of PFM EMG amplitude along time-points shows heterogeneity among studies in the literature [51,52]. Previous studies have suggested that this heterogeneity arises mainly due to electrode movement, which contaminate the signal with motion artifact and change the population of motor units recorded, making it difficult to evaluate the same motor units across different time points [52]. In addition, some studies have assessed raw EMG amplitudes which turns the external validity and results comparisons unfeasible.

This novel cohort study evaluated PFM activity in pregnant women with and without GDM at three distinct time points during and after delivery. We argue that the strengths of the study were that (i) we only included continent pregnant women; (ii) we excluded from analysis participants who did not complete the cohort entirely; (iii) only high-quality EMG was included on the analysis, confirmed by high SNR and absence of signal artifacts; (iv) we assessed many different parameters, including traditional amplitude and timing parameters and the assessment of the full RMS EMG waveform, in an attempt to perform a comprehensive assessment of the motor strategies during PFM contractions; and (v) EMG amplitude of each subject was normalized by the maximal voluntary activation to allow comparisons between groups and time-points.

Nevertheless, we also acknowledge some limitations in our study, which should be taken into consideration in future studies. First, we had a relatively high dropout rate, which is a common problem in cohort studies and randomized controlled trials assessing pregnant women [53], probably underpinned by the major changes in women's life that accompany pregnancy and delivery. Second, the assessment of vaginal pressure or force, concomitant with EMG would have been valuable to assess changes in force-generating capacity, and allow more reliable estimates of maximal voluntary contractions. [54] Third, employment of intravaginal high-density surface electromyography to allow other such as the number of motor units action potentials by the decomposed signal. [55] Finally, the use of vaginal probes with suction, designed to minimize movement artefacts and ensure optimal electrode alignment with the muscle fiber direction, are likely to enhance the technical quality of the EMG recordings.

CONCLUSION

Our findings show impaired PFM motor control strategies on pregnant women with GDM compared to non-GDM during execution of 1-sec Flick and 10-sec Hold contractions during pregnancy and 18-24 months postpartum. Taken together, these results suggest that differences on motor behaviour of GDM women arises in late pregnancy and exacerbate on postpartum.

RESEARCH IMPLICATIONS

To the best of our knowledge this is the first study to provide information about PFM neuromuscular strategy of women GDM in a long-term follow-up. Further studies should be necessary to investigate the influence of this strategy on PFM strength and pelvic floor dysfunctions. This additional information should be important to delineate preventive and therapeutic strategies on this population.

Acknowledgments

The authors would like to give our great appreciation to the pregnant women who participated in this study.

Data Availability

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

Supporting information

S1 Fig. Glazer Protocol plots showing full signal and intravaginal probe image (A), protocol segment tasks (B) and contraction time window with all performed tasks (C).

S2 Fig. Example EMG recording of the Flick task from a representative subject, illustrating the EMG variables used in the analyses.

S3 Fig. GDM women's screening, diagnosis, enrollment, follow-up analysis and reasons for signal exclusion from analysis.

S4 Fig. Group average and SD of the RMS EMG during the 1-sec Flick and 10-sec Hold PFM contraction tasks from Glazer protocol. Before averaging, the EMG patterns from each subject was expressed as percentage of the peak recorded during the 1-sec Flick contractions. Positive contrasts indicate that GDM < non-GDM.

REFERENCES

1. Barbosa AMP, Adriano Dias GM, Calderon IMP, Rudge MVC, Witkin S. Urinary incontinence and vaginal squeeze pressure two years post-cesarean delivery in primiparous women with previous gestational diabetes mellitus. *Clinics*. 2011;66: 1341–1345. doi:10.1590/S1807-59322011000800006
2. Piculo F, Marini G, Vesentini G, Morceli G, Damasceno DC, Sobrevia L, et al. Pregnancy-specific urinary incontinence in women with gestational hyperglycaemia worsens the occurrence and severity of urinary incontinence and quality of life over the first year post partum. *Eur J Obstet Gynecol Reprod Biol*. 2020;252: 336–343. doi:10.1016/j.ejogrb.2020.06.036
3. Kim C, McEwen LN, Sarma A V., Piette JD, Herman WH. Stress Urinary Incontinence in Women with a History of Gestational Diabetes Mellitus. *J Women's Heal*. 2008;17: 783–792. doi:10.1089/jwh.2007.0616
4. Sangsawang B. Risk factors for the development of stress urinary incontinence during pregnancy in primigravidae: A review of the literature. *Eur J Obstet Gynecol Reprod Biol*. 2014;178: 27–34. doi:10.1016/j.ejogrb.2014.04.010
5. Chuang C-M, Lin I-F, Horng H-C, Hsiao Y-H, Shyu I-L, Chou P. The impact of gestational diabetes mellitus on postpartum urinary incontinence: a longitudinal cohort study on singleton pregnancies. *BJOG An Int J Obstet Gynaecol*. 2012;119: 1334–1343. doi:10.1111/j.1471-0528.2012.03468.x
6. Vesentini G, Barbosa AMP, Damasceno DC, Marini G, Piculo F, Matheus SMM, et al. Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women. Li L, editor. *PLoS One*. 2020;15: e0231096. doi:10.1371/journal.pone.0231096
7. Marini G, Piculo F, Vesentini G, Damasceno DC, Delella FK, Calderon IMP, et al. The influence of hyperglycemia on the remodeling of urethral connective tissue in pregnant rats. *Eur J Obstet Gynecol Reprod Biol*. 2018;221: 81–88. doi:10.1016/j.ejogrb.2017.12.032
8. Marini G, Piculo F, Vesentini G, Barbosa AMP, Damasceno DC, Matheus SMM, et al. Effects of short-term severe and long-term mild STZ-induced diabetes in urethral tissue of female rats. *Neurourol Urodyn*. 2016; n/a-n/a. doi:10.1002/nau.22974
9. Marini G, Pascon Barbosa AM, Damasceno DC, Michelin Matheus SM, De Aquino Castro R, Castello Girão MJB, et al. Morphological changes in the fast vs slow fiber profiles of the urethras of diabetic pregnant rats. *Urogynaecologia*. 2011;25. doi:http://dx.doi.org/10.4081/uj.2011.e9
10. Piculo F, Marini G, Barbosa AMP, Damasceno DC, Matheus SMM, Felisbino SL, et al. Urethral striated muscle and extracellular matrix morphological characteristics among mildly diabetic pregnant rats: translational approach. *Int Urogynecol J*. 2014;25: 403–15. doi:10.1007/s00192-013-2218-4
11. Zwolsman S, Kastelein A, Daams J, Roovers J-P, Opmeer BC. Heterogeneity of cost estimates in health economic evaluation research. A systematic review of stress urinary incontinence studies. *Int Urogynecol J*. 2019;30: 1045–1059. doi:10.1007/s00192-018-3814-0
12. Barbosa AMP. Prevalência e fator de risco para incontinência urinária e disfunção do assoalho pélvico dois anos após Diabete Melito gestacional. *Rev Bras Ginecol e Obs*. 2007;29. doi:10.1590/S0100-72032007000400010
13. Kim C, McEwen LN, Sarma A V., Piette JD, Herman WH. Stress Urinary

Incontinence in Women with a History of Gestational Diabetes Mellitus. *J Women's Heal.* 2008;17: 783–792. doi:10.1089/jwh.2007.0616

14. Van Geelen H, Ostergard D, Sand P. A review of the impact of pregnancy and childbirth on pelvic floor function as assessed by objective measurement techniques. *International Urogynecology Journal.* Springer London; 2018. pp. 327–338. doi:10.1007/s00192-017-3540-z

15. Vesentini G, Marini G, Piculo F, Damasceno DC, Matheus SMM, Felisbino SL, et al. Morphological changes in rat rectus abdominis muscle induced by diabetes and pregnancy. *Brazilian J Med Biol Res.* 2018;51. doi:10.1590/1414-431x20177035

16. Vesentini G, Barbosa AMP, Floriano JF, Felisbino SL, Costa SMB, Piculo F, et al. Deleterious effects of gestational diabetes mellitus on the characteristics of the rectus abdominis muscle associated with pregnancy-specific urinary incontinence. *Diabetes Res Clin Pract.* 2020;166: 108315. doi:10.1016/j.diabres.2020.108315

17. Prudencio CB, Rudge MVC, Pinheiro FA, Sartorão Filho CI, Nunes SK, Pedroni CR, et al. Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. Palatnik A, editor. *PLoS One.* 2019;14: e0223261. doi:10.1371/journal.pone.0223261

18. Sartorão Filho CI, Pinheiro FA, Prudencio CB, Nunes SK, Takano L, Enriquez EMA, et al. Impact of gestational diabetes on pelvic floor: A prospective cohort study with three-dimensional ultrasound during two-time points in pregnancy. *Neurourol Urodyn.* 2020; nau.24491. doi:10.1002/nau.24491

19. De Souza Caroci A, Riesco MLG, Da Silva Sousa W, Cotrim AC, Sena EM, Rocha NL, et al. Analysis of pelvic floor musculature function during pregnancy and postpartum: a cohort study. *J Clin Nurs.* 2010;19: 2424–2433. doi:10.1111/j.1365-2702.2010.03289.x

20. Laycock J, Jerwood D. Pelvic floor muscle assessment: the PERFECT scheme. *Physiotherapy.* 2001;87: 631–642.

21. American Diabetes Association. Standards of Medical Care in Diabetes—2015. *Diabetes Care.* 2015;38: S1–S2. doi:10.2337/dc15-S001

22. Henderson JW, Wang S, Egger MJ, Masters M, Nygaard I. Can Women Correctly Contract Their Pelvic Floor Muscles Without Formal Instruction? *Female Pelvic Med Reconstr Surg.* 2013;19: 8–12. doi:10.1097/SPV.0b013e31827ab9d0

23. Friedman S, Blomquist JL, Nugent JM, McDermott KC, Muñoz A, Handa VL. Pelvic muscle strength after childbirth. *Obstet Gynecol.* 2012;120: 1021–8. doi:http://10.1097/AOG.0b013e318265de39

24. Glazer HI. Glazer Intrapelvic SEM:G Assessment for the Diagnosis & Treatment of Post Radical Prostatectomy Urinary Incontinence. SURFACE ELECTROMYOGRAPHY (SEMG). Biofeedback Federation CIC; 2000.

25. Hermens H, B F, Merletti R, Stegeman D, Blok J, Rau G, et al. European recommendations for surface electromyography. *Roessingh Res Dev.* 1999;8: 13–54.

26. Hacad CR, Glazer HI, Zambon JPC, Burti JS, Almeida FG. Is There Any Change in Pelvic Floor Electromyography During the First 6 Months After Radical Retropubic Prostatectomy? *Appl Psychophysiol Biofeedback.* 2015;40: 9–15. doi:10.1007/s10484-015-9271-3

27. Madill SJ, Harvey MA, McLean L. Women with stress urinary incontinence demonstrate motor control differences during coughing. *J Electromyogr Kinesiol.* 2010;20: 804–812. doi:10.1016/j.jelekin.2009.10.006

28. Hodges PW, Bui BH. A comparison of computer-based methods for the determination of onset of muscle contraction using electromyography.

- Electroencephalogr Clin Neurophysiol. 1996;101: 511–519.
29. SALOMONI SE, MARINOVIC W, CARROLL TJ, HODGES PW. Motor Strategies Learned during Pain Are Sustained upon Pain-free Reexposure to Task. *Med Sci Sport Exerc.* 2019;51: 2334–2343. doi:10.1249/MSS.0000000000002059
 30. McKay JL, Welch TDJ, Vidakovic B, Ting LH. Statistically significant contrasts between EMG waveforms revealed using wavelet-based functional ANOVA. *J Neurophysiol.* 2013;109: 591–602. doi:10.1152/jn.00447.2012
 31. G V, R ED, LAR R, F P, G M, GAR F, et al. Pelvic floor and abdominal muscle cocontraction in women with and without pelvic floor dysfunction: a systematic review and meta-analysis. *Clinics.* 2019;74. Available: <https://pubmed.ncbi.nlm.nih.gov/31778432/>
 32. Koenig I, Eichelberger P, Luginbuehl H, Kuhn A, Lehmann C, Taeymans J, et al. Activation patterns of pelvic floor muscles in women with incontinence while running: a randomized controlled trial. *Int Urogynecol J.* 2021;32: 335–343. doi:10.1007/s00192-020-04334-0
 33. Yang JM, Yang SH, Huang WC, Tzeng CR. Factors affecting reflex pelvic floor muscle contraction patterns in women with pelvic floor disorders. *Ultrasound Obstet Gynecol.* 2013;42: 224–229. doi:10.1002/uog.12457
 34. Barbosa AMP, Marini G, Piculo F, Rudge CVC, Calderon IMP, Rudge MVC. Prevalence of urinary incontinence and pelvic floor muscle dysfunction in primiparae two years after cesarean section: cross-sectional study. *Sao Paulo Med J.* 2013;131: 95–9.
 35. Brown JS, Vittinghoff E, Lin F, Nyberg LM, Kusek JW, Kanaya AM. Prevalence and Risk Factors for Urinary Incontinence in Women With Type 2 Diabetes and Impaired Fasting Glucose: Findings from the National Health and Nutrition Examination Survey (NHANES) 2001-2002. *Diabetes Care.* 2006;29: 1307–1312. doi:10.2337/dc05-2463
 36. Fayyad AM, Hill SR, Jones G. Prevalence and risk factors for bothersome lower urinary tract symptoms in women with diabetes mellitus from hospital-based diabetes clinic. *Int Urogynecol J Pelvic Floor Dysfunct.* 2009;20: 1339–1344. doi:10.1007/s00192-009-0949-z
 37. Cao N, Gu B, Gotoh D, Yoshimura N. Time-Dependent Changes of Urethral Function in Diabetes Mellitus: A Review. *Int Neurourol J.* 2019;23: 91–99. doi:10.5213/inj.1938050.025
 38. Christ GJ, Bushman W, Fraser MO. Impact of Diabetes and Obesity on the Prostate and Urethra: Implications to Improved Bladder Dysfunction Understanding and Treatment. *J Urol.* 2009;182. doi:10.1016/j.juro.2009.07.085
 39. Krhut J, Zachoval R, Rosier PFWM, Shelly B, Zvara P. ICS Educational Module: Electromyography in the assessment and therapy of lower urinary tract dysfunction in adults. *Neurourol Urodyn.* 2018;37: 27–32. doi:10.1002/nau.23278
 40. Agochukwu-Mmonu N, Pop-Busui R, Wessells H, Sarma A V. Autonomic neuropathy and urologic complications in diabetes. *Auton Neurosci.* 2020;229: 102736. doi:10.1016/j.autneu.2020.102736
 41. Zhang YX, Xu HN, Xia ZJ, Wu B. Analysis of clinical interventional strategy for women with urinary incontinence complicated with diabetes mellitus. *Int Urogynecol J.* 2012;23: 1527–1532. doi:10.1007/s00192-011-1572-3
 42. Jian F, Pan H, Zhang Z, Lin J, Chen N, Zhang L, et al. Sphincter electromyography in diabetes mellitus and multiple system atrophy. *Neurourol Urodyn.* 2015;34: 669–674. doi:10.1002/nau.22639

43. Prudencio CB, Rudge MVC, Pinheiro FA, Sartorão Filho CI, Nunes SK, Pedroni CR, et al. Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. Palatnik A, editor. PLoS One. 2019;14: e0223261. doi:10.1371/journal.pone.0223261
44. Luginbuehl H, Baeyens J-P, Taeymans J, Maeder I-M, Kuhn A, Radlinger L. Pelvic floor muscle activation and strength components influencing female urinary continence and stress incontinence: A systematic review. *Neurourol Urodyn*. 2015;34: 498–506. doi:10.1002/nau.22612
45. Srinivasan D, Mathiassen SE. Motor variability in occupational health and performance. *Clin Biomech*. 2012;27: 979–993. doi:10.1016/j.clinbiomech.2012.08.007
46. Allen MD, Doherty TJ, Rice CL, Kimpinski K. Physiology in Medicine: neuromuscular consequences of diabetic neuropathy. *J Appl Physiol*. 2016;121: 1–6. doi:10.1152/jappphysiol.00733.2015
47. Besomi M, Hodges PW, Clancy EA, Van Dieën J, Hug F, Lowery M, et al. Consensus for experimental design in electromyography (CEDE) project: Amplitude normalization matrix. *J Electromyogr Kinesiol*. 2020;53: 102438. doi:10.1016/j.jelekin.2020.102438
48. Moser H, Leitner M, Baeyens J-P, Radlinger L. Pelvic floor muscle activity during impact activities in continent and incontinent women: a systematic review. *Int Urogynecol J*. 2018;29: 179–196. doi:10.1007/s00192-017-3441-1
49. Leitner M, Moser H, Eichelberger P, Kuhn A, Radlinger L. Pelvic floor muscle activity during fast voluntary contractions in continent and incontinent women. *Neurourol Urodyn*. 2019;38: 625–631. doi:10.1002/nau.23911
50. Gray M. Traces: Making Sense of Urodynamics Testing - Part 3: Electromyography of the Pelvic Floor Muscles (Abstract Only): Special Series on Urodynamics. *Urol Nurs*. 2011;31: 31. doi:10.7257/1053-816x.2012.31.1.31
51. Grape HH, Dederling Å, Jonasson AF. Retest reliability of surface electromyography on the pelvic floor muscles. *Neurourol Urodyn*. 2009;28: 395–399. doi:10.1002/nau.20648
52. Auchincloss CC, McLean L. The reliability of surface EMG recorded from the pelvic floor muscles. *J Neurosci Methods*. 2009;182: 85–96. doi:10.1016/j.jneumeth.2009.05.027
53. De Souza Caroci A, Riesco MLG, Da Silva Sousa W, Cotrim AC, Sena EM, Rocha NL, et al. Analysis of pelvic floor musculature function during pregnancy and postpartum: a cohort study. *J Clin Nurs*. 2010;19: 2424–2433. doi:10.1111/j.1365-2702.2010.03289.x
54. Madill SJ, Harvey M-A, McLean L. Women with stress urinary incontinence demonstrate motor control differences during coughing. *J Electromyogr Kinesiol*. 2010;20: 804–812. doi:10.1016/j.jelekin.2009.10.006
55. Merletti R, Holobar A, Farina D. Analysis of motor units with high-density surface electromyography. *J Electromyogr Kinesiol*. 2008;18: 879–890. doi:10.1016/j.jelekin.2008.09.002

