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ORIGINAL ARTICLE

Relaxin-2 during pregnancy according to glycemia, continence status, and pelvic floor muscle function

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Abstract

Introduction and hypothesis To investigate relaxin-2 concentration comparing gestational diabetes mellitus (GDM) and non-GDM patients during pregnancy according to urinary incontinence (UI) and pelvic function status.

Methods This is a cross-sectional study evaluating 282 pregnant women from 24 weeks of gestation. The participants were divided into two groups, non-GDM and GDM, according to American Diabetes Association's diabetes mellitus gestational threshold. In addition, according to subanalysis, both groups were subdivided according to the presence of pregnancy-specific urinary incontinence: non-GDM continent, non-GDM incontinent, GDM continent, and GDM incontinent. All participants filled in questionnaires on clinical, obstetric, and urinary continence status (International Consultation on Incontinence Questionnaire-Short Form, ICIQ-SF, and Incontinence Severity Index, ISI), followed by pelvic floor muscle evaluation by the PERFECT scheme in which strength, endurance, and speed of contractions were evaluated.

Results Serum relaxin-2 concentrations were significantly lower in pregnant women with pregnancy-specific urinary incontinence in both non-GDM and GDM patients, but GDM showed the lowest concentration. In addition, the stratification of the groups according to pelvic floor muscle strength showed that pregnant patients with GDM and modified Oxford scale 0–2 had significantly lower levels than those who were non-GDM and GDM with Modified Oxford Scale 3–5. Relaxin-2 level was much lower in GDM incontinent pregnant women with MOS 0–2 compared to the other three groups.

Conclusions Lower relaxin-2 concentration was associated with the presence of pregnancy-specific urinary incontinence, but the combination of GDM, pregnancy-specific urinary incontinence, and lower levels of pelvic floor strength led to lower levels of relaxin-2 compared to the other three groups.

Keywords Gestational diabetes · Obstetrics · Pelvic floor · Pregnancy · Relaxin-2

Introduction

Urinary incontinence (UI) is a silent but prevalent event [1]. Pregnancy-specific urinary incontinence (PSUI) is a term we propose to describe the urinary incontinence that appears during pregnancy. The pathophysiology is multifactorial, and many gaps in the literature have not yet been clarified, including the inclusion in a scientific glossary. The first-line treatment for UI is pelvic floor muscle (PFM) training [2]; therefore, PFM function is one of the main points of interest, and all other possible conditions that modify its function and/or morphology should be investigated. Gestational diabetes mellitus (GDM) can damage muscular tissue, causing atrophy, thinning, disorganization, and co-localization of fast

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and slow fibers [3], consequently impairing PFM morphology [4] and function [5].

In addition, during pregnancy complicated by hyperglycemic events, relaxin concentration is less dependent on the trimester [6]. Experimental studies in 1926, conducted by Hisaw, demonstrated that the injection of a serum induced relaxation of the pelvic ligaments. Later, in 1930, the active substance was named relaxin, which is part of the insulin superfamily of peptide hormones. Since then, studies have been conducted on animals, and from the mid-1980s onwards, human relaxin has been the focus of much research. Although relaxin is recognized as a pregnancy-specific hormone, it is present at lower and variables levels throughout life and has physiological targets in other organs that are important for insulin action (e.g., the pancreas, liver, and muscle) [7].

During pregnancy, the physiological action of relaxin on the cervix, vagina, and uterus plays a role in the preparation of the birth canal [8]. Connective and muscular tissue compositions are also impacted by relaxin, which may also be implicated in the skeletal muscle healing process by regulating inflammation, tissue remodeling, and fibrosis [9]. Cell culture studies suggest that relaxin administered at physiological levels may have an effect on soft tissue remodeling (ligament fibrocartilage, articular cartilage, tendon, and dermis) [10].

The integral theory implies that, for a physiological function, the pelvic structures should work in harmony to modulate possible pelvic overload; since relaxin promotes the process of ligamentous laxity, this process may counteract muscle forces [11]. Therefore, it is widely known in clinical practice that higher levels of relaxin are a risk factor for pelvic dysfunction (PD) such as UI [12]. Although this mechanistic explanation is reasonable, clinical research on relaxin concentration dosage and UI assessment is limited and inconclusive; thus, its impact on PFM function is not yet known.

The possible interactions of this hormone with GDM and its impact on PFM function and on PD should be compared and critically analyzed for further guidance in future studies on the pathophysiology of UI during pregnancy. Our hypothesis was that GDM could be used as a predictor of relaxin-2 concentrations, while the presence of PSUI and level of pelvic floor function would be affected by the mediating action of different relaxin-2 concentrations. Therefore, our primary aim was to investigate relaxin concentration between GDM and non-GDM during pregnancy according to UI status. The secondary aim was to investigate the subgroups according to pelvic function status.

Materials and methods

Study design, participants, and group composition

This was a cross-sectional study approved by the Institutional Ethics Committee of Botucatu Medical School of Sao Paulo State University (protocol no. CAAE 82225617.0.0000.5411). All participants were informed about the study procedures and signed the consent form after careful explanation of all research procedures.

