UNIVERSIDADE ESTADUAL PAULISTA
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Giovana Vesentini

Repercussões da hiperglicemia gestacional e incontinência urinária gestacional no músculo reto abdominal: matriz extracelular, expressão proteica e ultraestrutura

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

Orientadora: Profa. Dra. Marilza Vieira Cunha Rudge
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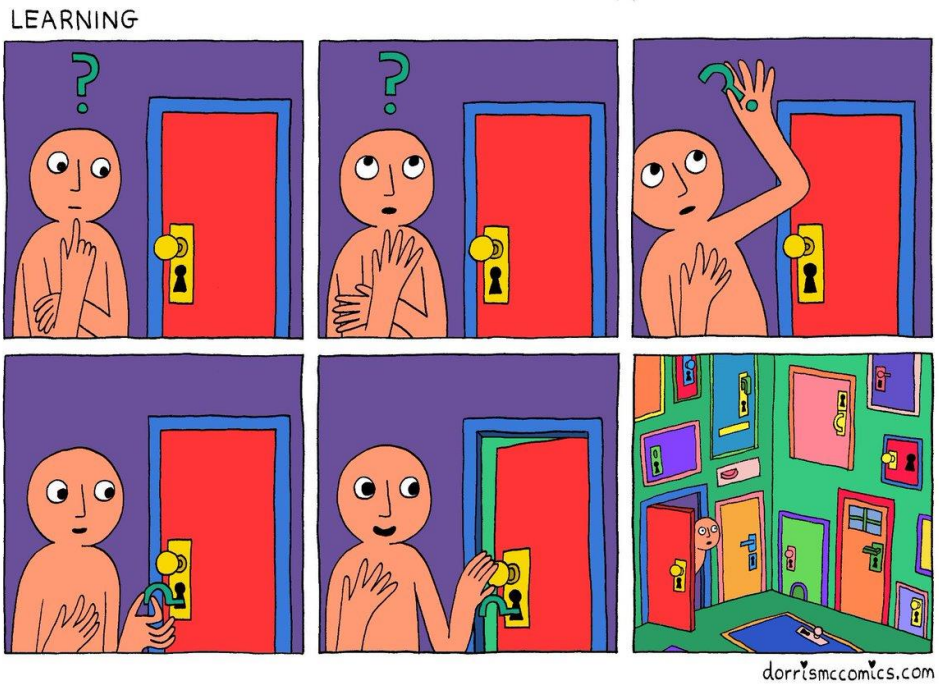
Sumário



Fonte: https://dorrismccomics.com/

Capítulo 1

Review Article

PELVIC FLOOR AND ABDOMINAL MUSCLE CO-CONTRACTION IN WOMEN WITH AND WITHOUT PELVIC FLOOR DYSFUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Running title: Co-contraction of PFM and abdominal muscle

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Author contributions

GV, RED and MVCR were involved in the conception and design of the review. GV and RED developed the search strategy. GV and LARR performed study selection and data collection. GV, RED, LARR and MVCR were involved in the data analysis. GV, RED, MVCR and AMPB were involved in the interpretation and discussion of results. GV drafted the manuscript, and RED, FP, GM, GARF, IMPC, AMPB and MVCR contributed to the drafting of the review. All authors approved the final version for publication.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ABSTRACT

Objective: There is an ongoing discussion about the abdominal muscle (AbM) and pelvic floor muscles (PFM) synergism. To investigate the co-contraction between AbM and PFM in women with or without pelvic floor dysfunction (PFD).

Method: The following databases were searched up to 21 December 2018: MEDLINE, EMBASE, LILACS, PEDro and CENTRAL. were searched up to. We accept any study that assess the co-contraction between PFM and AbM in women with and without PFD. Two reviewers independently screened eligible articles and extracted data. The outcomes were extracted and analyzed as continuous variables with random effect models.

Results: Twenty studies were included. Meta-analysis did not show difference in women with and without PFD. However, a sensitivity analysis suggests co-contraction of the transversus abdominis (TrA) during PFM contraction in healthy women (SMD −1.02 [95% CI −1.90 to −0.14], p = 0.02; I^2= not applicable; very low quality of evidence). Women with PFD during contraction of PFM showed co-contraction of the obliquus internus (OI) (SMD 1.10 [95% CI 0.27 to 1.94], p = 0.01; I^2= not applicable; very-low quality of evidence), and obliquus externus (OE) (SMD 2.08 [95% CI 1.10 to 3.06], p < 0.0001; I^2= not applicable; very low quality of evidence).

Conclusions: Increased co-contraction of the TrA may be associated with maximal contraction of PFM in women without PFD. On the other side, there is likely an increased co-contraction with OI and OE in women with PFD.

Keywords: pelvic floor disorders; abdomino-pelvic muscles; pelvic floor function; synergism

INTRODUCTION

Pelvic floor dysfunction (PFD) refers to a group of disturbances in the pelvic floor muscles (PFM) or connective tissues usually associated to pelvic organ prolapse, urinary and/or anal incontinence, sexual dysfunction, and pelvic pain (1). Treatment-related costs are estimated to correspond to an annual spending of 12 billion dollars and are projected to increase every year (2) with a considerable prevalence according to the population and definition used (3). The estimated prevalence is reported to be 25% to 46% in high-income (4), low-income and middle-income countries (5). This is a common disease that affects women at all ages, leading to a severe impact on their lives and consuming considerable health resources (4).

Researchers have been reporting strategies, such as the use of a model of abdominal muscle (AbM) training to stimulate tonic PFM activity (6). This scientific evidence is based on the idea of synergistic co-contraction of the PFM and AbM, which occurs during normal activities (7, 8). Although there is an established literature highlighting that PFM and AbM interaction is usually present in asymptomatic women (9), clinical practice guidelines for conservative management of PFD (10, 11) demonstrated that the AbM remains a neglected aspect of care. The addition of AbM training might improve clinical outcomes for patients with PFD (12) and restore the normal PFM function. The lack establishment in the co-activation between PFM and AbM in women with PFD might reflect the lack of robust evidence that exercise regimens other than PFM training would potentially add benefits to conservative management of PFD (13).

The understanding of co-contraction among AbM and PFM could be valuable for alternative strategies of PFM exercises to promote continence. In this systematic review, we investigate the co-activity of AbM – transversus abdominis (TrA), rectus abdominis

(RA), obliquus internus (OI), and obliquus externus (OE) – and PFM in women with or without PFD. We hypothesized that women with PFD would show decreased co-activity of the AbM or PFM during maximal voluntary contraction (MVC) of PFM or AbM compared to women with no history of PFD.

MATERIALS AND METHODS

Adhering to the Preferred Reporting Items for Systematic Reviews and Meta-analyses - PRISMA (14) and Meta-analysis of Observational Studies in Epidemiology - MOOSE (15) guidelines, this review was registered on PROSPERO (CRD42017055462).

Eligibility criteria

- Study design: any observational study (cohort, cross-sectional, comparative cross-sectional) or any baseline subset of data provided by randomized controlled trials, as we are avoiding interaction effects due to any applied interventions. Studies that aimed to assess the reliability of scoring systems for the investigation of co-contraction of the muscles under investigation in this review, as well as studies that provided information on our predefined outcomes were also included;
- Participants: women with or without PFD, with urinary incontinence (UI), pelvic organ prolapse (POP), and pelvic pain;
- Interventions: any voluntary contraction of PFM that recorded the co-contraction of AbM (TrA, RA, OI, and OE) and vice versa;
- Outcomes:
    - The co-contraction of AbM (TrA, RA, OI, and OE) and PFM was measured by surface electromyography (EMG), ultrasonography (US), digital palpation scale, or perineometer;

We also considered any indirect assessment of the muscle contraction.

We excluded full-text peer-review studies that evaluated AbM and PFM in resting activity.

Data source and searches

Using the Medical Subject Headings (MeSH), based on combination of terms "female urinary incontinence," "continent," "pelvic floor," "abdominopelvic musculature," and "abdominal muscle," we ran the search strategy in MEDLINE (1980 to 21st December 2018), EMBASE (1980 to 21st December 2018), PEDro (1999 to 21st December 2018), LILACS (1982 to 21st December 2018), and CENTRAL (1999 to 21st December 2018). No language restriction was applied. This strategy was similar for the other databases and executed until December 21, 2018 (Appendix 1).

Selection of studies

Two reviewers (GV and LARR) independently screened all titles and abstracts identified by the literature search, obtained full-text articles of all potentially relevant records, and evaluated them. Disagreements were resolved through discussion or by consulting a third person (RED).

Data extraction

Data from included studies were summarized in a standardized data extraction with participant demographics, inclusion and exclusion criteria, co-contraction measurement methods, muscles studied and outcomes. Two reviewers (GV and LARR) extracted the sample size, means and standard deviations (SD). When SD data were unavailable, we estimated the SD using the standard error according to the recommendations of the Cochrane Handbook (16).

If data regarding methods or results was incomplete, we attempted to contact the authors for further information. Moreover, when we found figures without data, we used the WebPlotDigitizer® (v. 3.8) for Windows to extract an estimation of the data from the

figures.

Risk of bias assessment

The risk of bias with a modified version of the Ottawa-Newcastle instrument was independently assessed by the reviewers (17). This tool includes confidence in assessment of exposure and outcome, adjusted analysis for differences between groups in prognostic characteristics and missing data (17). When information regarding risk of bias or other aspects of methods or results was unavailable, we attempted to contact study authors for additional information.

Certainty of evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used to rate the certainty of the evidence for each outcome measure as high, moderate, low, or very low (18). Detailed GRADE guidance was according to the following: imprecision (19), inconsistency (20), indirectness (21) and the results are summarized in a Table of evidence profile.

Data synthesis and statistical analysis

We analyzed the outcomes as continuous variables with random effect models on the results from the muscles investigated (TrA, RA, OI, and OE). Since the assessment of co-contraction in the included studies were measured in different ways (e.g. US and EMG) the individual scales were aligned to point in the same direction and we calculated the standardized mean difference (SMD) along with the respective confidence interval (CI) of 95%, using the extracted means and SDs (16). A positive SMD values indicated higher co-contraction of the evaluated muscle in the PFD group compared to the asymptomatic group, and a negative SMD indicated higher co-contraction of the evaluated muscle in the asymptomatic group compared to the PFD group.

We also conducted sensitivity analyses to test the robustness of those results.

When data was obtained from RCTs and the results was provided separately by intervention and control groups, we calculated the baseline mean and SD based on the mean and SD from the studies. Furthermore, when studies provided both left and right sides of the AbM, we also calculated the mean and SD based on the mean and SD provided for both sides.

We calculated the heterogeneity across studies using the I^2 statistic and the p-value for the Chi-square test using Review Manager software (RevMan version 5.3; Nordic Cochrane Centre, Cochrane).

RESULTS

Search results

Figure 1 presents the PRISMA flow diagram of identifying eligible studies based on title and abstract screening. After assessment of 93 full texts, we included 20 studies included in the systematic review with subset of data provided by one RCT (22) one prospective (23) and 18 cross-sectional studies (8, 9, 24-39) with a total of 468 participants. The inter-observer agreement for screening was substantial (kappa 0.82).

Study characteristics

The sample size of the studies ranged from three (26) to 44 (31) participants. Typical participants were aged from 19 (25) to 66 (34) years old. (Table 1). From a total of 20 included studies, four (8, 9, 32, 39) recorded the activity of all AbM (TrA, RA, OI, and OE) during PFM contraction and 19 studies provided instruction to contract PFM and recorded the AbM co-activity (8, 9, 22-35, 37-39). Fifteen studies (8, 9, 22-24, 26, 28, 31-37, 39) reported MVC of PFM. Three studies (23, 27, 34) considered the standing position for the assessment of the co-activity, and other eleven studies the supine position (8, 9, 22, 25, 29-31, 35-38). Four studies (28, 32, 33, 39) considered different positions –

standing, sitting and supine, and one did not report the position for the assessment of co-activity (26). Fifteen studies (8, 9, 23, 25, 26, 28, 30-35, 37-39), measured the contraction by EMG, four (22, 24, 27, 36) by US, and one (29) by visual inspection and digital palpation scale (Table 2).

Risk of bias assessment

Figure 2 describe the risk of bias summary of the studies that compare two groups. Six observational studies compare women with and without PFD. The main problems with the studies was referred to follow-up (24, 29, 31, 34, 36, 38), information regarding co-interventions (24, 29, 31, 34, 36, 38), assessment of outcome (24, 29, 34, 36, 38) and exposure (31, 36, 38). Tables 3 detail the description for each study.

Outcomes

Meta-analysis of transversus abdominis muscle co-contraction when PFM contract

Results from two studies (24, 36) with a total of 52 participants assessing the co-contraction by US, failed to show difference in the co-contraction of TrA in women with and without PFD (SMD −0.61 [95% CI −1.41 to 0.20], p=0.14; I^2= 41%) (Figure 3). However, a plausible sensitivity analysis, excluding the Arab *et al.* (2011) (24) study, yields results that were inconsistent with the primary analysis, showing higher co-activity of TrA during MVC of PFM in women without PFD (SMD −1.02 [95% CI −1.90 to −0.14], p = 0.02; I^2= not applicable) (Figure 4).

Certainty of evidence was rated down to low because of serious limitations on high risk of bias, indirectness due to evaluation of only one PFD (UI) (Figure 3) and different ages, and imprecision (Table 4).

Meta-analysis of rectus abdominis muscle co-contraction when PFM contract

Results from three studies (31, 34, 38) with a total of 128 participants was unable to demonstrated difference of the co-contraction of RA between normal pelvic floor

women and PFD (UI) women (SMD −2.05 [95% CI −6.51 to 2.42], p=0.37; I^2= 98%) (Figure 3). Furthermore, the sensitivity analysis, excluding the Madill *et al.* (2009) study (31), showed results that were inconsistent with the primary analysis, with higher co-contraction of RA during MVC of PFM in women with PFD, however with no statistical significance (SMD 0.89 [95% CI -0.03 to 1.82], p = 0.06; I^2= 63%) (Figure 4).

Certainty of evidence was rated down to very low because of serious limitations on high risk of bias, inconsistency due to high heterogeneity (Figure 3), indirectness due to evaluation of only one PFD (UI), different assessments of UI and different ages, and imprecision (Table 4).

Meta-analysis of obliquus internus abdominis muscle co-contraction when PFM contract

Results from three studies (24, 31, 38) with a total of 118 participants showed no difference between normal pelvic floor women and PFD (UI) women (SMD −0.47 [95% CI −2.38 to 1.44], I^2= 95%; p=0.63) (Figure 3). However, a plausible sensitivity analysis, excluding the Madill *et al.* (2009) study (31) and Arab *et al.* (2011) (24), presented results that were inconsistent with the primary analysis, showing a higher mean of co-contraction in women with PFD (UI) compared with normal pelvic floor women (SMD 1.10 [95% CI 0.27 to 1.94], p = 0.01; I^2= not applicable) (Figure 4).

Certainty of evidence was rated down to very low because of serious limitations on inconsistency due to high risk of bias, high heterogeneity (Figure 3), indirectness due to evaluation of only one PFD (UI), different assessments of UI and different ages, and imprecision (Table 4).

Meta-analysis of obliquus externus abdominis muscle co-contraction when PFM contract

Results from two studies (31, 38) with a total of 98 participants failed to show

difference between normal pelvic floor women and PFD women (SMD 0.01 [95% CI −4.00 to 4.03], p=1.00; I^2= 98%) (Figure 3). However, a plausible sensitivity analysis, excluding the Madill *et al.* (2009) study (31), demonstrated results that were inconsistent with the primary analysis, showing a higher mean of co-contraction in PFD (UI) women compared with normal pelvic floor women (SMD 2.08 [95% CI 1.10 to 3.06], p < 0.0001; I^2= not applicable) (Figure 4).

Certainty of evidence was rated down to very low because of serious limitations on inconsistency due to high heterogeneity (Figure 3), indirectness due to high risk of bias, evaluation of only one PFD (UI), different assessments of UI and different ages, and imprecision (Table 4).

DISCUSSION

Main findings

This systematic review investigating the co-contraction of AbM and PFM in women with or without PFD identified 20 studies. Therefore, it might provide evidence of synergism between PFM and TrA, RA, OI and OE, i.e., the co-contraction of PFM and AbM occur during both voluntary contraction of the pelvic floor and abdominal muscle contractions. The studies showed a co-contraction of abdominal muscles during contraction of PFM in women with no history of symptoms of PFD, with PFD, and both. Meta-analysis of data from five cross-sectional studies assessed the synergism of TrA, RA, OI, and OE during MVC of PFM. As the primary meta-analysis failed to show any difference between women with and without PFD, we performed a sensitivity analysis to minimize the heterogeneity of data. Our sensitivity analysis showed a different co-contraction pattern according to the four AbM considered. The co-contraction between TrA and PFM in asymptomatic women showed a higher activation compared to

symptomatic women. However, women with PFD, such as UI, demonstrated an increased co-contraction of AbM (RA, OI, and OE) compared to women without PFD, suggesting an altered mechanism.

One study (24) was excluded for a sensitivity analysis on co-contraction of TrA and OI, because it did not report the position of women during the measurement. Also, prior to the testing, as participants were trained until the correct performance of contraction of PFM, we believe that the training before the measurement may have affected the data provided. Furthermore, another study (31) was not included in a sensitivity analysis of RA, OI, and OE. Although this study had the highest sample size, women with PFD were classified as mild and severe UI, according to the severity of urine leakage. Moreover, the EMG data provided were smoothed by computing root mean square. In this sensitivity analysis of RA, the I^2 value, previously at 100%, reduced to 0% when this study (31) was removed. Moreover, the results from the sensitivity analysis in OI and OE reached a statistical significance favoring the PFD group.

Strengths and limitations

The strengths of our study include our unique analysis of the influence of each one of the four muscles from the abdominal wall during maximal and submaximal contraction of PFM. Additionally, we have provided evidence of different synergism between AbM and PFM in women with and without PFD.