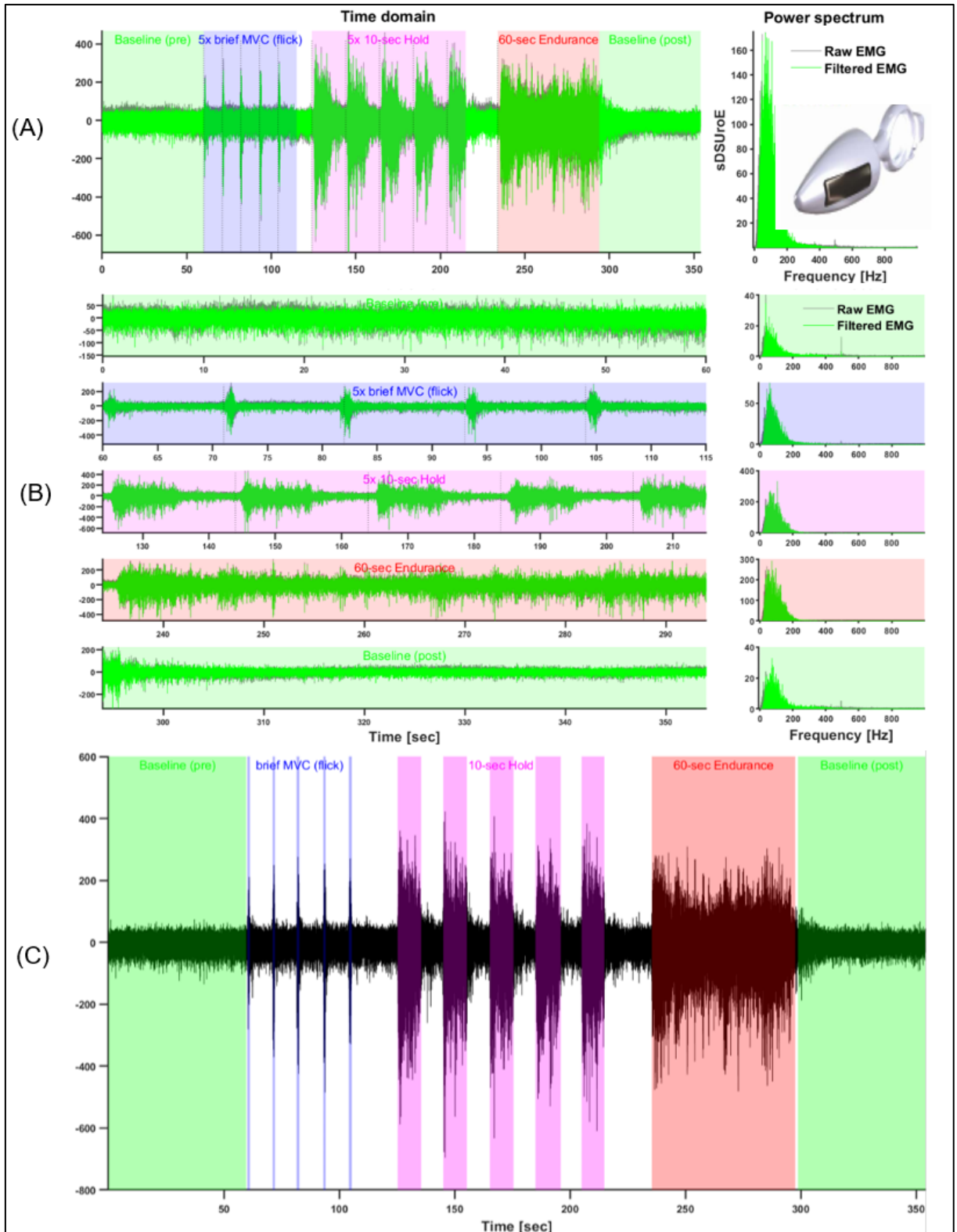


Figure 1. Glazer Protocol plots showing full signal and intravaginal probe image (A), protocol segment tasks (B) and contraction time window with all performed tasks (C).

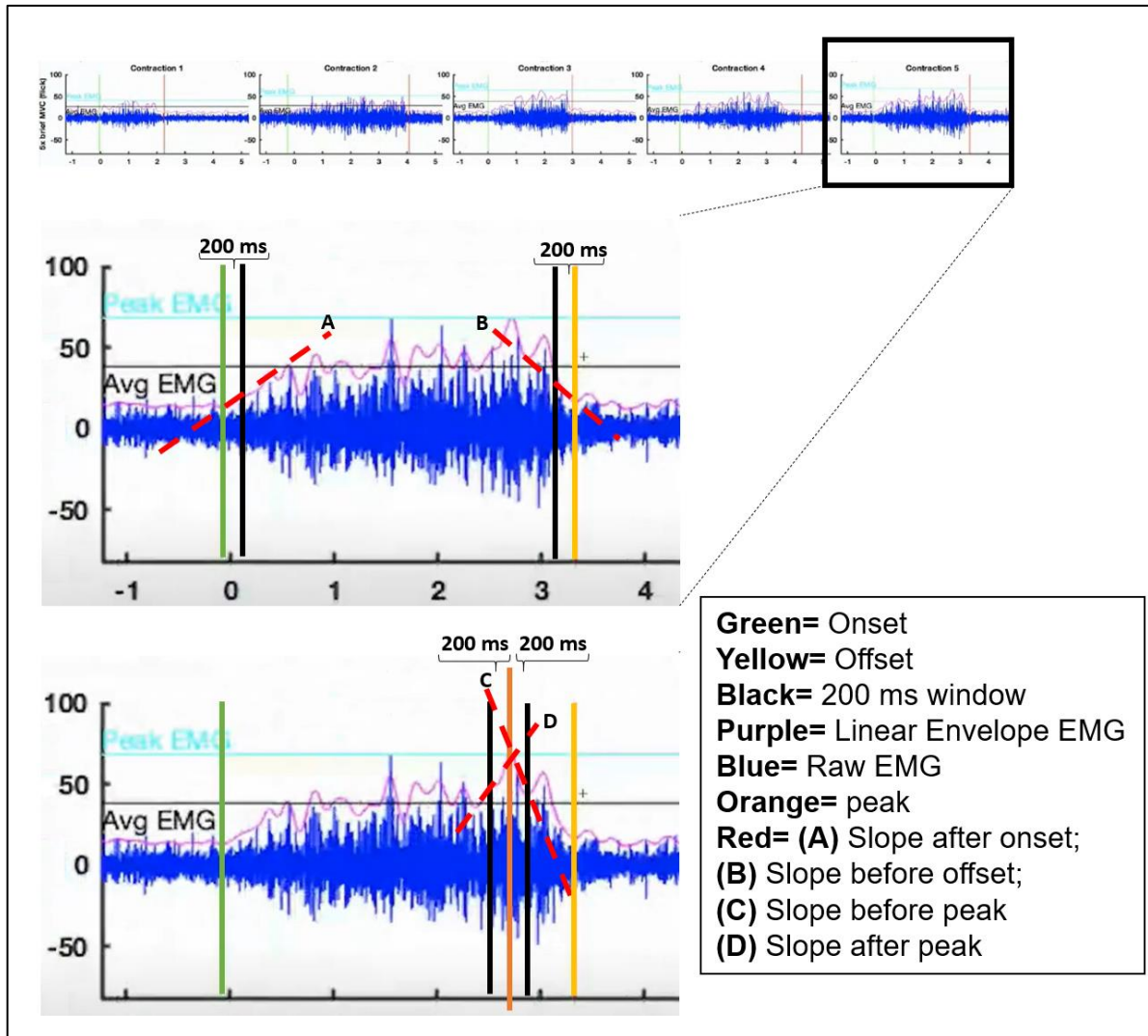


Figure 2. Example EMG recording of the Flick task from a representative subject, illustrating the EMG variables used in the analyses.

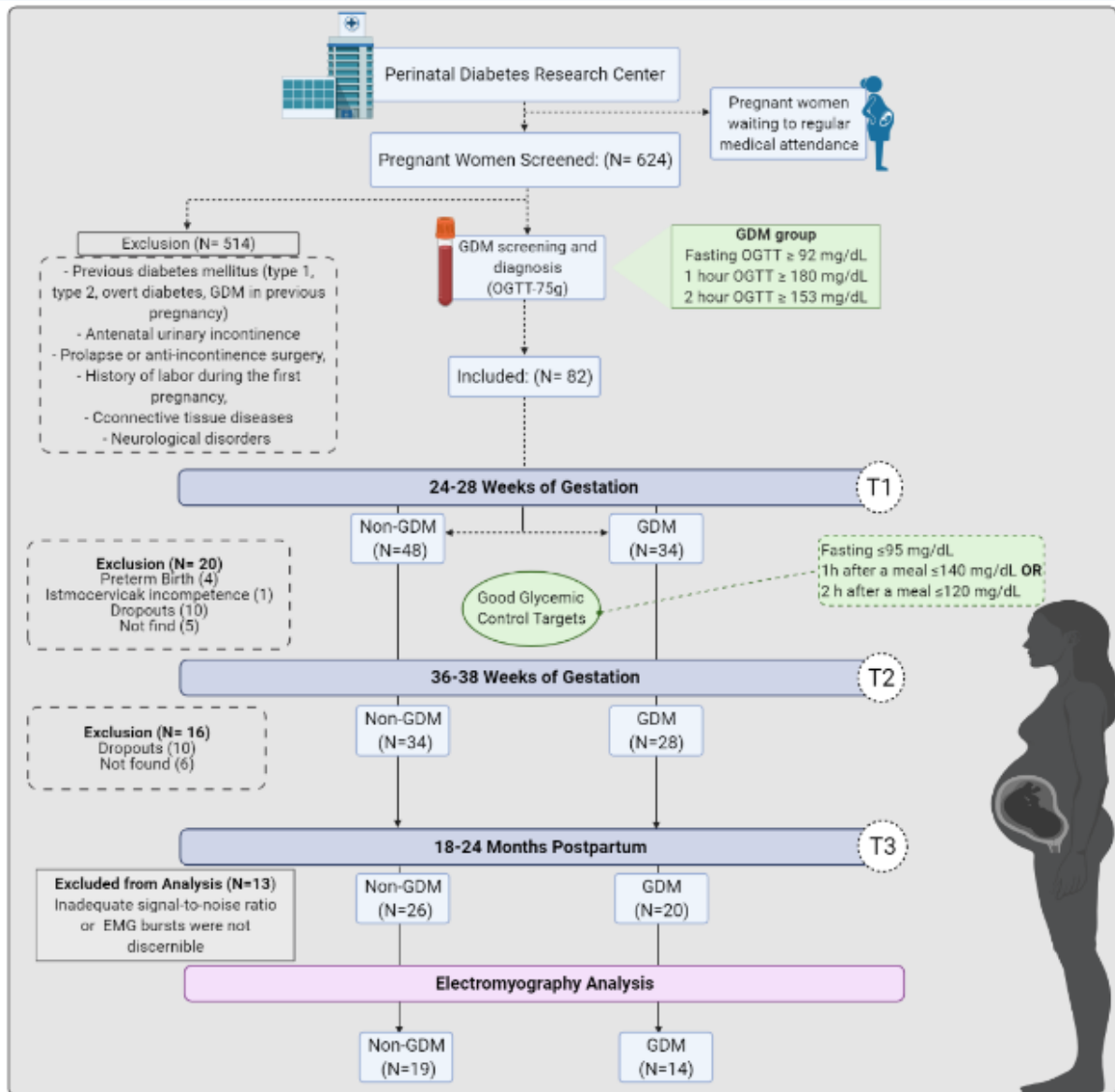


Figure 3. GDM women's screening, diagnosis, enrollment, follow-up analysis and reasons for signal exclusion from analysis.

Table 1. Average participant characteristics for non-GDM and GDM groups along time-points.

Variable		non-GDM (n=19)	GDM (n=14)	p*
Ethnicity	Caucasian	13 (68.4%)	7 (50%)	.472
	Other	6 (31.6%)	7 (50%)	
Smoking in pregnancy		0 (0.0)	0 (0.0)	1.000
Smoking postpartum		0 (0.0)	0 (0.0)	1.000
Education level–min. High School		7 (36.8%)	4 (28.6%)	.453
Diabetes postpartum		0 (0.0)	0 (0.0)	1.000
Age (years) ¹		26 (18-39)	29 (18-40)	.529
BMI (kg/T2) pre-pregnancy		23.6 (19.1-30.7)	25.2 (18.5-34.7)	.900
BMI (kg/T2) at 24-30 weeks		26.4 (19.1-32.9)	25.9 (21.6-37.4)	.843
BMI (kg/T2) at 36-38 weeks		28.4 (21.2-34.0)	27.7 (22.8-38.7)	.928
BMI (kg/T2) post-partum		24.6 (17.1-35.2)	24.2 (18.3-36.6)	.957
Weeks of Gestational ¹		26.0 (24.2-29.0)	27.0 (24.0-29.0)	.506
Weeks of Gestational ²		36.0 (35.3-38.0)	36.0 (35.0-38.0)	.843
Postpartum time		24.0 (18.1-24.0)	19.5 (18.0-24.0)	.123
Delivery Mode	C-Section	14 (73,3%)	11 (78,6%)	.746
	Vaginal	5 (26,3%)	3 (21,4%)	
Newborn weight at birth (grams)		3100 (2205-4100)	3150 (2560-3935)	.577
Blood glucose (mg/dL) ¹		84 (65-90)	88 (76-98)	.077
OGTT (mg/dL) – fasting ¹		76.0 (71.7-90.0)	92.0 (76.0-124.0)	.000
OGTT – 1h (mg/dL) ¹		122.0 (76.7-163.0)	152.0 (82.0-211.0)	.012
OGTT – 2h (mg/dL) ¹		110.0 (64.6-148.0)	138.5 (72.0-179.0)	.019

Non-GDM, non-gestational diabetes mellitus group; GDM, gestational diabetes mellitus group; BMI, body mass index; OGTT, oral glucose tolerance test. ¹evaluation at 24–30 weeks of gestation; ²evaluation at 36–38 weeks of gestation. ³Evaluation at 18-24 months postpartum. Data are presented in median (minimum - maximum) or absolute frequency (n) and percentage (%). *Based on Mann–Whitney U, Chi-square and Fischer’s exact. significance p <0.05. P-values represent the results from the relevant statistical tests.

Table 2. Group mean \pm standard deviation (across subjects) of the parameters extracted from the EMG signals at each task of the Glazer protocol. Results are presented from the 2-way generalized linear model (GLM) using factors Group (non-GDM, GDM) and Time-Point (T1: 24–30 weeks of gestation, T2: 36–38 weeks of gestation, T3: 18-24 months postpartum) as factors, with repeated measures on Time-Point.