All subjects met the following inclusion criteria: pregnant women at between 24 to 40 weeks of gestation; singleton pregnancy; 18–40 years of age; had not received PFM training or any musculoskeletal PFM treatment before or during pregnancy. Pregnant women were ineligible if they had a clinical diagnosis of diabetes (type I or II, or overt diabetes in previous pregnancy), pre-pregnancy history of UI, more than two pregnancies, previous vaginal delivery, previous organ prolapse or incontinence surgery, difficulty in understanding or following the command to contract their PFM, history of neurological diseases, visible genital prolapse, cervical isthmus incompetence, smoking history, preterm birth or abortion or if they withdrew their consent during the cohort.

Recruitment was carried out at the Perinatal Diabetes Research Center (PDRC) of the Botucatu Medical School, UNESP, Brazil, between 2018 and 2020. After giving their written consent, participants were asked to answer a questionnaire about personal and anthropometric details. Then, the clinical and obstetric history was collected, followed by blood collection.

Gestational screening for diabetes mellitus

The diagnosis guidelines proposed by American Diabetes Association were used to identify patients with GDM [13] using the 75-g oral glycemic tolerance test (75g-OGTT) at 24–30 weeks of gestation. The results were collected in the digital medical records. The participants were allocated to the GDM group if they presented fasting glycemic levels ≥ 92 mg/dl, 1 h ≥ 180 mg/dl, or 2 h ≥ 153 mg/dl. Participants who had lower levels comprised the non-GDM group.

Urinary incontinence questionnaire

The questionnaires were given on the same day as relaxin dosage. To determine continence status, the first question of the Brazilian version of the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) and International Incontinence Index (ISI) were used. [14]. If the participant answered "never" to the standard question "How often do you leak urine?," they were considered

continent; any other answer was framed as incontinent. The final score ranges from 0 to 21 and was stratified according to severity and bothersomeness as slight (1–5 points), moderate (6–12 points), severe (13–18 points), and very severe (19–21 points) [15]. The groups were composed according to glycemic condition, as described above and according to the presence of incontinence: non-gestational diabetes mellitus continent group (non-GDM-C), non-gestational diabetes mellitus incontinent group (non-GDM-PSUI), gestational diabetes mellitus continent group (GDM-C), and gestational diabetes mellitus incontinent group (GDM-PSUI).

Pelvic floor muscle function assessment

A single trained physiotherapist (CBP) with 4 years of experience in PFM evaluation conducted the assessment. After emptying their bladder, participants were instructed to lie down on an examination table in the supine position with their lower limbs flexed. Explanation of the anatomy and function of PFM was provided. Bidigital vaginal palpation was performed, and approximately 4 to 6 cm of the fingers was inserted inside the vaginal introitus and positioned at the posterior vaginal wall. Moderate pressure was applied to assist in the initiation of the appropriate muscle contraction. The patients were requested to voluntarily contract the PFMs with the following verbal instruction: "squeeze the vaginal muscles and hold them as hard as possible, as if you were holding the urine, until I tell you to relax." Co-contraction of the adductor and gluteus, hip movements, and expulsion movements were corrected [5]. Power (strength), endurance, and speed aspects of the PERFECT scheme were determined [16]. For strength classification, the Modified Oxford Scale (MOS) was used, ranging from 0 (no discernible muscle contraction) to 5 (strong PFM contraction against resistance applied to the posterior vaginal wall) to assess PFMs. For data analysis, MOS was stratified according to the posterior wall action, meaning that participants who scored 0–2 were not able to elevate the posterior vaginal wall and those with 3–5 performed a better contraction with elevation of the posterior vaginal wall. Endurance was evaluated as time in seconds (up to 10 s) that maximum voluntary contraction (MVC) force was held before it was deemed (through palpation) to have reduced by ≥ 35%. Fast contraction was evaluated as the number of times the MVC force (determined by MOS) was repeatedly achieved at 1-s intervals. The participant was instructed to contract and relax the muscles as quickly and strongly as possible until muscle fatigue (up to 10 s) [16].

Blood collection and relaxin analysis

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

Sample size estimation

The sample size calculation was performed a priori using G*Power. Since no previous research has performed the measurement proposed by this study, for the calculations we considered a one-way analysis of variance test, power of 0.80, probability of error α of 0.05, and effect size of 0.25. According to the study design, four groups were considered for the calculation (non-GDM-C, non-GDM-PSUI, GDM-C, and GDM-PSUI); the estimated sample size required was 180 participants (45 in each group).

Statistical methods

IBM SPSS Statistics for Windows software, version 20.0 (IBM Corp., Armonk, NY, USA), was used for statistical analysis. Study population characteristics were expressed as numbers and percentages for categorical variables and median, and minimum and maximum for continuous variables. Chi-square test or Fisher's exact test was applied to compare the nominal data between groups. Comparisons among the four groups were performed by Kruskal-Wallis H test, followed by Dunn-Bonferroni multiple comparisons; for two-group comparisons, Mann-Whitney U tests were performed. Differences were considered statistically significant at $p < 0.05$.

