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FACULDADE DE MEDICINA

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Caracterização da incontinência urinária específica da gestação por ensaio de miografia *ex vivo* no músculo reto abdominal: Estudo de corte transversal aninhado a coorte do Projeto DIAMATER

Pregnancy-specific urinary incontinence characterization by rectus abdominis muscle ex vivo myography assay: Cross-sectional study nested within the DIAMATER Project cohort

Dissertação apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, como parte dos requisitos para obtenção do título de Mestre em Tocoginecologia

Orientadora: Prof.ª Emérita Marilza Vieira Cunha Rudge
Coorientadores: Prof.ª Dra. Angélica Mércia Pascon Barbosa
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Botucatu, 25 de fevereiro de 2022

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Em primeiro lugar a **Deus**, por sempre guiar e iluminar o meu caminho e por ter me dado força, fé e perseverança para a concretização deste trabalho.

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Epígrafe

“Conheça todas as teorias, domine todas as
técnicas, mas ao tocar uma alma
humana, seja apenas outra alma humana”.

Carl Jung

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Lista de abreviações

Português e Inglês

Lista de abreviações em Português

CEP	Comitê de Ética em Pesquisa
CIDPN	Centro de Investigação do Diabete Perinatal
DM	diabetes mellitus
DMG	*diabetes mellitus* gestacional
Dra.	doutora
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
FFC	Faculdade de Filosofia e Ciências
FMB	Faculdade de Medicina de Botucatu
IU	incontinência urinária
IU-EG	incontinência urinária específica da gestação
MAP	músculos do assoalho pélvico
MRA	músculo reto abdominal
Prof.ª	professora
UNESP	Universidade Estadual Paulista

Lista de abreviações em Inglês

%	percentage
ADA	American Diabetes Association
BMI	body mass index
Cm	centimeter
Cm^2	square centimeter
DM	diabetes mellitus
GDM	Gestational Diabetes Mellitus
ICS	International Continence Society
IUGA	International Urogynecological Association
Max	maximum
Min	minimum
PDRC	Perinatal Diabetes Research Center
PFM	pelvic floor muscles
PSUI	Pregnancy-specific urinary incontinence
UI	urinary incontinence

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Seção 1

Trajetória Acadêmica

Iniciei a minha formação acadêmica em 2002, ingressando no curso de Fisioterapia pela Faculdade Santa Terezinha - CEST, em São Luís - MA. Nesses 4 anos e meio pude me dedicar intensamente, aprender e amadurecer como profissional, me apaixonando ainda mais pela fisioterapia.

Atuei durante 8 anos (2008-2016) no Hospital e Maternidade Marly Sarney (HMMS), referência em atendimento no estado do Maranhão, onde, após a graduação, tive o primeiro contato com gestantes de risco habitual e alto risco.

Fui coordenadora da Clínica de Saúde Integrada entre 2012 e 2016, além de ter trabalhado em diversas clínicas e estúdios em São Luís e em Parnaíba - PI.

Tive a oportunidade de imergir em uma nova cultura e aperfeiçoar o idioma (inglês) quando morei no Canadá, de fevereiro de 2016 a fevereiro de 2017, pois além de ter participado de voluntariados, pude estudar e trabalhar.

Ao retornar, iniciei a pós-graduação em Fisioterapia Pélvica e Uroginecologia Funcional e, em seguida, Fisioterapia Dermatofuncional, tendo realizado os dois trabalhos de conclusão de curso com a temática “Saúde da Mulher”, o que reacendeu o meu desejo de voltar a atuar nessa área. Considero que esses dois títulos foram essenciais para o impulso e continuidade da minha jornada acadêmica.

Em 2019, antes de entrar oficialmente no PPG em Tocoginecologia como mestranda, integrei o grupo de pesquisa “Diabete e Gravidez – Clínico e Experimental” da Faculdade de Medicina de Botucatu (FMB – Unesp), liderado pela Profa. Emérita Marilza VC Rudge, participando de reuniões, treinamentos e coletas, me permitindo, assim, realizar o mestrado em um período menor que o habitual.

Em 2021 iniciei o mestrado pelo Projeto Temático FAPESP DIAMATER, "*The Diamater Study Group*", sob a orientação da Profa. Emérita Marilza Rudge e coorientação da Profa. Dra. Angélica Barbosa e do Dr. David Reyes.

No decorrer do ano, além de dar seguimento ao meu projeto, participei de eventos internacionais (Anexo 1), como "*Diabetes, Obesity and Pregnancy Outcomes*", "*Obesity Inflammasome as a Risk for Developing Gestational Diabetes*" e "*Placental Adaptation in Gestational Diabetes and Obesity*" e fui contemplada com uma bolsa Treinamento Técnico 3 (TT3) da FAPESP (Anexo 2), com o projeto intitulado: "Treino e aperfeiçoamento na execução do preparo e análise da contratilidade *ex vivo* do músculo reto abdominal de gestantes", sob orientação da Profa. Emérita Marilza Rudge.

Foi possível ainda colaborar em 4 artigos, sendo um publicado e demais em fase de submissão para revistas de alto impacto.

Artigo publicado: *Effectiveness of the pelvic floor muscle training on muscular dysfunction and pregnant specific urinary incontinence in pregnant women with gestational diabetes mellitus: A systematic review protocol* (Anexo 3).

Tenho o intuito de concluir o mestrado, continuar me aperfeiçoando e adquirir conhecimento constante pensando nos benefícios para a saúde das mulheres. Além disso, almejo fazer a diferença no cenário acadêmico e científico, dando continuidade aos estudos por meio do Doutorado.

Seção 2
Contextualização

Incontinência urinária específica da gestação e sua relação com os músculos abdominais

A incontinência urinária (IU), queixa frequente em todo o mundo, causa angústia e constrangimento, além de gastos significativos, tanto para o indivíduo quanto para a sociedade. As estimativas de prevalência variam de acordo com a definição de incontinência e a população analisada. No entanto, há consenso universal sobre a importância do problema em termos de sofrimento humano e custo econômico (1,2).

De acordo com a *International Urogynecological Association* (IUGA) e a *International Continence Society* (ICS) pode transcorrer de sintoma isolado, como na incontinência urinária de esforço (ao tossir, espirrar ou no esforço físico) (3) ou associado, quando a incontinência urinária de urgência é acompanhada de urgência e frequência (4).

A IU é definida por qualquer perda involuntária de urina. Da mesma forma, quando iniciada na gestação estabelece a IU específica da gestação (IU-EG), identificada por autorrelato (5), e com incidência de 26% (6) a 41,8% (7). Embora a fisiopatologia da IU-EG ainda não esteja totalmente compreendida, a literatura traz como causa as alterações mecânicas e hormonais ocorridas neste período (2)

Mais de um terço das mulheres vivenciam essa perda indesejada no segundo e no terceiro trimestre da gestação e, cerca de um terço, nos primeiros três meses após o parto (8), podendo afetar negativamente na qualidade de vida (QV) (9) e na função sexual (10). Embora durante a gestação afete duas em cada três mulheres, apenas uma em cada oito procura ajuda profissional. Os principais motivos variam desde mínimo incômodo até a ideia de que a IU se

resolverá sozinha (11,12).

A cavidade abdominopélvica é formada por músculos, ossos e estrutura aponeurótica, que circunda os órgãos pélvicos e abdominais. Dentre os músculos que compõe esta região, encontram-se os músculos reto abdominal (MRA) e o músculos do assoalho pélvico (MAP), que estão predominantemente envolvidos na estabilização lombo-pélvica e continência urinária (13–19). O MRA tem atividade contrátil sinérgica com os músculos do assoalho pélvico no trabalho de parto e no mecanismo de continência urinária (20–22).

A contração sinérgica vem sendo observada em mulheres assintomáticas (23,24). Por outro lado, ainda não existe consenso na literatura se mulheres com IU apresentam ou não resposta sincrônica dos MAP e dos músculos abdominais (25–27). Alguns autores (27–31) sugerem que a presença dos sintomas miccionais esteja relacionada à ação descoordenada entre estes músculos, caracterizada pela reposta muscular dos MAP inferior à resposta muscular abdominal, bem como a lentidão entre o tempo de ativação destes músculos (18,32–34).

Estudos indicam a participação efetiva do MRA em mulheres com continência urinária e demonstraram que quando ausente ou enfraquecidos apresentam sintomas de IU. A ação coordenada destes músculos controla e modula a pressão intra-abdominal e desempenha papel relevante no controle da postura, dos movimentos do tronco e da estabilidade pélvica, além de responder as solicitações respiratórias, aumento de pressão intra-abdominal e participar da manutenção da continência urinária (29,35).

Durante a gravidez, o corpo da gestante passa por diversas adaptações fisiológicas. Como consequência ao permitir o crescimento uterino,

há estiramento do MRA, ocorrendo separação dos feixes dos MRA em relação a linha alba, podendo dar origem à diástase do músculo reto abdominal, considerado patológico quando o afastamento é superior a três centímetros (36,37). Sua modificação de comprimento pode resultar em alteração em sua capacidade de produzir o torque, interferindo no vetor de força muscular (38) e repercutindo negativamente no pós-parto (36).

Evidências de revisão sistemática demonstraram que a co-contração do MAP e dos músculos abdominais ocorre tanto na contração voluntária do assoalho pélvico quanto nas manobras abdominais e que o músculo reto abdominal (MRA) deve participar dos mecanismos de continência urinária (23). A partir desses resultados, a dificuldade ética na obtenção de amostras do MAP no parto vaginal sem episiotomia pode ser contornada por MRA na cesárea com indicação obstétrica, permitindo estudos detalhados da contratilidade muscular (39–41).

Além das alterações estruturais do MRA, que devem impactar na atividade contrátil, a avaliação da contratilidade *ex vivo* é desafio a ser vencido: é músculo sem tendão, que se estende ao longo do abdome até a sínfise púbica (20,42–45), o que dificulta a realização da técnica de miografia (46–48). Por ser músculo estriado, o principal foco das pesquisas tem sido as células musculares contráteis (49).

Nenhum estudo foi desenvolvido com enfoque na análise conjunta das características da contração *ex vivo* do MRA *e* suas respectivas alterações estruturais (potencial mediadora) em mulheres com IU-EG (variável de exposição) na ocorrência de IU pós-cesárea (variável de desfecho). Assim, justificam-se novas investigações envolvendo esses mediadores estruturais e

funcionais do MRA para melhor compreensão do papel da IU-EG e sua associação com a IU pós-parto. Os resultados obtidos permitirão estabelecer estratégias, com base científica, para o tratamento adequado dessas mulheres na gestação e pós-parto.

Esta dissertação de mestrado é um dos subprojetos da coorte DIAMATER que está desenvolvimento desde 2016, na busca da consolidação da tríade DMG-IU-EG-MHG e sua potencial associação com a IU pós-parto. Como outros subprojetos, tem como base o "*Integrated Diamater Conceptual Model*" (Figura 1).

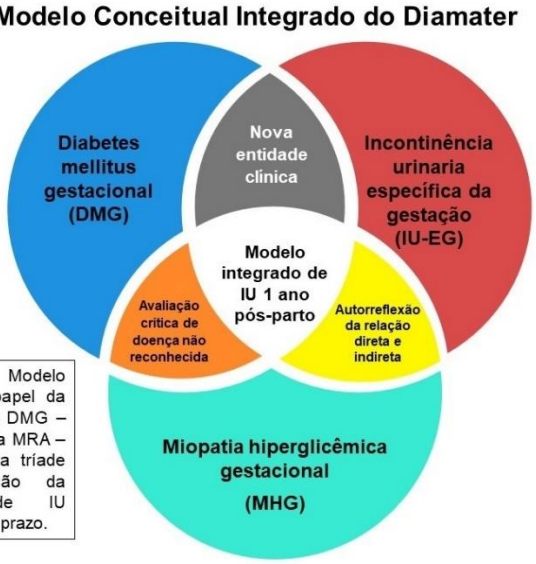

Figura 1 – Modelo Conceitual do papel da integração entre DMG – IU-EG e miopatia MRA – MAP como nova tríade na determinação da prevalência de IU materna a longo prazo.

Portanto, o objetivo deste trabalho foi caracterizar a IU-EG em mulheres com tolerância normal à glicose por ensaio de miografia *ex vivo* no músculo reto abdominal coletado durante o parto cesáreo, com indicação obstétrica.

A partir dos resultados deste estudo pretende-se contribuir para o estabelecimento de ações de rotina de diagnóstico, prevenção e de tratamento das alterações do MRA de gestantes com IU-EG. Embasado na literatura e em dados de pesquisas prévias, acredita-se encontrar alterações anátomo-funcionais do MRA nos grupos com IU-EG.