The primary limitation of our review is the low evidence because of study limitations. We identified a small number of studies with a small number of participants, resulting in high CIs, therefore, these findings should be carefully interpreted. EMG results should be cautiously interpreted because most studies used surface electrodes and this may contaminate data and distort their interpretation from the muscles surrounded (40). Additionally, the data processing of EMG studies is widely different, mostly by the

position of electrodes, position of evaluation, and type of data normalization.

Another limitation of this review was given the insufficient number of included studies, we were not able to carry out the complete statistical analysis. Publication bias were not assessed because there were <10 eligible for each outcome in a meta-analysis (16).

Relation to prior work

Although previous systematic reviews have shown evidence of co-contraction between PFM and AbM (41, 42), investigators had not previously conducted a comparison between women with normal pelvic floor and PFD involving all four muscles of the abdominal wall (TrA, RA, OI, and OE). Furthermore, to our knowledge, there is no meta-analysis publication about the co-contraction between PFM and the four AbM.

The first systematic review related to this theme focuses only in the combined training of TrA and PFM to treat UI and included five studies (41). Another previous systematic review focuses only on healthy women and included ten studies (42). In contrast, our search found 20 studies, and only five could be included in the meta-analyses. Our much larger analyses, including 468 women, provided more precisely the biomechanics of the communication between the abdominopelvic muscles both in normal pelvic floor and PFD. Furthermore, we have also been able to detect the influence of each one of the four muscles of the abdominal wall in the PFM contraction.

Implications

PFD is very common among women worldwide and it has become an increasing socioeconomic problem with prejudicial public health consequences, including symptoms that could lead to a significant decrease in quality of life and disability (43). While the prevalence of PFD is high, many factors involved in PFD are often poorly recognized or understood. Knowing the pathways related to PFD in detail is a main goal

to seek the tools to prevent or correct these disorders (44). Our findings were able to suggest a mechanism of PFD according to changes in the biomechanics caused by the increased AbM activation strength or by recruitment timing activation associated with different co-activity mechanisms according to the AbM and PFM.

In our view, there is a plausible biomechanical explanation to support higher co-activation levels of AbM during MVC of PFM. In asymptomatic women, the co-activation between TrA and PFM showed a higher activation compared to symptomatic women. However, the pattern of activation of the other AbM is different in time and strength in symptomatic women. During the contraction of PFD, there is a rapid and stronger co-activity of RA, OI, and OE. This higher strength co-activity of the these AbM could implicate in an increase of intra-abdominal pressure that, added to the insufficient PFM contraction, would increase the PFD.

Pereira *et al*. (45) has proposed a theory explaining the synergism between TrA and PFM. The abdomino-pelvic cavity has a static function of containment of the viscera and interacts with the PFM. The fibers from TrA are prolonged by the transverse perineal muscle because these muscles belong to the same muscle chain. This is an important conclusion for rehabilitation therapy, since numerous studies focus only in the TrA strengthening to demand higher muscular strength of PFM (22, 27, 35, 36). The knowledge of synergism among the PFM and AbM may provide useful tool to assessment of PFM and teaching of PFM exercises.

Our results show a synergism between AbM and PFM in women with and without PFD in different positions of evaluation. However, the studies included in this review had no standardized methods for selecting the participants, sample size, EMG, and US measurement, which limits the reliability of the findings. Very low-quality evidence suggests an association between the co-contraction of the abdominal muscles when PFM

contract either on normal pelvic floor or on PFD women and should be interpreted with caution. Further research is needed to provide better understanding of the co-contraction between the PFM and AbM.

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FIGURE CAPTIONS

Figure 1. Flowchart of the studies included in this review.

Figure 2. Risk of bias assessment. We considered "probably high risk of bias" as "definitely high risk of bias" (red color) and "probably low risk of bias" as "definitely low risk of bias" (green color).

Figure 3. Forest plot showing the co-activity of transversus abdominis, rectus abdominis, obliquus internus and obliquus externus muscles during maximal pelvic floor muscle contraction.
CI = Confidence interval; PFD = Pelvic floor dysfunction.

Figure 4. Sensitivity analysis of co-activity of transversus abdominis (without the Arab *et al.* 2011 study), rectus abdominis (without the Madill *et al* 2009 study), obliquus internus (without the Madill *et al* 2009 study) and obliquus externus (without the Madill *et al* 2009 study) muscles when the pelvic floor muscle contracts.
CI = Confidence interval; PFD = Pelvic floor dysfunction.

Table 1. Study characteristics related to study design, location, number of participants, mean age, inclusion and exclusion criteria.

Author, year	Study design	Location	No. participants	Mean age	Inclusion criteria	Exclusion criteria
With Pelvic Floor Dysfunction						
Bø et al., 2009	Cross-sectional	Europe	13	46.5	Consecutive women, at their first consultation in an ongoing randomized clinical trial on PFMT to reduce POP	Inability to understand the Norwegian language and contract the PFM; nulliparous or less than 12m pp; previous pelvic surgery; chronic lung disease, or stage 0 and 4 POP measured by the POP quantified
Tajiri et al., 2014	Randomized control trial	Asia	15	52	Women who had experienced one or more SUI events in the last 1 m	NR
Without Pelvic Floor Dysfunction						
Bø et al., 1990	Cross-sectional	Europe	3	31.6	Physical therapists; aged 30-33 y; extensive experience in correct PFM contractions	NR
Bø et al., 1994	Cross-sectional	Europe	6	19.5	Women aged 19-21 y; nulliparous; no history of UI, neurological disease, or urinary tract infection; exercising regularly more than 3×/week	NR
Sapsford et al., 2001	Cross-sectional	Australia	7	49.3	Parous women with a history of vaginal deliveries	History of PFD or LBP; abdominal or pelvic surgery; neurological or respiratory condition; regularly performing sit-ups or abdominal muscle training

Neumann et al., 2002	Cross-sectional	Australia	4	34	Nulliparous women aged 25-42 y, who were tested on two occasions 1 week apart	Skinfold thickness of >2.5 cm; history of LBP; known or suspected pregnancy; UI; urinary tract or vaginal infection; surgery involving incision of the left abdominal wall
Madill et al., 2006	Cross-sectional	Canada	15	36.3	Continent women aged 21-60 y; not pregnant; had not given birth in the previous 12 months; in good general health	History of DM, neurological conditions, or autoimmune CT disorders; used any medications to treat or known to exacerbate UI; previous history of SUI
Thompson et al., 2006a	Cross-sectional	Australia	13	37	Women aged 20–55 y and premenopausal or on HRT and consistent PFM exercise technique	History of urinary tract or vaginal infection; known or suspected pregnancy; surgery involving incision of the abdominal wall; obesity; history of LBP or sporting activities; neurological disorders; inability to understand English
Madill et al., 2008	Cross-sectional	Canada	15	36.3	Women aged 21–60y; no history of SUI; not pregnant or had not given birth in the previous 12m and in good general health	History of DM; neurological conditions; autoimmune CT disorders; used any medications to treat or known to exacerbate UI; history of SUI
Junginger et al., 2010	Cross-sectional	NR	9	42	Volunteers without PFM disorders; aged 32-59 y; with height 157–174 cm and weight 57-72 kg	History of LBP; hip or abdominal surgery or history of PFD and of laparotomy
Strupp et al., 2011	Cross-sectional	Central and South America	34	28.1	Willingness to participate in the study and ability to contract the PFM and perform the AHT correctly	Unable to contract AbM and PFM correctly; pregnancy; neurological disease; autoimmune CT disorder or PFD

Chmielewska et al., 2015	Cross-sectional	Europe	19	23.6	Continent women aged 19-28 y	SUI; pregnancy; childbirth(s); pelvic surgery; DM; hypertension; neurological abnormalities; urinary tract infection; elevated temperature; practicing professional sport; spinal pain; obesity
Silva et al., 2016	Prospective	Central and South America	25	24.76	Women aged 18–35y; no history of UI	Virgin women; abdominal-pelvic surgeries; metabolic disorders; presence of myopathies and collagen diseases, neurological disorders, cognitive disturbance and physical limitations; previous PFM training; inability to contract PFM
Ithamar et al., 2018	Cross-sectional	Central and South America	30	25.77	women aged 18-35y; BMI between 18.50-24.99kg/m²; abdominal skinfold ≤ 3 cm; with active or irregularly active physical activity	Abdominal or pelvic surgery; pregnancy; metabolic disorders; smoking; neurological; respiratory or cardiac disease; PFD or menstrual dysfunctions
Both						
Devreese et al., 2004	Cross-sectional	Europe	C: 40 I: 40	C: 50.9 I: 48.4	Patients were referred by the Hospital for an individual pelvic floor exercise program	Subjects with a vaginal, urethra, or bladder infection, neurological disorders, LBP or pregnancy

Thompson et al., 2006b	Cross-sectional	Australia	C: 13 I: 13	C: 37 I: 38	Inclusion criteria for both groups were women aged 20-55 y and premenopausal or on HRT and consistent PFM exercise technique	History of urinary tract or vaginal infection; known or suspected pregnancy; surgery involving incision of the abdominal wall; obesity; history of LBP or sporting activities; neurological disorders; inability to understand English
Madill et al., 2009	Cross-sectional	Canada	C: 28 I: 44	C: 46.8 I: 49.65	C and SUI women aged 21-60 y; not pregnant and not given birth in the last 12m; in good general health.	Previous gynecological or continence surgery, POP greater than stage 2; intrinsic sphincter deficiency; history of DM; neurological conditions; autoimmune CT disorders; used any medications to treat or known to exacerbate UI
Arab et al., 2011	Cross-sectional	Asia	C: 10 I: 10	C: 41.66 I: 38.47	Women with IU, premenopausal, or HRT. Asymptomatic females, matched in age and body mass index and with no symptoms of UI.	Pregnancy and given birth in previous 12m; neurological or respiratory disorders; severe LBP; POP greater than stage 2; surgery of the abdominal or pelvic regions
Tajiri, 2011 et al.,	Cross-sectional	Asia	C: 25 I: 7	C: 45.8 I: 50.1	Primiparous women	Not reported
Ptaszkowski et al., 2015	Cross-sectional	Europe	C: 14 I: 16	C: 66.1 I: 63.9	Control group: no history of SUI; UI group: history of SUI.	Inability to contract the PFM; previous gynecological and abdominal surgery; neurologic condition; contraindications to measurements such as infection, menstruation, and allergy to nickel; other symptoms of PFD

Abbreviations: NR: not reported; C: continent; I: incontinent; No.: number; PFM: pelvic floor muscle; PFD: pelvic floor dysfunction; PFMT: pelvic floor muscle training; AbM: abdominal muscle; LBP: low back pain; HRT: hormone replacement therapy; DM: *Diabetes mellitus*; UI: urinary incontinence; POP: pelvic organ prolapse; CT: connective tissue; y: years; m: months; pp: postpartum; SUI: stress urinary incontinence; cm: centimeters; Kg: kilograms; AHT: abdominal hypopressive technique; BMI: body mass index; cm: centimeters

Table 2. Study characteristics related to population, co-activity, and assessed outcomes.

Author, year	Instruction of co-activity	Maximal Voluntary Contraction (PFM)	Measurement of correct PFM contraction	Position tested	Measurement of contraction	PFMC Measurement and variable assessed	Muscle tested
With Pelvic Floor Dysfunction							
Bø et al., 2009	Activity of PFM during TrA contraction.	NR	Inward lift and squeeze of pelvic openings and vaginal palpation.	Standing	US	Axial plane of minimal hiatal dimensions. Area measured as cm^2.	PFM
Tajiri et al., 2014	Recorded TrA during PFM contraction.	Yes	Verbal orientation	Supine	US	Not applicable	AbM
Without Pelvic Floor Dysfunction							
Bø et al., 1990	Activity of RA during PFM contraction.	Yes	Perineal and vaginal palpation; observation of movement and vaginal pressure measurements.	NR	EMG	Balloon catheters. Results specifications not described.	AbM
Bø et al., 1994	Activity of PFM during abdominal contraction.	NR	Vaginal palpation; observation and vaginal pressure measurements.	Supine	EMG	Needle EMG. Results specifications not described	PFM
Sapsford et al., 2001	Activity of TrA, RA, OI, OE and PFM was recorded during PFM contraction in three different lumbar spine positions.	Yes	Vaginal palpation.	Supine	EMG	Intra-vaginal probe Using NEEN HealthCare. %MVC-normalized EMG amplitudes.	AbM
Neuman et al., 2002	Activity of TrA, OI, and PFM was recorded. The subjects performed PFM and abdominal contraction.	Yes	Perineal and vaginal palpation; observation of movement and vaginal pressure measurements.	Supine and Standing	EMG	Vaginal surface EMG. %MVC-normalized EMG amplitudes.	PFM and AbM

Madill et al., 2006	Activity of TrA, RA, OI, OE and PFM was recorded during PFM contraction.	Yes	Squeezing around the vagina and visible cephalad movement of the perineum, without breath holding.	Supine	EMG	Modified Femiscan™ EMG probe. %MVC-normalized EMG amplitudes.	AbM
Thompson et al., 2006a	Activity of RA, OI, OE, and PFM was recorded during PFM contraction and valsalva.	Yes	Vaginal palpation.	Supine	EMG	Intra-vaginal probe Using NEEN HealthCare. %MVC-normalized EMG amplitudes.	PFM and AbM
Madill et al., 2008	Activity of TrA, RA, OI, and OE was recorded during PFM contraction.	Yes	EMG and pressure and observation of the perineum.	Supine, Sitting, and Standing	EMG	Modified Femiscan™ EMG probe. %MVC-normalized EMG amplitudes.	AbM
Junginger et al., 2010	Activity of TrA and PFM was recorded during abdominal and PFM contraction.	No	Confirmed by EMG.	Supine	EMG	Intra-vaginal probe Periform®. %MVC-normalized EMG amplitudes.	PFM and AbM
Strupp et al., 2011	Activity of TrA and PFM was recorded. The subjects performed AHT and PFM contraction.	Yes	Inspection and vaginal palpation.	Supine	EMG	Intra-vaginal probe Chattanooga Group®. MVEA-normalized EMG.	PFM and AbM
Chmielewska et al., 2015	Measurement of TrA and RA during PFM contraction.	Yes	Confirmed by EMG.	Supine, Sitting, and Standing	EMG	Small diameter intra-vaginal probe. %MVC-normalized EMG amplitudes.	AbM
Silva et al., 2016	Activity of TrA/OI during PFM contraction. Activity of PFM during TrA/OI contraction.	Yes	Vaginal palpation; orientation of how to effectively contract PFM	Standing	EMG	Endovaginal sensor PhysioMed Services®%. MVC-normalized EMG amplitudes.	PFM and AbM
Ithamar et al., 2018	Activity of TrA/OI, RA, OE and PFM during AHT	Yes	Verbal orientation of how to effectively contract PFM	Supine, Standing and quadruped	EMG	Intra-vaginal probe. %MVC-normalized EMG amplitudes.	PFM
			Both				

Devreese et al., 2004	PFM during abdominal contraction.	NR	Inward observation of the perineum and vaginal palpation	Supine	Other	Digital palpation. Scoring system	PFM
Thompson et al., 2006b	Activity of RA, OI, OE, and PFM was recorded during PFM contraction and valsalva.	NR	Vaginal palpation.	Supine	EMG	Intra-vaginal probe Using NEEN HealthCare. %MRC-normalized EMG amplitudes.	PFM and AbM
Madill et al., 2009	Activity of RA, OI, and OE was recorded during PFM contraction.	Yes	Visible cephalad movement of perineum.	Supine	EMG	Modified Femiscan™ EMG probe. RMS-MVC EMG amplitudes.	AbM
Arab et al., 2011	Activity of TrA and OI was recorded during PFM contraction.	Yes	Lifting of the bladder base on transabdominal US.	Not reported	US	Not applicable.	AbM
Tajiri et al., 2011	TrA during PFM contraction.	Yes	NR	Supine	US	Not applicable	AbM
Ptaszkowski et al., 2015	RA during PFM contraction.	Yes	Confirmed by physiotherapist.	Standing	EMG	Life-care Vaginal Probe PR-02. RMS-MVC EMG amplitudes..	PFM and AbM

Abbreviations: NR: not reported; AbM: abdominal muscle; PFM: pelvic floor muscle; TrA: transversus abdominis; RA: rectus abdominis; OI: obliquus internus abdominis; OE: obliquus externus abdominis; EMG: Electromyography; US: Ultrasonography; IAP: intra-abdominal pressure; MVC: maximal voluntary contraction; MRC: maximal reference contraction; MVEA: maximal voluntary electrical activity; RMS: root mean square.

Table 3. Risk of bias assessment of the included studies.

Author, year	Was selection of exposed and non-exposed cohorts drawn from the same population?	Can we be confident in the assessment of exposure?	Can we be confident that the outcome of interest was not present at start of study?	Did the study match exposed and unexposed for all variables that are associated with the outcome of interest or did the statistical analysis adjust for these prognostic variables?	Can we be confident in the assessment of the presence or absence of prognostic factors?	Can we be confident in the assessment of outcome?	Was the follow up of cohorts adequate?	Were co-Interventions similar between groups?
Devreese et al., 2004	Definitely low risk	Probably low risk	Probably low risk	Probably high risk	Definitely low risk	Probably high risk	Definitely high risk	Probably high risk
Thompson et al., 2006b	Probably high risk	Probably high risk	Probably low risk	Probably low risk	Definitely low risk	Probably high risk	Definitely high risk	Probably high risk
Madill et al., 2009	Probably low risk	Probably high risk	Probably low risk	Probably low risk	Definitely low risk	Definitely low risk	Definitely high risk	Probably high risk
Arab et al., 2011	Probably low risk	Probably low risk	Probably low risk	Probably low risk	Probably low risk	Probably high risk	Definitely high risk	Probably high risk
Tajiri, 2011 et al.,	Probably high risk	Probably high risk	Probably low risk	Probably high risk	Definitely low risk	Probably high risk	Definitely high risk	Probably high risk
Ptaszkowski et al., 2015	Definitely low risk	Probably low risk	Probably low risk	Probably low risk	Definitely low risk	Probably high risk	Definitely high risk	Probably high risk

Table 4. GRADE evidence profile for cross-sectional studies: women without pelvic floor dysfunction *versus* women with pelvic floor dysfunction.