Results

A total of 2432 consecutive participants were enrolled for recruitment at the Perinatal Diabetes Research Center (PDRC); 2104 were not included. A total of 328 met the inclusion criteria and completed all stages of the research. Among these, 46 participants were excluded from the final analysis: 15 because of missing data, 15 because of undetectable relaxin-2, and 16 because OGTT-75g was not available. Thus, 282 participants were successfully included in this study. Of these, 186 were non-GDM and were divided according to continence status: 81 were continent and 105 had PSUI; 96 had GDM and were divided into 46 continent

and 50 with PSUI (Fig. 1). The baseline characteristics of 282 participants are summarized in Table 1. The age of GDM-PSUI patients was similar that in the other groups, the pre-gestational BMI was higher compared to the non-GDM-C group, and the gestational BMI was higher compared to the non-GDM-C and GDM-C patients.

The OGTTs (fasting; 1 h and 2 h), as expected, were different between the non-GDM and GDM groups. The groups were matched for gestational age, maternal weight gain, ethnicity, and previous cesarean section. The prevalence of PSUI was statistically similar ($p = 0.485$) between the non-GDM (56.5%) and GDM (52.1%) groups.

When relaxin-2 concentrations were compared between the non-GDM and GDM groups, excluding the stratification by continence status, the analysis showed similar levels of 510.5 (58.7–2563.1) and 437.9 (76.3–3369.7) pg/ml ($p =$

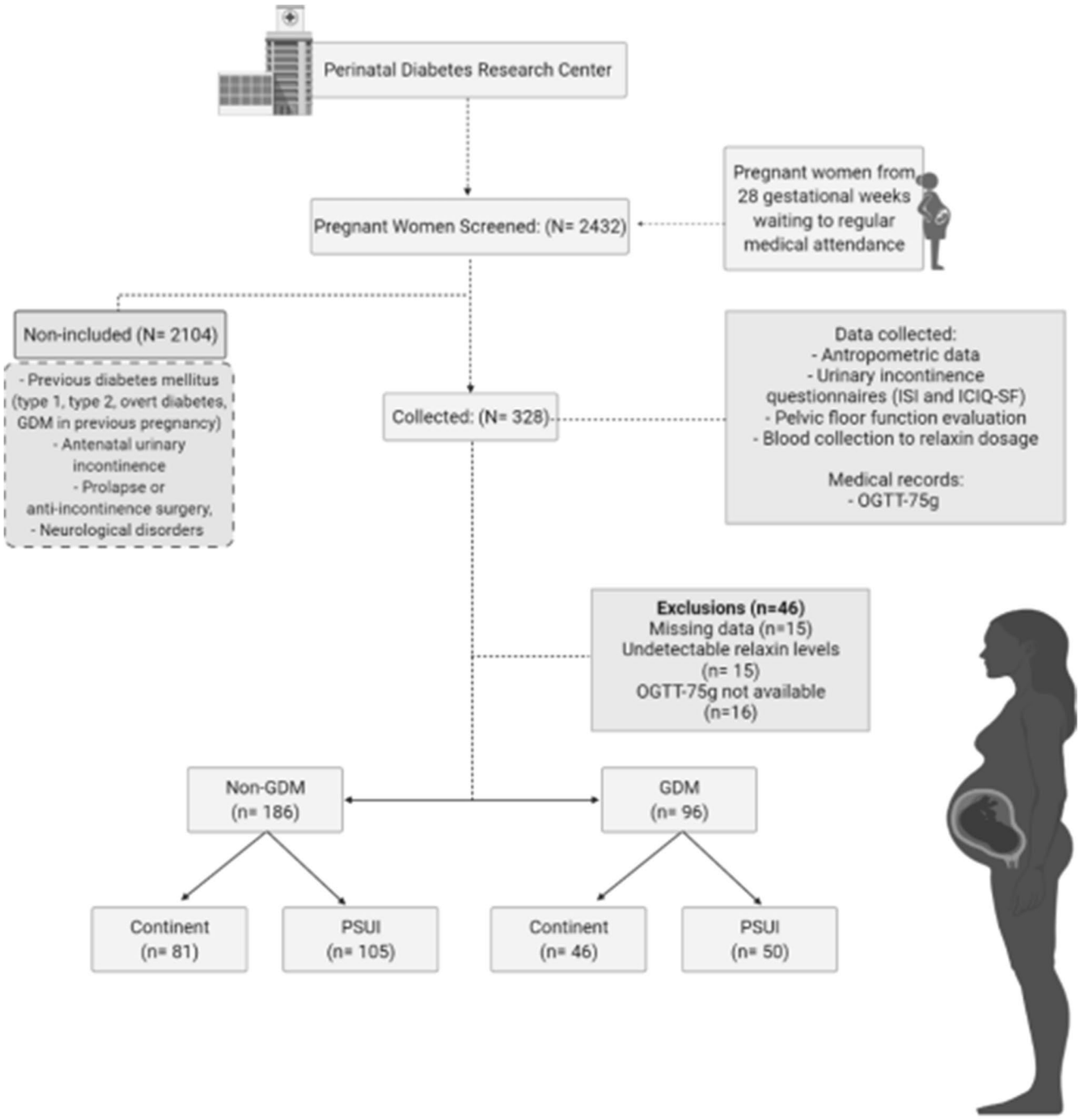

Fig. 1. Flow chart indicating the distributions of study participants according to glycemic and continence status