Espera-se que os resultados obtidos nesta pesquisa auxiliem a prática clínica, ampliando as áreas de investigação e de intervenção, contribuindo na saúde da gestante com IU-EG. Será possível divulgar os resultados deste estudo com apresentação de trabalhos em eventos e periódicos de relevância científica e destacar, nacional e internacionalmente, a UNESP.

Diante de todos os impactos e circunstâncias que a IU pode ocasionar na vida da mulher, os estudos comprovam a necessidade de a qualidade de vida ser considerada como parâmetro primordial da avalição, no qual as ações dos profissionais de saúde durante o planejamento e orientação do tratamento vão se centrar, revelando-se essenciais para o processo de recuperação. Logo, esses profissionais devem priorizar desenvolver uma assistência focada na promoção da saúde e em processos terapêuticos, com intuito de interferir na redução das repercussões negativas e suas complicações, bem como melhor direcionar a prática clínica nos serviços de saúde.

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Seção 3

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Ex vivo myo-mechanical assay of pregnancy - Isolated Rectus Abdominis Muscle in Pregnancy-Specific Urinary Incontinence: Cross-sectional study nested within the Diamater Project cohort.

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ABSTRACT

Introduction: Pregnancy-Specific Urinary Incontinence (PSUI) is a predictor variable of long-term UI. We have previously demonstrated that Rectus Abdominis Muscle (RAM) myopathy is the underlie mechanism. Selective *ex vivo* myo-mechanical assay (MMA) of RAM is unknown in healthy pregnant women with PSUI. **Aims:** Understand the relationship between PSUI and continent pregnant women with normal glucose tolerance and explore an established protocol for qualitative and quantitative peak and graph analysis of fresh RAM samples acquired at C-section from mothers with normal glucose tolerance with and without (the control group) PSUI, applying *ex vivo* myography. **Method:** A cross-sectional study nested within a cohort was performed using ex vivo myography assay in 87 mothers with normal glucose tolerance with (n= 48) and without (n = 39) PSUI. General data were extracted from database. RAM samples were collected at C-section for *ex vivo* myography qualitative and quantitative analysis (initial baseline, final baseline, peak, strength, and duration time). Statistical analysis: Continuous variable results were expressed as mean and standard deviation and categories by number and percentage (%). **Results:** We found no overall specific quantitative parameters of window analysis and contractile response of RAM *ex vivo* MMA. A progressive and continuous decline was mostly confined to the peak and strength in both groups. Qualitative contractile response of RAM *ex vivo* MMA using peak and strength parameters allow us to demonstrate three behaviors of contractile response of RAM under electrical stimulation: progressive decrease in strength; sudden muscular arrest and asynchrony (disorderly; messy) register with ups and downs. **Conclusion:** Deep quantitative analysis of RAM ex vivo analysis did not benefit the results to differentiate both groups. Qualitative analysis improved the differentiation between both groups by demonstrating a tendency of loss the progressive fall of the peaks observed in control group.

Keywords – pregnancy; urinary incontinence; rectus abdominis muscle; myography; *ex vivo* analysis.

1 INTRODUCTION

Pregnancy is one of the main risk factors for long-term urinary incontinence (UI), regardless the Cesarean (C-section) or vaginal delivery route (1–5). This development is due to problems which leads to disorders in skeletal muscle structure required to maintain urinary continence (6). Women who experience UI for the first time during pregnancy, termed pregnancy-specific UI (PSUI) (3) have been shown to be more likely to develop long UI (3,4).

PSUI is a disease undervalued during prenatal care assistance and not reported in obstetric classic textbooks (7,8). This terminology was described in 2002 by Hvidman *et al*. (3) and its etiology is related to hormonal factors of pregnancy.

Previously, we demonstrated that GDM is a strong predictor of pelvic floor muscle (PFM) dysfunction and UI for up two years postpartum (2) having rectus abdominis muscle (RAM) or PFM hyperglycemic-related myopathy as a potential mediator (9). Although, it is unknown if PSUI in mothers with normal glucose tolerance exhibits similar myopathy (9). In this context, selective *ex vivo* myo-mechanical assay (MMA) of these muscle lesions is considerably unknown in healthy pregnant women with PSUI.

PSUI and its association with the long-term UI

The incidence of PSUI ranges from 26% (10) to 41.8% (11). The question of how to prevent long-term UI has led to growing interest in the impact of various potential risk factors for this disorder. The processes that lead to PSUI play an important role for understanding the future occurrence of periods with UI episodes during a woman's adult life (long UI) (3). PSUI considerably increases, up to 67%,

the prevalence of UI in subsequent pregnancies (12–16). This demonstrates that this signal, overlooked in prenatal care, should be carefully investigated. Previous clinical findings (2) showed that the overall prevalence rates of PSUI and UI 2 years postpartum in primiparous women after C-section were 31.6 and 18.4%, respectively. Stress UI was the most common pattern during the first year postpartum, which is consistent with previous inquiries (17,18). Early findings have shown that obesity (19), hormonal status (20), risk of PSUI (17) and risk of postpartum pelvic floor dysfunction are associated with a clinical history of PSUI (21).

Ex vivo myo-mechanical assay

Ex vivo MMA would be an important tool in the approach to evaluating myo-mechanical properties of the skeletal muscle associated with PSUI, as it reflects both endogenous parameters and exogenous exposure. *Ex vivo* MMA is a quantitative method for measuring force generation and fatigability in freshly muscle. The *ex vivo* MMA of isolated muscle was described by Oishi *et al.* (22) to assess the *in vivo* effects of therapeutic interventions for the treatment of muscle disease and for muscle function in experimental models. This technology has been showed to a potential utility as a primary diagnostic of vascular damage (23–26). The ex vivo MMA technique is an important method to obtain such information. It consists in excising a small sample of muscle tissue, mounting the muscle in a strip myograph (Model 820MS – Danish Myo Technology®, Ann Arbor, Michigan, USA) and the measurement of initial and final baseline, contraction peak and time of relaxation, strength duration and fatigue (loss of viability) using electrodes and square pulse stimulator (Grass Model S48, Danish

Myo Technology®, Michigan, USA).

Feasibility of *ex vivo* MMA as a diagnostic tool

The RAM, a striated muscle without tendon, which extends along the abdomen to the pubic symphysis, undergoes physiological adaptations during pregnancy (27–31). As a striated muscle, the main focus of research has been on contractile muscle cells (32). RAM has synergistic contractile activity with pelvic floor muscles in labor and in urinary continence mechanism (28,33,34).

The importance of analyzing the RAM can be highlighted by the fact that some complications during pregnancy, such as GDM, can cause severe morphological changes (35–37) leading to atrophy and, consequently, long-UI up to two years after C-section (2,9,29,35,38).

However, it is unknown whether RAM, a skeletal muscle without tendon, might be evaluated using *ex vivo* MMA. It is also unknown whether isolated **RAM** in PSUI can demonstrate different patterns of myo-mechanical parameters compared to continent pregnant women with normal glucose tolerance. This procedure may elucidate whether myo-mechanical property is involved in this process.

Thus, our hypothesis is that the contractile muscle response may elucidate the pathophysiology of PSUI and predict pathways to long UI. As a result, the study of the contractile response in a very sensitive and precise manner was chosen as an adequate technique for analyzing the contractile response throughout its course (stimulation, contraction and relaxation). Therefore, the present study aimed to understand the relationship between PSUI and continent pregnant women with normal glucose tolerance. We had three questions: 1. How

well do pregnancy isolated RAM *ex vivo* MMA values correlates to PSUI in non-GDM healthy pregnant women; 2. Is there a shift in the RAM characteristics at the beginning and end of each electrical stimulus in *ex vivo* MMA of pregnant women with and without PSUI; and 3. Is there a decline of these contractile performance of the ex vivo MMA trial of pregnant women with and without PSUI until the final experiment?

To better understand the state of PSUI and isolated RAM *ex vivo* MMA values being connected, the present study aimed, in addition to understanding the relationship between PSUI and continent pregnant women with normal glucose tolerance, to explore an established protocol for qualitative and quantitative peak and graph analysis of fresh RAM samples acquired at C-section from pregnant women with and without PSUI, as part of an extensive cohort study, applying *ex vivo* MMA

We analyzed the quantitative and qualitative ex vivo MMA patterns of the 10 first and 10 last waves of each electrical stimulus from pregnant women with PSUI and the control group at the same gestational age.

2 METHOD

Study design and location

Cross-sectional study nested within the developing cohort in the DIAMATER Thematic project – “Gestational triad cohort: hyperglycemia, urinary incontinence and clinical, molecular and omic profile of hyperglycemic myopathy in the prediction of incontinence and muscle dysfunction and translational research with biodevice for muscle regeneration in rats”. The project was previously approved by the research ethics committee of Botucatu Medical

School and the National Research Ethics Committee (CONEP) under the number CAAE: 82225617.0.0000.5411 (9).

DIAMATER project and its subprojects are hosted by Sao Paulo State University (UNESP), Botucatu Medical School (FMB), Botucatu, SP, Brazil. The physical organization responsible for its development is the Perinatal Diabetes Research Center (CIDP). The monitoring and obstetric care of pregnant women including evaluations and sample collection were performed at the Obstetrics Service of Clinical Hospital from Botucatu Medical School (HCFMB) and at the Clinical Research Unit (UPECLIN). The samples technical and laboratory storage and processing were developed at the laboratories of Experimental Research Unit (UNIPEX). All these units belong to FMB/Unesp assistance and research complex. For this specific project, clinical and laboratory data were obtained from an existing proprietary database and continuously fed with new participants. Respective data and samples were included from March/2018 to December/2021.

The study was performed in accordance with the ethical guidelines and regulations of the Declaration of Helsinki. All participants provided written informed consent before participating in the study.

Study subjects and groups

Eligibility, inclusion, and discontinuation criteria

The selection and invitation for voluntary participation of the women were carried out from the 36th week of pregnancy.

Inclusion criteria and eligibility

Women aged 18 years or older were considered eligible; primiparous (nulliparous) or secundiparous (primiparous) with previous C-section; single pregnancy; usual risk; submitted to the hyperglycemia screening and diagnosis protocol between 24-28 weeks, with a negative response (39,40); with full intellectual capacity and without the need to develop any stage of the study. Among the recommendations, from 37 weeks, were included with the prenatal indication and the parts (C-section of 37 weeks and which are included in the offer).

Discontinuation criteria

At any time, the subjects included may withdraw their consent to participate in the study, without prejudice to the standard obstetric care of the service. Thus, women who withdrew their consent to participate, those who spontaneously evolved to vaginal or premature delivery and cases with possible loss of clinical and laboratory data, including samples that were inappropriate for processing and analysis, were discontinued.

Sample size calculation

We considered the observational, cross-sectional study and data from previous studies, specific to the research group, which showed PSUI in 31.6% in normoglycemic pregnant women. With a significance level of 95% and statistical sample power of 80% (1-beta, probability of detection), at least 40 participants were included in each of the groups.

Study groups

The groups were defined as: control (mothers with normal glucose tolerance and continent; n = 39) and PSUI (mothers with normal glucose tolerance and incontinent; n = 48). n = describes the number of patients in each

group.

Independent Variable

The independent variable was the presence or absence of PSUI.

PSUI diagnosis

PSUI is defined as any onset of new urinary leakage during pregnancy (3) initiated in the current pregnancy (41). Participants who gave positive responses were identified as having UI, following the definition set by the International Continence Society (41).

Control Variables

They were defined by characteristics of pregnancy and delivery (maternal and fetal/neonatal), potentially related to independent variables (PSUI). Among the mothers, age; parity; nutritional status, characterized by the pre-gestational body mass index (BMI), of special interest the overweight and obesity classes, and weight gain during pregnancy; glycemic status, gestational glycemic mean and glycated hemoglobin (Hb) levels at childbirth. Among those related to the fetus/newborn: gestational age at delivery; birth weight; weight/gestational age ratio. These data are already standardized in the DIAMATER project (9) and are detailed below:

• Maternal age: <20 years (adolescent), 20 - 35 years (adult) and ≥ 35 years (advanced age);

• Women parity: zero (primiparous) and one (secondiparous);

• Pre-gestational BMI: overweight (BMI ≥ 25 Kg/m2) and obesity (BMI ≥ 30 Kg/m^2);

• Weight gain: difference between the initial weight and the final weight assessed during pregnancy;

• Gestational Glycemic mean - arithmetic mean of blood glucose levels evaluated in the glycemic profiles performed: adequate (MG < 120 mg/dL) and inadequate (MG ≥ 120 mg/dL) (39,40,42–45);

• Glycated Hb at delivery: adequate (Hb ≤ 6.5%) and inadequate (Hb > 6.5%);

• Gestational age at delivery - defined in gestational weeks: term (37 to 41 completed weeks) and post-term (from 42 incomplete weeks onwards);

• Birth weight (BW) - weight/gestational age ratio, of special interest: LGA (BW ≥ P90) and macrosomic (BW ≥ 4000g

Dependent Variables

The qualitative and quantitative results obtained from the *ex vivo* MMA on RAM samples were divided in two experimental groups.