EMG Variables	non-GDM (19)			GDM (14)			General Linear Model		
	T1 time point	T2 time point	T3 time point	T1 time point	T2 time point	T3 time point	P Between Groups	P Interaction Group vs time points	P Time Points
60-sec pre-baseline (rest)									
Average (%)	8,5 \pm 8,0	7,7 \pm 5,0	8,3 \pm 6,2	6,2 \pm 3,9	5,8 \pm 3,2	8,3 \pm 3,1	.461	.760	.664
Peak (%)	21,9 \pm 19,4	16,8 \pm 10,2	18,0 \pm 11,8	17,1 \pm 11,7	13,9 \pm 6,1	24,4 \pm 8,8	.907	.411	.415
Amplitude SD (%)	2,5 \pm 1,8	2,3 \pm 1,4	2,3 \pm 1,4	2,1 \pm 1,4	1,7 \pm 0,6	3,4 \pm 1,4	.951	.258	.264
Amplitude CV (%)	32,7 \pm 6,2	30,0 \pm 8,7	30,3 \pm 6,8	33,6 \pm 6,3	32,6 \pm 10,3	42,0 \pm 14,9	.096	.199	.310
Task Duration	57,1 \pm 4,9	59,7 \pm 0,6	59,4 \pm 0,5	58,6 \pm 2,2	59,4 \pm 0,4	58,5 \pm 0,4	.854	.347	.201
SNR	1,5 \pm 1,0	1,3 \pm 0,6	1,3 \pm 0,4	1,2 \pm 0,4	1,5 \pm 0,6	1,2 \pm 0,2	.833	.466	.889
1-sec phasic (Flicks)									
Average (%)	50,0 \pm 9,0	52,2 \pm 4,7	51,9 \pm 5,9	55,2 \pm 4,7 ^a	52,7 \pm 5,3	49,1 \pm 5,2 ^a	.558	.022	.233
Peak (%)	83,6 \pm 11,1	87,9 \pm 5,6	86,8 \pm 6,4	89,2 \pm 5,0	86,9 \pm 6,1	84,0 \pm 5,7	.711	.063	.495
Amplitude SD (%)	20,5 \pm 3,2 ^b	23,0 \pm 3,0 ^b	22,2 \pm 3,3	22,6 \pm 2,4	22,1 \pm 3,0	21,7 \pm 2,9	.806	.043	.287
Amplitude CV (%)	42,1 \pm 8,3	44,7 \pm 7,0	43,6 \pm 7,0	41,6 \pm 6,3	42,8 \pm 7,6	44,7 \pm 7,3	.825	.626	.290
Time from onset to peak (sec)	0,9 \pm 0,2 ^{&}	0,8 \pm 0,2	0,8 \pm 0,2 ^{&}	0,9 \pm 0,3 [*]	0,8 \pm 0,3	0,7 \pm 0,2 [*]	.978	.146	.001
Time from peak to offset (sec)	1,0 \pm 0,3	0,7 \pm 0,2	0,8 \pm 0,3	0,9 \pm 0,3	0,8 \pm 0,3	0,9 \pm 0,2	.442	.172	.052
Task Duration	1,8 \pm 0,4 ^{&§}	1,5 \pm 0,3 ^{&}	1,5 \pm 0,5 [§]	1,8 \pm 0,4 ^{*£}	1,7 \pm 0,3 [*]	1,5 \pm 0,2 [£]	.650	.306	.000
Slope after onset (%/sec)	111,7 \pm 30,8 ^{c&}	165,7 \pm 52,3 ^{&}	152,9 \pm 55,5	174,5 \pm 62,5 ^{c*}	186,1 \pm 64,2 [*]	169,3 \pm 67,9	.043	.117	.033
Slope before offset (%/sec)	-96,3 \pm 64,6 ^{de}	-149,7 \pm 61,9 ^d	-142,8 \pm 63,4 ^e	-130,5 \pm 69,4	-124,7 \pm 53,6	-115,4 \pm 43,2	.741	.012	.110
Slope before peak (%/sec)	100,9 \pm 57,1	125,3 \pm 55,3	131,0 \pm 38,1	118,2 \pm 50,7	116,6 \pm 67,0	120,8 \pm 38,1	.971	.423	.367
Slope after peak (%/sec)	-116,8 \pm 46,9	-148,4 \pm 71,1	-114,9 \pm 42,0	-99,5 \pm 32,8	-116,1 \pm 41,1	-129,0 \pm 38,3	.254	.174	.162
SNR	21,1 \pm 15,1	24,6 \pm 13,6	28,4 \pm 36,3	22,8 \pm 16,8	30,9 \pm 22,0	14,8 \pm 10,8	.701	.157	.437

Continua na próxima página

EMG Variables	non-GDM (19)			GDM (14)			General Linear Model		
	T1	T2	T3	T1	T2	T3	P	P	P
	time point	time point	time point	time point	time point	time point	Between Groups	Interaction Group vs time points	Time Points
10-sec hold									
Average (%)	52,2±15,5	56,4±20,0	52,9±16,6	48,0±10,6	51,4±17,3	46,7±18,3	.241	.962	.437
Peak (%)	101,2±27,9	106,3±32,3	99,7±21,5	95,2±10,8	98,2±18,6	95,9±29,2	.268	.941	.729
Amplitude SD (%)	17,8±5,6	19,0±5,7	17,5±4,5	17,6±3,0	18,1±3,4	16,4±5,4	.491	.916	.383
Amplitude CV (%)	35,7±9,1	35,6±8,3	35,3±9,5	38,1±8,2	38,0±11,0	38,2±14,4	.361	.988	.996
Time from onset to peak (sec)	3,1±1,8	2,4±1,7	2,9±2,0	2,1±1,6	3,2±2,3	2,2±1,8	.548	.019	.715
Time from peak to offset (sec)	7,0±1,8	7,8±1,8	7,4±2,0	8,2±1,5	7,0±2,2 ^f	8,4±1,7 ^f	.408	.009	.313
Task Duration	10,1±0,5 ^{&}	10,2±0,4 [§]	10,4±0,3 ^{&§}	10,3±0,4 [*]	10,2±0,4 [£]	10,5±0,4 ^{*£}	.189	.516	.026
Slope after onset (%/sec)	116,9±57,4	144,7±57,0	151,1±50,3	144,1±45,2	160,7±62,4	147,2±71,1	.397	.397	.126
Slope before offset (%/sec)	-81,7±61,0	-83,6±44,9	-97,4±67,1	-60,0±29,4	-80,0±58,8	-75,1±45,1	.266	.626	.356
Slope before peak (%/sec)	121,8±47,3 ^{&}	136,9±59,8	142,2±55,0 ^{&}	125,4±53,9 [*]	124,9±53,2	171,8±76,6 [*]	.588	.310	.042
Slope after peak (%/sec)	-129,1±62,6	-123,7±48,4	-130,9±37,8	-105,1±32,5	-107,6±43,1	-123,9±75,5	.190	.781	.577
SNR	25,8±22,3	23,7±17,4	26,1±26,8	17,0±13,2	23,8±14,0	9,7±4,4	.053	.186	.414
60-sec endurance									
Average (%)	38,7±14,5	48,7±28,3	39,9±14,1	38,4±11,7	37,3±10,2	40,2±36,5	.540	.602	.790
Peak (%)	116,8±50,0	114,9±58,6	109,1±12,8	92,7±16,7	93,4±22,4	111,8±104,5	.337	.673	.915
Amplitude SD (%)	17,3±6,6	18,4±10,8	18,3±4,5	13,4±4,7	13,4±3,1	14,8±13,5	.091	.943	.894
Amplitude CV (%)	46,8±13,9	38,8±10,5	51,3±20,5	36,1±11,6	37,0±9,2	42,8±21,9	.249	.391	.030
Task duration	60,9±1,0	60,4±1,0	60,3±1,4	59,5±2,4	60,7±1,6	59,2±3,0	.174	.244	.333
SNR	14,0±12,0	17,9±12,7	13,0±10,2	10,7±6,5	16,4±7,2	7,2±4,4	.275	.698	.067
60-sec post-baseline (rest)									
Average (%)	10,0±7,7	9,0±3,3	8,4±2,4	7,0±0,6	3,5±0,9	10,4±4,5	.234	.137	.235
Peak (%)	25,0±12,3	22,6±7,8 ^{&}	24,2±12,5 ^{&}	17,5±3,7	9,1±2,1 [*]	33,2±14,3 [*]	.304	.054	.031
Amplitude SD (%)	3,4±1,5	3,2±1,0 ^f	3,3±1,7	2,4±0,4	1,2±0,1 ^f	3,6±1,4	.044	.176	.138
Amplitude CV (%)	46,8±33,6	37,2±10,1	37,9±11,1	33,8±6,2	34,8±6,2	36,7±11,2	.322	.753	.782
Task duration	56,2±3,6	56,9±1,6	57,9±0,8	55,7±0,3	57,3±1,9	55,1±1,7	.158	.269	.532
SNR	2,0±1,4	1,4±0,6	1,5±0,9	1,3±0,4	1,1±0,2	1,4±0,4	.273	.729	.622

non-GDM, non-gestational diabetes mellitus group; GDM, gestational diabetes mellitus group; SD, standard deviation; CV, coefficient of variation; %, percentage; Sec, seconds; same letters and symbols indicates differences detected by post-hoc (Bonferroni) contrasts test; p value < 0.05.

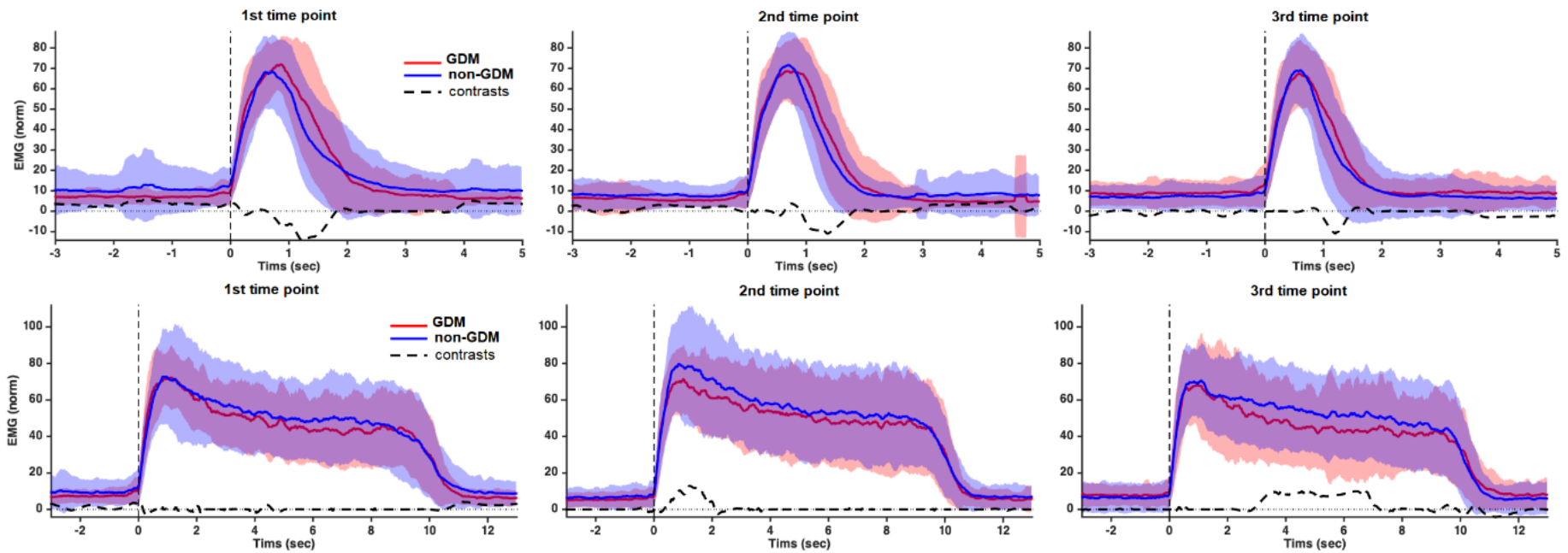


Figure 4. Group average and SD of the RMS EMG during the 1-sec Flick and 10-sec Hold PFM contraction tasks from Glazer protocol. Before averaging, the EMG patterns from each subject was expressed as percentage of the peak recorded during the 1-sec Flick contractions. Positive contrasts indicate that GDM < non-GDM. Source: Diamater Study Group.

Artigo 2

Seção redigida como Artigo Original de acordo com as normas de publicação da revista *Neurourology and Urodynamics* para a qual está em processo de submissão.

Qualis A1 MED III – FI: 2,037 <https://onlinelibrary.wiley.com/journal/15206777>.

Relaxin-2, gestational diabetes, urinary incontinence, and pelvic floor muscle function: a cross-sectional study

Caroline B. Prudencio^{1‡}, Sthefanie K. Nunes^{1‡}, Fabiane A. Pinheiro^{1*}, Carlos I. Sartorão Filho^{1*}, Guilherme T. A. Nava^{3*}, Cristiane Rodrigues Pedroni^{2*}, Marilza V. C. Rudge^{1•}, Angélica M. P. Barbosa^{1,2•}, Diamater Study Group[#]

¹São Paulo State University (Unesp), Medical School, Botucatu, Brazil, Department of Gynecology and Obstetrics, Botucatu Medical School, Universidade Estadual Paulista (UNESP), Brazil;

² São Paulo State University (Unesp), School of Philosophy and Sciences, Marília, Brazil,

³ Department of Physiotherapy and Occupational Therapy, Universidade Estadual Paulista (UNESP), Brazil.

‡ CBP and SKN are first authors on this work.

• MVCR and AMPB are last authors on this work.

* These authors contributed equally to this work.

#Diamater Study Group - Rudge MVC, Barbosa, AMP, Caldeiron IMP, Souza FP, Berghmans B, de Bie R, Thabane L, Junginger B, Graeff CFO, Magalhães CG, Costa RA, Lima SAM, Kron-Rodrigues MR, Felisbino S, Barbosa W, Campos FJ, Bossolan G, Corrente JE, Nunes HRC Abbade J, Rossignoli PS, Pedroni CR, Atallah AN, Di Bella ZIKJ, Uchoa SMM, Hungaro MA, Mareco EA, Sakalem ME, Martinho N, Hallur LSR, Reyes DRA, Alves FCB, Marcondes JPC, Prudencio CB, Pinheiro FA, Sartorão CI, Quiroz SBCV, Pascon T, Nunes SK, Catinelli BB, Reis FVDS, Oliveira RG, Barneze S, Enriquez EMA, Takano L, Carr AM, Magyori ABM, Iamundo LF, Carvalho CNF, Jacomin M, Avramidis RE, Silva AJB, Orlandi MIG, Dangió TD, Bassin HCM, Melo JVF, Takemoto MLS, Menezes MD, Caldeirão TD, Santos NJ, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Garcia GA, Nava GTA, Pascon C, Bussaneli DG, Nogueira VKC, Rudge CVC, Piculo F, Prata GM.

* Corresponding Author: Angélica Mércia Pascon Barbosa

Botucatu Medical School, Univ Estadual Paulista (UNESP), Department of Gynecology and Obstetrics, Botucatu, Brazil. Email: angelicapascon@gmail.com.

Short Title: Gestational Diabetes and Relaxin

Financial Disclosure: The authors have no potential conflicts of interest related to this study. Funding received: Supported by Sao Paulo Research Foundation protocol number 2016/01743-5. Brazilian Federal Agency for Support and Evaluation of graduate Education/SD (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES/DS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ABSTRACT

Aims: To investigate relaxin-2 concentration between DMG and non-DGM pregnant according to UI and pelvic function status. **Methods:** This is a cross-sectional study which evaluate 282 pregnant from 24 weeks of gestation. The participants were divided into two groups, non-GDM and GDM, according to American diabetes Association's diabetes mellitus gestational threshold. In addition, according to sub analysis the both groups were subdivided according to the presence of pregnancy specific urinary incontinence (PSUI): non-GDM-C (non-GDM continent), non-GDM-PSUI (non-GDM incontinent), GDM-C (DMG continent), GDM-PSUI (GDM incontinent). All participants carried out questionnaires about clinical, obstetric and urinary continence status ("International Consultation on Incontinence Questionnaire - Short Form"- ICIQ-SF and the "Incontinence Severity Index" -ISI). Followed by pelvic floor muscle evaluation by the PERFECT scheme which strength, endurance and fast contraction were evaluated. **Results:** Serum relaxin-2 concentrations were significantly lower in pregnant with PSUI both in non-GDM and GDM pregnancies, but GDM showed the lowest concentration. In addition, the stratification of the groups according to PFM strength showed that pregnancy with GDM and MOS 0-2 had significantly lower levels than non-GDM and GDM with MOS 3-5. Relaxin-2 level was intensely lower in GDM-PSUI pregnant with MOS 0-2 when compared with the other 3 groups. **Conclusion:** The relaxin-2 concentration was related to the presence of PSUI, but the combination between GDM and PSUI and lower levels of pelvic floor strength leads to lower levels of relaxin-2 when compared to the other 3 groups.

INTRODUCTION

The urinary incontinence (UI) is a silent but prevalent event during pregnancy (1). The pathophysiology is multifactorial and many gaps still unclear in the literature. The first line treatment is the pelvic floor muscle (PFM) training (2), so the PFM function is one of the main points of interest and all possible conditions that modify function or morphology should be investigated. The gestational diabetes mellitus (GDM) is capable to damage the muscular tissue causing atrophy, thinning, disorganization, and co-localization of fast and slow fibers (3) and as consequence impairing the PFM morphology (4) and function (5).

In addition, during pregnancy complicated by hyperglycaemic events, the relaxin-2 concentration is higher independent of the trimester. (6) Experimental studies in 1926 conducted by Hisaw, demonstrated the injection of serum induced a relaxation of the pelvic ligaments, later in 1930 the active substance was named relaxin, which is a member of the insulin superfamily of peptide hormones. Since then, studies were conducted in animals and from mid-80's the human relaxin was focus of many researches. Although relaxin is recognized as a pregnancy specific hormone, it is presented in lower and variables levels lifelong and has physiological targets in other organs that are important for insulin action: the pancreas, liver and muscle. (7)

During pregnancy, the physiological action on the cervix, vagina and uterus plays a role on the preparation of the birth canal. (8) The connective and muscular composition are also impacted by relaxin-2 which may be implicated in the skeletal muscle healing process by regulating inflammation, tissue remodeling, and fibrosis. (9) Cell culture studies suggest that relaxin-2 administered at physiological levels can influence soft tissue remodeling (ligament fibrocartilage, articular cartilage, tendon, and dermal). (10)

The integral theory implies that for a physiological function, the pelvic structures should be working in harmony to modulate the possible pelvic overload, since relaxin-2 promote lax ligaments process, it may invalidate the muscle forces. (11) For this reason it is widely spread on the clinical practice, that higher relaxin-2 levels is a risk factor to pelvic disorders such as UI. (12) Although this mechanistic explanation is reasonable, clinical research dosing relaxin-2 concentration and assessing UI are limited and inconclusive and its impact on PFM function is not known yet.

The possible interactions of this hormone with DMG and its impact on PFM function and PD deserve to be collated and critically analyzed for further direction in the next studies on the pathophysiology of UI during pregnancy. Therefore, the aim is to investigate relaxin-2 concentration between DMG and non-DGM pregnant according to UI and pelvic function status in a larger number of pregnant.

METHODS

Study design, participants and group composition

This was cross-sectional study approved by the Institutional Ethical Committee of Botucatu Medical School of Sao Paulo State University (Protocol Number CAAE 82225617.0.0000.5411). All participants were informed of the study procedures and signed a consent form after careful explanation of all research procedures.

All subjects met the following inclusion criteria: pregnant women in any time between 24 to 40 weeks of gestation; singleton pregnancy; 18–40 years of age; had not received PFM training or any musculoskeletal PFM treatment previously or during pregnancy. The exclusion criteria were clinical diagnose of diabetes (type I or II, or overt diabetes in previous pregnancy), history of UI, have had more than two

pregnancies, previous vaginal delivery, previous prolapse or incontinence surgery, failure to understand or follow of the command to contract PFM, history of neurological diseases, visible genital prolapse, cervical isthmus incompetence, smoking, preterm birth, abortion, as well as participants who withdrew their consent during cohort.