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

	Non-GDM-C ($n = 81$)	Non-GDM-PSUI ($n = 105$)	GDM-C ($n = 46$)	GDM-PSUI ($n = 50$)	p value
Age (years)	26 (18-38)	24 (18-39)[a]	29 (18-41)[a]	26 (18-41)	0.022
Gestational age	28 (24-38)	28 (24-38)	29.5 (24-38)	31.5 (24-38)	0.086
Pre-pregnancy BMI	23.6 (16.8-42.5)[a]	26.3 (16.8-44.4)	26.2 (18.7-35.9)	28.7 (18.5-48.4)[a]	0.000
BMI gestational	27.5 (18.7-44.3)[a]	29.2 (18.7-49.3)	28.2 (20.4-38.9)[b]	33.9 (21.6-49.5)[ab]	0.001
Weight gain (kg)	7.5 (-1.0-29.0)	7 (-21.0-27)	7.4 (-26-18.8)	7.2 (-32-17)	0.792
OGTT-75g fasting (mg/dl)	74 (50-90)	73 (58-87)	86.5 (64-124)	93 (73-119)	0.000
OGTT-75g 1 h (mg/dl)	108 (62-167)	112 (42-166)	145 (82-220)	151.5 (88-235)	0.000
OGTT-75g 2 h (mg/dl)	97 (51-151)	97 (49-143)	131 (72-205)	144.5 (72-217)	0.000
Caucasian	65 (80.2%)	83 (79%)	32 (69.6%)	37 (74%)	0.491
Prior cesarean delivery	9 (11.1%)	27 (25.7%)	9 (19.6%)	12 (24.0%)	0.086

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

0.216). Tests comparing groups by continence status showed a significant difference ($p \leq 0.001$). The GDM-PSUI group showed lower relaxin levels than the GDM-C ($p = 0.027$) and non-GDM-C ($p = 0.001$) groups. In addition, the non-GDM-PSUI group had lower relaxin levels compared to the non-GDM-C group ($p = 0.023$) (Table 2 and Fig. 2).

The characteristics of PSUI in the non-GDM and GDM groups are shown in Table 2. The leakage volume in the ICIQ-SF questionnaire showed that the GDM group had more (moderate/large) urine loss ($p = 0.013$), and the GDM group had leakage episodes more frequently (several times a day/all the time) ($p = 0.018$). The impact on quality of life and total scores in both groups was comparable.

There were differences between groups regarding the strength evaluated by palpation. In the groups with PSUI, the prevalence of pregnant women with MOS between 0–2 was higher than in the continent groups, regardless of glycemic status. However, in other aspects of PFM function, such as endurance and fast contractions, all groups were comparable.

Serum relaxin-2 concentrations were lower in pregnant women with GDM whose MOS was lower (0–2) compared to the non-GDM and GDM groups whose MOS was 3–5. When groups were stratified by UI status, the concentration was significantly lower in the GDM-PSUI group compared to the other three groups (Table 3).

Discussion

To the best of our knowledge, this is the first study to evaluate the interaction among GDM, PSUI, pelvic floor strength, and serum relaxin-2 profile during pregnancy. The main finding provides evidence that serum relaxin-2 concentrations were significantly lower in pregnant women with PSUI in both the non-GDM and GDM groups in which GDM showed the lowest concentration. In addition, the stratification of the groups according to PFM strength showed that pregnant women with GDM and MOS 0-2 had significantly lower levels than non-GDM and GDM with MOS 3–5. The relaxin-2 level was markedly lower in GDM-PSUI pregnant women with MOS 0-2 compared to the other three groups.

Our main aim was quantifying the relaxin-2 concentrations in the presence of GDM and incontinence. There are no data available considering how BMI and maternal age influence relaxin-2 concentration, but it is important to consider that our groups diverged regarding maternal age and pre-gestational and gestational BMI. These differences should be addressed to the group composition, since in general maternal age and higher BMI are the risk factors for GDM [17] and should be considered the incontinence pathophysiology [18].

DeLancey [19] and Petros [11] highlight the importance of the functional balance between connective tissues and PFM for the continence process. According to hormonal theory, estrogen, progesterone, and relaxin-2 are the three hormones most commonly associated with UI [20]. Relaxin is recognized as an anti-fibrotic hormone. Relaxin's action facilitates collagen degradation as it promotes changes in its concentration and remodels the matrix metalloproteinases, gelatinases, collagenases, and alpha smooth muscle in addition to decreasing the gene expression of collagen I and III and inter-collagen fibril interactions leading to increased collagen fibril sliding and ligament length [21].