	Quality assessment					**Summary of findings**				**Certainty in estimates**
						Study event rates MD (SD)		**Mean difference**	**Anticipated absolute effects**	**OR**
No. of participants (studies)	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Women without PFD**	**Women with PFD**	**(95% CI)**	**Risk in women without PFD***	**Risk in women with PFD***	**Quality of evidence**
	Co-contraction activity of transversus abdominis muscles when PFM contract										
52 (2)	Serious limitation[a]	Not serious limitation[b]	Serious limitation[d]	Serious limitation[e]	Undetectable	2.5 (0.4)**	2.1 (0.3)**	−0.61 (−1.42 to 0.20)	The mean rate of co-activity of transversus abdominis muscles was 2.5	The mean rate of co-activity of transversus abdominis muscles in the exposed group was on average 0.61 lower (1.42 lower to 0.20 higher)	⊕⊕OO LOW
	Sensitivity analysis of co-contraction activity of transversus abdominis muscles when PFM contract										
32 (1)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Not serious limitation[e]	Undetectable	2.5 (0.4)**	2.1 (0.3)**	−1.02 (−1.90 to -0.14)	The mean rate of co-activity of transversus abdominis muscles was 2.5	The mean rate of co-activity of transversus abdominis muscles in the exposed group was on average 1.02 lower (1.9 lower to 0.14 lower)	⊕OOO VERY LOW
	Co-contraction activity of rectus abdominis muscle when PFM contract										
128 (3)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Serious limitation[e]	Undetectable	6 (4)***	8 (5)***	−2.05 (−6.51 to 2.42)	The mean rate of co-activity of rectus abdominis muscle was 6	The mean rate of co-activity of rectus abdominis in the exposed group was on average 2.05 lower (6.51 lower to 2.42 higher)	⊕OOO VERY LOW
	Sensitivity analysis of co-contraction activity of rectus abdominis muscle when PFM contract										

56 (2)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Not serious limitation[e]	Undetectable	6 (4)***	8 (5)***	0.89 (-0.03 to 1.82)	The mean rate of co-activity of rectus abdominis muscle was 6	The mean rate of co-activity of rectus abdominis in the exposed group was on average 0.89 higher (0.03 higher to 1.82 higher)	⊕⊕OO LOW
	Co-contraction activity of obliquus internus abdominis muscle when PFM contract										
118 (3)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Serious limitation[e]	Undetectable	23 (3)****	18 (2)****	−0.47 (−2.38 to 1.44)	The mean rate of co-activity of obliquus internus muscle was 0.23	The mean rate of co-activity of obliquus internus in the exposed group was on average 0.47 lower (2.38 lower to 1.44 higher)	⊕OOO VERY LOW
	Sensitivity analysis of co-contraction activity of obliquus internus abdominis muscle when PFM contract										
26 (1)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Not serious limitation[e]	Undetectable	26 (18)***	57 (34)***	1.10 (0.27 to 1.94)	The mean rate of co-activity of obliquus internus muscle was 26	The mean rate of co-activity of obliquus internus in the exposed group was on average 1.10 higher (0.27 higher to 1.94 higher)	⊕⊕OO LOW
	Co-contraction activity of obliquus externus abdominis muscles when PFM contract										
98 (2)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Serious limitation[e]	Undetectable	30 (5)****	21 (4)****	0.01 (−4.00 to 4.03)	The mean rate of co-activity of obliquus externus muscle was 30	The mean rate of co-activity of obliquus externus in the exposed group was on average 0.01 higher (4.00 lower to 4.03 higher)	⊕OOO VERY LOW
	Sensitivity analysis of co-contraction activity of obliquus externus abdominis muscle when PFM contract										
26 (1)	Serious limitation[a]	Serious limitation[c]	Serious limitation[d]	Not serious limitation[e]	Undetectable	9 (4)***	37 (18)***	2.08 (1.10 to 3.06)	The mean rate of co-activity of obliquus externus muscle was 9	The mean rate of co-activity of obliquus externus in the exposed group was on average 2.08 higher (1.10 higher to 3.06 higher)	⊕⊕OO LOW

Abbreviations: MD: mean difference; SD: standard deviation; PFD: pelvic floor dysfunction; CI: Confidence interval.
*Cross-sectional studies started from high quality evidence because of the nature of the clinical question.

[**]The estimated risk control was taken from the mean estimated control risk from the Tajiri (2011) study (35).
[***]The estimated risk control was taken from the mean estimated control risk from the Thompson (2006b) study (37).
[****] The estimated risk control was taken from the mean estimated control risk from the Madill (2009) study (30).
[a]Issues related to exposure and outcome assessments, follow-up period and co-interventions
[b]There may not be considerable heterogeneity (I^2 <50%)
[c]There is considerable heterogeneity (I^2>75%).
[d]Included studies with only one pelvic floor dysfunction.
[e]95% Confidence interval for absolute effects include clinically important significance and no significance.

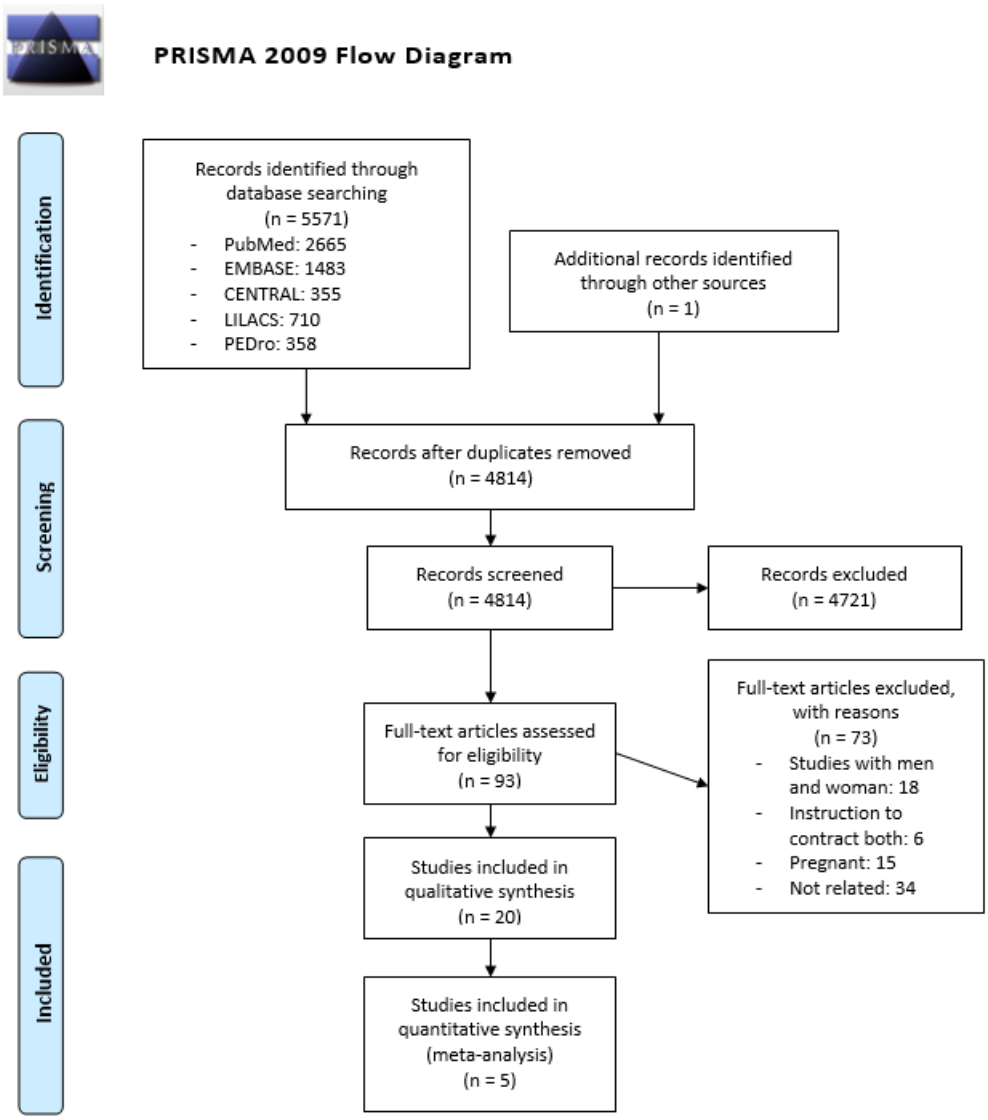
PRISMA 2009 Flow Diagram
Identification
Screening
Eligibility
Included
Records identified through database searching (n = 5571)
- PubMed: 2665
- EMBASE: 1483
- CENTRAL: 355
- LILACS: 710
- PEDro: 358
Additional records identified through other sources (n = 1)
Records after duplicates removed (n = 4814)
Records screened (n = 4814)
Records excluded (n = 4721)
Full-text articles assessed for eligibility (n = 93)
Full-text articles excluded, with reasons (n = 73)
- Studies with men and woman: 18
- Instruction to contract both: 6
- Pregnant: 15
- Not related: 34
Studies included in qualitative synthesis (n = 20)
Studies included in quantitative synthesis (meta-analysis) (n = 5)

Figure 1

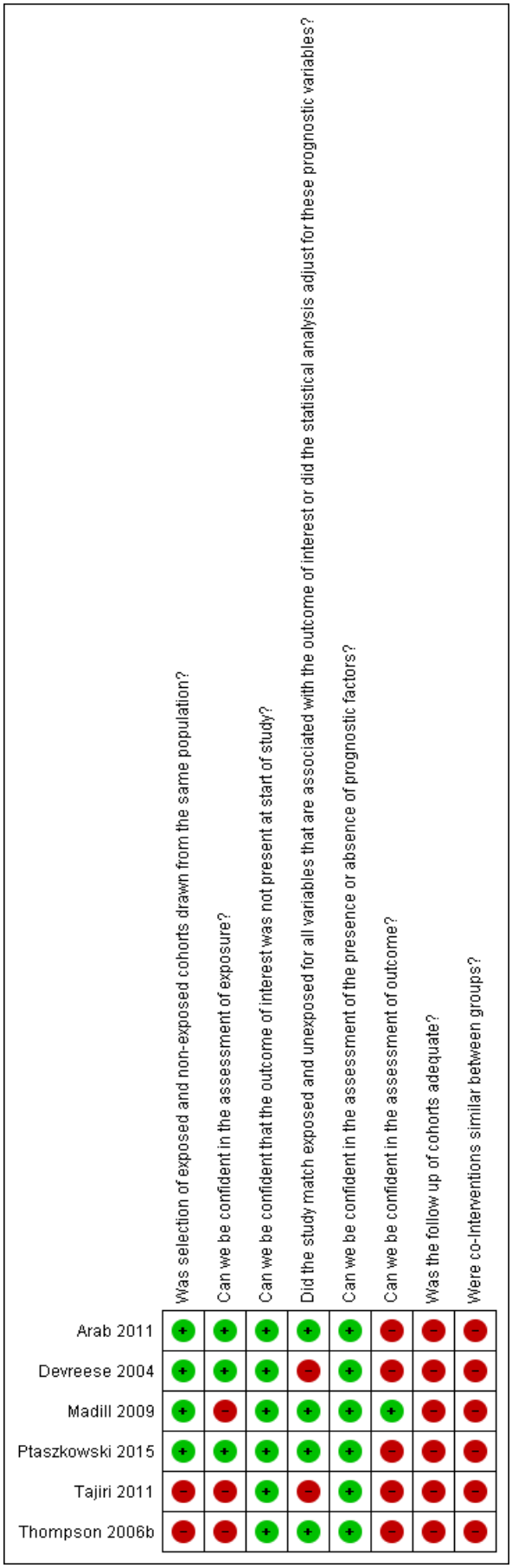
Was selection of exposed and non-exposed cohorts drawn from the same population?
Can we be confident in the assessment of exposure?
Can we be confident that the outcome of interest was not present at start of study?
Did the study match exposed and unexposed for all variables that are associated with the outcome of interest or did the statistical analysis adjust for these prognostic variables?
Can we be confident in the assessment of the presence or absence of prognostic factors?
Can we be confident in the assessment of outcome?
Was the follow up of cohorts adequate?
Were co-Interventions similar between groups?
Arab 2011
Devreese 2004
Madill 2009
Ptaszkowski 2015
Tajiri 2011
Thompson 2006b

Figure 2

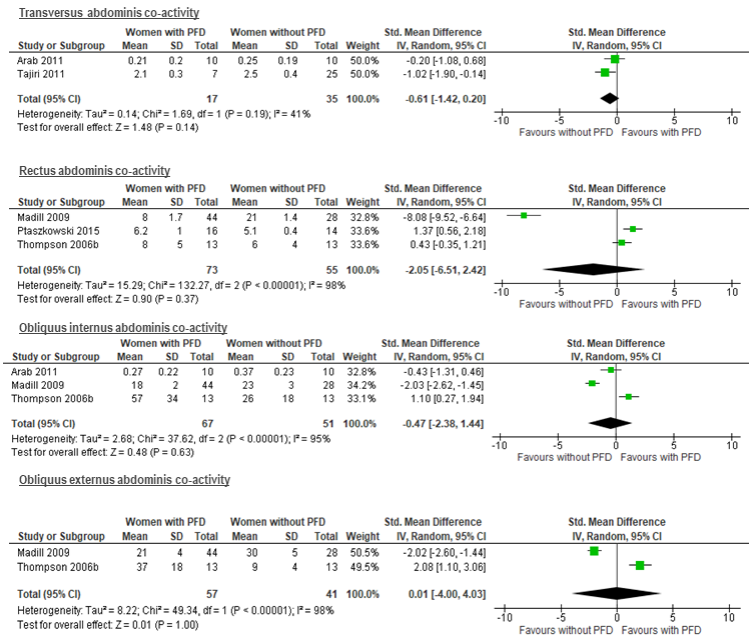
Transversus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Arab 2011 0.21 0.2 10 0.25 0.19 10 50.0% -0.20 [-1.08, 0.68]
Tajiri 2011 2.1 0.3 7 2.5 0.4 25 50.0% -1.02 [-1.90, -0.14]
Total (95% CI) 17 35 100.0% -0.61 [-1.42, 0.20]
Heterogeneity: Tau² = 0.14; Chi² = 1.69, df = 1 (P = 0.19); I² = 41%
Test for overall effect: Z = 1.48 (P = 0.14)
-10 -5 0 5 10
Favours without PFD Favours with PFD
Rectus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Madill 2009 8 1.7 44 21 1.4 28 32.8% -8.08 [-9.52, -6.64]
Ptaszkowski 2015 6.2 1 16 5.1 0.4 14 33.6% 1.37 [0.56, 2.18]
Thompson 2006b 8 5 13 6 4 13 33.6% 0.43 [-0.35, 1.21]
Total (95% CI) 73 55 100.0% -2.05 [-6.51, 2.42]
Heterogeneity: Tau² = 15.29; Chi² = 132.27, df = 2 (P < 0.00001); I² = 98%
Test for overall effect: Z = 0.90 (P = 0.37)
-10 -5 0 5 10
Favours without PFD Favours with PFD
Obliquus internus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Arab 2011 0.27 0.22 10 0.37 0.23 10 32.8% -0.43 [-1.31, 0.46]
Madill 2009 18 2 44 23 3 28 34.2% -2.03 [-2.62, -1.45]
Thompson 2006b 57 34 13 26 18 13 33.1% 1.10 [0.27, 1.94]
Total (95% CI) 67 51 100.0% -0.47 [-2.38, 1.44]
Heterogeneity: Tau² = 2.68; Chi² = 37.62, df = 2 (P < 0.00001); I² = 95%
Test for overall effect: Z = 0.48 (P = 0.63)
-10 -5 0 5 10
Favours without PFD Favours with PFD
Obliquus externus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Madill 2009 21 4 44 30 5 28 50.5% -2.02 [-2.60, -1.44]
Thompson 2006b 37 18 13 9 4 13 49.5% 2.08 [1.10, 3.06]
Total (95% CI) 57 41 100.0% 0.01 [-4.00, 4.03]
Heterogeneity: Tau² = 8.22; Chi² = 49.34, df = 1 (P < 0.00001); I² = 98%
Test for overall effect: Z = 0.01 (P = 1.00)
-10 -5 0 5 10
Favours without PFD Favours with PFD