The recruitment was done on Perinatal Diabetes Research Center (PDRC) of Botucatu Medical School/UNESP/Brazil, between 2018 and 2020. After giving their written consent, the participants were invited to answer a questionnaire with personal, clinical and obstetric historic and anthropometric details. Followed by blood collection.

Diabetes Mellitus Gestational Screening

The diagnosis guidelines proposed by American Diabetes Association were used to identify patients with GDM (13) using the 75g oral glycemic tolerance test (75g-Oral glucose tolerance test (75g-OGTT) at 24-30 gestational weeks. The results were consulted at the digital medical records. The presence of GDM were confirmed if they presented fasting glycemic levels ≥ 92 mg/dL or 1 hour ≥ 180 mg/dL or 2 hours ≥ 153 mg/dL.

Urinary Incontinence Questionnaire

The questionnaires were applied in the same day of the relaxin-2 dosage. The first question of the Brazilian version of International Consultation on Incontinence Questionnaire - Short Form (ICIQ-SF) and International Incontinence Index (ISI) was used to determine the continence status. (14) If the participant answered never for the standard question "How often do you leak urine" they were considered continent, any other answer was framed as incontinent. The final score ranges from 0 to 21 and was

stratified according to severity and bothersomeness as slight (1–5 points), moderate (6–12 points), severe (13–18 points), and very severe (19–21 points). (15) The groups were composed according glycaemic and continence status: non-GDM-C: non-gestational diabetes mellitus continent group; non-GDM-PSUI: non-gestational diabetes mellitus incontinent group; GDM-C: gestational diabetes mellitus continent group; GDM-PSUI: gestational diabetes mellitus incontinent group.

Pelvic Floor Function Assessment

A single trained physiotherapist (CBP) with 4 years of experience in PFM evaluation conducted the assessment. After emptying their bladder, participants were asked to lie down on the stretcher in supine position with lower limbs flexed. Explanation about the anatomy and function of PFM was provided. Digital vaginal palpation was performed approximately 4 cm to 6 cm inside the vagina and positioned at the posterior part of the vagina. Moderate pressure was applied to assist in the initiation of the appropriate muscle contraction. PFM voluntary contraction was requested by giving the verbal instruction “squeeze the vaginal muscle and hold them as hard as possible, as if you were holding urine until I say to relax”. Co-contraction of the adductor, gluteus, hip movements, or expulsion movements were rectified. (5) The power (strength), endurance and fast topics from perfect scheme was extracted. (16) For the strength classification we used the modified Oxford scale (MOS) range 0 (No discernible muscle contraction) to 5 (Strong resistance can be applied to the elevation of the posterior vaginal wall) to assess PFM. To data analysis we stratified MOS according to the posterior wall action, so we considered that participants who performed 0-2 were not able to contract posterior vaginal wall and 3-5 performed a better contraction with posterior portion of the PFM. For endurance, the length of time,

up to 10 seconds, was recorded until the strength reduced by 35% or more. For fast, the number (up to 10) of one-second MVCs was assessed. (16)

Blood collection and Relaxin-2 analysis

After the questionnaires and physical exams, 1 ml of blood was collected with a vacutainer Serum Separation Transport Tube (SST) and the samples were allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. The sample was stored at -80° C until the analytical procedures. The relaxin analyses were completed by the same trained technicians. Samples were analyzed blinded for outcome in duplicate using the Human relaxin-2 DuoSet enzyme linked immune-sorbent assay (ELISA), according to the manufacturer's instructions (DY2804-05, R&D Systems, Abingdon, United Kingdom). The samples were diluted 1:5. The ELISA kit has an intra and inter-assay coefficient of variance of 4.7% and 10.2% respectively.

Sample size estimation

The sample size calculation was performed *a priori* using the G*Power. Considering that any other previous study performed the measurement proposed by this study. We considered to the calculations a one-way analysis of variance test, power of 0.80, probability of error α 0.05, effect size of 0,25. According to the study design, it was considered for the calculation 4 groups (non-GDM-C, non-GDM-PSUI, GDM-C and GDM-PSUI), the estimated sample size required was at 180 participants (45 in each group).

Statistical methods

The software IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA) was used to statistical analysis. Study population characteristics were expressed as numbers and percentages for categorical variables and median and minimum and maximum for continuous variables. The Chi-square test or Fisher's exact test was applied to compare the nominal data between groups. Comparisons between 4 groups were performed by a The Kruskal-Wallis H test, followed by Dunn-Bonferroni multiple comparisons and for 2 groups comparisons Mann-Whitney U test were performed. Differences were considered statistically significant if $p < 0.5$.

RESULTS

A total of 2432 consecutive participants were enrolled for recruitment at Perinatal Diabetes Research Center (PDRC), 2104 were not included. A total of 328 matched the inclusion criteria and completed all research steps. Among these, 46 participants were excluded of the final analysis, due to missing data, undetectable relaxin-2, OGTT-75g not available. Thus, 282 participants were successfully included in this study. 186 were non-GDM and were divided according to continence status, 81 were continent and 105 with PSUI; 96 were GDM which divided into 46 continent and 50 with PSUI. (Figure 1)

Table 1 summarizes the baseline characteristics of the 282 participants. The age of GDM-PSUI were similar to other groups, the BMI pre gestational were higher compared to non-GDM-C and the BMI gestational were higher compared to non-GDM-C and GDM-C. The OGTT (fasting, 1 hour and 2 hours), as expected, were different between non-GDM and GDM group. The groups were paired concerning gestational age, maternal weight gain, ethnicity, and previous C-section. The prevalence of PSUI

were statistically similar ($p=.485$) among non-GDM (56,5%) and GDM (52,1%) groups.

When the relaxin-2 concentrations were compared between non-GDM and GDM groups excluding the continence status the analysis showed similar levels 642,7 (58,7-2563,1) and 636,4 (76,3-3369,7) pg/mL ($p=.216$). The tests comparing the groups by the continence status showed significant $p= <.001$. The GDM-PSUI showed lower relaxin-2 levels than GDM-C ($p=.027$) and non-GDM-C ($p=.001$) groups. In addition, the non-GDM-PSUI group showed lower relaxin-2 levels compared to non-GDM-C group ($p=.023$) (table 2 and figure 2).

The characteristics of PS-UI in non-GDM and GDM groups are shown in Table 2. The leakage volume on the ICIQ-SF questionnaire showed that GDM group showed higher amount (moderate/large) of urine loss ($p= .013$) and GDM had leakage episodes more frequent (several times a day/all the time) ($p=.018$). The impact on quality of life and total scores in both groups showed comparable.

There were differences between groups concerning the strength evaluated during palpation, the groups presenting PSUI the frequency of pregnant who performed lower (MOS: 0-2) were higher than the continent groups independently of glycaemic status. Concerning endurance and fast contractions they were comparable.

The relaxin-2 serum concentrations were lower in GDM pregnant whom the MOS was lower (0-2) compared to non-GDM and with GDM pregnant who MOS were (3-5). When we stratified the groups by UI status the concentration showed significantly lower in GDM-PSUI when compared with the 3 other groups.

DISCUSSION

To the best of our knowledge, this is the first study to evaluate the interaction between GDM, PSUI, pelvic floor strength and serum relaxin-2 profile during

pregnancy. The main finding was that serum relaxin-2 concentrations were significantly lower in pregnant with PSUI both in non-GDM and GDM pregnancies, but GDM showed the lowest concentration. In addition, the stratification of the groups according to PFM strength showed that pregnancy with GDM and MOS 0-2 had significantly lower levels than non-GDM and GDM with MOS 3-5. Relaxin-2 level was intensely lower in GDM-PSUI pregnant with MOS 0-2 when compared with the other 3 groups.

DeLancey (17) and Petros (11) highlight the importance of the functional balance between connective tissues and PFM to continence process. In addition, according to hormonal theory, estrogen, progesterone, and relaxin-2 are the three most common hormones associated with UI. (18) Relaxin-2 is recognized as anti-fibrotic hormone. The relaxin's action facilitates collagen degradation, promoting changes in the concentration of collagen and remodeling the matrix metalloproteinases, gelatinases, collagenases, and alpha smooth muscle. Besides, decreasing gene expression for collagen I and III. In addition, decreases inter-collagen fibril interactions leading to increased collagen fibril sliding and ligament length. (19)

Although in clinical practice and even in academic field the concept of higher relaxin-2 levels leads to pelvic dysfunction, particularly, UI, articles correlating it with dosing relaxin-2 concentrations are few and with poor methodological quality and small sample, thus, not so there is not enough data to allow this statement (20,21). In our study we found that pregnant with PSUI in both groups GDM and non-GDM presented lower levels of relaxin-2 in comparison to continent groups, contradicting the higher levels hypothesis. No studies were found comparing non-GDM and GDM groups regarding or not continence status. Nevertheless, to allow further comparisons we selected studies which included non-GDM pregnant. Previous study which failure to show significant differences between pregnant with and without PFMD, but showed in

a prospective approach that the PFMD group decreased concentrations faster from 24 to 28 weeks. (20) Another study showed that women with UI had lower relaxin-2 concentrations compared to continent groups in different time points before 20 weeks of gestation but not in the following weeks. (22)

Our findings also showed that the GDM-PSUI group showed even lower concentrations. As external contrasts with literature are not possible due to the present innovative analyses. The possible rational line to be followed is that due to the antifibrotic action of relaxin-2 and knowing that its action are similar between rats and human, we hypothesize that lower relaxin-2 levels may explain the fact that on experimental rat studies the combination between pregnancy and hyperglycemia promote fibrosis process on urethral muscle. (23)

A previous study including GDM pregnant women were conducted and showed that in GDM group comparing with non-GDM the relaxin-2 concentration was higher during 12 weeks of gestation. Although interesting, the sample selection differed regarding glycemic threshold and the control group selection were not fully addressed. (24)

The literature is very restricted about the link between PFM function and hormonal status in general and during pregnancy it is, apparently, unknown. We choose MOS because it had highly interrater reliability ($r = 0.947$; $p < 0.001$) and stratified in 0-2 and 3-5, because 0-2 comprehended women who were not able to perform a contraction against pubic bone and cranial movement and those (3-5) who were. The relaxin-2 levels were even lower if we stratified the sample according in GDM pregnant who graded MOS= 0-2 compared with GDM with MOS= 3-5 and with non-GDM group. When the GDM group were stratified by continence status, the GDM-PSUI had significantly lower levels compared to other 3 groups.

Fibrosis is regarded a major factor in lower urinary tract dysfunctions (25) if we consider that there is some plausibility to support that the GDM-PSUI maybe have intensely connective and morphological muscle alterations (3,26), this could lead us to consider further investigations of the influence of lower relaxin-2 levels, especially in this group. This sounds promising since recent studies showed in animal model the capacity of fibrosis reversion and increased detrusor force generation on bladder by relaxin-2. (25)

The strength from this study was address convergent subjects (GDM, PSUI and PFM function) together, used 3 points from OGTT-75g to classify groups, a high-quality relaxin-2 kit was used to dosage, pregnant were selected during gestational weeks which is expect to relaxin-2 be stable (27) and participants had similar characteristics concerning obstetric history among the groups. Concerning the limitations as this study is an observational study, causality cannot be determined, should be interesting to consider for future studies perform dosages earlier and during different time points and we suggest to other authors consider include an objective PFM function tool.

CONCLUSION

Our findings showed that in the presence of the PSUI the relaxin-2 serum concentrations were lower both in non-GDM and GDM pregnant and it is remarkable specially in GDM-PSUI group. In addition, pregnant with GDM who presented a lower PFM strength had lower relaxin-2 levels comparing to GDM pregnant with better PFM function. When the continence status was taking together the GDM-PSUI with lower PFM strength presented even lower levels than all the others 3 groups.

RESEARCH IMPLICATIONS

Dichotomic observation underlying possible explanations diabetes leads to a fibrosis process, the relaxin-2 in the other hand leads to anti-fibrotic process. Further studies should be necessary to investigate the influence along time and determine the strength of the connection between lower levels of relaxin-2 PFM impairment and fibrosis on GDM group, mainly in GDM-PSUI. As the relaxin-2 are a favorable hormone used to enhance fibrotic conditions, it could be a useful new therapeutic tool to preventive and therapeutic strategies, depends on new future investigation.

Acknowledgments

The authors would like to give our great appreciation to the pregnant women who participated in this study.

Data Availability

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

REFERENCES

1. Piculo F, Marini G, Vesentini G, Morceli G, Damasceno DC, Sobrevia L, et al. Pregnancy-specific urinary incontinence in women with gestational hyperglycaemia worsens the occurrence and severity of urinary incontinence and quality of life over the first year post partum. *Eur J Obstet Gynecol Reprod Biol* [Internet]. 2020 Sep;252:336–43. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0301211520304036>
2. Woodley SJ, Lawrenson P, Boyle R, Cody JD, Mørkved S, Kernohan A, et al. Pelvic floor muscle training for preventing and treating urinary and faecal incontinence in antenatal and postnatal women [Internet]. Vol. 2020, *Cochrane Database of Systematic Reviews*. John Wiley and Sons Ltd; 2020 [cited 2020 Sep 28]. Available from: <http://doi.wiley.com/10.1002/14651858.CD007471.pub4>
3. Vesentini G, Barbosa AMP, Damasceno DC, Marini G, Piculo F, Matheus SMM, et al. Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women. Li L, editor. *PLoS One* [Internet]. 2020 Apr 3;15(4):e0231096. Available from: <https://dx.plos.org/10.1371/journal.pone.0231096>
4. Sartorão Filho CI, Pinheiro FA, Prudencio CB, Nunes SK, Takano L, Enriquez EMA, et al. Impact of gestational diabetes on pelvic floor: A prospective cohort study with three-dimensional ultrasound during two-time points in pregnancy. *Neurourol Urodyn* [Internet]. 2020 Aug 28;nau.24491. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/nau.24491>
5. Prudencio CB, Rudge MVC, Pinheiro FA, Sartorão Filho CI, Nunes SK, Pedroni CR, et al. Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. Palatnik A, editor. *PLoS One* [Internet]. 2019 Nov 7;14(11):e0223261. Available from: <https://dx.plos.org/10.1371/journal.pone.0223261>
6. Steinetz B., Whitaker P., Edwards JR. Maternal relaxin concentrations in diabetic pregnancy. *Lancet*. 1992 Sep;340(8822):752–5.
7. Feijóo-Bandín S, Aragón-Herrera A, Rodríguez-Penas D, Portolés M, Roselló-Lletí E, Rivera M, et al. Relaxin-2 in Cardiometabolic Diseases: Mechanisms of Action and Future Perspectives. *Front Physiol* [Internet]. 2017 Aug 18;8. Available from: <http://journal.frontiersin.org/article/10.3389/fphys.2017.00599/full>
8. Goldsmith LT, Weiss G. Relaxin in Human Pregnancy. *Ann N Y Acad Sci* [Internet]. 2009 Apr;1160(1):130–5. Available from: <http://doi.wiley.com/10.1111/j.1749-6632.2008.03800.x>
9. Ferlin A, De Toni L, Sandri M, Foresta C. Relaxin and insulin-like peptide 3 in the musculoskeletal system: from bench to bedside. *Br J Pharmacol* [Internet]. 2017 May 5;174(10):1015–24. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/bph.13490>

10. Ng HH, Shen M, Samuel CS, Schlossmann J, Bennett RG. Relaxin and extracellular matrix remodeling: Mechanisms and signaling pathways. *Mol Cell Endocrinol* [Internet]. 2019 May;487:59–65. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0303720719300218>
11. Petros PEP, Ulmsten UI. An integral theory of female urinary incontinence. *Acta Obstet Gynecol Scand*. 1990 Jan;69(S153):7–31.
12. Sangsawang B. Risk factors for the development of stress urinary incontinence during pregnancy in primigravidae: A review of the literature. *Eur J Obstet Gynecol Reprod Biol* [Internet]. 2014;178:27–34. Available from: <http://dx.doi.org/10.1016/j.ejogrb.2014.04.010>
13. American Diabetes Association. Standards of Medical Care in Diabetes—2015. *Diabetes Care* [Internet]. 2015 Jan 1;38(Supplement_1):S1–2. Available from: <http://care.diabetesjournals.org/cgi/doi/10.2337/dc15-S001>
14. Tamanini JTN, Dambros M, D’Ancona CAL, Palma PCR, Rodrigues Netto Jr N. Validação para o português do “International Consultation on Incontinence Questionnaire - Short Form” (ICIQ-SF). *Rev Saude Publica* [Internet]. 2004 Jun;38(3):438–44. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0034-89102004000300015&lng=pt&nrm=iso&tlng=pt
15. Klovning A, Avery K, Sandvik H, Hunskaar S. Comparison of two questionnaires for assessing the severity of urinary incontinence: The ICIQ-UI SF versus the incontinence severity index. *Neurourol Urodyn* [Internet]. 2009 Jun;28(5):411–5. Available from: <http://doi.wiley.com/10.1002/nau.20674>
16. Laycock J, Jerwood D. Pelvic floor muscle assessment: the PERFECT scheme. *Physiotherapy*. 2001;87(12):631–42.
17. Ashton-miller JA, Delancey JOL. Functional Anatomy of the Female Pelvic Floor. *Ann N Y Acad Sci* [Internet]. 2007 Feb 15;1101(1):266–96. Available from: <http://doi.wiley.com/10.1196/annals.1389.034>
18. Kristiansson P, Samuelsson E, Schoultz B Von, Svärdsudd K. Reproductive hormones and stress urinary incontinence in pregnancy. *Acta Obstet Gynecol Scand* [Internet]. 2001 Jan;80(12):1125–30. Available from: <http://doi.wiley.com/10.1080/j.1600-0412.2001.801209.x>
19. Ellen C, Travis A, Laurie W, Frances F S, Sandra J S. Optimal Paradigms for Measuring Peak Serum Relaxin in Eumenorrhic, Active Females. *Reprod Med Int*. 2018;1(2).
20. Harvey M-AE, Johnston SL, Davies GAL. Mid-trimester serum relaxin concentrations and post-partum pelvic floor dysfunction.