Collection and techniques for obtaining data

Clinical and laboratory data of pregnancy and delivery

Personal, clinical and laboratory data on pregnancy and childbirth/newborn related to this project were extracted from the DIAMATER project database (9), which the team of researchers constantly feeds.

Data collection on PSUI

PSUI is defined as any onset of new urinary leakage during pregnancy Participants are interviewed and asked whether in the current pregnancy they had lost urine or control of even a small leakage of urine with activities such as coughing, lifting weights, or exercising or if they felt the urge or pressure to urinate and were unable to go to the bathroom quickly enough (3,38,41). Pregnant women who reported PSUI were asked to complete the Brazilian version of the International Consultation on Incontinence Questionnaire-Urinary Incontinence-

Short Form (ICIQ-SF) (46). (47).

Collection and preparation of RAM samples

The collection of RAM samples was standardized in the DIAMATER project, being performed by the medical staff from the HC-FMB/UNESP, at the time of C-section with obstetric indication, after fetal extraction (Figure 1).

Immediately after resection, the samples were delivered to researchers team, previously trained, to be dissected and free of fat and connective tissue. After this procedure, the flaps were prepared according to the technique to be performed (9).

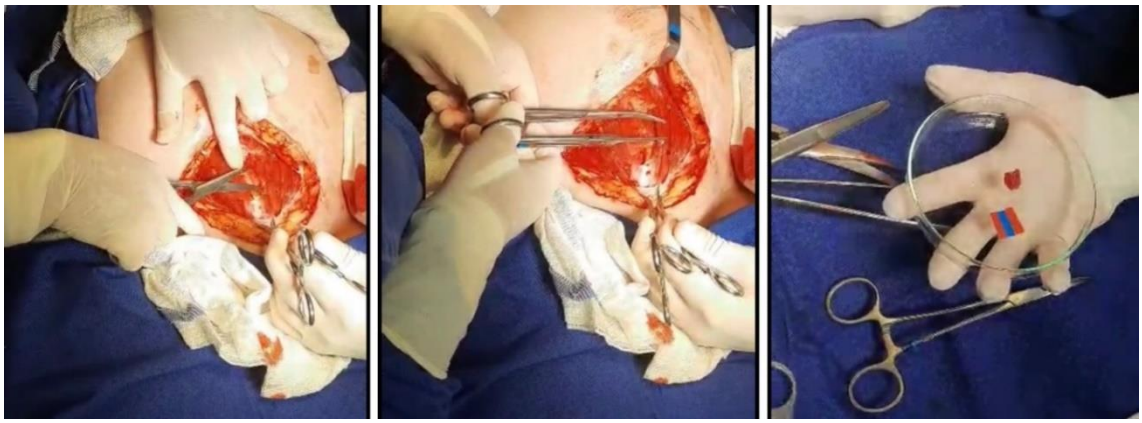


Figure 1. Collection of RAM at the time of C-section

Ex vivo MMA in RAM

Immediately after resection, a 1 cm^2 sample was collected; fat and connective tissue were removed and then placed in a Falcon tube containing 5mL of Krebs solution (118.5 µM NaCl, 30 µMKCl, 290 µM NaHCO3, 9 µM MgSO4, 9 µM KH2PO4, 20 µg CaCl2, 5.5 g D-glucose and 300 µM L-arginine, pH 7.4) previously oxygenated with carbogen gas (95% O_2 and 5% CO_2) and kept at 4°C until analysis. The time between sample collection and the beginning of *ex vivo*

MMA was a maximum of 30 minutes.

Myograph – Equipment to perform ex vivo MMA

This study used the DMT-myograph system (model 820MS DMT-Danish Myo Technology®, Ann Arbor, Michigan, USA) (Figure 2) to assess RAM contractile responses under electrical stimulation. The chamber contains hooks on which the muscles are mounted by the edges. One end of the chamber contains the force transducer that converts kinetic energy (muscle responses, under electrical stimulation) into an electrical signal that can be recorded and analyzed with PowerLab equipment (PowerLab Data Acquisition System, AD Instruments, São Paulo, Brazil) and LabChart software (LabChart 8 for Windows, AD Instruments, São Paulo, São Paulo, Brazil). The equipment was calibrated according to the manufacturer's recommendations and according to training obtained from the DTM Company (Ann Arbor, Michigan, USA)

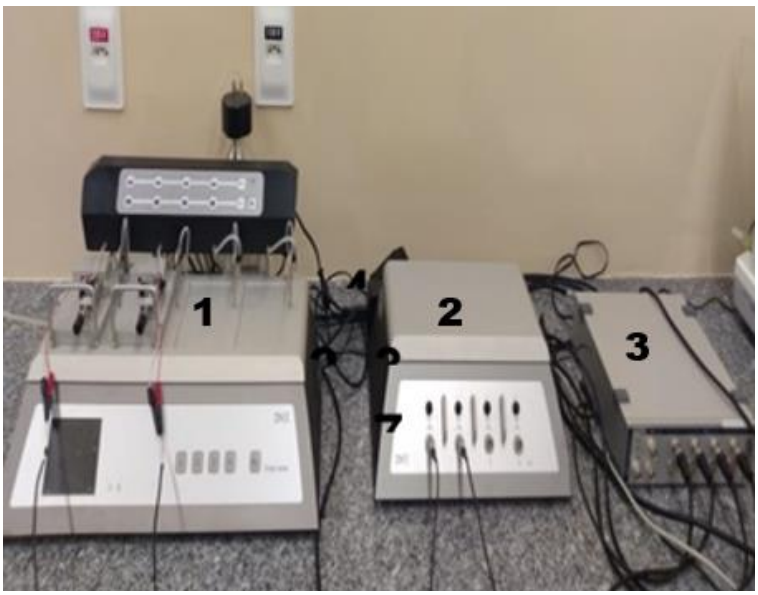


Figure 2. 1- Myograph (Model 820MS - Danish Myo Technology®, Ann Arbor, Michigan, USA); 2- LabChart software (LabChart 8 for Windows, AD Instruments, São Paulo, São Paulo, Brazil) and 3- Stimulator machine (Grass Model S48, Danish Myo Technology®, Michigan, USA)

Muscle assembly method and myography performance

The settings adjusted on the stimulation equipment were based on previous literature (22,48), which used other skeletal muscles with tendons and tissues (49–51). This study was based on the standardization performed by the Diamater group (data not disclosed) of the records of myographic activity, and they were carried out in five sequential steps (Figure 3) (Reyes *et al*. 2022 submitted) and described below.

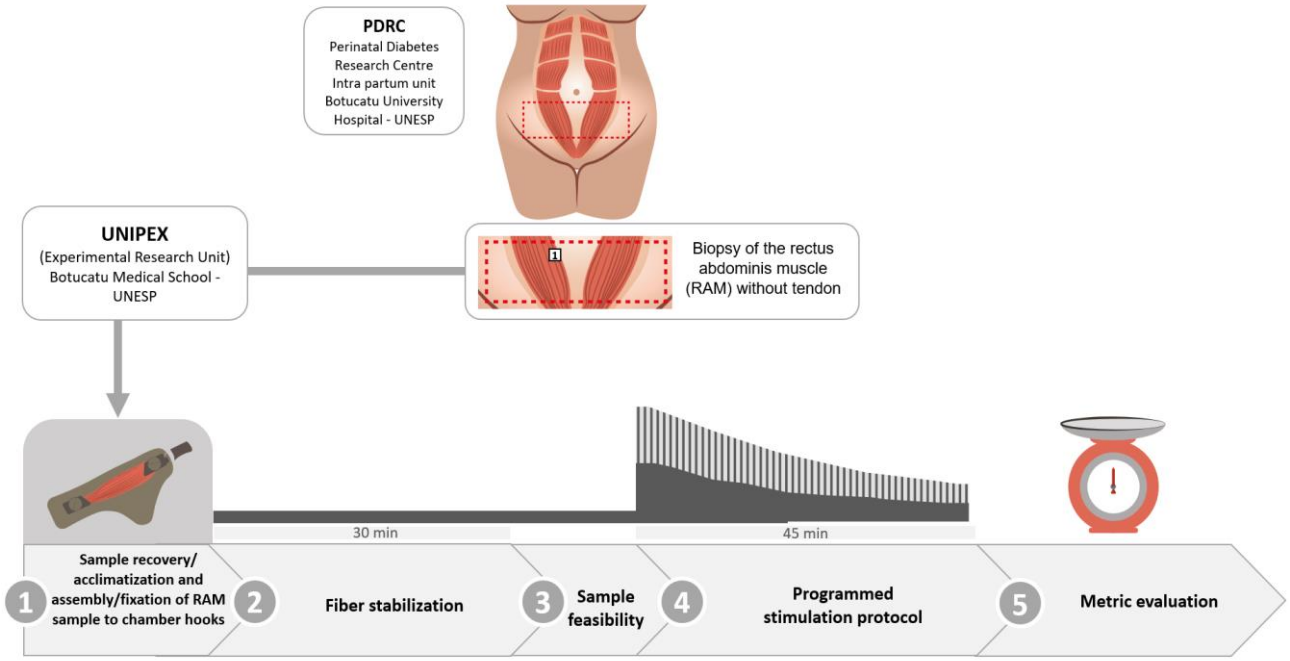


Figure 3. Schematic diagram showing the 5 steps of experiment performed using RAM without tendon (Reyes *et al*. 2022 submitted)

1st step - Sample recovery/acclimatization and assembly/fixation of RAM sample to chamber hooks: An isolated organ bath was performed in Krebs solution with carbogenic gas for 15 minutes, previously heated to 37°C, to recover the mechanical properties of the muscle. The RAM fragment was mounted vertically, following the arrangement of the fibers, in the myograph chamber filled with 4mL

of Krebs solution with continuous gas flow and minimal bubbling, which provides essential gases to maintain muscle in conditions that simulate the organic environment.

2nd step - Fiber stabilization: the Krebs solution was changed twice at 15 min intervals, with a fixed tension force of 10 mN applied at each Krebs change. To carry out the 3rd and 4th stages, electrical stimulation was performed using platinum electrodes, placed parallel to the longitudinal axis, at 0.8 cm from the muscle tissue and integrated into the myographic cells, which provide electrical stimuli through the electrodes, by a series of predefined quadratic waves, due to its characteristic digital pulse (0-1). These conditions allow the observation of minimum and maximum points (dual pulse wave, gradient, ramp, sine, and triangle), controlled by MyoD analog output software (Danish Myo Technology®, Michigan, USA), with voltage-controlled (20V) computer software and constant current management. To capture muscle responses with greater sensitivity and precision, muscle stimulation was performed with stimulation equipment (Grass Model S48, Danish Myo Technology®, Michigan, USA);

3rd step – Sample feasibility: to verify the feasibility, electrical stimulation was performed with three stimuli of biphasic wave force of 10mN, voltage of 20V, pulse width of 25mS. Approximate run time of 2 minutes

4th step – Programmed stimulation protocol: the application of the continuous alternating current stimulation protocol was performed with a fixed pulse train. Pulse voltage (volts): 20V, pulse width (milliseconds): 25ms, pulse interval (milliseconds): 60ms, power: 10mN, pulse frequency (Hertz): 11.8Hz, pause between pulse trains (milliseconds): 500 ms, pulse trains in group: 500, repeat group: 10 times, pause between groups of trains (milliseconds): 60000ms, total

run time: 45 minutes.

5th step - Metric evaluation: After the stimulation, the equipment was turned off and the parameters of muscle sample, length (cm), width (cm) and weight (g) were determined. In the analysis of the results, the average of three measurements performed to normalize the results was considered. A digital caliper (STARFER®, Vargem Grande do Sul, SP, Brazil) was used to measure muscle length and a digital analytical balance (model SHIMADZU® ATX224 Kern ABT 220-4NM, KYOTO, JAPAN) was used to weigh the fragment. Muscle weight was recorded in grams (dry weight).