Figure 3

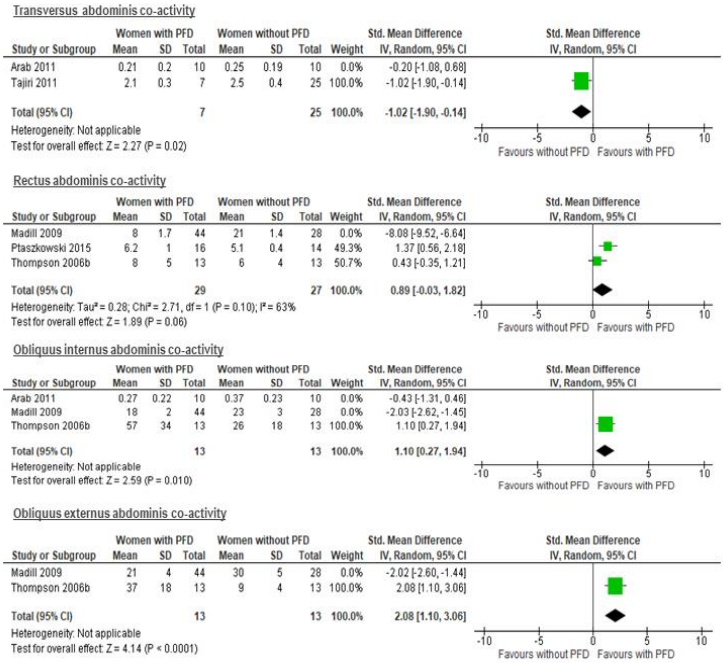
Transversus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Arab 2011 0.21 0.2 10 0.25 0.19 10 0.0% -0.20 [-1.08, 0.68]
Tajiri 2011 2.1 0.3 7 2.5 0.4 25 100.0% -1.02 [-1.90, -0.14]
Total (95% CI) 7 25 100.0% -1.02 [-1.90, -0.14]
Heterogeneity: Not applicable
Test for overall effect: Z = 2.27 (P = 0.02)
-10 -5 0 5 10
Favours without PFD Favours with PFD
Rectus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Madill 2009 8 1.7 44 21 1.4 28 0.0% -8.08 [-9.52, -6.64]
Ptaszkowski 2015 6.2 1 16 5.1 0.4 14 49.3% 1.37 [0.56, 2.18]
Thompson 2006b 8 5 13 6 4 13 50.7% 0.43 [-0.35, 1.21]
Total (95% CI) 29 27 100.0% 0.89 [-0.03, 1.82]
Heterogeneity: Tau² = 0.28; Chi² = 2.71, df = 1 (P = 0.10); I² = 63%
Test for overall effect: Z = 1.89 (P = 0.06)
-10 -5 0 5 10
Favours without PFD Favours with PFD
Obliquus internus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Arab 2011 0.27 0.22 10 0.37 0.23 10 0.0% -0.43 [-1.31, 0.46]
Madill 2009 18 2 44 23 3 28 0.0% -2.03 [-2.62, -1.45]
Thompson 2006b 57 34 13 26 18 13 100.0% 1.10 [0.27, 1.94]
Total (95% CI) 13 13 100.0% 1.10 [0.27, 1.94]
Heterogeneity: Not applicable
Test for overall effect: Z = 2.59 (P = 0.010)
-10 -5 0 5 10
Favours without PFD Favours with PFD
Obliquus externus abdominis co-activity
Women with PFD
Women without PFD
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Madill 2009 21 4 44 30 5 28 0.0% -2.02 [-2.60, -1.44]
Thompson 2006b 37 18 13 9 4 13 100.0% 2.08 [1.10, 3.06]
Total (95% CI) 13 13 100.0% 2.08 [1.10, 3.06]
Heterogeneity: Not applicable
Test for overall effect: Z = 4.14 (P < 0.0001)
-10 -5 0 5 10
Favours without PFD Favours with PFD

Figure 4

Appendix 1. Search strategy.

(Women OR woman OR female OR Women's Groups OR Women's Group OR Women Groups OR Women Group OR healthy women OR healthy woman OR incontinent OR incontinent women OR incontinent woman OR urinary incontinence in women OR Female Urinary Incontinence OR continent OR continent women OR continent woman OR urgency urinary incontinence OR Urinary Stress Incontinence OR stress urinary incontinence OR stress urinary OR UUI OR SUI OR MUI OR Urinary Urge Incontinence OR Urinary Reflex Incontinence OR Urge Incontinence OR mixed urinary incontinence OR Urinary Bladder Disease OR Urinary Bladder Diseases OR Urinary Reflex Incontinence) AND ((Pelvic Floor OR Pelvic Diaphragm OR Pelvic Diaphragms OR Pelvic Floor Disorders OR Pelvic Floor Disorder OR Pelvic Floor Disease OR Pelvic Floor Diseases OR pelvic floor dysfunction OR pelvic floor dysfunctions OR Pelvic Floor muscle OR Pelvic Floor muscles OR Urinary Incontinence OR abdomino-pelvic musculature OR perineal musculature OR Perineum OR perineums OR perineal function OR pelvic floor contraction OR pelvic floor muscle contractions OR co-contraction OR muscle synergism OR muscle co-contraction OR co-activity OR co-activity muscle) AND (Abdominal Muscles OR Abdominal Muscle OR Abdomen OR Abdomens OR abdomino-pelvic musculature OR transversus abdominis OR Rectus Abdominis OR Rectus Muscle of Abdomen OR Abdomen Rectus Muscle OR Abdomen Rectus Muscles OR external obliques OR external oblique OR internal obliques OR internal oblique OR abdominal muscle contractions OR synergistic co-contraction of abdominal muscles OR synergism co-contraction of abdominal muscles OR co-contraction OR muscle synergism OR muscle co-contraction OR co-activity OR co-activity muscle))

Capítulo 2

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

Short Title: Changes in rectus abdominis muscles caused by diabetes and pregnancy in rats and women

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Abstract

BACKGROUND AND OBJECTIVE: In the present study, we compared the effects of diabetic pregnancy in rectus abdominis muscle (RAM) of human and rats. We hypothesized that relative to other models, our animal model best resembles alterations in rectus abdominis muscle of women with Gestational Diabetes (GDM).

METHOD: For the mild hyperglycemic pregnant (MHP) rats, newborns rats (n=10/group) received streptozotocin subcutaneous injection (100 mg/kg body weight). Three months old rats were mated, and at the end of pregnancy, the mothers were sacrificed for tissue harvesting. Pregnant women who underwent the GDM diagnosis and treatment were allocated into two groups non-GDM (n=10) and GDM (n=8). The muscle samples were obtained at C-section in GDM and non-GDM women as well as in MHP rats and non-MHP rats. For both studies, the RAM was removed and processed for the immunohistochemical analysis of fast and slow fibers and picrosirius red staining.

RESULTS: No statistically significant differences were found between groups regarding any variables of materno-fetal characteristics for both rats and women. However, significant alterations were observed in the RAM. Diabetes in pregnancy increased slow fiber and decreased the fast fiber number and area of rats and women.

CONCLUSION: Our results indicated that pregnancy-associated with diabetes-induced similar structural adaptations in the RAM of women and rats with slight alterations in fiber type number and area. These findings suggest that this animal model can be used for studying the effects of pregnancy-associated diabetes in fiber structure of RAM.

Keywords: Animal model; Collagen; Skeletal muscle; Histology; Diabetic myopathy

Introduction

Diabetes mellitus (DM) is a global health concern. Women that develop gestational diabetes (GDM) are more willing to develop type 2 diabetes later in life (1). Pregnancy causes an insulin-resistance state and when an increase in insulin secretion cannot meet the needs of such a pregnancy-induced insulin resistance status, GDM develops (2). DM is defined as a group of metabolic diseases associated with a hyperglycemic state due to metabolic or genetic malfunction in insulin release (3). DM has also been associated with diabetic myopathy a deficiency of healthy muscle maintenance (4). Diabetic myopathy is a universal complication of diabetes and is related to the loss of muscle mass and strength (i.e., sarcopenia and dynapenia) (4-6).

Skeletal muscle is a heterogeneous tissue and its structure is composed of different fiber types, all of which are characterized by myosin heavy chain isoforms (7). Anatomically, mammalian skeletal muscle is composed of two major fiber types - slow and fast; that differ in terms of size, metabolism and contractile properties (8). Another component of skeletal muscle is the extracellular matrix (ECM) with major roles in muscle fiber force transmission, maintenance, and repair (9). The skeletal muscle is known to play a key role in skeletal muscle in locomotion and glucose homeostasis (10).

Although significant improvements been made over the past decade to improve GDM care, women with the condition still prone to a substantial risk of developing urinary incontinence (11). The consequences persist not only during pregnancy but up to 2 years post-partum (12, 13). Translational studies from the ‘‘bedside to bench’’ with analysis of urethral tissue of female rats was conducted by our research group using Streptozotocin (STZ) rat model (14-19). The hyperglycemia status is manifested by necrosis of the pancreatic β-cells caused by STZ (20). However, animal, dose, route of administration and life period of induction are key factors in the glycemic intensity (14). Either higher or lower hyperglycemic levels showed impairment of the urethral tissue (15, 16, 18). Likewise, we investigated the changes in rectus abdominis muscle (RAM) in the same animal models and discovered that RAM and urethral muscle are subjected to similar morphological changes related to diabetic myopathy (19). Henceforth, the “bench-to bed” approach was started to investigate the morphological changes of RAM in GDM women.

Therefore, we hypothesize that GDM women and mild hyperglycemic pregnant (MHP) rats should exhibit similar morphological changes in RAM tissues. The objective of this study is to compared the effects of diabetic pregnancy in RAM of human and rats using histological and

immunohistochemical techniques. The comparative evaluation may be considered a reliable tool for future research in the field of GDM related to new therapeutical approaches.

Materials and Methods

Ethics statement

All animal experiments complied with the Institutional Animal Care and Use Committee in the Faculdade de Medicina de Botucatu - UNESP with applicable regulations and recommendations from Brazilian authorities and approved the study (protocol 1003-2013).

For human study, all participants presented their written informed consent before participation. Participants were recruited at the University Hospital (Perinatal Diabetes Research Center), São Paulo State University (UNESP), Brazil. The study was registered in the Brazilian National Research Registry platform (Plataforma Brasil) and approved by the National Committee for Ethics in Research (CONEP) (CAAE: 26142614.0.0000.5411 and CAAE: 82225617.0.0000.5411) following the guidelines of the Declaration of Helsinki on human experimentation.

Animal model

Female and male Wistar (around 12–13 weeks-old and 250–300 g) rats were obtained from the same source, Multidisciplinary Center for Biological Investigation (Campinas, SP, Brazil). Animals were housed in a facility with constant temperature (22±2°C) and humidity (55±5%) on a controlled 12h light–12h dark cycle with food and water *ad libitum*. After one week of acclimatization, the dams were mated to obtain female newborns. The female offspring, on the first day of life, were randomly assigned to two groups (n=10/group): the mild hyperglycemic pregnant (MHP) group, which received STZ (SIGMA Chemical Company, St. Louis, MO, USA), diluted in 0.1 M citrate buffer (pH 4.5) at a dose of 100 mg/kg by subcutaneous route (21), or non- mild hyperglycemic pregnant (non-MHP) group, which received the same dose of citrate buffer. At adult stage (around 12-13 weeks-old) these rats were kept overnight with adult male rats. The first day of gestation (GD0) was determined by examining the vaginal smear, and the rats were caged singly. An oral glucose tolerance test (OGTT) was performed on the 17^{th} day of pregnancy to assess the development of altered glucose metabolism (22). Blood glucose concentrations were measured by a One-Touch Ultra glucometer (LifeScan, Johnson and Johnson®, Milpitas, CA, USA), and the values were expressed as mg/dL. At the end of pregnancy (GD21), the dams were euthanized by sodium thiopental injection (Thiopentax®, Brazil 80 mg/kg dose). The lower third of RAM was exposed, dissected, and removed. The

edges were reduced, and the sample was wrapped in talc, frozen in liquid nitrogen and kept at -80 °C. Around 500 mg of RAM were obtained from a total of 20 rats per group. The data analyses of morphometric, immunohistochemical, maternal and fetal of the rats were published by Vesentini et al (19).

Participant selection

Pregnant women were screened for GDM between 24–28 weeks' gestation and were diagnosed according to ADA criteria using a 75 g-OGTT test (23, 24). Women with known type 1 or type 2 DM, preterm delivery (<37 weeks of gestation), multiple pregnancies and known fetal anomaly were excluded. All GDM women underwent the same treatment in Perinatal Diabetes Research Center (PDRC). This protocol guidance includes adequate nutrition from nutritionists, motivation to exercise regularly and insulin associated with dietary advice and exercise. Around 500 mg of rectus abdominis skeletal muscle were obtained from a total of 18 pregnant women undergoing C-section and were categorized into non-GDM group (n=10) and GDM group (n=8). Sections of skeletal muscle were obtained after delivery and stripped off from visible adipose and connective tissues. The sample was then wrapped in talc, snap-frozen in liquid nitrogen and stored at -80 °C.

Histological examination, immunohistochemical staining, and morphometric analysis

Either rats or maternal frozen muscle specimens were cut into 10-μm-thick cross-sections using a cryostat (Leica CM 1800). The cross-sections were fixed on microscope glass slides in cold acetone for 10 minutes and were stained for hematoxylin and eosin (H&E), picrosirius red and were processed for immunohistochemical analyses. The slides were examined by light microscopy and photographed (DMR, Leica® coupled with a CCD-IRIS/RGB digital camera, Sony®). The rats' images published by Vesentini et al were re-analyzed (i.e., morphometric area of fiber types and collagen and fiber number) (19).

The Picrosirius red stain was done to determine the tissue area of red-stained (collagen). For this analysis, ten sections/sample for quantitative morphometric analysis of collagen area (20× magnification) were analyzed using the software Image J (National Institutes of Health, USA). For immunohistochemistry staining of fast and slow-type skeletal muscle fibers, the sections were incubated with antibodies directed towards WB-myosin heavy chain, fast (WB-MHCf) Novocastra (rats, 1:120; human, 1:160) and WB-MHC slow (WB-MHCs) Novocastra (rats, 1:180; human, 1:120). A quantitative method was performed for fiber type area and number, as described previously (19).

Statistical analysis

Categorical data were described by percentages and assessed by chi-square tests.

Continuous data were described by their means ± standard deviations (SD) and compared by t-tests (clinical characteristics of participants, rats fetal weight, fiber area), ANOVA (rat maternal weight day 0 and 21), Poisson (fiber type number) and OGTT results were calculated using total area under the curve (AUC) (25) and compared by t-tests. Statistical significance was set as a *p*-value < 0.05. All analyses were performed by SAS for windows, v.9.3 (Statistical Analysis System Institute Inc., USA).

Results

Table 1 displays the social-demographic and clinical characteristics of pregnant women. No statistically significant difference was found between groups regarding any of the variables. Table 2 shows no between-groups difference of maternal and fetal weight of the rats. The main characteristics showed the homogeneity of the sample for both pregnant women and rats. GDM and MHP groups (GDM = 18428.6 ± 1963.2; MHP = 20142 ± 5194.6), presented higher AUC with elevated glucose levels throughout the OGTT test compared to control groups (non-GDM = 12303.3 ± 2547.6; non-MHP = 9662.5 ± 1339.2) (Figure 1).

During pregnancy, the RAM morphometric analysis of non-GDM and non-MHP groups showed that the predominant RAM fiber type population is represented by the fast fiber and not the slow fiber (non-GDM = fast 66.98 ± 10.75, slow 33.02 ± 15.32; non-MHP = fast 87.71 ± 6.24, slow 12.29 ± 16.69) (Figure 2). Despite maintaining the predominance of RAM fast fiber number in GDM and MHP rats (GDM = 57.93 ± 8.22, p=0.0012; MHP = 77.58 ± 6.63, p<.0001) there is an increase of slow fiber numbers (GDM = 42.07 ± 9.65, p<.0001; MHP = 77.58 ± 6.63, p<.0001) compared to controls. Immunohistochemistry staining markers of fast and slow-type skeletal muscle area in non-GDM pregnant women and in non-MHP rats confirmed the predominance of fast fiber area (non-GDM = 4544.82 ± 825.54; non-MHP = 3363.29 ± 773.51) associated with lower slow fiber area (non-GDM = 2820.89 ± 509.23; non-MHP = 1273.63 ± 233.9). Notwithstanding the maintenance of fast fibers area predominance in GDM and MHP rats, it was associated with a decrease in the fast fiber area either in pregnant women or rats (GDM = 2895.8 ± 459.2, p<.0001; MHP = 2878.35 ± 640.3, p<.0001). The slow fiber area presented different patterns. While for GDM there was a decrease of slow fiber area (GDM = 1908.3 ± 294.3, p<.0001), for pregnant rats there was an increase in slow fiber area (MHP = 1324.85 ± 286.46, p=0.0178)

The collagen area in the GDM group (15208.3 ± 4181.2) was significantly reduced compared with the non-GDM group (25194.2 ± 7579.1, p<.0001). On the other side, there

were no differences in the collagen area between the rat groups (non-MHP = 35150.7 ± 4010.3; MHP = 34701.1 ± 6078.7, p=0.5376) (Figure 3).

Discussion

To the best of the authors' knowledge, no studies have been conducted to compare the structural changes induced in RAM tissues of MHP rats and GDM. To determine whether rats can be used as an established model for studying DM and devising therapeutical approaches for DM a the morphological and immunohistochemical characteristics of RAM were evaluated. Our findings revealed that among patterns of pregnant diabetic myopathy in rats and women, diabetes during pregnancy significantly impacted the structural characteristics of the RAM tissue. Despite this, the number of dominant fast fiber number in RAM samples was maintained in both women and rats. Our results showed that diabetes during pregnancy modify the RAM fiber type number and decrease the fast fiber area. Moreover, in rats, there is no change in the collagen area when comparing MHP and non-MHP rats. Putting together both findings, lead us to a picture of RAM submitted to a diabetic environment characterized as a muscle with a decrease of fast fiber number and area and increase of slow fiber number. Although MHP and GDM present similar changes regarding fast fiber number and area, slow fiber number, the collagen area in GDM decreases. Comparison of the results demonstrates that RAM is most vulnerable to histological architecture changes due to GDM in humans. The alterations in the muscle fiber pattern of RAM could influence the functionality of this muscle either in GDM or MHP rats.