21. Zaman I, Swaminathan R, Brackenridge A, Sankaralingam A, McGowan B. Assessment of relaxin levels in pregnant women with gestational diabetes mellitus. *Endocr Abstr.* 2014 Apr;
22. Kristiansson P, Samuelsson E, Von Schoultz B, Svärdsudd K. Reproductive hormones and stress urinary incontinence in pregnancy. *Acta Obstet Gynecol Scand.* 2001;80(12):1125–30.
23. Piculo F, Marini G, Barbosa AMP, Damasceno DC, Matheus SMM, Felisbino SL, et al. Urethral striated muscle and extracellular matrix morphological characteristics among mildly diabetic pregnant rats: translational approach. *Int Urogynecol J.* 2014 Mar;25(3):403–15.
24. Alonso Lopez Y, Dereke J, Landin-Olsson M, Strevens H, Nilsson C, Hillman M. Plasma levels of relaxin-2 are higher and correlated to C-peptide levels in early gestational diabetes mellitus. *Endocrine [Internet].* 2017 Sep 29;57(3):545–7. Available from: <http://link.springer.com/10.1007/s12020-017-1354-x>
25. Andersson K-E, Fry C, Panicker J, Rademakers K. Which molecular targets do we need to focus on to improve lower urinary tract dysfunction? ICI-RS 2017. *Neurourol Urodyn [Internet].* 2018 Jun;37(S4):S117–26. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/nau.23516>
26. G V, R ED, LAR R, F P, G M, GAR F, et al. Pelvic floor and abdominal muscle cocontraction in women with and without pelvic floor dysfunction: a systematic review and meta-analysis. *Clinics [Internet].* 2019 [cited 2020 Jul 14];74. Available from: <https://pubmed.ncbi.nlm.nih.gov/31778432/>
27. Petersen LK, Vogel I, Agger AO, Westergård J, Nils M, Uldbjerg N. Variations in serum relaxin (hRLX-2) concentrations during human pregnancy. *Acta Obstet Gynecol Scand.* 1995 Jan;74(4):251–6.

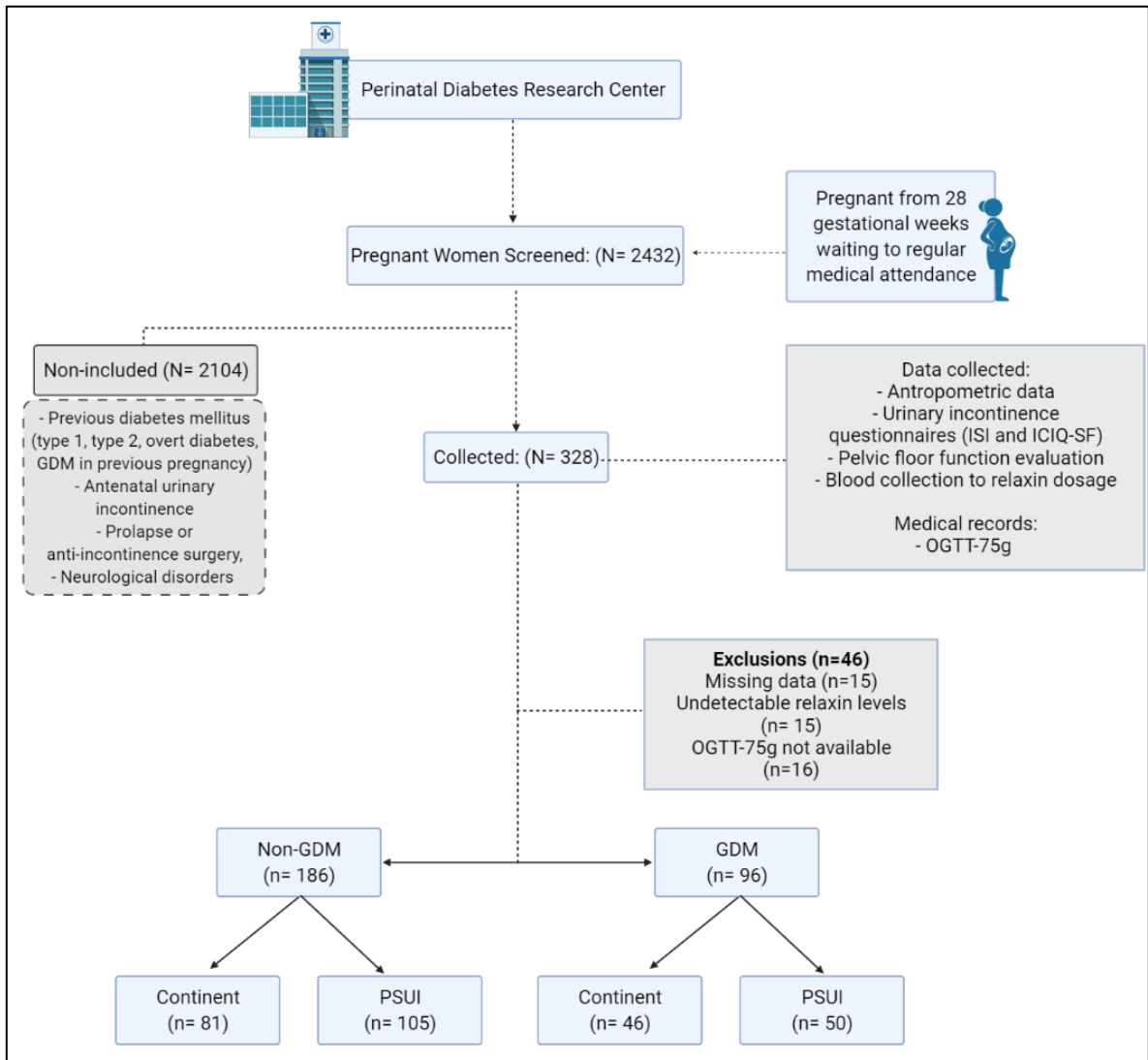
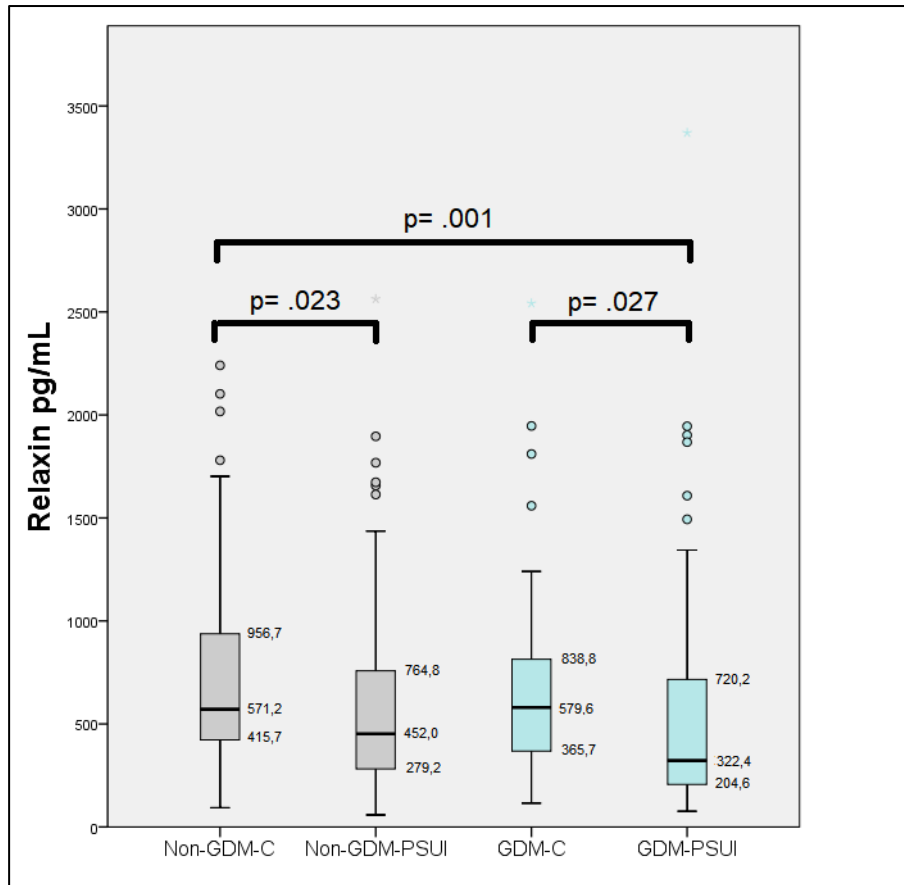


Figure 1. Flow chart indicating the distributions of study participants according to glycaemic and continence status.

Table 1. Baseline characteristics of study participants according to glycaemic and continence status.

	Non-GDM-C (n= 81)	Non-GDM-PSUI (n=105)	GDM-C (n= 46)	GDM-PSUI (n=50)	p value
Age (years)	26 (18-38)	24 (18-39) ^a	29 (18-41) ^a	26 (18-41)	,022
Gestational age	28 (24-38)	28 (24-38)	29,5 (24-38)	31,5 (24-38)	,086
Pre-pregnancy BMI	23,6 (16,8-42,5) ^a	26,3 (16,8-44,4)	26,2 (18,7-35,9)	28,7 (18,5-48,4) ^a	,000
BMI gestational	27,5 (18,7-44,3) ^a	29,2 (18,7-49,3)	28,2 (20,4-38,9) ^b	33,9 (21,6-49,5) ^{ab}	,001
Weight gain (kg)	7,5 (-1,0-29,0)	7 (-21,0-27)	7,4 (-26-18,8)	7,2 (-32-17)	,792
OGTT-75g fasting (mg/dL)	74 (50-90)	73 (58-87)	86,5 (64-124)	93 (73-119)	,000
OGTT-75g 1 hour (mg/dL)	108 (62-167)	112 (42-166)	145 (82-220)	151,5 (88-235)	,000
OGTT-75g 2 hours (mg/dL)	97 (51-151)	97 (49-143)	131 (72-205)	144,5 (72-217)	,000
Caucasian	65 (80,2%)	83 (79%)	32 (69,6%)	37 (74%)	,491
Prior cesarean delivery	9 (11,1%)	27 (25,7%)	9 (19,6%)	12 (24,0%)	,086

n: sample; BMI: body mass index; kg: kilograms; OGTT-75g: oral glucose tolerance test of 75 grams; ^{a,b}equal letters represent post-hoc differences; Kruskal-Wallis test, followed by Dunn's multiple comparisons and chi-square test. Non-GDM-C: non-Gestational diabetes mellitus continent group; Non-GDM-PSUI: non-Gestational diabetes mellitus incontinent group; GDM-C: Gestational diabetes mellitus continent group; GDM-PSUI: Gestational diabetes mellitus incontinent group; p < 0.05 significant difference between the four groups.



Significant p-values of post-hoc Dunn-Bonferroni for between group multiple comparisons were showed on the figure with black lines between group. Median, 25th and 75th percentiles; pg/mL: picogram/millilitres; Non-GDM-C: non-Gestational diabetes mellitus continent group; Non-GDM-PSUI: non-Gestational diabetes mellitus incontinent group; GDM-C: Gestational diabetes mellitus continent group; GDM-PSUI: Gestational diabetes mellitus incontinent group; p < 0.05 significant difference.

Figure 2. Median ± 25th - 75th percentiles of relaxin-2 serum concentration according to glycaemic and continence status during pregnancy.

Table 2. Pregnancy specific-urinary incontinence questionnaires and pelvic floor function according to groups.

	Non-GDM-C (n= 85)	Non-GDM-PSUI (n=111)	GDM-C (n= 47)	GDM-PSUI (n=54)	p- value
ICIQ-SF					
Amount of urine lost	-	-	-	-	
A small amount	-	79 (75,2%)	-	13 (26%)	.013
A moderate amount	-	25 (23,8%)	-	14 (28%)	
A large amount	-	1 (1,0%)	-	11 (22%)	
Frequency of urine lost	-	-	-	-	
About once a week or less often	-	36 (34,3%)	-	11 (22%)	.018
Two or three times a week	-	32 (30,5%)	-	1 (2%)	
About once a day	-	21 (20,0%)	-	7 (14%)	
Several times a day	-	16 (15,2%)	-	38 (76%)	
All the time	-	0	-	5 (10%)	
QoI (0-10)	-	7 (0-10)	-	7 (0-10)	.400
ICIQ-SF mean score (0-21)	-	12 (3-18)	-	12 (3-18)	.998
ISI					
ISI score (1-12)	-	3 (1-12)	-	3,5 (1-9)	.333
Severity (ISI)	-	-	-	-	
Slight	-	20 (18,2%)	-	7 (14%)	.725
Moderate	-	79 (75,2%)	-	38 (76%)	
Severe	-	6 (5,7%)	-	5 (10%)	
Pelvic floor function					
	(n= 70)	(n= 95)	(n= 43)	(n= 59)	
MOS (0-5)					.003
0	7 (8,6%)	9 (8,6%)	2 (4,3%)	3 (6%)	
1	12 (14,8%)	19 (18,1%)	8 (17,4%)	13 (26%)	
2	14 (17,3%)	35 (33,3%)	3 (6,5%)	15 (30%)	
3	19 (23,5%)	18 (17,1%)	12 (26,1%)	15 (30%)	
4	12 (14,8%)	10 (9,5%)	11 (23,9%)	3 (6%)	
5	6 (7,4%)	4 (3,8%)	7 (15,2%)	0	
MOS (stratified)					
0-2	33 (40,7%)	63 (60%)	13 (28,3%)	31 (62%)	.000
3-5	37 (45,7%)	32 (30,5%)	30 (65,2%)	18 (36%)	
Endurance (0-10 sec)	4 (0-10)	3 (0-10)	4 (0-10)	3 (0-10)	.125
Endurance (stratified)					
0-5 seconds	51 (63%)	72 (68,6%)	29 (63%)	41 (82%)	.322
6-10 seconds	19 (23,5%)	23 (21,9%)	14 (30,4%)	8 (16%)	
Repetition (stratified)					
0-5 repetitions	34 (42%)	48 (45,7%)	12 (26,1%)	25 (50%)	.536
5-10 repetitions	36 (44,4%)	44 (41,9%)	31 (67,4%)	24 (48%)	

Significant p-values of the Mann-Whitney U test and chi-square test. Median (minimum-maximum); n(%); n= sample; sec: seconds; ICIQ-SF: International Consultation on Incontinence Questionnaire-Short Form; ISI: Incontinence Severity Index; QoL: quality of life; Non-GDM-C: non-Gestational diabetes mellitus continent group; Non-GDM-PSUI: non-Gestational diabetes mellitus incontinent group; GDM-C: Gestational diabetes mellitus continent group; GDM-PSUI: Gestational diabetes mellitus incontinent group; p < 0.05 significant difference.

Table 3. Relaxin-2 serum concentrations regarding pelvic floor strength according to different groups stratifications.

	MOS (0-2) pg/mL	MOS (3-5) pg/mL	<i>p</i> value*
Not stratified			
All sample (n= 282)	458 (75-2563)	529 (76-3370)	.254
Stratified by glycemc status			
Non-GDM (n=165)	506 (75-2563)	509 (89-1500)	.689
GDM (n=102)	361 (96-1947)	587 (76-3370)	.029
<i>p</i> value**	.023	.210	
Stratified by glycaemic and continence status			
Non-GDM-C (n=70)	537 (93-2102) ^c	560 (125-1500)	.729
Non-GDM-PSUI (n=95)	471 (75-2563) ^b	434 (89-1435)	.413
GDM-C (n=43)+	613 (198-1947) ^a	587 (115-2542)	.526
GDM-PSUI (n=59)	260 (96-1902) ^{a,b,c}	625 (76-3370)	.059
<i>p</i> value***	.001	.201	

MOS: Modified Oxford Scale; n: sample; pg/mL: picogram per decilitre; * Comparisons between stratified modified oxford scale within each group (Mann-Whitney U); median (minimum-maximum); **Comparisons according to each stratified score from modified oxford scale between Non-GDM and GDM ***Comparisons according to each stratified score from modified oxford scale between Non-GDM-C, Non-GDM_PSUI, GDM-C and GDM-PSUI (Kruskal Wallis H; post hoc Dunn-Bonferroni); MOS: modified oxford scale; **p* < 0.05 significant difference.