Contractile response analysis of ex vivo MMA

a) Force-frequency relationship - maximum peak vs stimulation frequency;

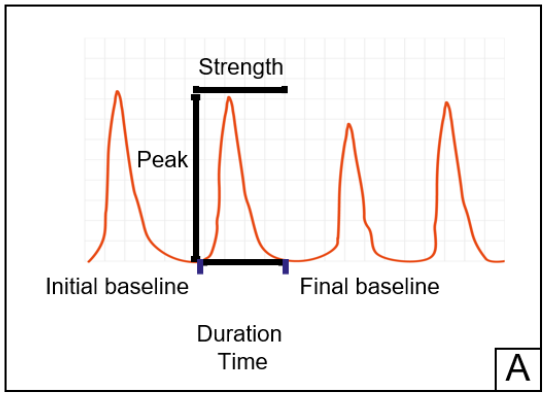
b) Force frequency vs. time - measured by analyzing the decrease in the muscular response of the muscle under traction of 10mN for 05 seconds;

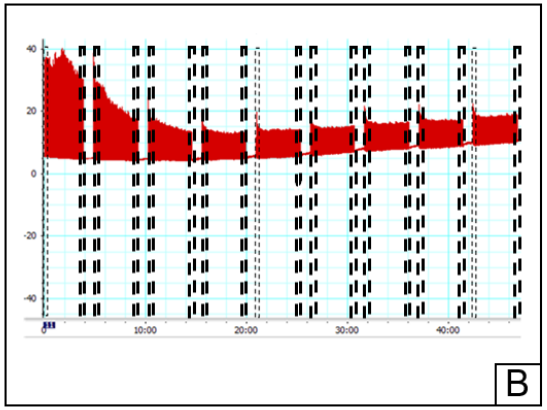
c) Low frequency fatigue - minimum strength vs time (46).

Analysis of the data obtained

The graphs observed in LabChart software were analyzed through 9-time windows of 5 minutes (windows); each window was subdivided into 2 periods of 5 seconds (initial and final) and all the contractions of the subdivisions were analyzed for initial baseline, final baseline, peak, strength, and duration time (Figure 4A).

The values obtained were calculated by means and standard deviation and plotted using the software GraphPad Prism 9. Figures 4B and 4C describes the data analysis methodology.


Strength
Peak
Initial baseline
Final baseline
Duration
Time
A


B

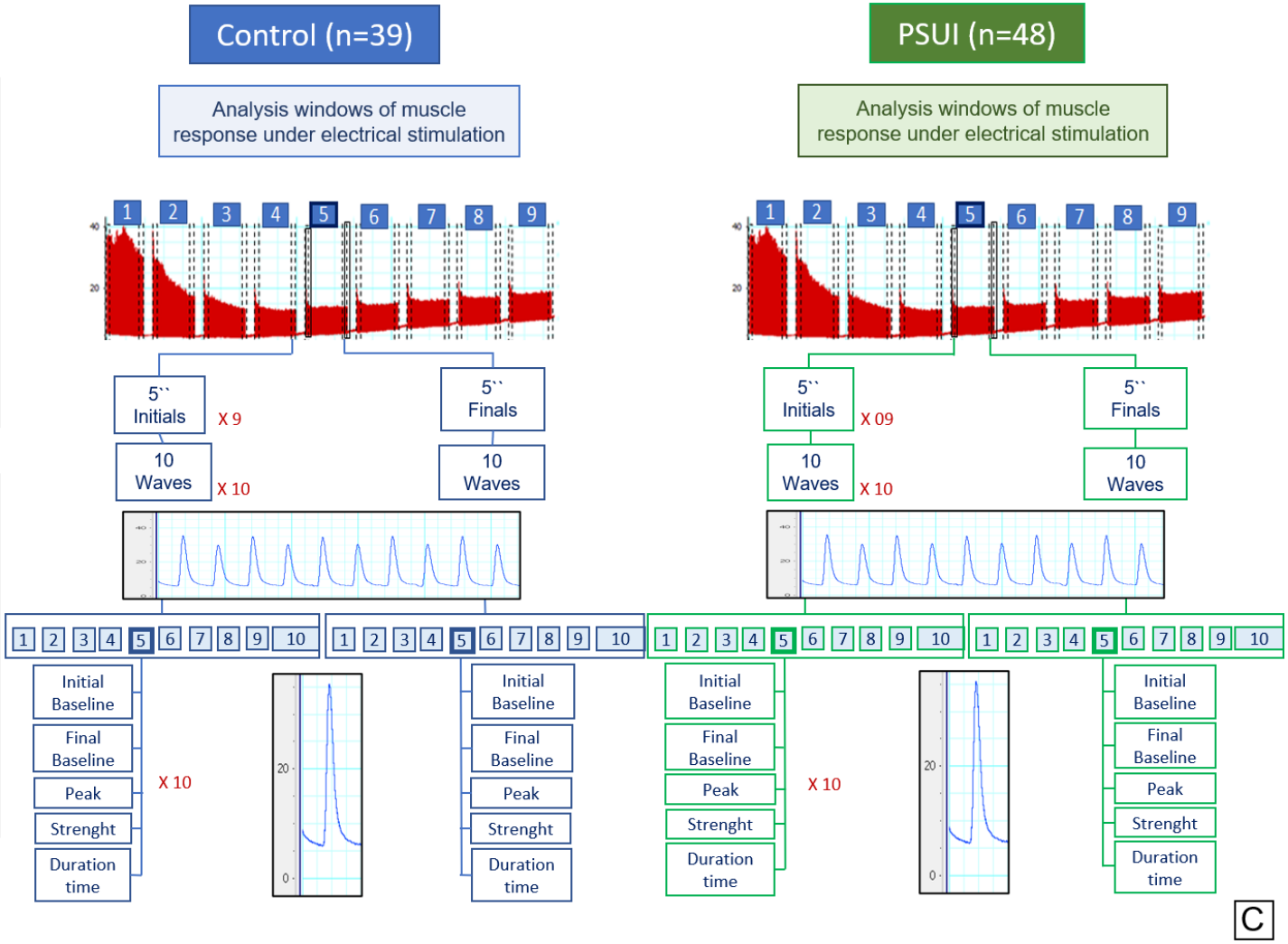

Control (n=39)
Analysis windows of muscle response under electrical stimulation
5`` Initials
X 9
10 Waves
X 10
5`` Finals
10 Waves
Initial Baseline
Final Baseline
Peak
Strenght
Duration time
X 10
PSUI (n=48)
Analysis windows of muscle response under electrical stimulation
5`` Initials
X 09
10 Waves
X 10
5`` Finals
10 Waves
Initial Baseline
Final Baseline
Peak
Strenght
Duration time
X 10
C

Figure 4. A - A schematic diagram of *ex vivo* MMA quantitative analysis: initial baseline, final baseline, peak, strength, and time duration; B - Windowing performed in first 5 initial and final seconds of each window of stimulation protocol and C - Step-by-step for extracting data from stimulation protocol

Statistical analysis

The obtained data *from ex vivo* MMA were evaluated according to a repeated measures model considering the interaction groups versus wave and groups versus windows using an adjustment in generalized linear model with gamma distribution for followed by the Wald multiple comparison test. Significance level was set at 5% or the corresponding p-value. All analyzes were performed using the program SAS for Windows, v.9.4.

3 RESULTS

The study included 315 mothers with normal glucose tolerance and asked about PSUI, recruited for the Diamater Study cohort. From this population, 145 were eligible for inclusion. From these, 87 pregnant women with normal glucose tolerance and RAM biopsy sample collected during C-section were successfully included for ex *vivo* MMA analysis: 39 in control group (mothers with normal glucose tolerance without PSUI), and 48 in PSUI group (mothers with normal glucose tolerance with PSUI) (Figure 5). According to the PSUI status, demographic characteristics are shown in Table 1.

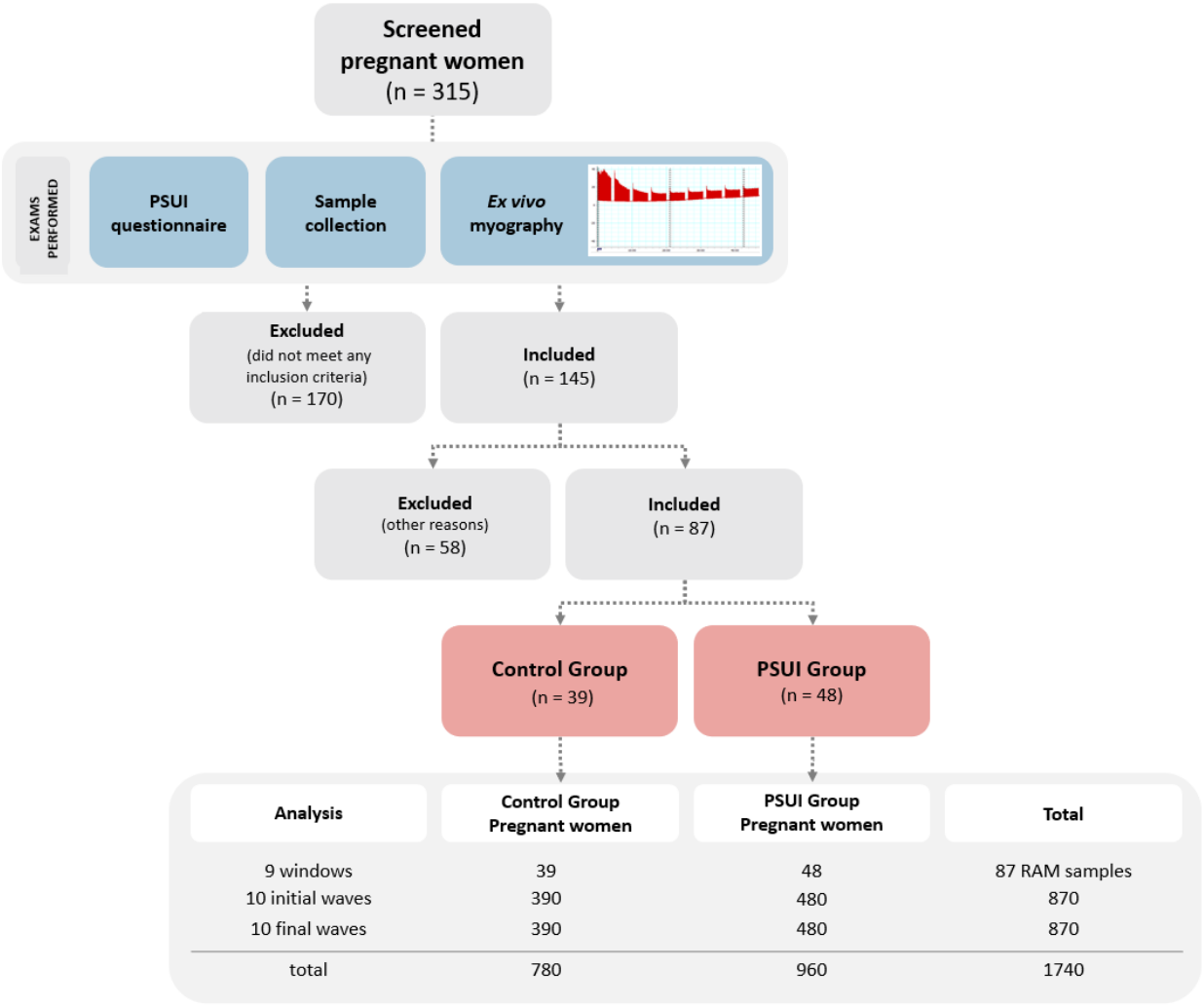


Figure 5. Flow chart indicating distributions of study participants at each group: control and PSUI.

Human data

In order to assess the influence of maternal and perinatal demographic characteristics and sample size on the skeletal muscle after *ex vivo* MMA, we previously analyzed these characteristics (Tables 1 and 2). Both groups are similar in quite all variables. Compared with control group, pregnant women with PSUI have lower weight gain during pregnancy ($p>0.015$).

Table 1. Characteristics of the study population according to urinary continence status control and PSUI groups.