The mammalian skeletal muscle is constituted by fiber types which differ according to their contraction and metabolism (26). Since the structure and histology of the abdominal wall muscles of rats are well characterized and similar to those of humans, they are an appropriate tissue model for studying physiological changes in the muscle (27). The RAM muscle is a typical glycolytic muscle with a predominance of fast fibers. The increase in abdominal content during pregnancy represents a physiological chronic stimulus in the muscle fibers of the abdominal wall (28). During pregnancy, there is an increase in slow fiber type in RAM muscle while being stretched during pregnancy (19, 28). This stretching is hypothesized to be a result of overload hypertrophy contributing to muscle tone and endurance (28). The duration and intensity of pregnancy morphological adaptations in muscle fiber depend on animal species, gestational period, number and size of fetuses (28-30). The characteristics of muscle overload in rat (i.e., weight gain and fetal weight) and women (i.e., weight gain and baby weight) present

no statistic difference, suggesting that the changes observed are related to hyperglycemic status.

Skeletal muscle atrophy is a complex molecular process that is yet to be understood. Reduced muscle fiber number and/or size is associated with a decrease in muscle function and can be caused by age (31), disuse (32) and illness (33). Strong evidence suggests that diabetes is associated with muscular changes such as reduced muscle strength (34), power (35), mass (36), quality (35) and endurance and fiber type switch (6, 37) termed as diabetic myopathy (6, 38). Our findings show that in the hyperglycemic environment, skeletal muscle in both rats and women decrease the number and area of fast fibers and increase the number of slow fibers. Furthermore, there is a decrease in fast fiber area. Diabetes is characterized by a fast-to-slow fiber type shift with preferential atrophy of fast glycolytic muscle fibers. The cause of the increase in the number of slow fibers related to diabetes is currently unknown. Studies suggest that slow fibers have a greater influence on muscle insulin action and glucose handling capacity (39). This might be related to a compensatory response of skeletal muscle due to hyperglycemia to regulate metabolic homeostasis. Slow fiber type has a higher turnover of protein synthesis and degradation, an oxidative profile with larger mitochondrial content, rich in myoglobin a greater insulin sensitivity and a higher content of GLUT4 expression compared to fast fiber (39, 40). Similar changes are seen in cancer cachexia (41), aging-related sarcopenia (42) and Huntington's Disease (43).

The major structural component of the skeletal muscle ECM is collagen (9). ECM is highly adaptative and therefore, capable of remodeling in response to physiological stimuli or disease (9). Studies have shown that during late-pregnancy in rats, there are marked alterations in ECM components in pelvic floor muscles (44), RAM (19) and vagina (45). These passive mechanical structures undergo significant maternal adaptations during pregnancy in preparation for parturition and birth (46). Previous studies show that diabetes is characterized by an increase in muscle collagen (47, 48). According to Kang (49), the inflammatory response associated with insulin resistance has extensive effects on increased collagen deposition and ECM remodeling. Although we hypothesized that pregnancy-associated with diabetes would result in fibrosis, here we report that the muscle collagen in rats and humans have yielded mixed findings. In rats, there is no change in collagen area whereas in humans, the collagen significantly decreases. It is not known whether the decrease in collagen observed in our studies was due to a decrease in synthesis, an increase in collagen degradation or the excessive muscle stretching caused by pregnancy. We speculate that, although pregnancy and diabetes are known to cause muscle fibrosis, the fact that they are in combination in a muscle that during pregnancy suffer substantial muscle strain altered variables associated with collagen synthesis. Further analysis is necessary.

An ideal animal model for GDM research has not yet been established. So, the results showing differences and similarities patterns of GDM and MHP rats enable us to suggest MHP rats as a preliminary prototype for future research in the field of diabetic myopathy related to new therapeutical approaches. Some limitations of this study are the use of a quadrupedal animal model that differs from human concerning the effect of gravity on the biomechanics of RAM, the size and number of fetus. However, they provide the opportunity to test hypotheses more rigorously and detailed in a controlled environment. Among the animal models available for research purposes, rodents stand out because they are small, it is possible to work within large numbers, cost-effective and the pregnancy period lasts 21 to 23 days (50). The abdominal wall of rats is similar to the morphology and architecture of humans (27). In this study, we present an animal model that it is comparable with glycemic levels of GDM in women and may, therefore, be applicable for future research to promote insights into the molecular mechanism and find for possible treatment for GDM and UI.

Conclusion

The present study is the first to show that RAM fast fiber predominance of pregnant groups is preserved in GDM and MHP rats. Furthermore, it shows that differences in RAM slow fiber pattern and collagen appear. The RAM slow fiber and collagen is decreased in GDM. No changes in collagen patterns were detected in RAM samples of MHP rats. The comparison of skeletal muscle fibers between GDM women and MHP rats revealed that each underwent profound similar architectural changes, suggesting that they have a comparable functional change in response to diabetes.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Ethical approval

All animal experiments complied with the Institutional Animal Care and Use Committee in the Faculdade de Medicina de Botucatu - UNESP with applicable regulations and recommendations from Brazilian authorities and approved the study (protocol 1003-2013).

The study was registered in the Brazilian National Research Registry platform (Plataforma Brasil) and approved by the National Committee for Ethics in Research (CONEP) (CAAE: 26142614.0.0000.5411 and CAAE: 82225617.0.0000.5411) following the guidelines of the Declaration of Helsinki on human experimentation.

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Figure legends

Figure 1. Oral Glucose Tolerance Test (OGTT) performed between 24–28 weeks for pregnant women and on the 17th day of pregnancy for rats. The area under the curve of each group is expressed as mean ± standard deviation. *$p < 0.05$ shows significant difference compared to control group (t-test).

Figure 2. Immunohistochemistry images of RAM fibers of fast and slow fibers in a transverse section. Non-GDM (slow 1, fast 2), GDM (slow 3, fast 4), non-MHP (slow 5, fast 6) and MHP (slow 7, fast 8). Results of the fiber type number (A) are expressed as percentages and area (B) are expressed as mean and standard deviation. Differences between groups for fiber type number were made using Poisson distribution. Differences between groups for fiber area were made using student t-test for non-GDM and GDM groups and gamma for non-MHP and MHP groups. *$p < 0.05$ shows a significant difference compared to control group. Abbreviations: GDM, Gestational Diabetes, MHP, mild hyperglycemic pregnant.

Figure 3. Picrosirius red staining images of RAM fibers of striated muscle (yellow) and collagen (red) transverse section. Non-GDM (1), GDM (2), non-MHP (3) and MHP (4). Differences between groups for collagen area were made using student t-test. *$p < 0.05$ shows a significant difference compared to the control group. Abbreviations: GDM, Gestational Diabetes, MHP, mild hyperglycemic pregnant; RAM, rectus abdominis muscle.

Table 1. Social-demographic and clinical characteristics of pregnant women.

	non-GDM (n=10) Mean (SD)	**GDM (n=8) Mean (SD)**	**p-value**
Age (years)	29.80 (5.03)	34.50 (6.07)	0.1052
Parity (n)	1.20 (0.79)	1.12 (1.45)	0.1430
Prepregnancy BMI (kg/m²)	33.63 (7.55)	29.91 (5.25)	0.3055
BMI at the end of gestation (kg/m²)	38.22 (6.26)	34.08 (4.30)	0.1498
Weight gain during pregnancy (kg)	12.11 (7.96)	10.46 (6.01)	0.5286
Ethnicity			
White (%)	6 (60%)	5 (62.5%)	0.4030
Educational level			
Primary	4 (40%)	3 (37.5%)	
High school	4 (40%)	4 (50%)	0.3229
University degree	2 (20%)	1 (12.5%)	
Hypertension			
Yes	5 (50%)	1 (12.5%)	0.2213
Newborn weight (g)	3408 (269.92)	3555.0 (335.4)	0.4533

Data presented as number (%) or mean ± standard deviation. Abbreviations: SD, standard deviation; BMI, body mass index. *$p < 0.05$ shows significant difference compared to the control group.

Table 2. Maternal and fetal weight analysis from animal groups.

	non-MHP (n=10) Mean (SD)	**MHP (n=10) Mean (SD)**	**p-value**
Maternal weight on day 0 (g)	257.33 (18.49)	254.53 (22.57)	0.9913
Maternal weight on day 21 (g)	374.61 (30.58)	349.39 (38.03)	0.0560
Fetal weight (g)	5.46 (0.58)	5.48 (0.61)	0.7714

Data presented as mean ± standard deviation. Abbreviations: SD, standard deviation. *p < 0.05 shows significant difference compared to control group.

Table 3. Synthesizes the morphological changes in the RAM of pregnant rats and women groups.

	MHP (Vesentini et al., 2018)	**DMG**
Collagen area	ns	↓
Fiber type area	↓ FAST	↓ FAST
	↑ SLOW	↓ SLOW
Fiber type number	↓ FAST	↓ FAST
	↑ SLOW	↑ SLOW

Abbreviations: ns, not significant

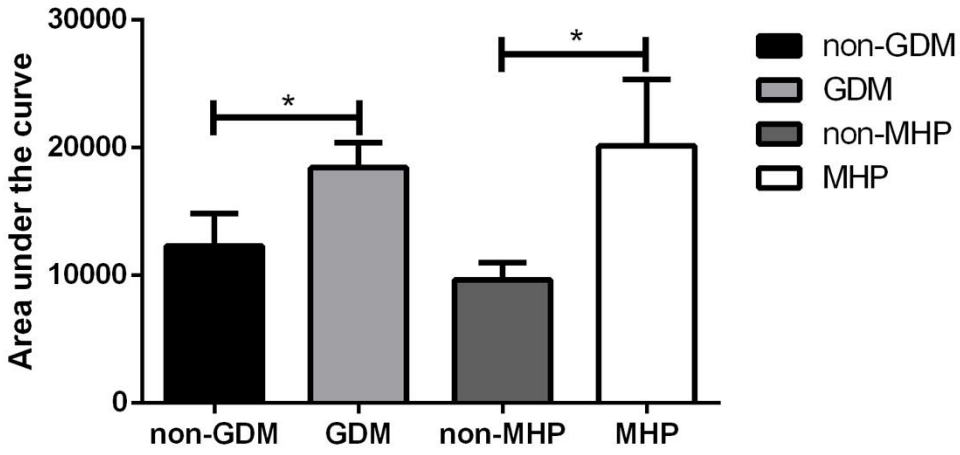
30000
20000
10000
0
Area under the curve
*
*
non-GDM
GDM
non-MHP
MHP
non-GDM
GDM
non-MHP
MHP

Figure 1

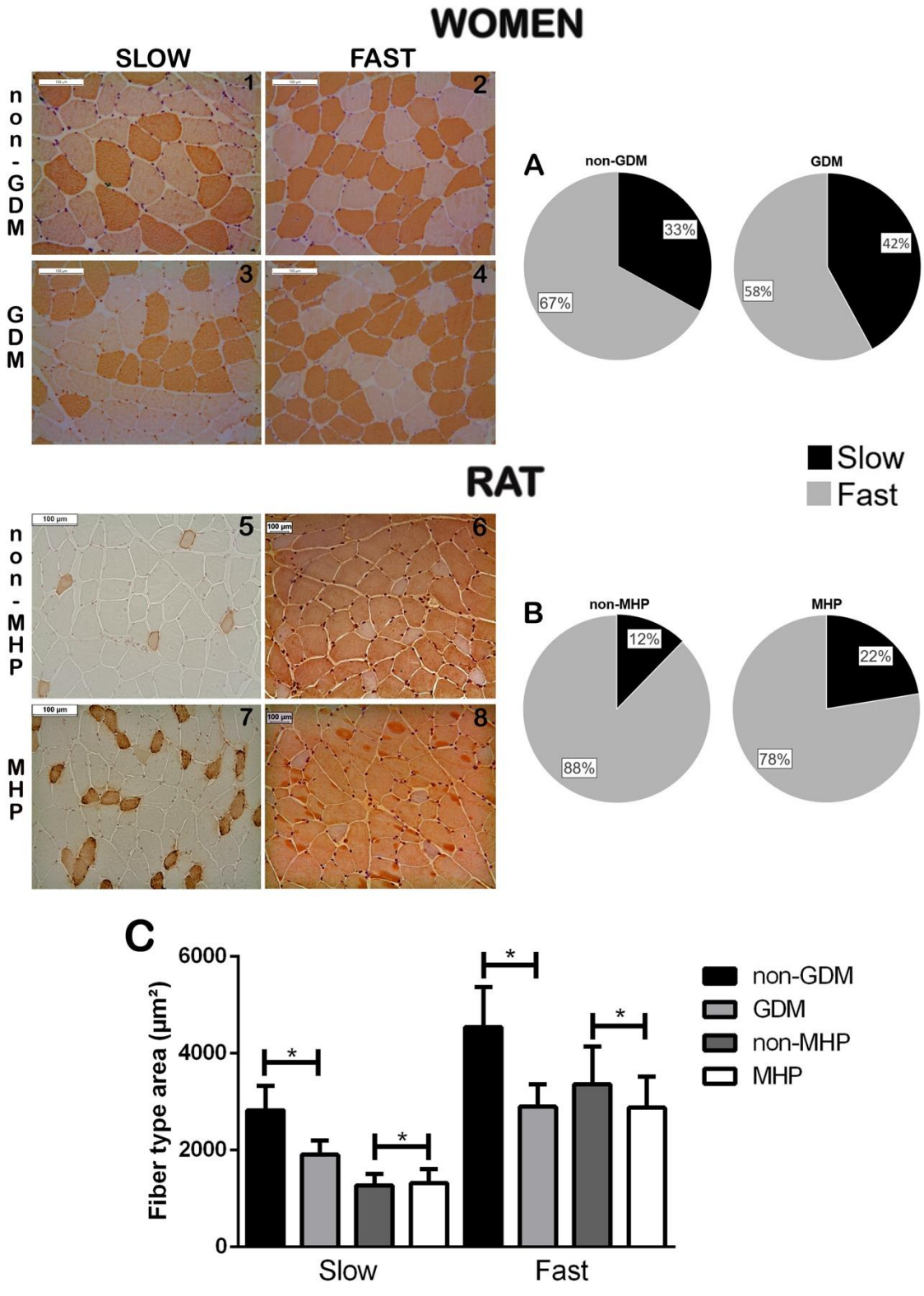
WOMEN
SLOW
FAST
non-GDM
GDM
1
2
3
4
A
non-GDM
GDM
33%
67%
42%
58%
Slow
Fast
RAT
non-MHP
MHP
5
6
7
8
100 µm
B
non-MHP
MHP
12%
88%
22%
78%
C
Fiber type area (µm²)
6000
4000
2000
0
Slow
Fast
*
non-GDM
GDM
non-MHP
MHP

Figure 2

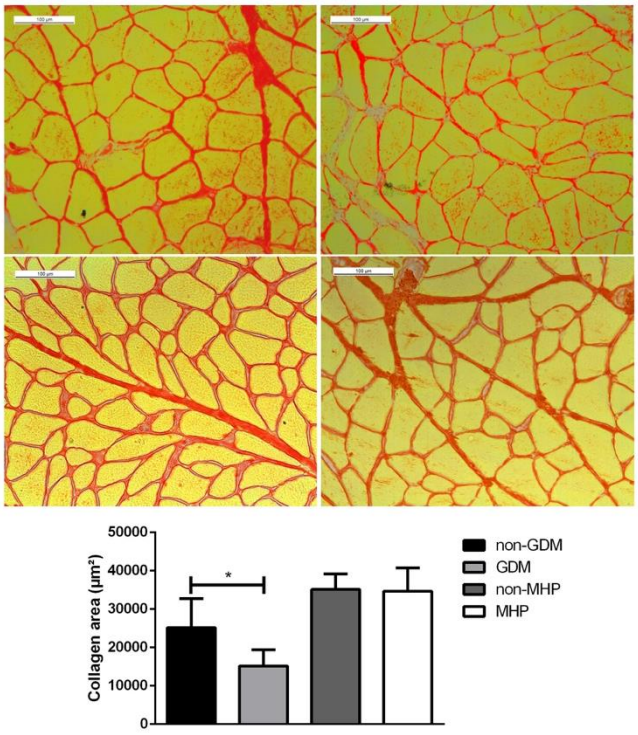
Collagen area (µm²)
50000
40000
30000
20000
10000
0
*
non-GDM
GDM
non-MHP
MHP

Figure 3

"It's good to have an end to a journey toward; but it is the journey that matters, in the end"

Ernest Hemingway

Capítulo 3

Rectus abdominis myopathy caused by gestational diabetes mellitus, an underlying mechanism for pregnancy-related urinary incontinence

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ABSTRACT

BACKGROUND: Gestational diabetes mellitus is a risk factor for long-term urinary incontinence. Recent findings have demonstrated that diabetic-pregnant rats exhibit intramuscular transformation and reorganization of the rectus abdominis muscle. However, human studies investigating diabetic myopathy in gestational diabetes are scarce. Given the importance of skeletal muscles in urinary continence, diabetic myopathy of the rectus abdominis muscle in gestational diabetic women could be related to the mechanisms of gestational diabetes associated with pregnancy-specific urinary incontinence.

OBJECTIVE: The goal of this study was to determine the influence of gestational diabetes mellitus on the rectus abdominis muscle structural characteristics, and - indirectly - on pregnancy-specific urinary incontinence.

STUDY DESIGN: Researchers harvested rectus abdominis muscle specimens during C-sections of patients with gestational diabetes and non-gestational diabetes, as well as pregnancy-specific urinary incontinence. The study was approved by the Institutional Research Bureau, considering our previous experimental results. The patients were divided into four groups, depending on their clinical conditions. Rectus abdominis muscles in gestational diabetes-associated pregnancy-specific urinary incontinent women (study group) were compared to those in non-gestational diabetes continent, non-gestational diabetes-associated pregnancy-specific urinary incontinent and gestational diabetes continent women (control groups). Muscle morphometry for fiber types and collagen fiber distribution was carried out using histochemistry and immunohistochemistry. Ultrastructural analysis of muscle fibers was performed using transmission electron microscopy. Tissue homogenates were used to analyze the protein expression of fiber types by western blotting, and type I and III collagen fibers were quantified using an enzyme-linked immunosorbent assay. Demographic variables were compared among groups by one-way ANOVA and Student t-test. The significance level was set to 5%. All statistical analyses were performed using SAS software.