Seção 4
Diameter Study Group

Pesquisadores Reponsáveis	Pesquisadores Associados
Profa. Emérita Marilza Vieira Cunha Rudge Profa. Titular Iracema de Mattos Paranhos Calderon Profa. Dra. Angélica Mérica Pascon Barbosa Profa. Dra. Fátima Pereira de Souza	PhD Raghavendra Hallur Lakshmana Shetty PhD David Rafael Abreu Reyes Dra Fernanda Cristina Bérghamo Alves Dr João Paulo de Castro Marcondes Dra Maíra Libertad Soligo Takemoto Dra Juliana Ferreira Floriano
Pesquisadores Nacionais Prof. Titular Carlos Frederico de Oliveira Graeff Prof. Titular Manoel João Batista Castello Girão Profa. Dra. Cláudia Garcia Magalhães Prof. Dr. Roberto Antonio de Araujo Costa Profa. Adjunto Silvana Andréa Molina Lima Profa. Dra. Meline Rossetto Kron Rodrigues Prof. Adjunto Sérgio Luis Felisbino Prof. Dr. Walnei Fernandes Barbosa Profa. Dra. Grasiela Bossolan Prof. Dr. José Eduardo Corrente Prof. Dr. Hélio Rubens de Carvalho Nunes Prof. Dr. Joelcio Francisco Abbade Profa. Dra Patrícia de Souza Rossignoli Profa. Dra. Cristiane Rodrigues Pedroni Prof. Titular Álvaro Nagib Atallah Profa. Dra. Zsuzsanna Ilona K. de Jarmy Di Bella Profa. Silvana Maria de Macêdo Uchôa Prof. Titular Marco Antonio Hungaro Duarte Prof. Dr. Edson Assunção Mareco Profa. Adjunta Marna Eliana Sakalem Profa. Dra. Natalia Miguel Martinho Fogaça Prof. Dr. Diego Giroto Bussaneli Profa. Titular Maeli Dal Pai Profa. Dra. Selma Maria Michelin Matheus Profa. Dra. Ana Karina Cristiuma De Luca Profa. Dra. Daisy Maria Fávero Salvadori Prof. Dr. Rondinelli Donizetti Herculano Prof. Dr. Spencer Luiz Marques Payao	Dra. Fernanda Piculo Dra. Gabriela Marini Prata Dra Cibele Viera Cunha Rudge Dr. Fabio Joly Campos Dr. Ícaro Putinhon Caruso Dr. Lucas Trevizani Rasmussen Dr Vinícius Krieger Costa Nogueira Ms. Caroline Baldini Prudencio Ms. Fabiane Affonso Pinheiro Ms. Carlos Izaias Sartorão Filho Ms. Sofia Beatriz Carolina Vega Quiroz Ms. Tawana Pascon Ms. Sthefanie Kenickel Nunes Ms. Bruna Bologna Catinelli Ms. Fabiana Vieira Duarte de Souza Reis Ms. Rafael Guilen de Oliveira Ms. Sarah Maria Barneze Costa Ms. Mariane de Oliveira Menezes Ms. Nilton José dos Santo Ms. Isabella Otenio de Lourenço Ms. Jéssica Maróstica de Sá Ms. Raissa Escandiusi Avramidis Ms Guilherme Thomaz de Aquino Nava Ms Talita Costa Domingues Eusebio Mario Amador Enriquez Jose Vitor Freitas Melo (<i>in memoriam</i>) Luiz Takano Aline Medolago Carr Gabriela Azevedo Garcia Adriely Bittencourt Morgenstern Magyori Carolina Neiva Frota de Carvalho Luana Fávaro Iamundo Henrique Caetano Mingoranci Bassin Carolina Neiva Frota de Carvalho Carolina Pascon Marques3 Michele Jacomin Ana Julia Bimbatti Silva Maiara Isabele Gonçalves Orlandi Tatiana Daniele Dangió
Pesquisadores Internacionais PhD Adonis Hijas PhD Luis Sobrevia Luarte PhD Bary Berghmans PhD Rob de Bie PhD Costanza Emanuelli PhD Baerbel Junginger PhD Antonio Musàro PhD Lehana Thabane	Apoio à Pesquisa Rita de Cássia Athanázio Cinthia Scolastico Cecilio



Seção 5
Anexos

Impact of gestational diabetes on pelvic floor: A prospective cohort study with three-dimensional ultrasound during two-time points in pregnancy

Carlos I. Sartorão Filho^{1,2} | Fabiane A. Pinheiro¹ | Caroline B. Prudencio¹ |
 Sthefanie K. Nunes¹ | Luiz Takano¹ | Eusebio M. A. Enriquez¹ |
 Maiara I. G. Orlandi¹ | Baerbel Junginger³ | Raghavendra L. S. Hallur¹ |
 Marilza V. C. Rudge¹ | Angélica M. P. Barbosa PT, PhD^{1,4}

¹Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

²Department of Medicine, Assis Municipality Educational Foundation (FEMA), Medical School, Fundação Educacional do Município de Assis (FEMA), Assis, São Paulo, Brazil

³Department of Gynecology, Pelvic Floor Center Charité, Charité University Hospital, Berlin, Germany

⁴Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marília, São Paulo, Brazil

Correspondence

Angélica M. P. Barbosa, PT, PhD, Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marília—SP 17525-900, Brazil.
 Email: angelicapascon@gmail.com

Funding information

Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 2016/01743-5

Abstract

Aim: To evaluate the pelvic floor (PF) biometry using three-dimensional ultrasound (US) at two-time points of gestational in pregnant women with gestational diabetes mellitus (GDM).

Methods: A prospective cohort study conducted at the Perinatal Diabetes Research Center including 44 pregnant women with GDM and 66 pregnant women without GDM at 24 to 28 weeks of gestation. Three-dimensional transperineal US was performed at 24 to 28 and 34 to 38 weeks of gestation in the lithotomy position at rest. The axial plane of the minimal Levator hiatal dimensions was used to determine Levator ani muscle and Hiatal area (HA) biometry at 24 to 28 and 34 to 38 weeks of gestation.

Results: Of the 110 pregnant women, 100 (90.9%) completed the follow-up at 34 to 38 weeks of gestation. The evaluation by US showed a negative biometric change between the two-time points, during pregnancy in women with GDM; in the HA (β coefficient: estimative of effect in biometric progression according to GDM diagnosis, using the non-GDM group as reference = -6.76 ; $P = .020$), anteroposterior diameter ($\beta = -5.07$; $P = .019$), and Levator ani thickness ($\beta = -12.34$; $P = .005$).

Conclusions: Pregnant women with GDM had a significantly lower than expected percentage of changes in biometry of Levator ani thickness and HA from 24 to 28 to 34 to 38 weeks of gestation when compared with the group of pregnant women without GDM. GDM alters the morphology of PF structures assessed by three-

Carlos I. Sartorão Filho, Fabiane A. Pinheiro, Caroline B. Prudencio, Sthefanie K. Nunes, Luiz Takano, Eusebio M.A. Enriquez, Maiara I. Orlandi, Baerbel Junginger, Marilza V. C. Rudge, and Angélica M.P. Barbosa contributed equally to this work.

Carlos I. Sartorão Filho and Fabiane A. Pinheiro are the first authors on this work.

Angélica M.P. Barbosa and Marilza V. C. Rudge are the last authors on this work.

← ACCEPT DECISION FOR IUJO: Ms. No. IUJO-D-21-00419R1 - [EMID:888d70bdab1dbd9c]



International Urogynecology Journal - Editorial Office <em@editorialmanager.com>

Dom, 18/07/2021 07:13

Para: Você



Ref.:

Ms. No. IUJO-D-21-00419R1

Is there a correlation between a 20-minute pad-test and subjective urine leakage amount?

International Urogynecology Journal

Dear Dr Baldini Prudencio,

I am pleased to inform you that your work has now been accepted for publication in International Urogynecology Journal.

Thank you for submitting your work to this journal.

With kind regards

Jaromir Masata, Ph.D., M.D., Prof.

International Urogynecology Journal



Contents available at ScienceDirect

Diabetes Research
and Clinical Practicejournal homepage: www.elsevier.com/locate/diabresInternational
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 Group¹**

^aPerinatal Diabetes Research Center, University Hospital, Botucatu Medical School, Univ Estadual Paulista_UNESP, São Paulo State, Brazil

^bSão Paulo State University (UNESP), Botucatu Medical School, Department of Gynecology and Obstetrics, Botucatu, Sao Paulo, Brazil

^cSão Paulo State University (UNESP), School of Philosophy and Sciences, Department of Physical Therapy and Occupational Therapy, Marília, São Paulo State, Brazil

^dSão Paulo State University (UNESP), Institute of Biosciences,

^eUniversidade Sagrado Coração, Department of Health Science

^fSão Paulo State University (UNESP), Institute of Biosciences,

^gSão Paulo State University (UNESP), Institute of Biosciences,

Acknowledgments

The authors wish to thank the patients who donated their RAM tissue within the context of this study. The authors are also grateful to all medical staff at the Maternity of University Hospital Botucatu Medical School UNESP for excellent patient care.

**** The Diamater Study Group:** Rudge Mvc, Barbosa, AMP, Caldeiron IMP, Souza FP, Hijas A, Sobrevia L, Palma MS, Graeff CFO, Arni RK, Herculano RD, Salvadori DF, Mateus S, Dal Pai Silva M, Magalhães CG, Costa RA, Lima SAM, Emanueli C, Felisbino SI, Barbosa W, Atallah A, Girão MJB, Di Bella Z, Uchoa SM, Payão S, Berghman B, De Bie R, Junginger B, Alves FCB, Rossignoli PS, Abbade J, Prudencio CB, Orlandi MIG, Gonçalves MI, Nunes SK, Catinelli BB, Quiroz S, Sarmento BV, Pinheiro FA, Sartório CI, Quiroz SBCV, Reyes DRA, Enriquez EMA, Oliveira RG, Floriano JF, Marcondes JPC, Sarmento, Costa SMB, Dangió TD, Pascon T, Hallur LSR, Melo JVF, Takano L, Reis FVDS, Caldeirão TD, Carr AM, Garcia GA, Rabadan GB, Bassin HCM, Suyama KS, Damasceno LN, Takemoto MLS, Menezes MD, Bussaneli DG, Nogueira, VKC, Lima PR, Lourenço IO, Marostica de Sá J, Megid RA, Caruso IP, Rasmussen LT, Prata GM, Piculo F, Vesentini G, Arantes MA, Ferraz GAR, Camargo LP, Kron MR, Corrente JE, Nunes HRC.

ARTICLE INFO

Article history:

Received 7 February 2020

Received in revised form

5 June 2020

Accepted 7 July 2020

Available online 15 July 2020

Keywords:

Pregnancy-specific urinary incontinence

Urinary incontinence

Gestational diabetes mellitus

Skeletal muscle

ABSTRACT

Aims: To evaluate characteristics of the rectus abdominis muscle associated with pregnancy-specific urinary incontinence. **Methods:** A total of 100 women with GDM associated with pregnancy-specific urinary incontinence (Western blotting) for the fibronectin expression at delivery were analyzed. **Results:** The GDM women had a higher expression of slow-twitch oxidized collagen; an increase in myofibrillar protein such as myofibrillar

* Corresponding author at: Univ Estadual Paulista (UNESP), Department of Gynecology and Obstetrics, Distrito de Rubião Júnior, s/n, Botucatu, SP 18618-970, Brazil.

E-mail address: marilzarudge@gmail.com (M.V.C. Rudge).

¹ DIAMATER Study Group Department of Gynecology and Obstetrics, School of Medicine of Botucatu, Univ Estadual Paulista_UNESP, São Paulo State, Brazil.

<https://doi.org/10.1016/j.diabres.2020.108315>

0168-8227/© 2020 The Authors. Published by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

RESEARCH ARTICLE

Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women

Giovana Vesentini^{1,2*}, Angélica M. P. Barbosa^{1,3}, Débora C. Damasceno², Gabriela Marini^{1,2,4}, Fernanda Piculo^{1,2}, Selma M. M. Matheus^{1,5}, Raghavendra L. S. Hallur^{1,2}, Sthefanie K. Nunes^{1,2}, Bruna B. Catinelli^{1,2}, Claudia G. Magalhães^{1,2}, Roberto Costa^{1,2}, Joelcio F. Abbade^{1,2}, José E. Corrente^{1,6}, Iracema M. P. Calderon^{1,2}, Marilza V. C. Rudge^{1,2}, **The DIAMATER Study Group[†]**



1 Perinatal Diabetes Research Center, University Hospital, Botucatu Medical School, Univ Estadual Paulista_UNESP, Botucatu, São Paulo, Brazil, **2** Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu Medical School, Botucatu, São Paulo, Brazil, **3** Department of Physiotherapy and Occupational Therapy, São Paulo State University (UNESP), School of Philosophy and Sciences, Marília, São Paulo, Brazil, **4** Department of Health Sciences, Universidade Sagrado Coração, Bauru, São Paulo, Brazil, **5** Department of Anatomy, São Paulo State University (UNESP), Institute of Biosciences, Botucatu, São Paulo, Brazil, **6** Department of Biostatistics, São Paulo State University (UNESP), Bioscience Institute, Botucatu, São Paulo, Brazil

OPEN ACCESS

Citation: Vesentini G, Barbosa AMP, Damasceno DC, Marini G, Piculo F, Matheus SMM, et al. (2020) Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women. PLoS ONE 15(4): e0231096. <https://doi.org/10.1371/journal.pone.0231096>

Editor: Lingjun Li, National University Singapore Yong Loo Lin School of Medicine, SINGAPORE

Received: September 24, 2019

Accepted: March 16, 2020

Published: April 3, 2020

Copyright: © 2020 Vesentini et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All data is available at <http://hdl.handle.net/11449/190666>.

Funding: The financial support provided by the Brazilian Public Health System (SUS) supported the treatment of all pregnant women. The authors gratefully acknowledge funding by São Paulo Research Foundation (FAPESP) (Grant No: #2016/09710-9 and #2016/01743-5). GV received a FAPESP scholarship (Grant No: #2014/26852-6).

Acknowledgments

The study was supported by the human resources and infrastructure of PDRC- Botucatu Medical School-UNESP. The authors thank Talisia Moretto and Gelson Rodrigues for technical assistance.

****The Diamater Study Group:** Rudge MVC (Universidade Estadual Paulista), Barbosa AMP (Universidade Estadual Paulista), Calderon IMP (Universidade Estadual Paulista), Souza FP (Universidade Estadual Paulista), Hijas A (Case Western Reserve University), Sobrevia L (Pontificia Universidad Catolica de Chile), Palma MS (Universidade Estadual Paulista), Graeff CFO (Universidade Estadual Paulista), Arni RK (Universidade Estadual Paulista), Herculano RD (Universidade Estadual Paulista), Salvadori DF (Universidade Estadual Paulista), Matheus S (Universidade Estadual Paulista), Dal Pai Silva M (Universidade Estadual Paulista), Magalhães CG (Universidade Estadual Paulista), Costa RA (Universidade Estadual Paulista), Lima SAM (Universidade Estadual Paulista), Emanuelli C (Imperial College London), Felisbino S (Universidade Estadual Paulista), Barbosa W (Universidade Estadual Paulista), Atallah A (Universidade Federal de São Paulo), Girão MJB (Universidade Federal de São Paulo), Di Bella Z (Universidade Federal de São Paulo), Uchoa SM (Universidade Católica de Pernambuco), Payão S (Faculdade de Medicina de Marília), Berghman B (Maastrich University), De Bie R (Maastrich University), Junginger B (Charité Universitätsmedizin Berlin), Alves FCB (Universidade Estadual Paulista), Rossignoli PS (Universidade Estadual Paulista), Abbade J (Universidade Estadual Paulista), **Prudencio CB (Universidade Estadual Paulista)**, Orlandi MIG (Universidade Estadual Paulista), Gonçalves MI (Universidade Estadual Paulista), Nunes SK (Universidade Estadual Paulista), Catinelli BB (Universidade Estadual Paulista), Sarmento

Results

No statistically significant differences in the maternal and fetal characters were observed between the groups in both rats and women. However, significant changes in RAM

RESEARCH ARTICLE

Prenatal exposure to gestational diabetes mellitus increases developmental defects in the enamel of offspring

Tawana Pascon¹, Angélica M. P. Barbosa^{1,2*}, Rita C. L. Cordeiro³, Diego G. Bussaneli³, Caroline B. Prudencio¹, Sthefanie K. Nunes¹, Fabiane A. Pinheiro¹, Grasiela Bossolan¹, Leandro G. Oliveira¹, Iracema M. P. Calderon¹, Gabriela Marini⁴, Marilza V. C. Rudge¹

1 Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu Medical School, Botucatu, São Paulo, Brazil, **2** Department of Physiotherapy and Occupational Therapy, São Paulo State University (UNESP), School of Philosophy and Sciences, Marília, São Paulo, Brazil, **3** Department of Pediatric Dentistry and Orthodontics, São Paulo State University (UNESP), Araraquara School of Dentistry, Araraquara, São Paulo, Brazil, **4** Health Sciences Center, University of the Sacred Heart (USC), Bauru, São Paulo, Brazil

* angelicapascon@gmail.com



Abstract

Background and objective

Gestational diabetes mellitus (GDM) is associated with short- and long-term maternal and perinatal repercussions. Our objective was to evaluate the long-term consequences of intra-uterine exposure to hyperglycemia on Developmental Defects of Enamel (DDE) in offspring.

Results

Overall, 50 children of women with GDM and 250 children of normoglycemic women participated, the latter serving as controls. Children were examined at the age between 3 and 12 years. In addition to physical examination, two independent observers examined and rated photographs to identify specific types of DDE in a blinded fashion. Among offspring of mothers with GDM, rates of DDE (all types combined) and hypoplasia (specific type) were significantly higher ($p < 0.001$, $p = 0.04$), in comparison to offspring of normoglycemic mothers. Considering only the affected teeth (1060 in GDM category; 5499 in controls), rates of DDE (all types combined) were significantly higher for total teeth ($p < 0.001$) and deciduous teeth ($p < 0.001$), but not permanent teeth. In specific types of DDE involving deciduous teeth, rates of demarcate opacity were significantly higher ($p < 0.001$; canine and 2nd mandibular molars) and hypoplasia ($p < 0.001$; 2nd maxillary molars and 2nd mandibular molars). In permanent teeth, the rate of diffuse opacity in association with GDM was significantly higher ($p < 0.001$; maxillary central incisors and 1st maxillary molars).

Conclusion

GDM was associated with the adverse effects of DDE on offspring. This study lays the foundation for future studies to determine the impact of GDM on long-term risk of DDE.