Although in clinical practice and even in academic fields there is a concept of higher levels of relaxin leading to pelvic dysfunction, in particular, UI, articles correlating it with the dosage of relaxin concentrations are scarce, have poor methodological quality, and have small sample sizes; thus, findings

Table 2. Urinary incontinence questionnaires and pelvic floor function according to groups

	Non-GDM-C ($n = 85$)	Non-GDM-PSUI ($n = 111$)	GDM-C ($n = 47$)	GDM-PSUI ($n = 54$)	p-value
ICIQ-SF					
Amount of urine lost	-		-		
A small amount	-	79 (75.2%)	-	13 (26%)	**0.013**
A moderate amount	-	25 (23.8%)	-	14 (28%)	
A large amount	-	1 (1.0%)	-	11 (22%)	
Frequency of urine lost	-		-		
About once a week or less often	-	36 (34.3%)	-	11 (22%)	**0.018**
Two or three times a week	-	32 (30.5%)	-	1 (2%)	
About once a day	-	21 (20.0%)	-	7 (14%)	
Several times a day	-	16 (15.2%)	-	38 (76%)	
All the time	-	0	-	5 (10%)	
Qol (0-10)	-	7 (0-10)	-	7 (0-10)	0.400
ICIQ-SF mean score (0-21)	-	12 (3-18)	-	12 (3-18)	0.998
ISI					
ISI score (1-12)	-	3 (1-12)	-	3.5 (1-9)	0.333
Severity (ISI)	-		-		
Slight	-	20 (18.2%)	-	7 (14%)	0.725
Moderate	-	79 (75.2%)	-	38 (76%)	
Severe	-	6 (5.7%)	-	5 (10%)	
Pelvic floor function	(n= 70)	(n= 95)	(n= 43)	(n= 59)	
MOS (0-5)					**0.003**
0	7 (8.6%)	9 (8.6%)	2 (4.3%)	3 (6%)	
1	12 (14.8%)	19 (18.1%)	8 (17.4%)	13 (26%)	
2	14 (17.3%)	35 (33.3%)	3 (6.5%)	15 (30%)	
3	19 (23.5%)	18 (17.1%)	12 (26.1%)	15 (30%)	
4	12 (14.8%)	10 (9.5%)	11 (23.9%)	3 (6%)	
5	6 (7.4%)	4 (3.8%)	7 (15.2%)	0	
MOS (stratified)					
0-2	33 (40.7%)	63 (60%)	13 (28.3%)	31 (62%)	**<0.001**
3-5	37 (45.7%)	32 (30.5%)	30 (65.2%)	18 (36%)	
Endurance (0-10 s)	4 (0-10)	3 (0-10)	4 (0-10)	3 (0-10)	0.125
Endurance (stratified)					
0-5 s	51 (63%)	72 (68.6%)	29 (63%)	41 (82%)	0.322
6-10 s	19 (23.5%)	23 (21.9%)	14 (30.4%)	8 (16%)	
Repetition (stratified)					
0-5 repetitions	34 (42%)	48 (45.7%)	12 (26.1%)	25 (50%)	0.536
5-10 repetitions	36 (44.4%)	44 (41.9%)	31 (67.4%)	24 (48%)	

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

are of limited value to support this statement [22, 23]. In our study, we found that pregnant women with PSUI in both the GDM and non-GDM groups presented lower levels of relaxin compared to continent groups, contradicting the higher levels hypothesis. No studies were found comparing non-GDM and GDM groups regarding continence status or lack of it.

Previous studies including pregnant women with GDM were conducted and showed that in the GDM group the relaxin concentration was higher during 12 weeks of gestation compared to a non-GDM group. Although interesting, sample selection differed in terms of glycemic threshold, and the control group selection was not fully addressed [24].