RESULTS: Gestational diabetes associated with pregnancy-specific urinary incontinence significantly impairs the rectus abdominis muscle. The gestational diabetic groups presented a significant increase in the number of slow fibers and slow-twitch oxidative fiber expression; a decrease in fiber area, fast fiber number, and collagen area; an increase in central nuclei; and ultrastructural alterations with focal lesion area, myeloid structures, sarcomere disorganization, and mitochondrial alteration. The pregnancy-specific urinary incontinence groups presented a considerable decrease in type I and III collagen content and collagen fiber localization.

CONCLUSIONS: Our data reveal morphological, biochemical and physiological changes in

the rectus abdominis muscle as a response to gestational diabetes mellitus. Furthermore, the results of fiber type number and area, increased centralized nuclei and substantial decrease in collagen localization and content in gestational diabetes mellitus associated with pregnancy-specific urinary incontinence. Importantly, the alterations found in muscle fibers are characteristic of gestational diabetes mellitus, while a decrease in collagen content is related to pregnancy-specific urinary incontinence.

Keywords: Collagen, Gestational diabetes mellitus, Rectus abdominis muscle, Skeletal muscle, Urinary incontinence.

INTRODUCTION

The link among previous gestational diabetes mellitus (GDM), and pregnancy-specific urinary incontinence (PSUI), and increased long-term urinary incontinence (UI) composes an understudied connection. Both GDM and UI have important social and economic implications, causing a significant increase in direct and indirect public health costs (1). They also tend to affect young women disproportionately. GDM is defined as the onset or first recognition of glucose intolerance during pregnancy and has been rapidly rising globally in the last decades (2). In addition to the increased risk of adverse neonatal outcomes, GDM has substantial implications for maternal health (3). Many studies on UI have been conducted (4), as it is highly prevalent in women with GDM (5-7). In fact, UI is a common complaint among pregnant women, with symptoms getting progressively worse through gestation (8). The term PSUI is used to define any urinary leakage starting during pregnancy (9). Women with GDM are more likely to develop PSUI (OR: 2.26; 95 % CI: 1.116-4.579) (5). Moreover, these UI symptoms have substantial long-term implications during the postpartum period (5, 6). There appears to be a four-fold increase in the risk of development of UI post-partum up to two years after childbirth (OR: 4.992; 95 % CI: 1.383-18.023) (5).

Animal studies indicate that pregnancy-associated diabetes may lead to harmful impacts on the urethra (10, 11). Such studies analyzed streptozotocin-induced pregnant rats using two different models (12). A comparison of urethral structural and biochemical parameters between models demonstrated detrimental changes in urethral striated muscles (10, 11). These models have also shown additional effects of diabetes, specifically in other muscles outside of the pelvis (13). To assess if these changes also occur in women, a large amount of tissue from the pelvic floor muscle (PFM) is necessary; however, ethical considerations prevent such studies from being conducted. Given this ethical limitation, we proposed to investigate other muscles involved in the continence mechanism. The abdominal muscles are implicated in the function of the PFM and, consequently, the closure of the urethra and continence (14). This rationale has been supported by recordings of the co-activity of the abdominal muscles transversus abdominis, obliquus internus abdominis, obliquus externus abdominis, and rectus abdominis muscle (RAM) during PFM contraction (15, 16).

The contractile properties of skeletal muscles depend on the distribution of myosin heavy chain (MHC) isoforms, which vary among slow-twitch oxidative, fast-twitch oxidative-glycolytic, and fast-twitch glycolytic fibers (17). The extracellular matrix (ECM) in skeletal muscles surrounds the muscle and muscle fibers and is responsible for muscle force transmission, maintenance, and repair (18). Changes in skeletal muscle may inhibit the progress

of some chronic diseases, such as Type-2 diabetes mellitus, highlighting the critical role of skeletal muscles in the pathogenesis of metabolic diseases (19). Such findings have motivated further investigations on the components of skeletal muscles affected by metabolic diseases, resulting in a growing body of evidence linking skeletal muscle impairment and the pathogenesis of GDM (20, 21).

Studying GDM allows us to better understand the long-term maternal consequences of UI and PFM dysfunction symptoms (5, 6). We tested the hypothesis that GDM insult induces PSUI, mediated by RAM myopathy. In this context, we investigated RAM samples obtained during C-sections with an integrative morphological, ultrastructural, and protein expression analysis of muscle fibers and extracellular matrix (ECM) to determine if GDM insult damaged and weakened the RAM. Understanding the consequences of GDM on morphology and biochemistry of fiber-type specification and ECM may help to elaborate future therapeutic treatments and improve human health.

METHODS

Research design and patients

This is a cross-sectional analysis of a cohort study among women in the third trimester of pregnancy attending the Perinatal Diabetes Research Center (PDRC), conducted between 2016 and 2018. The PDRC is located in the Clinical University Hospital of Botucatu Medical School (UNESP), a public tertiary referral health unit in southeast Brazil, which is financially supported by the Brazilian Public Health System (SUS). This study was approved by the Botucatu Medical School Research Ethics Committee of the institution (CAAE: 41570815.0.0000.5411 and CAAE: 82225617.0.0000.5411). The study objectives and protocol were explained to each potential participant. We obtained signed informed consent from all patients included in the study, according to the ethical resolution of the Brazilian Health Council (Resolution 466/2012) (22).

GDM Diagnosis. The diagnostic protocol for GDM in PDRC consists of a 75 g oral glucose tolerance test (OGTT) (23, 24) and a glycemic profile between 24 and 28 weeks of pregnancy (25, 26). All hyperglycemic pregnant women were diagnosed with GDM. After this diagnosis, all women were informed that glycemic control is essential to avoid complications for themselves and their children in the future. All women with GDM underwent the same treatment protocol, including nutritionist recommendations, encouragement to exercise regularly, and the use of insulin when necessary (25).

PSUI Diagnosis. PSUI is defined as any new urinary leakage onset during pregnancy (9). The

participants were asked to answer ‘‘yes’’ or ‘‘no’’ as to whether they experienced PSUI. If a positive answer was given, the participant was identified as having UI, following the definitions set by the International Continence Society (27).

Women with singleton pregnancies who were screened for GDM were recruited at 34 weeks of pregnancy. Additionally, only women who had previously undergone a cesarean section were included in the study. We considered the following as exclusion criteria: pre-pregnancy UI, known diabetes mellitus (DM) type 1 or type 2, pre-term delivery (<37 weeks of gestation), multiple pregnancies and any known fetal anomaly. Data on UI symptoms, maternal demographics characteristics, anthropometric measures, and previous obstetric history were collected. The sample comprised of 92 women who met the inclusion criteria. Patients were distributed into four groups, based on the collected information: (i) GDM UI: with GDM associated PSUI; GDM C: with GDM and no PSUI; non-GDM UI: no GDM and with PSUI; non-GDM C: no GDM no PSUI (“C” means continent).

Collection of RAM, Preparation, and Fixation

RAM samples (up to 500 mg) were obtained during C-section from consenting women who delivered healthy, singleton infants at term (>37 weeks of gestation). Indications for performing a C-section were: breech presentation, previous C-sections, and maternal and/or fetal indications. Dissections and assessment of samples were performed after delivery, consisting of cutting up the visible adipose and connective tissues. After this procedure, RAM tissue sections for protein expression were snap-frozen in liquid nitrogen and stored at –80 °C for further analysis. For structural examination, samples were rolled in talcum powder and snap-frozen in liquid nitrogen and stored at –80 °C. For ultrastructure analyses, samples were immersed in Karnovsky’s fixative.

Histological examination, immunohistochemical stain, and morphometric analysis

Frozen RAM specimens were cut using a cryostat (Leica CM 1800, Germany) into 10-µm cross-sections, mounted on glass slides and fixed in cold acetone for 10 minutes for histochemical or immunohistochemical staining. RAM tissue sections were stained with hematoxylin and eosin (H&E) following standard protocols for histological analysis and centrally located nuclei analysis. RAM collagen fiber distribution was visualized using Picrosirius Red staining, by rehydrating the sections and incubating them with Picrosirius red solution for 10 minutes. Finally, the slides were mounted and visualized in light microscopy (DMR, Leica® coupled with a CCD-IRIS/RGB digital camera, Sony®). The collagen fiber area

and centrally-located nuclei were quantified (10 random fields per participant) using the ImageJ software (National Institutes of Health, USA).

For immunohistochemical analysis of the RAM tissue, sections were incubated with antibodies for fast (mouse; 1:160; 1 mL NCL-MHCf; Novocastra) or slow (mouse; 1:120; 1 mL NCL-MHCs; Novocastra) fibers, followed by secondary antibodies (anti-mouse IgG, HRP-linked Antibody 7076S, Cell Signaling). For staining, the samples were incubated with 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) and counter-stained with hematoxylin. Morphometric analysis was performed, as previously described by Vesentini et al (13). All RAM images were analyzed by a blinded investigator.

Ultrastructural analyses

The RAM tissues were cut into small strips and fixed for 24 hours in a solution of Karnovsky's fixative. Tissues were then post-fixed with 1% osmium tetroxide and the specimens were embedded in epoxy resin. Ultra-thin sections were obtained at a longitudinal orientation using a diamond knife, and then evaluated by transmission electron microscopy using a Philips CM 100 (Philips, USA). All images were recorded using a Olympus Bx41, SC30 camera (Olympus, Japan).

Muscle homogenization and western blotting

The excised and snap-frozen RAM muscle samples were collected. The RAM samples were stored at –80°C until the analysis. The samples were then mechanically homogenized in extraction buffer RIPA (Millipore, Billerica, MA, USA), protease inhibitor cocktail (Sigma CO, Saint Louis, MO, USA), and Triton X-100 (Sigma CO, Saint Louis, MO, USA) using a Polytron homogenizer (Kinematica, Lucerne, Switzerland). After the centrifugation of the homogenate, proteins were extracted from the supernatant and protein concentrations were determined using a colorimetric assay. Equal amounts of protein (35 μg) were heated at 100 °C for 5 min in the sample-loading buffer, then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions, and finally transferred onto nitrocellulose membranes (Millipore Corp, Bedford, MA, USA). The membranes were then blocked in 3% bovine serum albumin in TBST (10 mM Tris–HCl pH 7.5, 150 mM NaCl, and 0.1% Tween-20) and left overnight in primary antibodies at 4 °C. The next day, the membranes were washed three times for 15 min in TBST and secondary antibodies (horseradish peroxidase (HRP)-conjugated polyclonal antibody) were added for 1 hour at room temperature. Blots were visualized using a chemiluminescence kit (Amersham ECL Select, GE Healthcare Life Science)

and the signals were captured using a CCD camera (ImageQuant LAS 4000 mini®; GE Healthcare™). Protein expression levels were obtained by analyzing the densitometry of the bands and expressed as arbitrary units. The expression levels of each protein were normalized to GAPDH values. The analysis was performed with the ImageJ software (Wayne Hasband, National Institute of Health). The primary antibodies used were: CnA (rabbit; 1:10000; ab109412; Abcam); CnB (mouse;1:10000; ab78114; Abcam); MyHCI (mouse; 1:1000; ab11083; Abcam); MyHCIIa (rabbit; 1:10000; ab104128; Abcam); MyCHIIx (rabbit; 1:500; ab124937; Abcam); and GAPDH antibody (rabbit; 1:1000; 14C10; Cell Signaling). The secondary antibodies used were: Anti-rabbit (1:2000; ab6721; Abcam) and Anti-mouse (1:2000; ab6728; Abcam).

ELISA analysis for type I and type III collagens content

The samples were homogenized in 1 mL of PBS and stored overnight at -20 °C. After this, the muscle homogenates were freeze-thawed twice and centrifuged at 5000 × g for 5 minutes at 2-8 °C. The supernatant was then obtained and stored in a freezer at −80 °C until the measurement of type I collagen (CBSE08082H-96T - CUSABIO, MD, USA) and type III collagen (CBSE04799H-96T - CUSABIO, MD, USA), done according to manufacturer's protocol.

Statistical analysis

SAS software for Windows, v.9.4 (Statistical Analysis System Institute Inc., USA) was used to analyze the data. Data were expressed as the mean ± standard deviation (SD) or the number of cases (%). One-way analysis of variance (ANOVA) and paired t-tests were used for intergroup comparisons. Poisson distribution was used for the percentage of fiber type numbers and central nuclei comparisons. Student's t-test was used to detect significant differences of protein expression (western blotting analysis) between groups. The statistical significance level was set at $p = 0.05$.

RESULTS

Table 1 presents the clinical and metabolic characteristics of the four groups of pregnant women included in the study (age, parity, body mass index, weight gain, oral glucose tolerance test, glycemic mean, glycated hemoglobin, hypertension, smoking, alcoholism, and newborn weight). *There were no* statistically *significant differences* between groups in any of the main

characteristics.

Comparison of RAM morphological analysis

The general morphology of muscular fibers showed polygonal forms with a peripheral nucleus and some central nuclei (Figure 1 A-D). The non-GDM C group showed muscular fibers with the largest diameter compared to the others. Regarding central nuclei morphometry, the GDM UI group has more than twice the number of muscle fibers with central nuclei (25.46 ± 15.27) compared to GDM C (16.62 ± 9.14), non-GDM UI (11.46 ± 6.84), and non-GDM C groups (11.39 ± 7; non-GDM C vs GDM UI: p <0.0001; non-GDM UI vs GDM UI: p = <0.0001; GDM C vs GDM UI: p = <0.0001). The GDM C group presented a significant increase in central nuclei compared to non-GDM C (p = 0.0007) and non-GDM UI groups (p = 0.0030) (Figure 1 E).

The morphometric analysis of collagen fiber area by Picrosirius red revealed differences in all groups (Figure 1 A-D). On the one side, the GDM UI group (12858.31± 3364.05) had the lowest collagen area compared to all control groups (GDM UI vs non-GDM UI: p<0.0001; GDM UI vs GDM C: p<0.0001; GDM UI vs non-GDM C: p<0.0001). On the other side, the non-GDM C group (32057.01 ± 4147.41) has the highest collagen area compared to all control groups (non-GDM C vs non-GDM UI: p<0.0001; non-GDM C vs GDM C: p<0.0001). In the non-GDM UI group (18331.31 ± 1654.43), collagen area was lower than that of the GDM C group (20797,89 ± 2248.44, non-GDM UI vs GDM C: p<0.001) (Figure 1 E). The collagen area of GDM groups was lower than in non-GDM groups.

The immunohistochemistry image analysis showed that muscle fiber types were organized in a mosaic form (Figure 2.1-8). The morphometric analysis showed that the GDM UI group had more slow fibers (41.15± 4.55) and less fast fibers (58.85± 5.44) than the non-GDM C group (slow: 30.46 ± 5.28, p = 0.0011; fast: 69.54 ± 7.98, p = 0.0114). Furthermore, compared to the non-GDM UI group (34.9 ± 4.83, p = 0.0379), the GDM UI group had a higher number of slow fiber, but the same amount of fast fibers (65.1 ± 6.6). There were no significant changes in fiber number in GDM C (slow: 43.64 ± 4.55; fast: 56.36 ± 5.1) and GDM UI comparison (slow: p = 0.4135; fast: p = 0.4839) (Figure 2 A-B).

Concerning fiber area morphometry, the GDM UI group presented smaller slow (1967.83 ± 258.98) and fast (2951.44 ± 439.38) fiber areas compared to the non-GDM-C group (slow: 2918.39 ± 496.42; fast: 4918.81 ± 889.73, p <0.0001) and the non-GDM UI group (slow: 2732.68 ± 505.17; fast: 4158.16 ± 523,93, p<0.0001). Moreover, the GDM UI group presented higher slow and fast fiber area compared to the GDM C group (slow: 1713.24 ± 295.95; fast:

2636.68 ± 424.74, p <0.0001) (Figure 2 C-D).

Ultrastructural analyses

Transmission electron microscopy revealed a well-defined morphology of the muscle fibers in the non-GDM C group, with a striated pattern of light and dark bands that characterize organized sarcomeres (Figure 3.1). The non-GDM UI group presented papillary projections on the surface of the muscle fiber membrane (Figure 3.2). The GDM C group presented with alterations of the Z line, resulting in disorganization of the myofibrillar pattern (Figure 3.3). Substantial ultrastructural alterations were found in the GDM UI group, with a focal lesion area. Moreover, we observed numerous myeloid structures, sarcomere disorganization, central nucleus, and alteration of mitochondria with structural abnormalities concerning size, shape, and internal matrix (Figure 3.4).

Comparison of protein expression levels

The western blot analysis showed no significant differences between groups in terms of the MyHCIIa (B), MyHCIIx (C), CnA (D) and CnB (E) expression levels (Figure 4). However, the MyHCI (A) expression level in the RAM was higher in the GDM C and GDM UI groups compared to the non-GDM C group ($p < 0.0082$ and $p < 0.0159$, respectively).

Type I and III Collagen content analysis

The concentration of type I collagen revealed significant between-group differences in all groups. The GDM UI group (2.38 ± 0.89) had the lowest content of type I collagen compared to all other groups (non-GDM C vs GDM UI: $p<0.0001$; non-GDM UI vs GDM UI: $p<0.0001$; GDM C vs GDM UI: $p<0.0001$). In the non-GDM UI group (5.43 ± 1.98), the type I collagen content was lower compared to that in the non-GDM C group (13.47 ± 6.09, $p<0.0001$). However, in the GDM C group (8.60 ± 3.96), the type I collagen content was higher compared to the non-GDM UI group ($p = 0.0035$), but lower compared to the non-GDM C group ($p = 0.0075$). In the same way, type III collagen content in the GDM UI group (22.52 ± 5.14) was significantly lower than in the non-GDM C (47.21 ± 16.37, $p<.0001$) and GDM C groups (38.94 ± 9.46, $p = 0.0280$). Type III collagen content in the non-GDM UI group (32.18 ± 8.56) was substantially lower compared to that in the non-GDM C group ($p = 0.0244$) (Figure 5). In the PSUI groups, type III collagen content was lower compared to that in the respective non-PSUI groups.