OPEN ACCESS

Citation: Pascon T, Barbosa AMP, Cordeiro RCL, Bussaneli DG, Prudencio CB, Nunes SK, et al. (2019) Prenatal exposure to gestational diabetes mellitus increases developmental defects in the enamel of offspring. PLoS ONE 14(2): e0211771. <https://doi.org/10.1371/journal.pone.0211771>

Editor: Víctor Sánchez-Margalet, Virgen Macarena University Hospital, School of Medicine, University of Seville, SPAIN

Received: March 18, 2018

Accepted: January 22, 2019

Published: February 27, 2019

Copyright: © 2019 Pascon et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

RESEARCH ARTICLE

Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study

Caroline B. Prudencio^{1*}, Marilza V. C. Rudge^{1e}, Fabiane A. Pinheiro^{1‡}, Carlos I. Sartorão Filho^{1‡}, Sthefanie K. Nunes^{1‡}, Cristiane R. Pedroni^{2‡}, Baerbel Junginger^{3‡}, Angélica M. P. Barbosa^{1,2e}

1 Department of Gynecology and Obstetrics, Botucatu Medical School, São Paulo State University (Unesp), Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil, **2** Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (Unesp), Universidade Estadual Paulista (UNESP), Marília, São Paulo, Brazil, **3** Gynecology Department, Charité University Hospital, Berlin, Germany

^e These authors contributed equally to this work.

[‡] These authors also contributed equally to this work.

* caroline_baldini@hotmail.com



OPEN ACCESS

Citation: Prudencio CB, Rudge MVC, Pinheiro FA, Sartorão Filho CI, Nunes SK, Pedroni CR, et al. (2019) Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. PLoS ONE 14(11): e0223261. <https://doi.org/10.1371/journal.pone.0223261>

Editor: Anna Palatnik, Medical College of Wisconsin, UNITED STATES

Received: March 7, 2019

Accepted: September 17, 2019

Published: November 7, 2019

Copyright: © 2019 Prudencio et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: This work received a scholarship from Brazilian Federal Agency for Support and Evaluation of graduate Education/SD (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES/DS to CBP. MVCR received financial assistance to develop the project from Sao Paulo Research Foundation protocol number 2016/

Abstract

Background and objective

Pelvic floor muscles are involved in postural stability, in maintenance intra-abdominal pressure, and on mechanical support for pelvic organ. Gestational Diabetes Mellitus' (GDM) pregnancies complicated by fetal macrosomia, large placenta and polyhydramnios contribute for abrupt and intense increase in maternal intra-abdominal pressure. Our objective was analyze the impact of GDM on pelvic floor muscle (PFM) electromyography (EMG) activity progress from 24–30 to 36–38 weeks of gestation. We conducted a prospective cohort study. PFM EMG was performed in nulliparous or primiparous women with one previous elective cesarean delivery and with or not GDM diagnosed by the American Diabetes Association criteria. A careful explanation of the muscle anatomy and functionality of the PFM was given before EMG assessment. The outcome measures were PFM recruitment and progress from 24–30 to 36–38 weeks of gestation analyzed by the normalized root mean square (RMS) during rest-activity, fast and hold pelvic floor muscle contraction.

Results

Fifty-two pregnant women were assigned to 2 groups: the GDM ($n = 26$) and normoglycemic (NG) ($n = 26$). The demographic and obstetric data showed homogeneity between the groups. PFM activity progress was decreased in rest-activity ($P = 0.042$) and hold contraction ($P = 0.044$) at 36–38 weeks of gestation in the GDM group relative to that in the NG group.

Impact of Gestational Diabetes Mellitus on Sexual Function: A Case–Control Study

Sthefanie K. Nunes, MSc,^{1,*} Cibele V.C. Rudge, PhD,^{1,*} Sofia C.B.V. Quiroz, MSc,¹
Raghavendra L. Hallur, PhD,¹ Caroline B. Prudencio, MSc,¹ Fabiane A. Pinheiro, MSc,¹
Carlos I. Sartorão Filho, MSc, MD,¹ Jon Odland, PhD,² Iracema M.P. Calderon, PhD,¹
Angélica M.P. Barbosa, PhD,^{1,3,*} and Marilza V.C. Rudge, PhD¹

Abstract

Background: The prevalence of gestational diabetes mellitus (GDM) is increasing worldwide, and this condition may be compromising female sexual function. However, there are controversial findings regarding the impact of GDM diagnosis and proposed treatments on sexual function during pregnancy. Therefore, this study seeks to elucidate the impact of GDM on sexual function in pregnant women by making a comparison between GDM and non-GDM groups using pregnancy sexual response inventory (PSRI).

Materials and Methods: A case–control study involved 303 [168 women without GDM (control group) and 108 women diagnosed with GDM (case group)] Brazilian pregnant women at the Perinatal Diabetes Research Centre-Universidade Estadual Paulista, Brazil. PSRI was used to collect the data. The sexual function was scored in 10 domains as composite and specific scores by domains, categorized into quartiles (0 < 25 “very low,” 25 < 50 “low,” 50 < 75 “high,” and 75–100 “very high”), for “before pregnancy” and “during pregnancy.” The obtained data were subjected to statistical analysis using Student’s *t*-, *F*-, and chi-square tests.

Results: GDM women (PSRI composite score < 50) are at risk of decreased sexual function during pregnancy, while non-GDM women are not at risk (PSRI composite score > 50). There were no significant differences in the sexual functions between the two groups before pregnancy ($p > 0.0001$). After GDM diagnosis and proposed treatment, the differences were significant ($p < 0.0001$), notably in the frequency, arousal, orgasm, satisfaction, and dyspareunia score.

Conclusions: This study showed that GDM diagnosis and proposed treatment resulted in decreased sexual functions during pregnancy.

Keywords: gestational diabetes mellitus, pregnancy, pregnancy sexual response inventory

Introduction

SEXUAL FUNCTION IN PREGNANT WOMEN has been attributed to several factors such as religious, sociocultural, relationships, physical, and psychological changes. Multiple studies have reported that pregnant women may experience impaired sexual function across all phases of the female sexual response cycle.^{1,2}

Gestational diabetes mellitus (GDM), defined as impaired glucose tolerance during pregnancy, has long been suspected of causing sexual dysfunction in pregnant women³ caused by

the sudden transformation of a normal pregnancy to a high-risk pregnancy without maternal clinical symptoms.

GDM is associated with maternal and fetal risks related to diagnosis, including strict glycemic control, altered well-being and fetal growth, timely decision-making and route of delivery, and increased short- and long-term complications for mother and newborn.⁴ To reduce this risk, women with GDM should change their habits and undergo rigorous medical follow-up. It is recommended to maintain adequate and controlled diet to ensure control of weight gain, active lifestyle, continuous monitoring of glucose level, and sometimes

¹Department of Gynecology and Obstetrics, Botucatu Medical School, São Paulo State University (UNESP), Botucatu, SP, Brazil.

²Department of Public Health and Nursing, Norwegian University of Science and Technology (NTNU), Trondheim, Norway.

³Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marília, SP, Brazil.

*These authors contributed equally to this work.



Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

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

Altered maternal metabolism during mild gestational hyperglycemia as a predictor of adverse perinatal outcomes: A comprehensive analysis[☆]

Marilza Vieira Cunha Rudge^{a,b,*}, Angélica Mercia Pascon Barbosa^{b,c}, Luis Sobrevia^{b,d,e,f}, Rafael Bottaro Gelaleti^b, Raghavendra Lakshmana Shetty Hallur^b, João Paulo Castro Marcondes^b, Daisy Maria Fávero Salvadori^g, Caroline Baldini Prudêncio^b, Claudia Garcia Magalhães^a, Roberto Costa^a, Joelcio Francisco Abbade^a, José Eduardo Corrente^h, Iracema de Mattos Paranhos Calderon^{a,b}, The Perinatal Diabetes Research Group¹

^a Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), CEP18618-687, São Paulo, Brazil

^b Graduate Program in Gynecology, Obstetrics, and Mastology, Botucatu Medical School (FMB), São Paulo State University (UNESP), São Paulo, Brazil

^c Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, Marília, São Paulo State University (UNESP), São Paulo, Brazil

^d Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile

^e Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Seville E-41012, Spain

^f University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Queensland, Australia

^g Department of Pathology, Botucatu Medical School (FMB), São Paulo State University (UNESP), São Paulo, Brazil

^h Biostatistics Department, Bioscience Institute, São Paulo State University (UNESP), São Paulo, Brazil

ARTICLE INFO

Keywords:

Mild gestational hyperglycemia
Gestational diabetes mellitus
Large for gestational age
Insulin resistance
Placenta
Cytokines
Apoptosis
Natural killer cells
Inflammatory mediators
IRS-1 gene
Gene expression
Moderator analysis

ABSTRACT

Mild gestational hyperglycemia (MGH), as assessed using the normal oral glucose tolerance test (OGTT) and detection of an altered glycemic profile, is associated with adverse perinatal outcome. This study described the results of 40 years of research conducted at the Perinatal Diabetes Research Centre at São Paulo State University (UNESP), Brazil, on the maternal MGH environment and placental markers. This study also described the unidirectional relationship between MGH and excessive fetal growth, also supplying moderator analysis. In addition to hyperglycemia, MGH is associated with an increased incidence of hypertension, metabolic syndrome, persistent insulin resistance after pregnancy, and high risk of developing type 2 diabetes mellitus (T2DM) after pregnancy. Structural changes and functional abnormalities resulting from MGH were observed in placenta. The fully adjusted model concluded that the predictor variable (MGH), which creates a complex environment for the fetus, has a direct effect on excessive birth weight and produces a z-score for ratios of birth weight to gestational age of ≥ 2 . Maternal age, pre-pregnancy BMI, number of previous pregnancies, numbers of prenatal visits, and 1 h OGTT are moderator variables that impact MGH and excessive fetal growth. These results show that maternal MGH has some characteristics associated with similar long-term T2DM development and similar adverse perinatal results to those of gestational diabetes mellitus (GDM) mothers, making it an intermediate maternal and placental marker between normoglycemic and GDM mothers.

[☆] This article is part of a Special Issue entitled: Membrane Transporters and Receptors in Pregnancy Metabolic Complications edited by Luis Sobrevia.

* Corresponding author at: Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, São Paulo, Brazil.

E-mail address: marilzarudge@gmail.com (M.V.C. Rudge).

¹ M.V. Rudge, I.M.P. Calderon, A.P. Barbosa, J. Abbade, R.A.A. Costa, C.G. Magalhães, D.F. Salvadori, R. Gelaleti, R.L.S. Hallur, J.P. Marcondes, J.F. Floriano, D.R.A. Reyes, L. Sobrevia, C.B. Prudêncio, F. Pículo, G. Marini, G. Vesentini, G. Morcelli, C.A. Negrato, H.D. Prazeres, S. Molina, M. Arantes, A.C. Cavassini, L. Kerche, A.K.C. De Luca, S. Corrêa-Silva, E. Bevilacqua, J.B. Moreli, L. Pietro, S. Daher, S. Fabio, A.C. Honorio-França, A.A. Queiroz, C.C.P. Hara, C.A.L. Boraschi, T.A.V.L. Pualetti, L. Jovanovic, A. Dias, A.N. Atallah, M.D. Ramos, M.A.M. Brasil, C.V.C. Rudge, A. Tristão, U. Del Nero, M. Mendonça, S.S. Witkin, C.I. Sartório Filho, S.K. Nunes, F.A. Pinheiro, S.V. Quiroz, T. Pascon, T.D. Caldeirão, A.P. Oliveira, B.F. Nicolosi, C.V. Bolognani, D.L.G. Fagundes, I.C.F. Llanos, J.M. Vernini, L.B.S.M. Reis, M.P. Sirimarco, N.M. Basso, N.A.Q. Maquesim, S.A.L.C. Silva, S.C. Silva, T.T. Scudeller, W. Ayach, A.P.M. Almeida, B.F.C.A. Nicolosi, C.P. Lima, D. Luminoso, F.C. Vasconcellos, G.A.R. Ferraz, H. Migoliaro, L.P. Camargo, M.L.S. Macedo, M.R.K. Rodrigues and P.H.O. Anézio.

<https://doi.org/10.1016/j.bbadis.2019.05.014>

Received 7 October 2018; Received in revised form 18 May 2019; Accepted 22 May 2019

0925-4439/ © 2019 Elsevier B.V. All rights reserved.

STUDY PROTOCOL

Open Access

Study protocol to investigate biomolecular muscle profile as predictors of long-term urinary incontinence in women with gestational diabetes mellitus



Marilza V. C. Rudge^{1*}, Fátima P. Souza², Joelcio F. Abbade¹, Raghavendra L. S. Hallur¹, João Paulo C. Marcondes¹, Fernanda Piculo^{1,3}, Gabriela Marini^{1,4}, Giovana Vesentini¹, Lehana Thabane^{5,6}, Steven S. Witkin^{7,8}, Iracema M. P. Calderon¹, Angélica M. P. Barbosa^{1,9}, and The Diamater Study Group

Abstract

Background: Pelvic floor muscles (PFM) and rectus abdominis muscles (RAM) of pregnant diabetic rats exhibit atrophy, co-localization of fast and slow fibers and an increased collagen type I/III ratio. However, the role of similar PFM or RAM hyperglycemic-related myopathy in women with gestational diabetes mellitus (GDM) remains poorly investigated. This study aims to assess the frequency of pelvic floor muscle disorders and pregnancy-specific urinary incontinence (PS-UI) 12 months after the Cesarean (C) section in women with GDM. Specifically, differences in PFM/RAM hyperglycemic myopathy will be evaluated.

Methods: The Diamater is an ongoing cohort study of four groups of 59 pregnant women each from the Perinatal Diabetes Research Centre (PDRC), Botucatu Medical School (FMB)-UNESP (São Paulo State University), Brazil. Diagnosis of GDM and PS-UI will be made at 24–26 weeks, with a follow-up at 34–38 weeks of gestation. Inclusion in the study will occur at the time of C-section, and patients will be followed at 24–48 h, 6 weeks and 6 and 12 months postpartum. Study groups will be classified as (1) GDM plus PS-UI; (2) GDM without PS-UI; (3) Non-GDM plus PS-UI; and (4) Non-GDM without PS-UI. We will analyze relationships between GDM, PS-UI and hyperglycemic myopathy at 12 months after C-section. The mediator variables to be evaluated include digital palpation, vaginal squeeze pressure, 3D pelvic floor ultrasound, and 3D RAM ultrasound. RAM samples obtained during C-section will be analyzed for ex-vivo contractility, morphological, molecular and OMICS profiles to further characterize the hyperglycemic myopathy. Additional variables to be evaluated include maternal age, socioeconomic status, educational level, ethnicity, body mass index, weight gain during pregnancy, quality of glycemic control and insulin therapy.

Discussion: To our knowledge, this will be the first study to provide data on the prevalence of PS-UI and RAM and PFM physical and biomolecular muscle profiles after C-section in mothers with GDM. The longitudinal design allows for the assessment of cause-effect relationships between GDM, PS-UI, and PFMs and RAMs myopathy. The findings may reveal previously undetermined consequences of GDM.

Keywords: Gestational diabetes mellitus, Hyperglycemic myopathy, Pelvic floor muscles, Rectus abdominis muscles, Urinary incontinence, Proteomics, Collagen, Electromyography, Transmission electron microscopy

* Correspondence: marilzarudge@gmail.com

¹Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), CEP18618-687, Sao Paulo, Brazil

Full list of author information is available at the end of the article



© The Author(s). 2020 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

ANEXO 2

Seleção pública para função docente

Publicado no DOE de 24-12-20, pg. 168 , Seção I

CAMPUS DE MARÍLIA
Faculdade de Filosofia e Ciências

EDITAL Nº 172/2020-STDARH-RESULTADO FINAL

A Diretora da Faculdade de Filosofia e Ciências da UNESP – Campus de Marília, por meio da área de Recursos Humanos, torna público o resultado final do concurso público de Provas e Títulos para contratação de 01 PROFESSOR SUBSTITUTO, por prazo determinado, em caráter emergencial para atender excepcional interesse público, no período letivo de 2020, e pelo prazo máximo de 5 meses, em 12 horas semanais de trabalho, sob o regime jurídico da CLT e legislação complementar, na disciplina: Estágio Supervisionado em Fisioterapia em Saúde Coletiva, junto ao Departamento de Fisioterapia e Terapia Ocupacional da Faculdade de Filosofia e Ciências do Câmpus de Marília, conforme segue:

CANDIDATOS HABILITADOS:

BEATRIZ MENDES TOZIM RG 469656657

EXAMINADOR / ANÁLISE CURRICULAR (P.2) / DIDÁTICA (P.1) / MÉDIA PONDERADA

PROF. DR. MARCOS EDUARDO SCHEICHER	18,60	8,70	9,10
DRª. AILA NARENE DAHWACHE CRIADO ROCHA	18,60	9,00	9,20
DRª. CRISTIANE PAIVA ALVES	18,60	8,40	9,00
MÉDIA FINAL / CLASSIFICAÇÃO: 9,10 / 1º lugar			

JULIANO FERREIRA ARCURI RG 43494774X

EXAMINADOR / ANÁLISE CURRICULAR (P.2) / DIDÁTICA (P.1) / MÉDIA PONDERADA

PROF. DR. MARCOS EDUARDO SCHEICHER	18,40	7,30	8,57
DRª. AILA NARENE DAHWACHE CRIADO ROCHA	18,40	6,40	8,27
DRª. CRISTIANE PAIVA ALVES	18,40	6,40	8,27
MÉDIA FINAL / CLASSIFICAÇÃO: 8,37 / 2º lugar			

CAROLINE BALDINI PRUDENCIO RG 362756910

EXAMINADOR / ANÁLISE CURRICULAR (P.2) / DIDÁTICA (P.1) / MÉDIA PONDERADA

PROF. DR. MARCOS EDUARDO SCHEICHER	11,20	9,80	7,00
DRª. AILA NARENE DAHWACHE CRIADO ROCHA	11,20	9,80	7,00
DRª. CRISTIANE PAIVA ALVES	11,20	10,00	7,07
MÉDIA FINAL / CLASSIFICAÇÃO: 7,02 / 3º lugar			

ANEXO 3

Cover Letter – Article 1

Cover Letter

Dear Dr.,

Please find enclosed our manuscript entitled "Gestational diabetes is associated with alteration on pelvic floor muscles activation pattern during pregnancy and postpartum: Prospective Cohort using electromyography assessment" which we request you to consider review the new version that met the reviewers' considerations for publication as an original research article in the Journal Plos One.