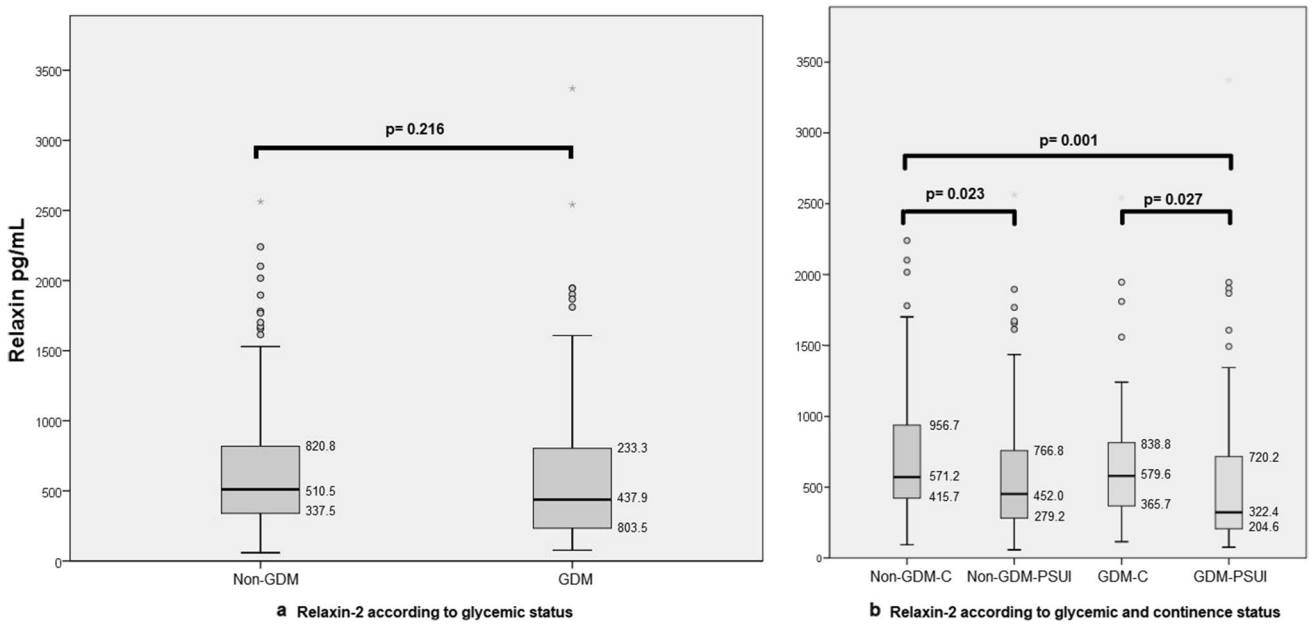

Fig. 2. Median ± 25th–75th percentiles of relaxin-2 serum concentration according to glycemic and continence status during pregnancy. Note: Significant *p*-values for post-hoc Dunn-Bonferroni tests for between-group multiple comparisons are shown in the figure with black lines between groups. Median, 25th and 75th percentiles; pg/ml: picogram/mililiters; Non-GDM-C: non-gestational diabetes mellitus continent group; Non-GDM-PSUI: non-gestational diabetes mellitus incontinent group; GDM-C: gestational diabetes mellitus continent group; GDM-PSUI: gestational diabetes mellitus incontinent group; $p < 0.05$ significant difference

Table 3. Serum relaxin-2 concentrations regarding pelvic floor strength according to different groups' stratifications

	MOS (0-2) pg/ml	MOS (3-5) pg/ml	*p* value*
Not stratified			
All samples ($n = 282$)	458 (75-2563)	529 (76-3370)	0.254
Stratified by glycemic status			
Non-GDM ($n = 165$)	506 (75-2563)	509 (89-1500)	0.689
GDM ($n = 102$)	361 (96-1947)	587 (76-3370)	**0.029**
p value**	**0.023**	0.210	
Stratified by glycemic and continence status			
Non-GDM-C ($n = 70$)	537 (93-2102)[c]	560 (125-1500)	0.729
Non-GDM-PSUI ($n = 95$)	471 (75-2563)[b]	434 (89-1435)	0.413
GDM-C ($n = 43$)	613 (198-1947)[a]	587 (115-2542)	0.526
GDM-PSUI ($n = 59$)	260 (96-1902)[a,b,c]	625 (76-3370)	0.059
p value***	**0.001**	0.201	

MOS: Modified Oxford Scale; *n*: sample; pg/ml: picogram per deciliter; *comparisons between stratified Modified Oxford Scale within each group (Mann-Whitney U); median (minimum-maximum); **comparisons according to each stratified score from Modified Oxford Scale between non-GDM and GDM. ***Comparisons according to each stratified score from Modified Oxford Scale among non-GDM-C, non-GDM_PSUI, GDM-C, and GDM-PSUI (Kruskal-Wallis H; post hoc Dunn-Bonferroni); MOS: Modified Oxford Scale; *$p < 0.05$ significant difference are in bold

Nevertheless, to allow additional comparisons, we selected studies which included non-GDM pregnant women. In this study, GDM and non-GDM groups with PFMD/PSUI had lower levels of relaxin-2, contradicting the opposite results from a previous study [22] with a small sample size, which failed to show significant differences between pregnant women with and without pelvic floor muscle dysfunction (PFMD). Nevertheless, this same study [22] showed with a prospective approach that concentrations in the PFMD group decreased more quickly from 24 to 28 weeks, which had some agreement with our hypothesis that lower levels or the drop in relaxin-2 levels could be associated with symptoms of PFMD. Another study showed that women with UI had lower relaxin concentrations compared to continent groups at different time points before 20 weeks of gestation, but not in the following weeks [25].

Higher glucose levels and presence of urinary incontinence presented by the GDM-PSUI group showed that this clinical combination leads to lower relaxin-2 concentrations. As comparisons with the literature are not possible because of the present innovative analyses, we hypothesized according to results from experimental rat studies showing that the combination of pregnancy and hyperglycemia promotes the fibrosis process in urethral muscle [26], which could be explained by the lower levels of relaxin-2, which is an antifibrotic hormone.