DISCUSSION

A common comorbidity of diabetes is skeletal muscle dysfunction, which compromises physical function. Despite the importance of skeletal muscles in whole-body metabolic control, studies investigating diabetic myopathy in GDM-associated PSUI are scarce, or even absent in the literature. To identify the underlying structural and biochemical mechanisms potentially influencing GDM on muscles involved in UI, we examined RAM samples of pregnant women who had undergone C-section. Using our aforementioned translational knowledge (10-13, 28), we demonstrate the RAM waste in GDM-associated PSUI. It characterizes the GDM associated PSUI RAM myopathy an unproved and unrelated complication of GDM (5-7). The parameters of RAM loss detected in GDM-associated PSUI suggest substantial damage to the RAM skeletal muscle. Our RAM samples exhibited a strong reduction in fast and slow fiber area, associated with a decrease and increase in fast and slow fiber number, respectively. We found a substantial increase in centrally-located nuclei and important ultrastructure alterations in muscle fibers, such as numerous myeloid structures, sarcomere disorganization, and morphologically abnormal mitochondria. The atrophy of RAM in GDM-associated PSUI suggests a reduction in skeletal muscle quality and may be referred to as “Pregnant hyperglycemic rectus abdominis myopathy”.

The loss of muscle mass and function caused by diabetes is commonly referred to as diabetic myopathy (29-31). Previous cohort studies report that diabetes triggers an accelerated loss of muscle mass, an effect attributed to diminished body protein metabolism (29, 30). Difficulties in maintaining a healthy muscle mass can affect patients’ life expectancy and quality of life, and such changes also result in major impacts on health costs (32). The proportion of central nuclei increased to 5 % in the GDM non-PSUI group, while in the GDM-associated PSUI group the value increased more than twice that of non-GDM groups. These findings suggest that GDM activates the regenerative response in RAM, as a response to muscle impairment. Supporting this, the analysis of fiber composition in GDM groups showed a significant decrease in fiber area and fast fiber number compared to non-GDM groups, suggesting a possible muscle atrophy phenotype in response to GDM.

We were unable to detect any differences in protein expression in fast-twitch oxidative MyHCIIa and fast-twitch oxidative-glycolytic (MyHCIIx) fiber types. These observations may indicate a different response of slow and fast muscle fiber to GDM. An increase in the number of slow fibers was identified in GDM non-PSUI and GDM-associated PSUI groups, and this finding was reinforced by the increase in protein expression of slow-twitch oxidative fibers. Studies demonstrated that insulin-stimulated glucose uptake is higher in slow fibers

than fast fibers (33, 34). Understanding these mechanisms is particularly relevant because skeletal muscles are responsible for most insulin-mediated glucose disposal (35). Although the influence of DM on mechanisms of intracellular signaling that control skeletal muscle fiber type has not been clarified, calcineurin is known to play a key role in skeletal muscle fiber type selection (36). The enzyme calcineurin is composed of two subunits: the catalytic calcineurin A and the regulatory calcineurin B. It has been implicated in a range of biological processes (e.g., vascular, neuronal, and cardiac development) (37-39), and evidence shows that the calcineurin signaling pathway is involved in the upregulation of genes encoding for slow fiber muscle (40). According to Ryder et al. (36), an increase in the expression of the slow muscle fiber phenotype could suppress the progression of chronic diseases such as diabetes, due to enhanced insulin action in skeletal muscles. However, we did not find differences in calcineurin A and B protein expression in this study.

The causes of UI are multifactorial. The most important finding of this study is that, although both GDM groups presented important changes in muscle fibers, a key factor distinguishing GDM women with and without PSUI is the intramuscular ECM. Kondo et al. (41), demonstrate that the ECM of the rectus fasciae of women with UI presented deteriorated integrity compared with continent subjects. These results are consistent with the epidemiological data suggesting that women with UI have altered ECM content (42-45). Alterations in intramuscular ECM affect normal muscle function, given that ECM bears the majority of passive loads, supports muscle fibers, and is the main determinant of muscle stiffness (18). The major structural protein in skeletal muscle ECM is collagen, and its main subtypes present in skeletal muscles are type I and type III (46). While type I collagen is responsible for tissue stiffness, type III collagen is related to tissue elasticity (47). With this in mind, alterations of collagen content compromise passive support for continence and can lead to compromised passive stability of the abdominal-pelvic dynamics, which, in turn, might contribute to the clinical manifestation of PSUI.

Intramuscular ECM showed an important decrease in collagen localization and type I and III collagen content in the RAM of PSUI women. These changes possibly represent a response to GDM, and presumably, as a consequence of such changes, the development of PSUI in pregnant women. To the best of our knowledge, this GDM RAM myopathy is an unprecedented finding in GDM-associated PSUI. Prior to UI symptoms, morphological changes appear in the PFM, such as the stretching and rupture of muscle and connective tissue, as well as a reduction of muscle tone, maximal strength and endurance of the muscle (48). Furthermore, it is well established that PFM voluntary contractions have been closely linked

with the recruitment of the abdominal muscles (15, 49-51). Thus, we hypothesized that women with GDM-related “rectus abdominis myopathy” are more likely to develop PSUI. Our findings support this conclusion.

Our results confirm that RAM is a plastic tissue that adapts its size and morphology according to external and internal stimuli (here represented by GDM). The GDM-related changes in muscular morphology lead to pregnant women developing PSUI. To the best of our knowledge, this is the first study in which the consequences of hyperglycemia and normoglycemia in pregnancy on the RAM of continent and incontinent women were compared. That being said, more research is required to confirm our findings and improve our understanding of this topic.

There were some limitations to the present study. First, the sample size of each group varied, given the difficulty in finding GDM women without PSUI. Second, the patients included in this study were not all primigravida, which might influence the results. Third, the RAM suffers from substantial strain (e.g., diastasis) during gestation. Diastasis recti abdominis is defined as a separation between the edges of the RAM at the linea alba (52). However, these limitations do not invalidate our results, as they also contribute to advancing knowledge in the field of urogynecology.

In conclusion, our data reveal that GDM-related RAM myopathy could be associated with PSUI. Future studies are clearly needed to confirm RAM myopathy as a key factor of GDM-associated PSUI and to discover novel therapies for muscle wasting pathologies, based on these findings.

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Table 1. Comparison of clinical features among GDM and non-GDM, with and without PSUI.

		non-GDM continent (non-GDM C) n = 20	non-GDM associated PSUI (non-GDM UI) n = 37	GDM continent (GDM C) n = 12	GDM-associated PSUI (GDM UI) n = 23	
	Age (years)	28.25 (5.73)	29.51 (5.63)	32.50 (5.00)	33.04 (5.46)a	
	Parity	1.15 (0.99)	1.03 (0.87)	1.67 (1.30)	1.30 (1.11)	
	Previous newborn weight (g)	2916.17 (1062.67)	2663.23 (1409.59)	3151.18 (1136.44)	2691.75 (1528.25)	
	Weight gain during pregnancy (kg)	14.38 (9.69)	11.99 (8.37)	11.68 (5.18)	9.76 (5.72)	
	Prepregnancy BMI (Kg/m^2)	29.74 (8.31)	31.00 (7.39)	30.28 (4.23)	32.76 (6.19)	
	Predelivery BMI (Kg/m^2)	35.37 (8.56)	35.68 (6.08)	34.69 (4.32)	36.58 (5.57)	
Oral Glucose Tolerance Test	Fasting Glucose (mg/dL)	73.32 (12.98)	72.73 (8.74)	91.42 (12.08)ab	103.78 (19.27)ab	
	1h (mg/dL)	111.83 (25.32)	112.84 (24.70)	166.70 (34.73)ab	178.89 (38.94)ab	
	2h (mg/dL)	95.67 (29.36)	101.09 (21.88)	142.50 (42.70)ab	172.72 (41.94)ab	
	Glicemic Mean (mg/dL)	83.94 (8.36)	82.20 (8.04)	99.09 (9.93)ab	105.31 (28.06)ab	
	HbA1c	5.09 (0.69)	5.01 (0.30)	5.56 (0.56)b	5.57 (0.65)b	
	White	15 (75%)	30 (81.08%)	9 (75%)	15 (65.22%)	0.5629‡
	Stable union/Married	18 (90%)	32 (86.49%)	9 (75%)	22 (95.66%)	0.1806‡
	Hypertension	8 (40%)	19 (51.35%)	4 (33.33%)	9 (39.13%)	0.6344‡
	Smoking	1 (5%)	4 (10.81%)	0 (0%)	1 (4.35%)	0.5304‡
	Alcoholism	0 (0%)	0 (0%)	0 (0%)	1 (4.35%)	0.3866‡
	Birth weight (g)	3239.00 (413.68)	3338.11 (675.36)	3658.75 (559.73)	3560.65 (407.26)	

Data are expressed as mean ± standard deviation or and number of subjects (percentage).;
‡ Chi-square test.
a $p<0.05$ – indicates significant difference compared to non-GDM C (Tukey-Kramer).
b $p<0.05$ - indicates significant difference compared to non-GDM UI (Tukey-Kramer).
PSUI: Pregnancy-Specific Urinary Incontinence; BMI: Body Mass Index; HbA1c: Glycated Hemoglobin.

Figure legends

Figure 1. (A-D) Histological sections of the RAM from one representative participant of each group stained with H&E. (E) Graph of the percentage of central nucleus of all groups. Groups were compared using Poisson distribution ($P < .05$). (a-d) Photomicrographs of transversal sections from one representative participant of each group of the muscular fibers (yellow) and collagen (red) stained with Picrosirius Red. (e) Graph of the mean and standard deviation of the collagen area of all groups. Groups were compared for collagen area using one-way ANOVA followed by Tukey-Kramer ($P < .05$). * indicates significant difference compared to non-GDM C group, § indicates significant difference compared to non-GDM UI, ‡ indicates significant difference compared to GDM C. Abbreviations: non-GDM C, Non-Gestational Diabetes Continent; non-GDM UI, Non-Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence; GDM C, Gestational Diabetes Continent; GDM UI, Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence.

Figure 2. Immunohistochemistry images of RAM fibers of fast and slow fibers in transversal sections (1-8). Graphs of the percentage of slow (A) and fast (B) fiber type number. Graphs of the mean and standard deviation values of the slow (C) and fast (D) fiber type area. Groups were compared for fiber type area using one-way ANOVA followed by Tukey-Kramer. Groups were compared for fiber type area using Poisson distribution ($P < .05$). * indicates significant difference compared to non-GDM C group, § indicates significant difference compared to non-GDM UI, ‡ indicates significant difference compared to GDM C. Abbreviations: non-GDM C, Non-Gestational Diabetes Continent; non-GDM UI, Non-Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence; GDM C, Gestational Diabetes Continent; GDM UI, Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence.

Figure 3. Electron micrographs of longitudinal sections of the RAM fibers; (M) mitochondria, (Z) Z-line, (↔) sarcomeres, (↑) papillary projections, (*) focal lesions areas, (MS) myeloid structure. Abbreviations: non-GDM C, Non-Gestational Diabetes Continent; non-GDM UI, Non-Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence; GDM C, Gestational Diabetes Continent; GDM UI, Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence.

Figure 4. Assessment of MyHCI (A), MyHCIIa (B), MyHCIIx (C) CnA (D) and CnB (E) protein expression in the RAM evaluated by western blotting. Quantification was performed by

Image J software. The values given are in arbitrary units expressed as mean and standard deviation. P < .05. Groups were compared for protein expression using student's t-test. * indicates significant difference compared to non-GDM C group. Abbreviations: non-GDM C, Non-Gestational Diabetes Continent; non-GDM UI, Non-Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence; GDM C, Gestational Diabetes Continent; GDM UI, Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence.

Figure 5. Type I and III collagen concentrations in tissue homogenate. Data presented as mass per milligram tissue wet weight. (mean ± SD). Values are expressed as mean and standard deviation. P < .05. Groups were compared for type I collagen using Gamma and for type III Collagen using one-way ANOVA followed by Tukey-Kramer. * indicates significant difference compared to non-GDM C group, § indicates significant difference compared to non-GDM UI, ‡ indicates significant difference compared to GDM C. Abbreviations: non-GDM C, Non-Gestational Diabetes Continent; non-GDM UI, Non-Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence; GDM C, Gestational Diabetes Continent; GDM UI, Gestational Diabetes-associated Pregnancy-Specific Urinary Incontinence.

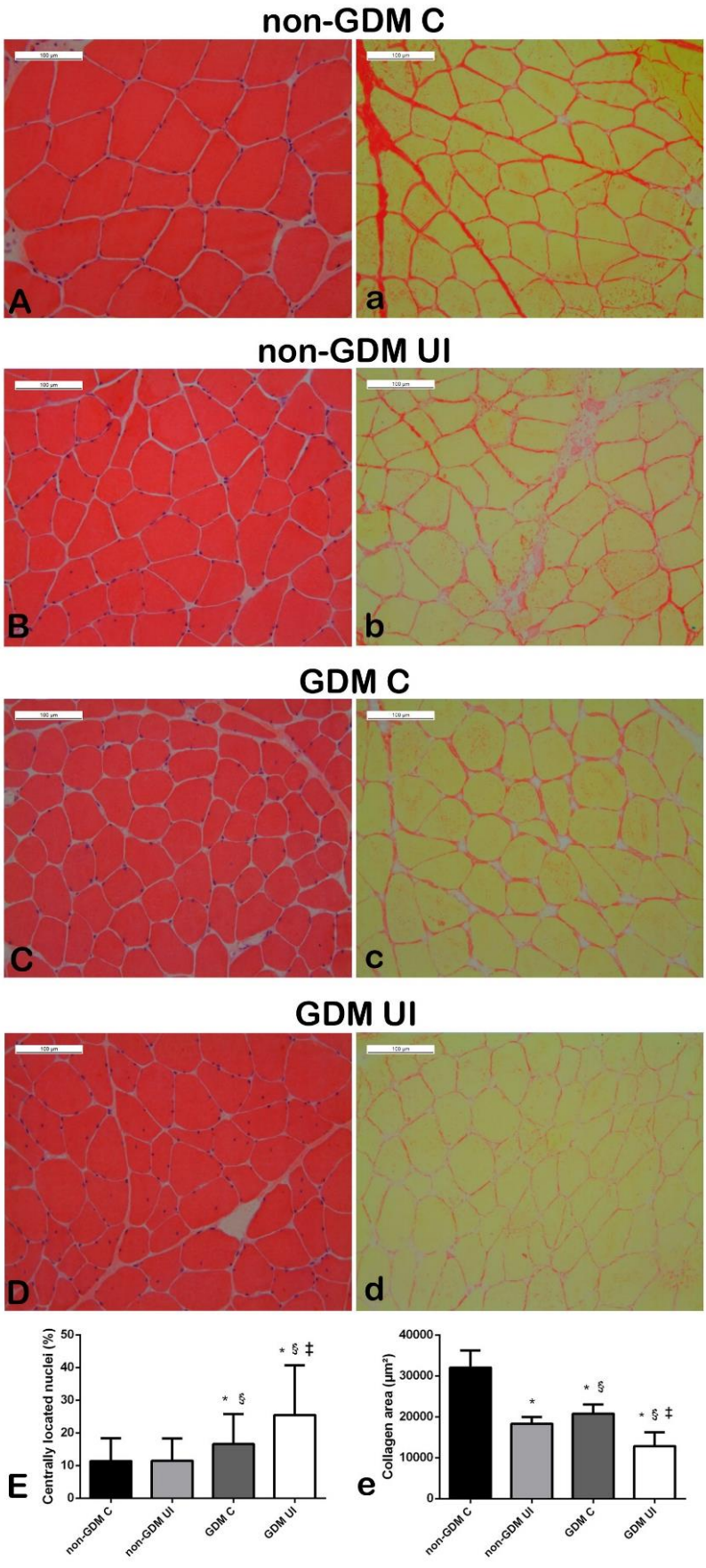
non-GDM C
A
a
non-GDM UI
B
b
GDM C
C
c
GDM UI
D
d
E
Centrally located nuclei (%)
0
10
20
30
40
50
* § ‡
* §
non-GDM C
non-GDM UI
GDM C
GDM UI
e
Collagen area (µm²)
0
10000
20000
30000
40000
*
* §
* § ‡
non-GDM C
non-GDM UI
GDM C
GDM UI