With respect to gestational diabetes mellitus (GDM), few studies have been conducted on deleterious effects on muscle and the consequences to muscle function. As proper pelvic floor function depends on the integrity and synergy of structures altered by GDM, GDM is hypothesized to decrease pelvic floor muscle (PFM) activity in pregnant women. In this study, we examined the effect of GDM on PFM electromyography (EMG) activity in pregnant women from 24–30 weeks of gestation, 36–38 weeks of gestation and 18-24 months of postpartum through a prospective cohort study. We performed EMG of PFM in nulliparous or primiparous women who had one previous elective cesarean delivery and GDM; we also performed glazer protocol analysis at the 3 time points to assess the progress of PFM activation pattern during resting and contractions. According to the demographic and obstetric data, homogeneity was observed between the groups. Alterations on different EMG PFM pattern were found on the GDM group.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership. The manuscript has been carefully reviewed by an experienced editor whose first language is English again and who specializes in editing papers written by scientists whose native language is not English. This manuscript has not been published elsewhere and is not under consideration by another journal. There are no conflicts of interest to declare.

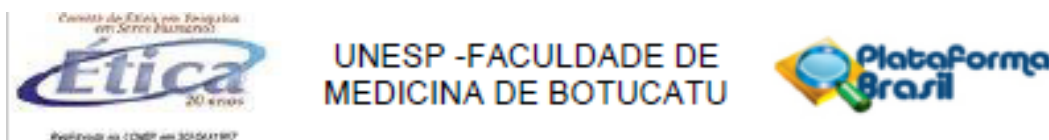
We look forward to hearing from you at your earliest convenience.

Sincerely,

Caroline Baldini Prudencio

ANEXO 4

Parecer consubstanciado do comitê de ética em pesquisa



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 Izalas 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 Bameze Costa/ Nível: Doutorado).

Pesquisador: Marilza Vieira Cunha Rudge

Área Temática:

Versão: 5

CAAE: 82225617.0.0000.5411

Instituição Proponente: Departamento de Ginecologia e Obstetrícia

Patrocinador Principal: FUNDAÇÃO DE AMPARO A PESQUISA DO ESTADO DE SÃO PAULO

DADOS DO PARECER

Número do Parecer: 3.747.338

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

Continuação do Parecer: 3.747.338

Apresentação do Projeto:

Trata a presente solicitação de emenda ao projeto em questão referente ao envio de amostras para análise em dois destinos diferentes, com a finalidade de aprendizado da técnica e maior sensibilidade/especificidade da técnica adotada.

Objetivo da Pesquisa:

Avallar a solicitação de emenda ao presente projeto para envio de amostras a dois destinos diferentes.

Avaliação dos Riscos e Benefícios:

Já avaliados.

Comentários e Considerações sobre a Pesquisa:

A pesquisadora solicita a emenda ao presente projeto para envio de amostras para dois destinos diferentes:

1) para Londres - UK: com a finalidade de envio de amostras já coletadas para o mesmo fim aprovado anteriormente por este CEP, sendo exclusivamente para aprendizado da técnica pela aluna de doutorado (Dra Juliana) com o grupo em questão. Os pesquisadores informam que não haverá alteração no projeto anteriormente aprovado.

2) Para Lincoln / Nebraska - EUA: com a finalidade de envio de amostras já coletadas para o mesmo fim aprovado anteriormente por este CEP, sendo para substituição da técnica anteriormente descrita (espectroscopia por RMN) pela técnica de 2 espectrômetro de massa, justificando maior especificidade e sensibilidade destas técnicas. Os pesquisadores informam que não haverá alteração no projeto anteriormente aprovado.

Os pesquisadores informam que o envio de amostras para o exterior, será feito por transportadora especializada], devendo seguir legislação vigente brasileira.

Considerações sobre os Termos de apresentação obrigatória:

Já avaliados.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

Após análise em REUNIÃO EXTRAORDINÁRIA, 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

Endereço: Chácara Butignoll, s/n	CEP: 18.618-970
Bairro: Rubião Junior	
UF: SP	Município: BOTUCATU
Telefone: (14)3880-1509	E-mail: cep@fmb.unesp.br

Documento 00 de 04

Continuação do Parecer: 3.747.338

Pesquisa FMB/UNESP, realizada em 19/11/2019, a EMENDA apresentada encontra-se APROVADA.

Atenciosamente,

Comitê de Ética em Pesquisa FMB/UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1440649_É2.pdf	23/09/2019 15:11:41		Acelto
Outros	OficioLondres.doc	23/09/2019 15:10:28	Marliza Vieira Cunha Rudge	Acelto
Outros	OficioenvioEUA.doc	23/09/2019 15:09:10	Marliza Vieira Cunha Rudge	Acelto
Outros	Oficioexplicativo_envio.docx	23/09/2019 15:06:38	Marliza Vieira Cunha Rudge	Acelto
Outros	Carta_resposta_comite_c.pdf	11/04/2019 13:41:35	Marliza Vieira Cunha Rudge	Acelto
Folha de Rosto	FolhaDeRostoAssinada_Sub_Proje_4_Sarah.pdf	29/01/2019 11:51:24	Sarah Maria Bameze Costa	Acelto
Outros	TermoDeAnuenciainstitucional_Sub_Proj_4_Sarah.pdf	29/01/2019 11:44:05	Sarah Maria Bameze Costa	Acelto
Projeto Detalhado / Brochura Investigador	Oficio_ao_comite_sub_proj_4_Sarah.pdf	29/01/2019 11:41:10	Sarah Maria Bameze Costa	Acelto
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_tematico_com_subprojeto_4_de_Sarah_Bameze.pdf	29/01/2019 11:35:52	Sarah Maria Bameze Costa	Acelto
Projeto Detalhado / Brochura Investigador	Projeto_doutorado_Sarah_Bameze_sub_projeto_4.pdf	29/01/2019 11:32:45	Sarah Maria Bameze Costa	Acelto
Outros	EAP_Marliza_Rudge.pdf	18/01/2018 17:27:08	Marliza Vieira Cunha Rudge	Acelto
Outros	Fluxograma_Tematico_CEP.pptx	26/11/2017 19:18:14	Marliza Vieira Cunha Rudge	Acelto
Outros	HRA.pdf	26/11/2017 19:09:17	Marliza Vieira Cunha Rudge	Acelto
Outros	termodeautorizacao_HRA.pdf	26/11/2017 19:01:53	Marliza Vieira Cunha Rudge	Acelto

Endereço: Chácara Butgnoll, s/n

Bairro: Rubião Junior

CEP: 18.618-970

UF: SP

Município: BOTUCATU

Telefone: (14)3880-1609

E-mail: cep@fmb.unesp.br

Continuação do Parecer: 3.747.338

Outros	coparticipacao_assis_municipio.jpeg	26/11/2017 18:53:44	Marliza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_3.doc	05/10/2017 23:41:43	Marliza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_2.docx	05/10/2017 23:41:04	Marliza Vieira Cunha Rudge	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_mae.docx	05/10/2017 23:39:04	Marliza Vieira Cunha Rudge	Aceito
Outros	Projeto_mae_Subprojetos.docx	05/10/2017 23:37:51	Marliza Vieira Cunha Rudge	Aceito
Orçamento	orcamento_tematico.pdf	05/10/2017 23:36:42	Marliza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_1.doc	05/10/2017 22:54:11	Marliza Vieira Cunha Rudge	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_tematico.docx	05/10/2017 22:48:47	Marliza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_FFC.pdf	21/09/2017 17:02:46	Marliza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Famema.jpeg	21/09/2017 17:02:11	Marliza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Araraquara.pdf	21/09/2017 17:00:09	Marliza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Bauru.pdf	21/09/2017 16:59:28	Marliza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_RioPreto.pdf	21/09/2017 16:58:52	Marliza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_RioClaro.pdf	21/09/2017 16:57:58	Marliza Vieira Cunha Rudge	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

BOTUCATU, 05 de Dezembro de 2019

Assinado por:
 Trajano Sardenberg
 (Coordenador(a))

Endereço: Chácara Butignoli, s/n
 Bairro: Rubião Junior CEP: 18.618-970
 UF: SP Município: BOTUCATU
 Telefone: (14)3880-1509 E-mail: cep@fmb.unesp.br

Seção 6
Apêndices

APÊNDICE 1

Termo de Consentimento Livre e Esclarecido (TCLE)

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

A sra. está sendo convidada a participar de uma pesquisa intitulada "Estudo de coorte prospectivo: nova triade 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 Sartorio Filho/ Nível: Doutorado);

() Subprojeto 3: Coorte prospectiva da triade 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).

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 fazem parte de um estudo com demais mulheres que também responderão nos 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 diabetes melito gestacional e também sem diabetes 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 aliquotas no Biorrepositorio. 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 Biorrepositorio. 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 a 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 retirá-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, a 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 contato 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 Chacara Butignelli s/nº em Rubião Junior – 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 deste 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: _____

Apêndice 2

Ficha de Avaliação

Data: ___/___/___	FICHA DE AVALIAÇÃO	Planilhado? () Sim () Não
ID: _____	PROJETO TEMÁTICO	Centro: _____
Nome: _____		D.N: _____
Idade: _____		
CRITÉRIOS DE INELEGIBILIDADE:		
() convite	() TCLE	() Diabete Tipo II
() Gemelar	() Diabete tipo I	() HAS ou HIG
() IU prévia a gestação		() cirurgia prévia IU
		() cirurgia prévia distopia
Dados Pessoais		
CPF: _____	RG: _____	Cartão SUS: _____
Endereço (nº/ complemento): _____		
Bairro: _____	Cidade/estado: _____	CEP: _____
Estado Civil: () Solteira () Casado () Amasiado () Divorciado () Viuva	Profissão: _____	
Escolaridade: () Analfabeto () Fundamental () Médio Completo () Médio Incompleto () Superior Comp		
() superior incomp	Etnia: () Asiática () branca () negra () parda () indígena	
Religião: () atea () Católica () Evangélica () Não tem () outra		Tel Residencial: _____
Tel Trabalho: _____	Celular: _____	E-mail: _____
Dados Clínicos		
() Cirurgia Abdominal	() Diabete Familiar	() Infecção Ativa HIV, Hep A, B, C e/ou sífilis
() ITU	() doenças desmielinizantes	() d. neurológicas
() neoplasias anteriores e atuais	() Tosse Crônica	() Constipação/Esforço
() I. Fecal	() HAS prévio	
() Bebe ___ doses/semana	() Fuma ___ cigarros/dia	() Drogas _____
() Trombofilia	() Ex físico 2x+/semana por 1h qual _____ tempo _____	() Outras _____
Dados Obstétricos		
Data Coleta: ___/___/___	Paridade: ___ G ___ C ___ V ___ A	Data Ultimo PV: ___/___/___
Data Ultimo PC: ___/___/___	Peso maior RN: _____	Maior ganho de peso: _____
Tempo TP: _____	() DMG prévia	() Gemelar Prévio
() Episiotomia n _____	() Outros _____	
Coleta 24-28 semanas		
DUM: ___/___/___	DPP: ___/___/___	SG: s d () Planejou a gestação () Preservativo
Peso atual: _____	Peso Prévio: _____	Altura: _____
() Placenta Prévia	() ITU	Outros: _____
Data Glicemia: ___/___/___	GJ: _____	Media Glicêmica: _____
GTT: _____; _____; _____	Hb: _____	
PG 8; 10; 12; 14; 16; 18	() Rastreamento Negativo	
Afirmar: "Muitas mulheres e gestantes se queixam que escapa urina sem querer e molham um pouco a calcinha ou chegam a molhar até mesmo a roupa."		
() Sim () quando espirrou, tossiu ou fez esforço? () mta vontade de urinar e não conseguiu chegar		
ISI: 1 _____ 2 _____	ICIQ: 1 _____ 2 _____ 3 _____ 4 _____	
() ativa sexualmente	1 _____ 2 _____ 3 _____ 4 _____ 5 _____ 6 _____ 7 _____ 8 _____	
9 _____ 10 _____ 11 _____ 12 _____ 13 _____ 14 _____ 15 _____ 16 _____ 17 _____ 18 _____ 19 _____		
Exame Físico		
P: _____ E: _____ R: _____ F: _____ E: _____ C: _____ T: _____	() Gluteo () Adutor () Abd	
() EMG () US	Justificativa: _____	
Coleta Sangue: Data ___/___/___	Horário: _____	Justificativa: _____
Ca: _____	Calcitonina: _____	PTH: _____
Vit D: _____	Vit A: _____	Glic: _____
K: _____	Insulina: _____	
Relaxina: _____	OCL7: _____	Fósforo: _____
Cortizol: _____	Etiqueta: L ___ G ___ ID ___ M ___	

Coleta 36-38 semanas ____/____/____					
SG: ____s ____d	Peso atual: _____	<input type="checkbox"/> ITU <input type="checkbox"/> Sangramento Vaginal		Outros: _____	
Data Glicemia: ____/____/____	GJ: _____	Media Glicemica: _____	GTT: _____; _____	Hb: _____	
PG 8: _____; 10: _____; 12: _____; 14: _____; 16: _____; 18: _____	<input type="checkbox"/> Rastreamento Negativo				
Afirmar: "Muitas mulheres e gestantes se queixam que escapa urina sem querer e molham um pouco a calcinha ou chegam a molhar até mesmo a roupa."					
<input type="checkbox"/> Sim <input type="checkbox"/> quando espirrou, tossiu ou fez esforço? <input type="checkbox"/> mta vontade de urinar e não conseguiu chegar					
ISI: 1 _____ 2 _____	ICIQ: 1 _____ 2 _____ 3 _____ 4 _____				

Data: ____/____/____ FICHA DE AVALIAÇÃO Planilhado? Sim Não
 ID: _____ PROJETO TEMÁTICO Centro: _____

<input type="checkbox"/> ativa sexualmente	1 _____	2 _____	3 _____	4 _____	5 _____	6 _____	7 _____	8 _____
9 _____	10 _____	11 _____	12 _____	13 _____	14 _____	15 _____	16 _____	17 _____
Exame Físico								
P: _____	E: _____	R: _____	F: _____	E: _____	C: _____	T: _____	<input type="checkbox"/> Gluteo <input type="checkbox"/> Adutor <input type="checkbox"/> Abd	
<input type="checkbox"/> EMG <input type="checkbox"/> US		Justificativa: _____						
Coleta Sangue: Data ____/____/____			Horário: ____:____			Justificativa: _____		
Ca: _____	Calcitonina: _____	PTH: _____	Vit D: _____	Vit A: _____	Glic: _____	K: _____	Insulina: _____	
Relaxina: _____	CCL7: _____	Fósforo: _____	Cortizol: _____		Etiqueta: L G ID M			

Coleta 18 meses ____/____/____								
ISI: 1 _____ 2 _____	ICIQ: 1 _____ 2 _____ 3 _____ 4 _____							
<input type="checkbox"/> ativa sexualmente	1 _____	2 _____	3 _____	4 _____	5 _____	6 _____	7 _____	8 _____
9 _____	10 _____	11 _____	12 _____	13 _____	14 _____	15 _____	16 _____	17 _____
Exame Físico								
P: _____	E: _____	R: _____	F: _____	E: _____	C: _____	T: _____	<input type="checkbox"/> Gluteo <input type="checkbox"/> Adutor <input type="checkbox"/> Abd	
<input type="checkbox"/> EMG <input type="checkbox"/> US		Justificativa: _____						
Coleta Sangue: Data ____/____/____			Horário: ____:____			Justificativa: _____		
Ca: _____	Calcitonina: _____	PTH: _____	Vit D: _____	Vit A: _____	Glic: _____	K: _____	Insulina: _____	
Relaxina: _____	CCL7: _____	Fósforo: _____	Cortizol: _____		Etiqueta: L G ID M			

Coleta 24 meses ____/____/____								
ISI: 1 _____ 2 _____	ICIQ: 1 _____ 2 _____ 3 _____ 4 _____							
<input type="checkbox"/> ativa sexualmente	1 _____	2 _____	3 _____	4 _____	5 _____	6 _____	7 _____	8 _____
9 _____	10 _____	11 _____	12 _____	13 _____	14 _____	15 _____	16 _____	17 _____
Exame Físico								
P: _____	E: _____	R: _____	F: _____	E: _____	C: _____	T: _____	<input type="checkbox"/> Gluteo <input type="checkbox"/> Adutor <input type="checkbox"/> Abd	
<input type="checkbox"/> EMG <input type="checkbox"/> US		Justificativa: _____						
Coleta Sangue: Data ____/____/____			Horário: ____:____			Justificativa: _____		
Ca: _____	Calcitonina: _____	PTH: _____	Vit D: _____	Vit A: _____	Glic: _____	K: _____	Insulina: _____	
Relaxina: _____	CCL7: _____	Fósforo: _____	Cortizol: _____		Etiqueta: L G ID M			
Descontinuidade: qual momento? _____ <input type="checkbox"/> óbito fetal <input type="checkbox"/> desistência <input type="checkbox"/> impossibilidade de consulta								