The literature is very restricted concerning the link between PFM function and hormonal status in general; during pregnancy it is apparently absent. We chose MOS because it has high interrater reliability (r = 0.947; $p < 0.001$). Laycock et al. [27] our analysis were based on the scale stratified into 0–2 and 3–5, with 0–2 indicating women who cannot perform a contraction resulting a movement against the pubic bone associated with cranial movement and those (3–5) who are able to do so. The relaxin-2 levels were even lower if we stratified the sample according to GDM pregnant women who scored MOS = 0–2 compared with GDM women with MOS = 3–5 and with the non-GDM group. When the GDM group was stratified by continence status, the GDM-PSUI had significantly lower relaxin-2 levels compared to the other three groups.

Fibrosis is considered an important factor in lower urinary tract dysfunctions [28] if we consider that there is some plausibility to support that GDM-PSUI patients may have intense connective tissue and morphological muscle alterations [3, 29]. This could lead us to consider further investigations on the influence of lower relaxin levels, especially in this group. This seems promising, as a recent study in an animal model has shown relaxin-2's potential for fibrosis reversal and increased detrusor force generation in the bladder [28].

The strengths of this study were approaching converging subjects (GDM, PSUI, and PFM function) together, using 3 points on the OGTT-75g to classify groups, using a high-quality relaxin kit for dosing, selecting pregnant women and assessing them during gestational weeks in which relaxin is expected to be stable [30], and having participants with similar characteristics concerning obstetric history among the groups. Regarding limitations, since this study is an observational study, causality cannot be determined. It would be interesting for future studies to use earlier dosages at different time points, and we suggest other authors consider including an objective tool for PFM function assessment.

Conclusion

Our findings showed that lower relaxin-2 concentration is associated with the presence of pregnancy-specific urinary incontinence in GDM and non-GDM groups but it is especially notable in the GDM-PSUI group. In addition, pregnant women with GDM who had lower PFM strength (MOS = 0–2), regardless of continence status, had lower relaxin-2 levels compared to GDM pregnant women with better PFM strength (MOS = 3–5). Considering also the continence status, the GDM-PSUI group with lower PFM strength (MOS = 0–2) presented even lower levels of relaxin-2 than the non-GDM-C, non-GDM-PSUI, and GDM-C groups.

Research implications

Contrasting physiological actions on the extracellular matrix affected the subjects of this article; while diabetes leads to a fibrosis process, relaxin leads to an anti-fibrotic process. Further studies are needed to investigate the influence over time and determine the strength of the connection between lower levels of relaxin on PFM impairment and fibrosis in the GDM population, especially in GDM-PSUI patients. As relaxin is a favorable hormone used to improve fibrotic conditions, it may be a useful new therapeutic tool for preventive and therapeutic strategies, requiring further investigation in the future.

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Diamater Study Group

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Declarations

Conflicts of interest None.