Variable	Control (n=39)	PSUI (n=48)	p-value
Age (years)	29.1±5.6	30.9±6.2	*0.181*
Gestacional age	38.5±0.9	38.5±1.0	*0.727*
Pre-pregnancy BMI	28.4±6.2	27.4±4.6	*0.459*
Gestacional BMI	34.1±5.7	31.4±5.3	*0.052*
Weight gain (kg)	15.3±7.3	10.5±8.37	*0.015***
Newborn weight (g)	3350.6±465.6	3285.2±433.8	0.727
Newborn's size	48.5±2.16	47.8±2.1	0.522
Primiparous	23 (59%)	22 (48%)	*0.537*
Education level – min. High School	32 (94.1%)	42 (95.4%)	*1.000*
Non-Caucasian	6 (21%)	4 (9%)	*0.288*

Data are expressed as means ± standard deviations or number of subjects and percentages p<0.05 – denotes significance between two groups using t-test for continuous variables and chi-square test** for weight gain. BMI: body mass index; kg: kilograms; g: grams; PSUI: pregnancy-specific urinary incontinence group.

RAM characteristics after *ex vivo* myography

The weight, width, and length of RAM samples at the end of *ex vivo* myography protocol displayed similar values (mean±s.d.) either in PSUI or in control groups (Table 2). There was no effect of samples weight, width, and length on ex vivo MMA results.

Table 2. RAM samples weight, width, and length in PSUI and control groups at the end of *ex vivo* MMA protocol.

Variables	Control (n=39)	PSUI (n=48)	p-value
Sample Weight (g)	0.3±0.2	0.3±0.2	*0.505*
Sample Width (cm)	0.4±0.2	0.5±0.2	*0.099*
Sample Length (cm)	1.2±0.3	1.3±0.3	*0.132*

n: sample; g: grams; cm: centimeters; independent T-test and chi-square test**. PSUI: pregnancy-specific urinary incontinence group; p < 0.05 significant difference between two groups.

***RAM ex vivo* MMA quantitative analysis**

For this quantitative analysis, a total of 1740 waves were evaluated with

five parameters as: initial baseline, final baseline, peak, strength, and duration time.

As there was no difference in RAM sample size, we were interested in observing if quantitative parameters of the waves of each window have responded differently in control and PSUI groups. First, there was no interaction among nine windows of both groups (Table 2). Second, we looked at the five measurements taken on the waves (initial baseline, final baseline, peak, strength, and duration time) at initial and final of each one of nine windows among two groups: with and without PSUI (Figure 4C).

3.1 Comparison between initial and final windows patterns of behavior inside each one of two groups: control and PSUI.

In order to assess the pattern of different parameters analyzed within each group, we followed the waves from initial and final characteristics in each one of the nine windows. In both groups, we observed an accelerated decrease in peak from the 1st window until 9th associated with a decline of strength from 1st to 3rd window. In control group duration time showed a slow and progressive decline with intermediate stabilizations from the five initial waves to final five waves in each window. In PSUI group, the duration time presented a slow and progressive decline from 1st to 5th window followed by stabilization until 9th window.

3.2 Comparisons of initial waves of nine windows between control and PSUI groups

As there was smaller small difference between the patterns of behavior between control and PSUI group, we were interested in observing if some specific

characteristics of the waves as initial baseline, final baseline, peak, strength, and duration time had responded differently in both groups in the initial and in the final waves from each window.

Initial baseline

Analyzing data in Table 3, for initial wave measurements, averages of initial baseline show a significant decrease from 1st (mean±s.d 8.19±4.22) window to others for control group; from 2nd to 6th (mean±s.d 6.89±4.64 to 6.49±5.86 respectively) window there is no difference in averages and from 7th to 9th (mean±s.d 5.05±8.09 to 4.84±9.07 respectively) window there was also no difference between averages. For PSUI group, there was no difference between means for windows. Between groups, there was a difference in means for 2nd and 3rd blocks associated with opposite results. For others, there was no difference in means between groups for other blocks.

Final baseline

A decrease in final baseline generated by twitch tension curve was observed from 1st to 2nd window and remain at constant values (mean±s.d. 8.43±4.31 to 7.06±4.68 respectively) in control group. In PSUI group the values remain constant from 1st to 9th window (mean±s.d. 7.95± 4.47 to 4.73±9.33 respectively).

Peak

A gradual and continuous loss of peak tension values from 1st to 8th windows in control group (mean±s.d. 14.06 ± 22.96 and 1.75 ±3.79 respectively). In PSUI group this progressive reduction of peak tension of twitch tension curve

occurs from 1st to 6th window and remained constant until ninth (mean±s.d. 11.97 ± 16.76 and 1.83 ±3.36 respectively).

Strength

During *ex vivo* myography assay**,** initial strength progressively decreased from 1st to 3rd window and remained in constant value until 9th window in control group. (mean±s.d. 6.58±8.89 and 2.81±3.99 respectively). In PSUI group all initial strength values stay in similar values since the 1st window until the 9th (mean± s.d. 5.02±6.24 and 3.02±12.86 respectively).

Duration time

A decrease in duration time of initial window was observed from 1st to 4th, remains constant until 7th, decreases in 8th, and remains constant in 9th in control group (mean ± s.d 0.40± 0.44; 0.28 ±0.20; 0.20 ± 0.21 and 0.12 ±0.19respectively). In PSUI group, duration time of initial window remains the same between 1st and 2nd windows, falls on 3rd and remains the same until 5th, falls on 6th and remains the same until 9th (mean±s.d., 0.40± 0.12; 0.34 ±0.18; 0.17 ± 0.21 respectively).

3.3 Comparisons of final waves of nine windows between control and PSUI groups

The control group lose feature of progressive reduction of initial moment from 1st to 9th of final windows. The PSUI group stay similar to initial moment with all similar windows. In both groups, final baseline, peak, strength, and duration were similar to initial windows.

Table 3. Comparison of five waves quantitative characteristics (initial baseline, final baseline, peak, strength, and duration time) according to initial and final of each one of nine windows among two pregnant groups: with and without PSUI. Comparison of initial and final waves of each window within each group: control and PSUI.

	Initial									**Final**										**Initial x final**	**Initial x final**
		Control				**PSUI**						**Control**				**PSUI**					**Control**	**PSUI**
Window	**Variable**	**Mean (n=390)**	**SD**			**Mean (n=480)**	**SD**			**p-value***	**Variable**	**Mean (n=390)**	**SD**			**Mean (n=480)**	**SD**			**p-value***	**p-value****	**p-value****
1	**Initial baseline**	8.19	4.22	a	A	10.01	5.98	a	A	0.2029	**Initial baseline**	7.78	4.49	a	A	10.04	7.80	b	A	0.9126	0.4145	0.942
2	**Initial baseline**	6.89	4.64	a	B	10.02	8.02	b	A		**Initial baseline**	6.83	5.44	a	A	10.80	10.66	b	A		0.9204	0.2696
3	**Initial baseline**	6.49	5.86	a	B	10.43	10.98	b	A		**Initial baseline**	6.25	7.12	a	A	10.87	12.58	b	A		0.7229	0.6307
4	**Initial baseline**	5.46	7.17	a	B	8.79	11.78	a	A		**Initial baseline**	5.75	8.16	a	A	9.14	12.64	a	A		0.6677	0.6985
5	**Initial baseline**	5.68	8.21	a	B	7.63	10.12	a	A		**Initial baseline**	5.53	7.47	a	A	8.22	11.93	a	A		0.8287	0.501
6	**Initial baseline**	6.24	9.51	a	B	7.54	12.23	a	A		**Initial baseline**	5.70	8.44	a	A	8.07	13.91	a	A		0.4814	0.5628
7	**Initial baseline**	5.05	8.09	a	C	7.79	13.79	a	A		**Initial baseline**	5.42	9.05	a	A	8.45	15.32	a	A		0.60	0.502
8	**Initial baseline**	5.23	9.09	a	C	8.74	15.36	a	A		**Initial baseline**	5.18	9.13	a	A	8.71	16.37	a	A		0.946	0.9731
9	**Initial**	4.84	9.07	a	C	8.31	15.88	a	A		**Initial**	4.72	9.31	a	A	8.12	16.42	a	A		0.8566	0.8541

	baseline										**baseline**											
1	**Final baseline**	8.43	4.31	a	A	10.21	6.06	a	A	0.1865	**Final baseline**	7.95	4.47	a	A	10.19	7.83	b	A	0.548	0.3464	0.957
2	**Final baseline**	7.06	4.68	a	B	10.22	8.05	b	A		**Final baseline**	6.88	5.45	a	A	10.90	10.68	b	A		0.7612	0.3453
3	**Final baseline**	6.60	5.95	a	B	10.55	11.04	b	A		**Final baseline**	6.33	7.17	a	A	10.96	12.62	b	A		0.6798	0.6549
4	**Final baseline**	5.51	7.13	a	B	8.93	11.88	a	A		**Final baseline**	5.78	8.17	a	A	9.25	12.73	a	A		0.6909	0.7342
5	**Final baseline**	5.71	8.23	a	B	7.68	10.19	a	A		**Final baseline**	5.55	7.49	a	A	8.28	11.97	a	A		0.8183	0.4992
6	**Final baseline**	6.27	9.51	a	B	7.58	12.27	a	A		**Final baseline**	5.75	8.54	a	A	8.07	13.88	a	A		0.5012	0.5988
7	**Final baseline**	5.06	8.12	a	B	7.82	13.81	a	A		**Final baseline**	5.44	9.08	a	A	8.46	15.33	a	A		0.5922	0.5188
8	**Final baseline**	5.23	9.08	a	B	8.77	15.38	a	A		**Final baseline**	5.15	9.10	a	A	8.73	16.38	a	A		0.9195	0.9754
9	**Final baseline**	4.87	9.12	a	B	8.33	15.88	a	A		**Final baseline**	4.73	9.33	a	A	8.14	16.45	a	A		0.8382	0.8569
1	**Peak**	14.06	22.96	a	A	11.97	16.76	a	A	0.0903	**Peak**	6.00	7.94	a	A	6.04	7.19	a	A	0.1156	<0.0001	<0.0001
2	**Peak**	6.80	10.11	a	B	7.24	9.22	a	B		**Peak**	3.88	5.23	a	B	3.64	4.24	a	B		<0.0001	<0.0001
3	**Peak**	4.80	7.62	a	C	4.55	5.65	a	C		**Peak**	2.68	4.21	a	C	2.59	3.23	a	C		<0.0001	<0.0001
4	**Peak**	3.22	5.81	a	D	3.20	4.53	a	D		**Peak**	2.29	3.87	a	D	2.22	3.02	a	D		0.0037	<0.0001
5	**Peak**	2.89	5.38	a	E	2.52	3.81	a	E		**Peak**	1.95	3.42	a	E	1.59	2.56	a	E		0.001	<0.0001
6	**Peak**	2.47	4.56	a	F	1.83	3.36	a	G		**Peak**	1.61	2.95	a	F	1.23	2.37	a	F		0.0003	<0.0001
7	**Peak**	2.03	4.2	a	G	1.63	3.21	a	G		**Peak**	1.36	2.70	a	G	1.15	2.40	a	F		0.0011	0.0006