Figure 1

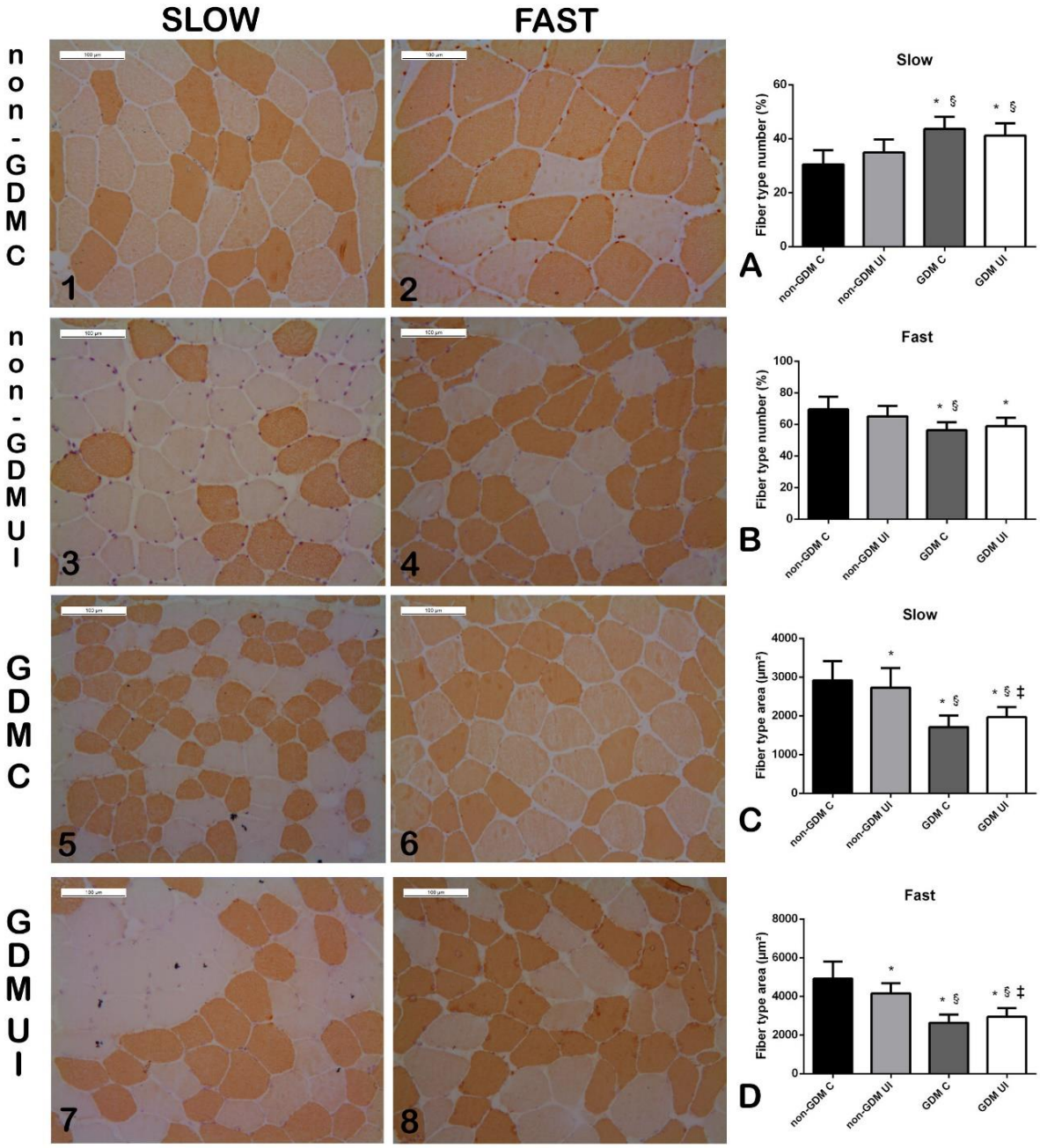
SLOW
FAST
non-GDMC
non-GDMUI
GDMC
GDMUI
1
2
3
4
5
6
7
8
Slow
Fiber type number (%)
A
Fast
Fiber type number (%)
B
Slow
Fiber type area (µm²)
C
Fast
Fiber type area (µm²)
D
non-GDM C
non-GDM UI
GDM C
GDM UI

Figure 2

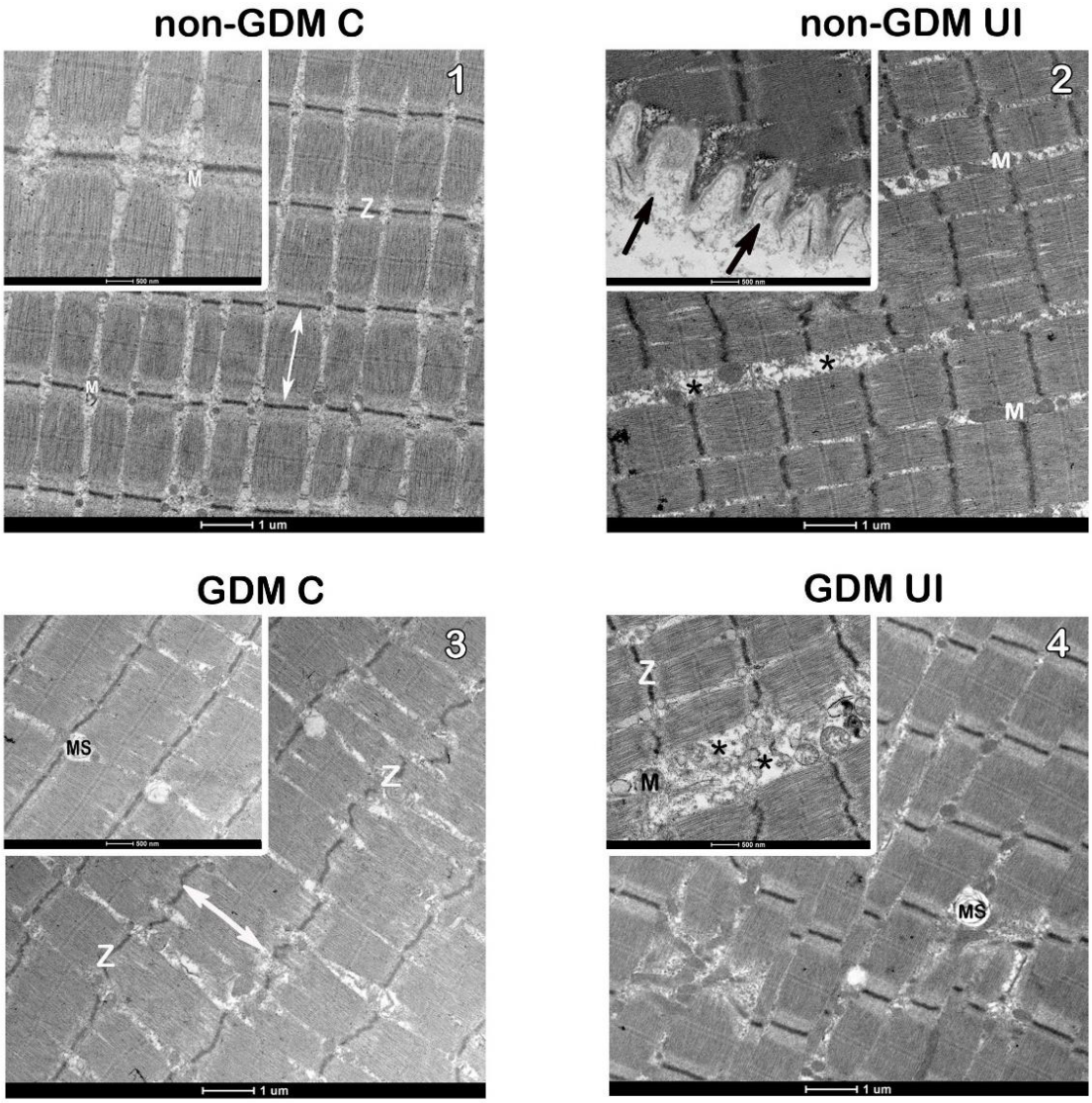
non-GDM C
1
M
Z
M
1 um
non-GDM UI
2
M
*
*
M
1 um
GDM C
3
MS
Z
Z
1 um
GDM UI
4
Z
*
*
M
MS
1 um

Figure 3

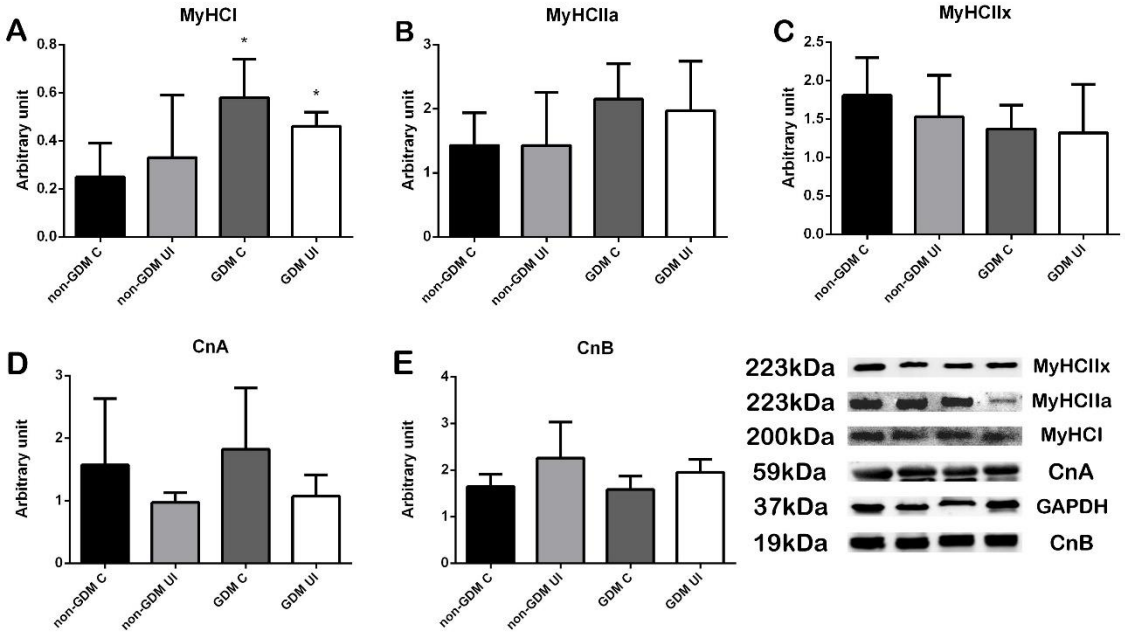
A
MyHCI
Arbitrary unit
0.8
0.6
0.4
0.2
0.0
*
*
non-GDM C
non-GDM UI
GDM C
GDM UI
B
MyHCIIa
Arbitrary unit
3
2
1
0
non-GDM C
non-GDM UI
GDM C
GDM UI
C
MyHCIIx
Arbitrary unit
2.5
2.0
1.5
1.0
0.5
0.0
non-GDM C
non-GDM UI
GDM C
GDM UI
D
CnA
Arbitrary unit
3
2
1
0
non-GDM C
non-GDM UI
GDM C
GDM UI
E
CnB
Arbitrary unit
4
3
2
1
0
non-GDM C
non-GDM UI
GDM C
GDM UI
223kDa
MyHCIIx
223kDa
MyHCIIa
200kDa
MyHCI
59kDa
CnA
37kDa
GAPDH
19kDa
CnB

Figure 4

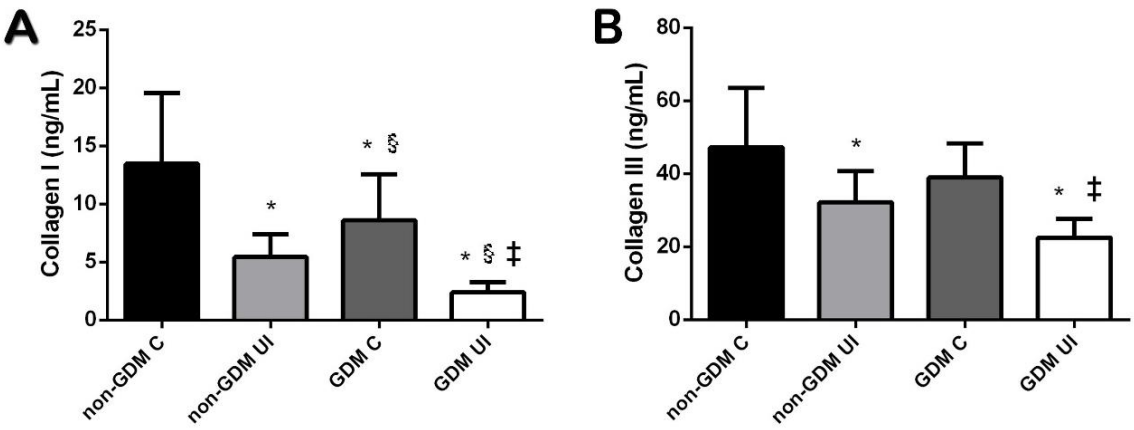
A
Collagen I (ng/mL)
25
20
15
10
5
0
*
* §
* § ‡
non-GDM C
non-GDM UI
GDM C
GDM UI
B
Collagen III (ng/mL)
80
60
40
20
0
*
* ‡
non-GDM C
non-GDM UI
GDM C
GDM UI

Figure 5

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: REPERCUSSÕES DA HIPERGLICEMIA GESTACIONAL E INCONTINÊNCIA URINÁRIA GESTACIONAL NO MÚSCULO RETO ABDOMINAL: MATRIZ EXTRACELULAR, EXPRESSÃO PROTEICA E ULTRAESTRUTURA

Pesquisador: Giovana Vesentini

Área Temática:

Versão: 4

CAAE: 41570815.0.0000.5411

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: 2.934.228

Apresentação do Projeto:

Trata-se de emenda do projeto intitulado “REPERCUSSÕES DA HIPERGLICEMIA GESTACIONAL SOBRE A CONTRATILIDADE, ULTRAESTRUTURA E EXPRESSÃO PROTEICA NAS FIBRAS MUSCULARES DO MÚSCULO RETO ABDOMINAL”, aprovado por este Comitê de Ética em 16 de março de 2015, com número de parecer 986.465.

A emenda propõe a alteração de uma das análises propostas inicialmente, a análise da actomiosina ATPase, considerando que o pesquisador contatado para auxiliar nesta análise específica mudou de país. Visto o tempo limitado para a apresentação do Relatório Final e levando em consideração que as amostras de MRA de gestantes diabéticas e não diabéticas já estão colhidas, bem como as evidências da literatura atual, optou-se pela adequação dos objetivos deste projeto, substituindo a análise da atividade da ATPase pela análise de componentes da Matriz Extracelular (MEC) em especial a análise de Colágeno I e III.

Consequentemente, houve necessidade de alteração no método de análise de dados e no título do projeto.

Em relação ao método, a técnica será realizada por kits de ELISA a saber: Collagen Type I (Código do produto: ABIN367624 Antibodies-Online Inc) e Collagen Type III (Código do produto: ABIN3666653 Antibodies-Online Inc), em parceria com o Prof. Dr. Sérgio Luiz Felisbino do Laboratório de Matriz Extracelular do Instituto de Biociências de Botucatu-UNESP.

Consequentemente às alterações citadas, o título do projeto passa a ser “REPERCUSSÕES DA HIPERGLICEMIA GESTACIONAL E INCONTINÊNCIA URINÁRIA GESTACIONAL NO MÚSCULO RETO ABDOMINAL: MATRIZ EXTRACELULAR, EXPRESSÃO PROTEICA E ULTRAESTRUTURA”.
Os pesquisadores esclarecem que as pacientes que já participaram da pesquisa serão contatadas e informadas a respeito das modificações sofridas e, se concordarem em participar, assinarão o novo modelo de Termo de Consentimento Livre e Esclarecido. Salientem, entretanto, que as modificações propostas não representam alterações na coleta de dados.

Objetivo da Pesquisa:
A emenda apresentada propõe mudanças nos objetivos da pesquisa, que passam a ser:

Objetivo Primário: Analisar a matriz extracelular, ultraestrutura e expressão proteica nas fibras musculares do músculo reto abdominal em gestantes hiperglicêmicas e
com incontinência urinária.

Objetivos Secundários:
- Quantificar e comparar a atividade da matriz extracelular Colágeno I e III do músculo reto abdominal entre os grupos;
- Identificar e comparar o padrão de expressão das proteínas MHC I, MHC IIa, MHC IIx/d, CnA e CnB entre os grupos de gestantes;
- Discriminar a ultraestrutura das fibras musculares entre os grupos.

Avaliação dos Riscos e Benefícios:
Considerando que a emenda proposta não representa alterações na coleta de dados e na forma de participação, os riscos e benefícios permanecem os mesmos, relatados em parecer de 16 de março de 2015 (Parecer 986.465).

Comentários e Considerações sobre a Pesquisa:
As alterações de que se trata essa emenda estão claramente descritas e justificadas. Houve a necessidade de alteração de uma das análises do material biológico coletado e, consequentemente dos objetivos e do título do trabalho.
Entretanto, as alterações propostas não representam ônus ou riscos/benefícios adicionais às participantes, uma vez que a coleta de dados não sofreu alterações. Inclusive, a coleta de dados está finalizada.
De qualquer forma, os pesquisadores se propõem a entrar em contanto e comunicar as

modificações às participantes, solicitando a assinatura de novo TCLE/TALE.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados todos os documentos obrigatórios:

- Folha de rosto devidamente assinada;
- Projeto de pesquisa com as alterações propostas na emenda;
- TCLEs e TALE em forma de convite, com linguagem acessível, contendo as informações necessárias e as adequações necessárias em função das modificações no projeto.

Conclusões ou Pendências e Lista de Inadequações:

Após análise, o Colegiado deliberou APROVAÇÃO da emenda ao projeto de pesquisa apresentada.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado em reunião ordinária do Comitê de Ética em Pesquisa da FMB/UNESP, realizada em 1º de outubro de 2018, o documento enviado na forma de “Emenda”, se encontra APROVADO, sem (com) necessidade de envio à CONEP.

Atenciosamente,

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

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1205211_E2.pdf	21/08/2018 15:42:50		Aceito
Declaração de Pesquisadores	Oficio_modificacao_CEP.pdf	21/08/2018 15:41:18	Giovana Vesentini	Aceito
Folha de Rosto	Folha_de_Rosto_Emenda.pdf	21/08/2018 15:39:41	Giovana Vesentini	Aceito
Outros	TCLE_Responsaveis.pdf	21/08/2018 12:02:41	Giovana Vesentini	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Maiores_de_18_anos.pdf	21/08/2018 11:59:13	Giovana Vesentini	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TALE_Menores_de_17.pdf	21/08/2018 11:59:05	Giovana Vesentini	Aceito
Projeto Detalhado / Brochura	Projeto_Modificado_Agosto2018.pdf	21/08/2018 11:51:57	Giovana Vesentini	Aceito

Continuação do Parecer: 2.934.228

Investigador	Projeto_Modificado_Agosto2018.pdf	21/08/2018 11:51:57	Giovana Vesentini	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BOTUCATU, 03 de Outubro de 2018

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador(a))

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