References

1. Piculo F, Marini G, Vesentini G, et al. Pregnancy-specific urinary incontinence in women with gestational hyperglycaemia worsens the occurrence and severity of urinary incontinence and quality of life over the first year post partum. Eur J Obstet Gynecol Reprod Biol. 2020;252:336–43. https://doi.org/10.1016/j.ejogrb.2020.06.036.
2. Woodley SJ, Lawrenson P, Boyle R, et al. Pelvic floor muscle training for preventing and treating urinary and faecal incontinence in antenatal and postnatal women. Cochrane Database Syst Rev. 2020;2020.
3. Vesentini G, Barbosa AMP, Damasceno DC, et al. Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women. PLoS One. 2020;15:e0231096. https://doi.org/10.1371/journal.pone.0231096.
4. Sartorão Filho CI, Pinheiro FA, Prudencio CB, et al. Impact of gestational diabetes on pelvic floor: A prospective cohort study with three-dimensional ultrasound during two-time points in pregnancy. Neurourol Urodyn nau. 24491. 2020. https://doi.org/10.1002/nau.24491.
5. Prudencio CB, Rudge MVC, Pinheiro FA, et al. Negative impact of gestational diabetes mellitus on progress of pelvic floor muscle electromyography activity: Cohort study. PLoS One. 2019;14:e0223261. https://doi.org/10.1371/journal.pone.0223261.
6. Steinetz B, Whitaker P, Edwards JR. Maternal relaxin concentrations in diabetic pregnancy. Lancet. 1992;340:752–5. https://doi.org/10.1016/0140-6736(92)92295-Q.
7. Feijóo-Bandín S, Aragón-Herrera A, Rodríguez-Penas D, et al. Relaxin-2 in Cardiometabolic Diseases: Mechanisms of Action and Future Perspectives. Front Physiol. 2017;8. https://doi.org/10.3389/fphys.2017.00599.
8. Goldsmith LT, Weiss G. Relaxin in Human Pregnancy. Ann N Y Acad Sci. 2009;1160:130–5. https://doi.org/10.1111/j.1749-6632.2008.03800.x.
9. Ferlin A, De Toni L, Sandri M, Foresta C. Relaxin and insulin-like peptide 3 in the musculoskeletal system: from bench to bedside. Br J Pharmacol. 2017;174:1015–24. https://doi.org/10.1111/bph.13490.
10. Ng HH, Shen M, Samuel CS, et al. Relaxin and extracellular matrix remodeling: Mechanisms and signaling pathways. Mol Cell Endocrinol. 2019;487:59–65. https://doi.org/10.1016/j.mce.2019.01.015.
11. Petros PEP, Ulmsten UI. An integral theory of female urinary incontinence. Acta Obstet Gynecol Scand. 1990;69:7–31. https://doi.org/10.1111/j.1600-0412.1990.tb08027.x.
12. Sangsawang B. Risk factors for the development of stress urinary incontinence during pregnancy in primigravidae: A review of the literature. Eur J Obstet Gynecol Reprod Biol. 2014;178:27–34. https://doi.org/10.1016/j.ejogrb.2014.04.010.
13. American Diabetes Association. Standards of Medical Care in Diabetes—2015. Diabetes Care. 2015;38:S1–2. https://doi.org/10.2337/dc15-S001.
14. Tamanini JTN, Dambros M, D'Ancona CAL, et al. Validação para o português do "International Consultation on Incontinence Questionnaire - Short Form" (ICIQ-SF). Rev Saude Publica. 2004;38:438–44. https://doi.org/10.1590/S0034-89102004000300015.
15. Klovning A, Avery K, Sandvik H, Hunskaar S. Comparison of two questionnaires for assessing the severity of urinary incontinence: The ICIQ-UI SF versus the incontinence severity index. Neurourol Urodyn. 2009;28:411–5. https://doi.org/10.1002/nau.20674.
16. Laycock J, Jerwood D. Pelvic floor muscle assessment: the PERFECT scheme. Physiotherapy. 2001;87:631–42.
17. Zhang Z, Yang L, Han W, et al. Machine learning prediction models for gestational Diabetes mellitus: Meta-analysis. J Med Internet Res. 2022;24:e26634. https://doi.org/10.2196/26634.
18. Pang H, Lv J, Xu T, et al. Incidence and risk factors of female urinary incontinence: a 4-year longitudinal study among 24 985 adult women in China. BJOG An Int. J Obstet Gynaecol. 2022;129:580–9. https://doi.org/10.1111/1471-0528.16936.
19. Ashton-Miller JA, Delancey JOL. Functional anatomy of the female pelvic floor. Ann N Y Acad Sci. 2007;1101:266–96. https://doi.org/10.1196/annals.1389.034.
20. Kristiansson P, Samuelsson E, Von Schoultz B, Svärdsudd K. Reproductive hormones and stress urinary incontinence in pregnancy. Acta Obstet Gynecol Scand. 2001;80:1125–30. https://doi.org/10.1080/j.1600-0412.2001.801209.x.
21. Ellen C, Travis A, Laurie W, et al. Optimal paradigms for measuring peak serum relaxin in eumenorrheic, active females. Reprod Med Int 1. 2018. https://doi.org/10.23937/rmi-2017/1710006.
22. Harvey M-AE, Johnston SL, Davies GAL. Mid-trimester serum relaxin concentrations and post-partum pelvic floor dysfunction. https://doi.org/10.1080/00016340802460321
23. Zaman I, Swaminathan R, Brackenridge A, et al. Assessment of relaxin levels in pregnant women with gestational diabetes mellitus. Endocr Abstr. 2014. https://doi.org/10.1530/endoabs.35.P377.
24. Alonso Lopez Y, Dereke J, Landin-Olsson M, et al. Plasma levels of relaxin-2 are higher and correlated to C-peptide levels in early gestational diabetes mellitus. Endocrine. 2017;57:545–7. https://doi.org/10.1007/s12020-017-1354-x.
25. Kristiansson P, Samuelsson E, Von Schoultz B, Svärdsudd K. Reproductive hormones and stress urinary incontinence in pregnancy. Acta Obstet Gynecol Scand. 2001;80:1125–30. https://doi.org/10.1034/j.1600-0412.2001.801209.x.
26. Piculo F, Marini G, Barbosa AMP, et al. Urethral striated muscle and extracellular matrix morphological characteristics among mildly diabetic pregnant rats: translational approach. Int Urogynecol J. 2014;25:403–15. https://doi.org/10.1007/s00192-013-2218-4.
27. Laycock J, Jerwood D. Pelvic floor muscle assessment: The PERFECT Scheme. Physiotherapy. 2001;87:631–42. https://doi.org/10.1016/S0031-9406(05)61108-X.
28. Andersson K-E, Fry C, Panicker J, Rademakers K. Which molecular targets do we need to focus on to improve lower urinary tract dysfunction? ICI-RS 2017. Neurourol Urodyn. 2018;37:S117–26. https://doi.org/10.1002/nau.23516.
29. Vesentini G, El Dib R, Righesso LAR, et al. Pelvic floor and abdominal muscle cocontraction in women with and without pelvic floor dysfunction: a systematic review and meta-analysis. Clinics. 2019;74.
30. Petersen LK, Vogel I, Agger AO, et al. Variations in serum relaxin (hRLX-2) concentrations during human pregnancy. Acta Obstet Gynecol Scand. 1995;74:251–6. https://doi.org/10.3109/00016349509024444.