8	**Peak**	1.75	3.79	a	H	1.49	3.23	a	G		**Peak**	1.19	2.41	a	H	1.04	2.43	a	F		0.0019	0.0004
9	**Peak**	1.41	3.30	a	H	1.40	3.29	a	G		**Peak**	1.02	2.27	a	H	0.99	2.17	a	F		0.008	0.0009
1	**Strength**	6.58	8.89	a	A	5.02	6.24	a	A	0.2416	**Strength**	3.64	4.12	a	A	3.59	5.18	a	A	0.8155	<0.0001	<0.0001
2	**Strength**	3.73	4.74	a	B	4.05	5.72	a	A		**Strength**	2.62	3.49	a	B	2.99	5.69	a	A		0.0002	0.0002
3	**Strength**	2.81	3.99	a	C	3.44	6.02	a	A		**Strength**	2.20	4.01	a	B	3.04	6.78	a	A		0.0179	0.1685
4	**Strength**	2.29	4.45	a	C	2.48	5.42	a	A		**Strength**	1.98	4.41	a	B	2.31	6.79	a	A		0.2102	0.4716
5	**Strength**	2.09	4.46	a	C	2.51	7.23	a	A		**Strength**	1.77	4.16	a	B	2.47	8.81	a	A		0.1671	0.9017
6	**Strength**	1.82	4.35	a	C	2.42	9.01	a	A		**Strength**	1.62	4.38	a	B	2.37	10.38	a	A		0.3376	0.8617
7	**Strength**	1.70	4.56	a	C	2.70	10.83	a	A		**Strength**	1.63	4.97	a	B	2.69	11.90	a	A		0.7444	0.9872
8	**Strength**	1.76	5.14	a	C	2.87	11.99	a	A		**Strength**	1.64	5.24	a	B	2.75	12.59	a	A		0.606	0.7272
9	**Strength**	1.29	4.59	a	C	3.02	12.86	a	A		**Strength**	1.17	4.81	a	C	2.94	13.24	a	A		0.4584	0.8287
											Strength											
1	**Duration time**	0.40	0.44	a	A	0.40	0.12	a	A	0.5287	**Duration time**	0.34	0.18	a	A	0.35	0.14	a	A	0.5344	0.0011	<0.0001
2	**Duration time**	0.32	0.18	a	B	0.38	0.16	a	A		**Duration time**	0.28	0.18	a	B	0.32	0.16	a	B		0.0067	<0.0001
3	**Duration time**	0.28	0.20	a	C	0.34	0.18	a	B		**Duration time**	0.22	0.21	a	C	0.27	0.18	a	C		0.0015	<0.0001
4	**Duration time**	0.20	0.21	a	D	0.27	0.28	a	B		**Duration time**	0.18	0.21	a	D	0.22	0.22	a	D		0.1627	0.0071
5	**Duration time**	0.18	0.21	a	D	0.22	0.22	a	B		**Duration time**	0.16	0.19	a	E	0.18	0.19	a	C		0.0784	0.0018
6	**Duration time**	0.17	0.21	a	D	0.17	0.21	a	C		**Duration time**	0.14	0.19	a	E	0.15	0.20	a	C		0.0151	0.1544
7	**Duration time**	0.15	0.35	a	D	0.16	0.20	a	C		**Duration time**	0.11	0.18	a	F	0.15	0.20	a	C		0.0055	0.3437
8	**Duration time**	0.12	0.19	a	E	0.16	0.20	a	C		**Duration time**	0.12	0.45	a	F	0.15	0.30	a	C		0.759	0.2601

9	**Duration time**	0.10	0.17	a	E	0.15	0.21	a	C		**Duration time**	0.09	0.16	a	F	0.13	0.19	a	C		0.3279	0.0613

Model adjustment in repeated measures evaluating group versus wave interaction.
*-p-value referring to group versus waves interaction test; **- p-value referring to comparison between initial and final measurements for each group;
Means followed by same lowercase letter (fixing waves and testing groups) do not differ at 5% level by Wald test;
Means followed by same lowercase letter (fixing groups and testing waves) do not differ at 5% level by Wald test.

3.4 Comparisons of initial and final waves in nine windows between control and PSUI groups

All these comparisons are in Table 3. In this analysis 1740 waves were analyzed.

Initial baseline

According to Table 4S, it is noted that for initial measurements, initial baseline variable means are decreasing but do not differ until wave 5 (mean±s.d. 6.90±8.24 to 6.96±8.24) for control group. From wave 6 to 9 (mean±s.d. 5.38±6.93 to 4.96±6.83 respectively), there was no difference between means and there is also no difference between waves 9 and 10 (mean±s.d. 4.96±6.83 to 4.85±6.77). For PSUI group, the behavior was similar. Despite this, no significant difference was observed between group means for each wave.

Final baseline

For final baseline, the behavior was the same, although there was a difference between groups up to wave 5 for control (mean ± s.d 7.05±8.26 to 7.02±8.19) and PSUI (mean ± s.d 9.98±12.94 to 9.90±12.92) groups. After wave 6 (mean ± s.d 5.44±6.97; 8.11±11.06, control and PSUI groups respectively), the group means do not differ. Again, no significant difference was observed between means for groups in each wave.

Peak

For peak, there is an oscillation of averages (increases and decreases for each wave) presenting significant differences in several averages for control

group. As for PSUI group, the behavior is also variable, but differences appear in 1st wave (mean±s.d. 5.60±9.84) for others, with no significant difference from 2nd to 5th (mean±s.d. 4.23±7.56 to 4.19±8.38 respectively) and from 6th to 9th (mean±s.d. 3.53±7.19 to 3.61±8.29 respectively). The 10th (mean±s.d. 3.18±6.76) differs from the others. Despite the differences between waves, there were no differences between groups for each wave.

Strength

For strength, there was a significant difference between means of 1st (mean±s.d. 3.32±5.99) wave for others, and 10th (mean±s.d. 2.11±4.58) wave for others in control group. There was no significant difference between means for waves in PSUI group. Again, no significant difference was observed for means between groups per wave.

Duration time

The averages for waves in duration do not differ from 1st to 5th (mean±s.d. 0.25±0.23 to 0.23±0.21) and from 6th to 10th (mean±s.d. 0.19±0.21 to 0.20±0.49) for control group. In PSUI group, there was a difference from 1st (mean±s.d. 0.31±0.30) to others, but there was no difference between averages from 2nd to 5th (mean±s.d. 0.27±0.21 to. 0.28±0.21 respectively) and from 6th to 10th (mean±s.d. 0.22± 0.21 to 0.21±0.20 respectively). Fixing waves there was no difference between groups again.

3.5 Comparisons of final waves of nine windows between control and PSUI groups

The same may be observed for final measurements.

3.6 Comparison between initial and final windows characteristics inside each one of two groups: control and PSUI.

For initial and final baseline, there were differences between control and PSUI groups from 1st to 5th wave, with no further differences in others. For peak again, there is an oscillation between averages for waves for both control and PSUI groups. For strength, there is no difference between averages for waves in each group and there is no difference in group averages for each wave. The duration fluctuates on average, showing some significant differences for both control and PSUI groups, although, again, there are no differences between averages for group in each wave.

The detailed Table with all results can be seen in supplementary material.

Qualitative analysis of *ex vivo* RAM myography of study groups

After carrying out all necessary statistical tests (Wald test, T-test and Chi-square test) an alert came to us that although the quantitative statistics, analyzing the entire population overall, there was no significant difference. However, Figures 6 A and B gave us an indication that the individual case-by-case analysis showed different behaviors (trends) within the whole population. Through this observation, we performed the qualitative analysis and describe 3 trends observed within the evaluated groups (control and PSUI).

Qualitative analyzes were performed by analyzing each patient using the GraphPad Prism software, version 9. Serial bar graphs were constructed for the analysis of the average number of peaks in 10s of each of the 9 analyzed windows (5s initial and final). In addition, the same was performed for the analysis

of contractile force (peak height).

Through detailed qualitative examination of *ex vivo* RAM myography three different patterns (tendencies of contraction response vs. applied electrical stimulus under electrical stimulation) categorized in each study group was observed as: (Figure 6):

1st pattern - Progressive decrease in strength

2nd pattern - Sudden muscular arrest

3rd pattern - Asynchrony (disorderly; messy) register with ups and downs

Control Group

1st pattern - From Figure 6 C and D, it was possible to observe that the participant showed 100% of response to electrical stimulus (every 5 seconds analyzed, 2 stimuli were applied, so 10 responses per period corresponds to 100%), but there was a progressive loss of strength.

2nd pattern - Figure 6 E and F shows a good response initially, in first three windows, but there is an abrupt stop in muscular response to electrical stimulus. There was also a difference in strength of contraction where it progressively increased.

3rd pattern - Figure 6 G and H shows a trend of progressive loss of response as well as progressive loss of strength and also a lower strength than that observed in C and D.

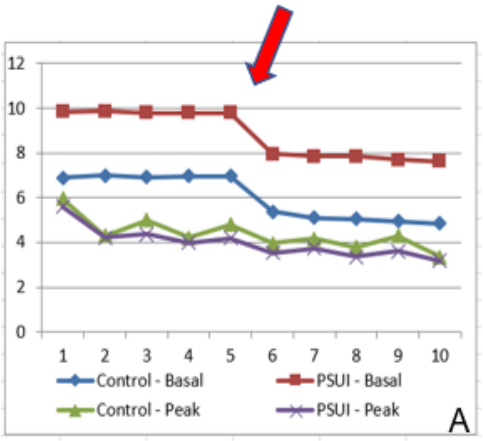
PSUI Group

1st - Figure 6 I and J presents the results of the 2nd group studied (PSUI). Although 100% response to electrical stimulation there are a lack of progressive

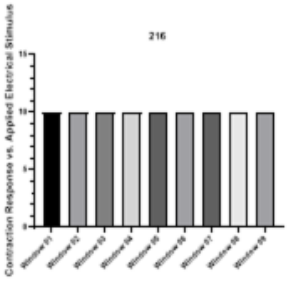
loss of strength observed in the control group. This result suggests an altered coordination in contraction force.

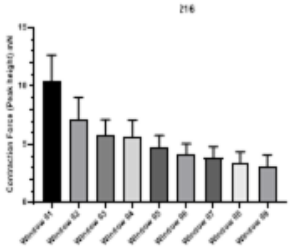
2nd - In Figure 6 K and L, some patients in PSUI group showed only a response in the first three windows analyzed, similar to control group. Although, a lack of coordination in strength was observed.

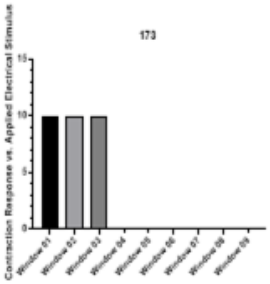
3rd - Figure 6 M and N shows that there is an abrupt and gradual decrease in muscle response as well as a progressive but intense decrease in strength.

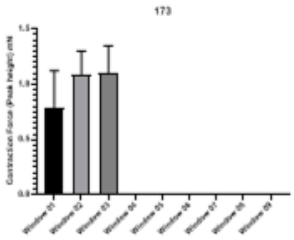


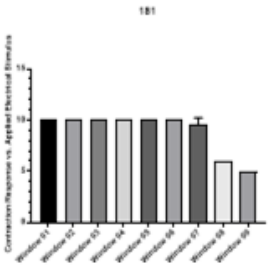
CONTROL

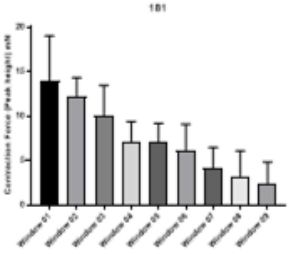


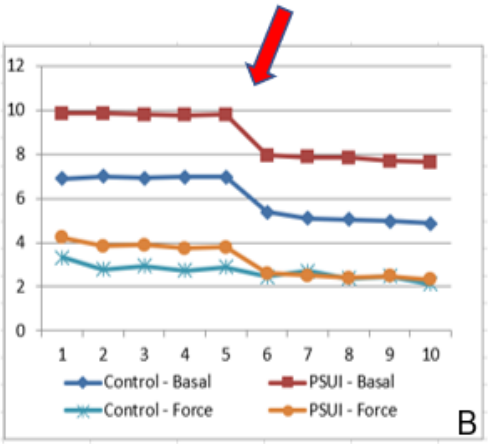




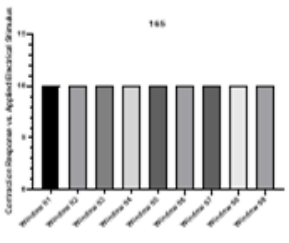


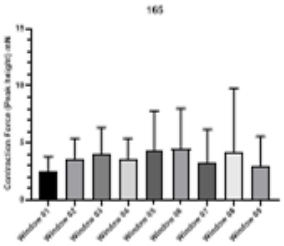


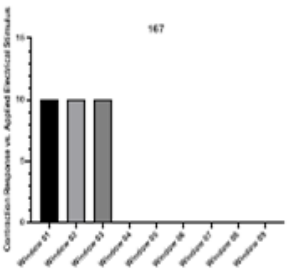


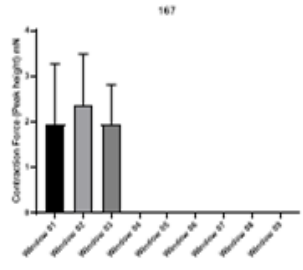


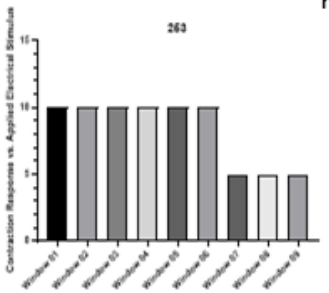
PSUI











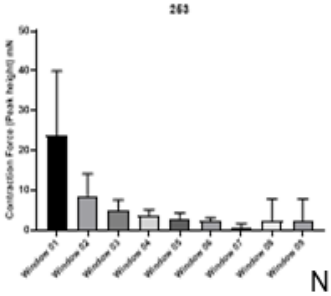
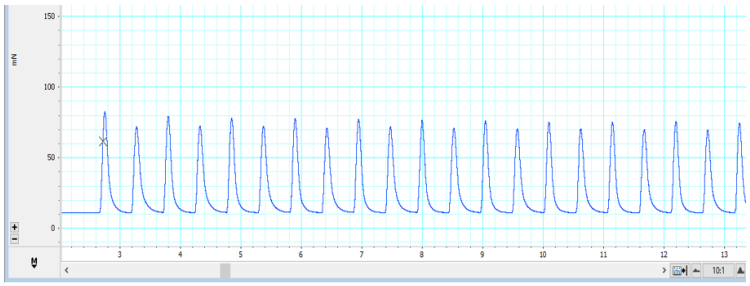
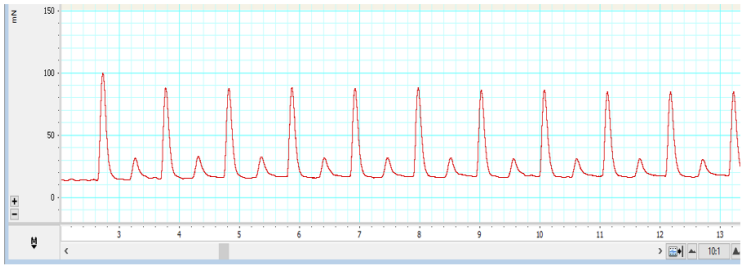


Figure 6. A and B show quantitative statistical data of peak and force in control and PSUI groups. The red arrows indicate regions where there is a change in behavior, although, the statistically significant differences were not found ($p<0.05$); C, E and G demonstrate contraction response vs. applied electrical stimulus in control group; D, F and H demonstrate contraction response vs. applied electrical stimulus in PSUI group; I, K and M demonstrate contraction force (peak height) mN in control group and J, L and N demonstrate contraction force (peak height) mN in PSUI group.

There are here some disagreements about the details of quantitative and qualitative results. Some reasons could be related to not consider in the quantitative analysis the occurrence of 2 types of wave records named by our group as Type 1 and Type 2. (Figure 7) (Reyes *et al*. 2022 submitted). Not having taken these differences into account may have been responsible for the very high values of the standard deviation and influenced the comparisons of means.



Type 1 - 48 (55%)



Type 2 - 39 (45%)

Groups	Type 1	Type 2	Total
Control	23 (59%)	16 (41%)	39 (100%)
PSUI	25 (52%)	23 (48%)	48 (100%)
total	48 (55%)	39 (45%)	87 (100%)

Figure 7. Type 1 and Type 2 of RAM wave records of ex vivo myography (Reyes *et al*. 2022 submitted)

4 DISCUSSION

This study of carrying out the ex vivo MMA of RAM, a muscle without tendon in pregnant women, performed by the Diamater group, is unprecedented. To the best of our knowledge, this is the first published study in PSUI pregnant women to combine qualitative and quantitative examination of *ex vivo* RAM myography. The feasibility of analyzing this muscle was demonstrated through its contractile response after receiving an electrical stimulus.

The *ex vivo* myo-mechanical assay (MMA) of RAM skeletal muscle is unknown in healthy pregnant women with PSUI. This fresh skeletal muscle samples from pregnant women analyzed by ex vivo MMA myography detailed its feasibility through a qualitative and quantitative assessment and demonstrate the following study's key findings:

1st, a five-parameter quantitative analysis of the window and waves of the RAM *ex vivo* myography assay reveals modest changes that are insufficient to distinguish pregnant women with and without PSUI; 2nd, quantitative measurement of peak and strength in both groups shows a progressive and persistent reduction in response to electrical stimuli in *ex vivo* experiment and 3rd, a qualitative wave analysis of RAM *ex vivo* myography assay using peak and strength parameters allow us to demonstrate in the PSUI RAM *ex vivo* myography a tendency of the loss of progressive fall of the peaks observed in control group differentiating both pregnant groups (PSUI and control).

Interpretation of results and comparison with existing literature

By definition, PSUI has been considered a transient condition, in prenatal

period. However, there are data to suggest that women with PSUI continue to be at an increased risk for postpartum and long UI (2,19). PSUI in women with gestational hyperglycemia worsens the occurrence and severity of UI, and the impact of UI on QoL over the first year postpartum (27). Meantime, the underlying mechanisms responsible for this stressful situation in pregnant women with normal glucose tolerance is undervalued and under investigated in the current literature.

We found that women with PSUI had similar reduced wave peak (contractile response) values in *ex vivo* myography from first to ninth window compared with women without PSUI. The others four parameters analyzed did not detect any significative difference. Previously, we demonstrated that this control group showed decreased collagen expression and area and decreased slow and fast fiber area (38). These findings suggest a possible interference in muscle contraction that we are unable to demonstrate by quantitative *ex vivo* myography.

One possible explanation may be the high standard deviation in statistical analysis. Another possible explanation may arise from the non-valuation of the 2 types of wave records named by our group as Type 1 and Type 2 (Reyes *et al*. 2022 submitted) in the quantitative analysis performed (Figure 7). Other studies have shown altered muscle morphology in women with UI, including muscle atrophy caused by pudendal nerve denervation and muscle loss caused by impairment of insertion points for the puborectalis muscle (52).These changes were related to damage to the pelvic nerves, pelvic muscles, and connective tissue attachments due to the delivery (53,54).

Surprisingly, the PSUI group gained less weight throughout pregnancy

than the control group, implying that PSUI is more likely to be caused by *in situ* muscle alterations rather than mechanical changes caused by increased fetal pressure and weight gain. Our results showed that control group presented an increase in 1.5-fold weight ($p<0.05$). Weight gain is also linked to the patient's lifestyle, metabolic and hormonal changes and disorders associated with increased fluid storage (e.g., swelling during pregnancy).

Regarding the characteristics of muscle, there was no difference between the weight, width and length of muscle biopsy collected for *ex vivo* analysis. This means that the same amount of muscle fibers was used in both groups. So, the muscle response accurately reflects muscle condition and not the amount of muscle fibers present in biopsy.

Qualitative analysis, and peak assay by *ex vivo* myography (Figure 6) differentiated control and PSUI groups. After each electric stimulus, control group shows a progressive decline of strength in contractile performance that seems to represent the physiological muscular response (Figure 6 C and D). Although, some RAM samples from control group presents declines in contractile performance sudden cease the muscle response to electrical stimulation. This may be due to muscle death during analysis. The sudden stop probably follows muscle death. This premature death may be due to some factors as: difficulties to obtaining RAM sample at C-section, transport conditions from maternity to experimental lab and even due to the assembly of the muscle in the equipment or caused by its own characteristics (Figure 6 E and F).

Some patients in the control group have a progressive decrease of electrical stimulation response, which is followed by a gradual loss of strength. (Figure 6 G and H). This could be due to the patient's physical condition, such as

maternal hypotonia, which causes a higher loss of response to electrical stimulation than those who have stronger muscles (lifestyle).

In the PSUI group, there was a significant difference in contraction force. However, with a lesser force and a big force disparity, a response trend similar to the control group (Figure 6 I and J) was found. It was not able to confirm the control group's observation of a decreasing strength trend. During the experiment, some patients in the PSUI group display an abrupt cessation of muscular response due to tissue death (Figure 6 K and L). A progressive loss of responsiveness and reduced contraction force were also noted in certain cases (Figure 6 M and N).

As a result, more studies are required to corroborate the reported patterns. These analyses revealed that the PSUI group had a lack of coordination, which may impact in urinary continence since the contraction does not occur properly as in healthy muscles.

More in-depth studies of muscle biomechanics need to be carried out to observe fatigue, relaxation time of this muscle and more details of muscle contraction over electrical stimulation. This new investigation will allow us to spot significant alterations in muscle biomechanics that could have biological implications in long UI.

Furthermore, this research lays the groundwork for understanding the biomechanics of RAM in pregnancies worsened by GDM, which has previously been linked to myopathy. Moreover, the addition of new groups and an increase in the number of patients per group will be critical in confirming the quantitative and qualitative statistical analyses.

Another important element to explore further is the appearance of tiny

peaks immediately after the bigger contraction peaks, in order to better understand this response pattern and determine whether or not it has biological importance.

Limitations

The present study is unprecedented, and *ex vivo* myography analyzes, and the comparisons are under development leading to difficult to carry out. However, we also recognize some limitations in our study, which should be considered in future studies. Some samples were mainly due to poor quality or unfeasibility of the collected RAM fragment, lack of standardization in literature, and muscle death, which, as mentioned earlier, can be due to several factors to be controlled.

5 CONCLUSION

In conclusion, the present study underlines a tendency of contraction force incoordination in PSUI group through an original evaluation by *ex vivo* MMA. In addition, control group shows a tendency towards “normal and physiological” contractile response expected for skeletal muscle. Another significant finding was the feasibility of this analysis, which is not yet described in literature for prenatal care management in the future.

We anticipate that with further analysis, these findings will be able to identify different pathways of the pathophysiological basis of PSUI by more accurately determining contractile characteristics in pregnant women using an *ex vivo* myography assay of RAM. Further studies are needed to establish whether this pattern of qualitative and quantitative waves in *ex vivo* MMA in PSUI pregnant

women persists and deteriorates after delivery and renders women with PSUI at increased long UI risk.

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The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the above institutions.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

The Supplementary Material for this article can be found online at:

https://drive.google.com/drive/folders/1B0H1TeIjGvM770l3XNrOQkU1Zl06HD1y?usp=sharing

Data Availability Statement

Data are available on reasonable request. All inquiries about access to data should be sent to: m.rudge@unesp.br

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Seção 4

Perspectivas Acadêmicas e Científicas

Pensar nas perspectivas acadêmicas e científicas na fase final do mestrado, me fez rever todo o árduo processo vivido até aqui e me estimulou ainda mais a cumprir esta etapa que exige tanta dedicação, às vezes, até além da nossa capacidade. A formação no mestrado até o momento, fortaleceu e ampliou a perspectiva de iniciar a carreira docente e de pesquisadora.

Para a continuidade da busca do conhecimento e colaboração para melhorar a assistência a mulher, a proposta é permanecer como aluna de Doutorado, com início em março de 2022, na mesma linha de pesquisa desta trajetória e continuar contribuindo na composição da caracterização da IU-EG e da miopatia diabética por estudo morfofuncional como preditores da IU pós-parto de gestantes com DMG.

Sinto-me honrada em continuar a fazer parte do *Diamater Study Group!*

Espero poder levar conhecimento e principalmente beneficiar as mulheres, ao longo desta nova etapa da minha vida.

Seção 5

Diamater Study Group

Abaixo segue nominalmente o grupo Diamater

Pesquisadores Nacionais

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
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 Girotto 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

Pesquisadores Internacionais

PhD Adonis Hijas
PhD Luis Sobrevia Luarte
PhD Bary Berghmans
PhD Rob de Bie
PhD Costanza Emanueli
PhD Baerbel Junginger
PhD Antonio Musàro
PhD Lehana Thabane

Pesquisadores Associados

PhD Raghavendra Hallur Lakshmana Shetty
PhD David Rafael Abreu Reyes
Dra Fernanda Cristina Bérgamo Alves
Dr. João Paulo de Castro Marcondes
Dra. Maíra Libertad Soligo Takemoto
Dra. Juliana Ferreira Floriano
Dra. Fernanda Piculo
Dra. Gabriela Marini Prata
Dra Cibele Vieria Cunha Rudge
Dra. Talita Costa Domingues
Dr. Fabio Joly Campos
Dr. Ícaro Putinhon Caruso
Dr. Lucas Trevizani Rasmussen
Dr. Vinícius Krieger Costa Nogueira
Dra. Caroline Baldini Prudencio
Dra. Fabiane Affonso Pinheiro
Dr. 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 Santos
Ms. Isabella Otenio de Lourenço
Ms. Jéssica Maróstica de Sá
Ms. Raissa Escandiusi Avramidis
Ms Guilherme Thomaz de Aquino Nava
Eusebio Mario Amador Enriquez
Jose Vitor Freitas Melo (*in memoriam)*
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 Pascon Marques
Michele Jacomin
Ana Julia Bimbatti Silva
Maiara Isabele Gonçalves Orlandi
Tatiana Daniele Dangió
Vitória Pascon Barbosa

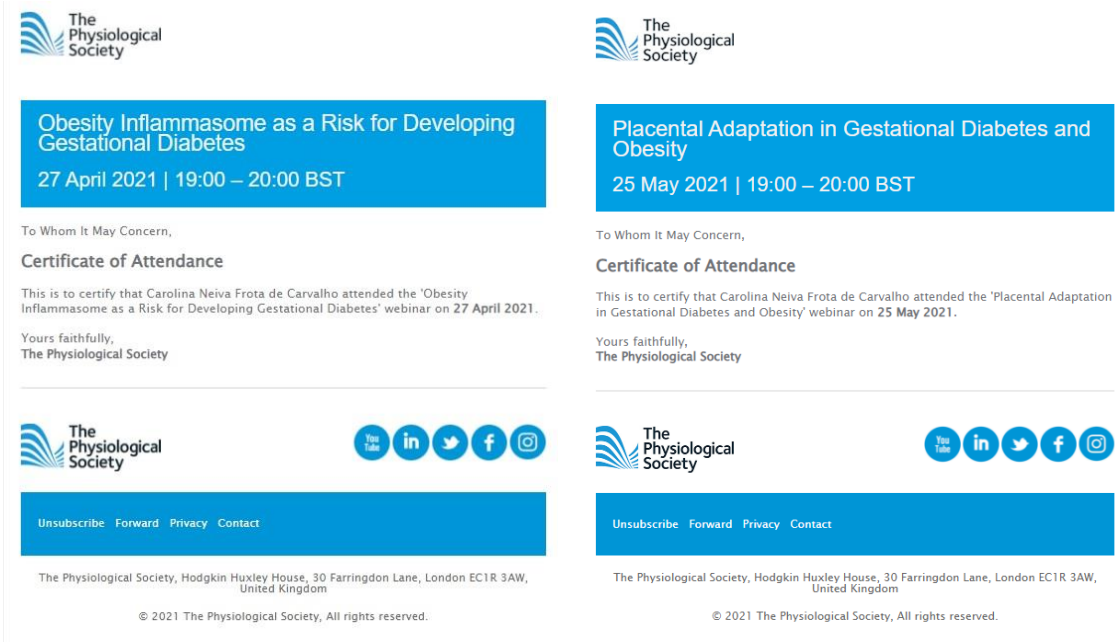
Apoio à Pesquisa

Rita de Cássia Athanázio
Cinthia Scolastico Cecilio

Seção 6

Anexos

Anexo 1 – Participação em eventos internacionais



The Physiological Society

Obesity Inflammasome as a Risk for Developing Gestational Diabetes

27 April 2021 | 19:00 – 20:00 BST

To Whom It May Concern,

Certificate of Attendance

This is to certify that Carolina Neiva Frota de Carvalho attended the 'Obesity Inflammasome as a Risk for Developing Gestational Diabetes' webinar on **27 April 2021**.

Yours faithfully,
The Physiological Society

The Physiological Society

Unsubscribe Forward Privacy Contact

The Physiological Society, Hodgkin Huxley House, 30 Farringdon Lane, London EC1R 3AW, United Kingdom

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The Physiological Society

Placental Adaptation in Gestational Diabetes and Obesity

25 May 2021 | 19:00 – 20:00 BST

To Whom It May Concern,

Certificate of Attendance

This is to certify that Carolina Neiva Frota de Carvalho attended the 'Placental Adaptation in Gestational Diabetes and Obesity' webinar on **25 May 2021**.

Yours faithfully,
The Physiological Society

The Physiological Society

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The Physiological Society, Hodgkin Huxley House, 30 Farringdon Lane, London EC1R 3AW, United Kingdom

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The Physiological Society

Diabetes, Obesity and Pregnancy Outcomes

29 June 2021 | 19:00 – 20:00 BST

To Whom It May Concern,

Certificate of Attendance

This is to certify that Carolina Neiva Frota de Carvalho attended the 'Diabetes, Obesity and Pregnancy Outcomes' webinar on **29 June 2021**.

Yours faithfully,
The Physiological Society

The Physiological Society

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Anexo 2 – Concessão Bolsa Treinamento Técnico

FAPESP SAGe

Processo

Identificação do Processo

Número do Processo	2021/03678-4 - BCO - Treinamento Técnico
Situação	Em Execução
Grupo de Financiamento	Bolsa no País
Linha de Fomento	Bolsas Concedidas como Itens Orçamentários em Auxílios / BCO - Treinamento Técnico - Fluxo Contínuo
Beneficiário	Carolina Neiva Frota de Carvalho
Responsável	Marilza Vieira Cunha Rudge
Data Início	01/05/2021
Duração	11 mês(es)
Período Total Usufruído	7 mês(es) / 22 dia(s)
Período Total Interrompido	-

Instituição de Pesquisa/Empresa	Faculdade de Medicina de Botucatu/FMB/UNESP
Departamento	Ginecologia e Obstetrícia
Data de Abertura	16/04/2021

Processo Vinculado

Número	Linha de Fomento	Beneficiário	Responsável	Título
2016/01743-5	Projeto de Pesquisa - Temático	Marilza Vieira Cunha Rudge	Marilza Vieira Cunha Rudge	Coorte da tríade gestacional hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica na predição de incontinência e disfunção muscular e pesquisa translacional com biodevice para regeneração muscular em ratas.

Resumo

Introdução: O grupo de pesquisa "Diabetes e Gravidez - Clínico e Experimental" da Faculdade de Medicina de Botucatu-Unesp, investiga desde 2006, a fisiopatologia de diabete gestacional (DMG) e da hiperglicemia gestacional leve (HGL) e suas relações com as disfunções musculares do assoalho pélvico (DMAP) e incontinência urinária (IU) em estudos clínicos e experimentais. Após análise crítica no grupo e com parceiros nacionais e internacionais dos resultados obtidos, verificaram-se grandes lacunas que precisam ser preenchidas com estudos pré-clínicos e clínicos mais complexos. Objetivos: Analisar a influência da associação entre hiperglicemia gestacional (HGG) considerando DMG e HGL, "IU específica da gestaçao" ("IUEG") e perfil (morfológico, funcional, bioquímico, molecular e ômico) da "miopatia hiperglicêmica gestacional" ("MHG") do músculo reto abdominal (MRA) e músculos do assoalho pélvico (MAPs); analisar a tríade HGG, "IUEG" e perfil da "MHG", a incidência de IU 6-12 meses pós-parto (PP) e perfil morfológico, funcional e bioquímico, dentro das quatro coortes constituídas pela combinação entre HGG e "IUEG"; avaliar o efeito do biodevice de látex de seringueira com células-tronco mesenquimais na regeneração do músculo uretral e do MRA após cesárea em ratas prenhes com diabete moderado no perfil morfológico, microvascular, molecular e ômico; e determinar a evidência científica, por meio da revisão sistemática, da efetividade dos exercícios sobre a DMAP e "IUEG" em gestantes com HG. Materiais e Métodos: No estudo clínico, as participantes serão selecionadas entre janeiro 2016 e dezembro de 2019 no Centro de Investigação do Diabete Perinatal da FMB UNESP, no Ambulatório de Gestação de Risco do Hospital Materno Infantil da FAMEMA e no Hospital Regional de Assis. O total de participantes será de 160 considerando as quatro coortes que serão constituídas. Será realizada análise do perfil bioquímico, obtido pelas análises sanguíneas dos níveis de CCL7, Relaxina, Ca, PTH, calcitonina, vitamina D, glicose e insulina; nos MAPs, serão realizados a Avaliação Funcional do Assoalho pélvico (AFA), Eletromiografia (EMG) e Ultrassonografia (US), nos MRA serão realizados EMG e US, no momento da cesárea a contratilidade ex vivo obtida pelo Miógrafo, e os perfis morfológico (ultraestrutura das fibras musculares e da matriz extracelular), molecular (Expressão Génica e Proteica das fibras musculares e da matriz extracelular), e Ômico (transcriptômica, proteômica e metabolômica). Para o estudo experimental, serão utilizadas ratas Wistar prenhes, divididas em grupos (n=20 cada) não diabéticas e diabéticas, que serão submetidas à instalação do biodevice de biomembrana de látex revestida de células-tronco mesenquimais, implantados na musculatura estriada uretral e MRA. O estudo de Revisão Sistemática seguirá as normas e será registado na Colaboração Cochrane. Na análise estatística com o software SPSS v21.0, as relações serão consideradas estatisticamente significativas de $p < 0,05$. Resultados esperados: Caracterizar a "MHG"; que a tríade proposta seja preditora de IU e DMAP 6-12 meses PP; definir marcadores para diagnóstico e em futuras terapias; evidenciar a primeira linha de intervenção preventiva ou reabilitadora para as disfunções musculares e "IUEG"; que a "IUEG" na HGG seja considerada e incluída como um problema de saúde pública e como intercorrência clínica, passando a representar uma nova ferramenta na definição do impacto materno de longo prazo; que o biodevice seja considerado para regeneração da musculatura em futuros estudos clínicos; e que esta abordagem translacional proposta trará esta pesquisa para a fronteira do conhecimento na área de Saúde da Mulher, uma vez que estão sendo aplicadas metodologias de análise inéditas na área que se complementam na abordagem de problemática de interesse médico/social.

Anexo 3 – Artigo publicado em periódico

PLOS ONE

REGISTERED REPORT PROTOCOL

Effectiveness of the pelvic floor muscle training on muscular dysfunction and pregnancy specific urinary incontinence in pregnant women with gestational diabetes mellitus: A systematic review protocol

Angélica Márcia Pascon Barbosa[1☯‡], Eusebio Mario Amador Enriquez[1☯‡], Meline Rossetto Kron Rodrigues[2☯], Caroline Baldini Prudencio[1☯], Alvaro Nagib Atallah[3☯], David Rafael Abreu Reyes[2☯], Raghavendra Lakshmana Shetty Hallur[2☯], Sthefanie Kenickel Nunes[2☯], Fabiana Affonso Pinheiro[2☯], Carlos Isaias Sarbório Filho[2☯], Gabriela Lopes Piemonte Andrade[2☯], Bary Berghmans[3☯], Rob de Bie[3☯], Silvana Andréa Molina Lima[1☯¶], Marilza Vieira Cunha Rudge[2☯¶], The Diamater Study Group[¶]

1 Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marília, São Paulo State, Brazil, 2 Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu, São Paulo State, Brazil, 3 Stricto Sensu Graduation Program in Nursing at UNIVERITAS, Guarulhos University (UNG), Guarulhos, São Paulo, Brazil, 4 Discipline of Evidence-Based Medicine, Paulista School of Medicine-Federal University of São Paulo (EPM-UNIFESP), São Paulo, Brazil, 5 Department of Physiotherapy, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, São Paulo State, Brazil, 6 Pelvic Care Center Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands, 7 Department of Nursing, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu, São Paulo State, Brazil

☯ These authors contributed equally to this work.
‡ AMPB and EMAE are first authors on this work. SAML and MVCR are last authors on this work.
¶ Membership of The Diamater Study Group is listed in the Acknowledgments.
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OPEN ACCESS

Citation: Barbosa AMP, Enriquez EMA, Rodrigues MRK, Prudencio CB, Atallah ÂN, Reyes DRA, et al. (2020) Effectiveness of the pelvic floor muscle training on muscular dysfunction and pregnancy specific urinary incontinence in pregnant women with gestational diabetes mellitus: A systematic review protocol. PLoS ONE 15(12): e0241962. https://doi.org/10.1371/journal.pone.0241962

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Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0241962

Abstract

Background

There is ample evidence that gestational diabetes mellitus has a direct influence on urinary incontinence and pelvic floor muscles. There are no standardized pelvic floor muscle exercise programs in the literature for the physiotherapy and differ in the type of exercise, intensity, type and duration of application, and the frequency and duration of treatment sessions. The aim of this systematic review will be to investigate that Pelvic Floor Muscle Training can prevent and/or decrease the pregnancy specific urinary incontinence in women with gestational diabetes mellitus or gestational hyperglycemia.

Methods

We will perform a systematic review according to the Cochrane methodology of Randomized Controlled Trials. An overall search strategy will be developed and adapted for Embase, MEDLINE, LILACS, and CENTRAL databases, with the date of consultation until

Anexo 4 - Aprovação Comitê de Ética em Pesquisa

UNESP -FACULDADE DE MEDICINA DE BOTUCATU

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

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

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

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

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

Pesquisador: Marilza Vieira Cunha Rudge
Área Temática:
Versão: 2
CAAE: 82225617.0.0000.5411
Instituição Proponente: Departamento de Ginecologia e Obstetrícia
Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 2.511.990

Apresentação do Projeto:

Trata-se de um projeto temático com 03 subprojetos.

Projeto Mãe: Estudo de coorte prospectivo: nova tríade gestacional (hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica) na predição de

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