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Ex vivo myo-mechanical assessment of isolated rectus abdominis muscle in pregnancy-specific urinary incontinence: a cross-sectional study nested within the Diamater cohort

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Abstract

Background: Rectus abdominis muscle (RAM) myopathy is the underlying mechanism of pregnancy-specific urinary incontinence (PSUI), which has been shown to be a predictor of long-term urinary incontinence (UI). However, there is limited knowledge about selective ex vivo myo-mechanical assessment (MMA) of the RAM in healthy pregnant women, particularly those with PSUI.

Results: Our analysis revealed no specific quantitative parameters from the window analysis or contractile response of the RAM in ex vivo MMA. A gradual and consistent decline was predominantly observed in the peak and strength parameters for both groups. The qualitative contractile response analysis of the RAM using peak and strength parameters revealed three distinct behaviors under electrical stimulation: a progressive decrease in strength, sudden muscular arrest, and asynchrony with erratic fluctuations. In-depth quantitative analysis of the RAM ex vivo did not yield substantial differences between the two groups. However, qualitative analysis proved valuable in distinguishing the groups, revealing a tendency toward the loss of the progressive peak declines observed in the control group.

Conclusion: This study highlights the complexity of RAM myopathy in the context of PSUI and its potential impact on UI outcomes.

Method: In this cross-sectional study nested within a cohort, we examined ex vivo myography assessments in 87 mothers, comprising 48 with PSUI and 39 without PSUI, all of whom exhibited normal glucose tolerance. General data were extracted from our database, whereas RAM samples were collected during C-section for qualitative and quantitative ex vivo myography analysis, with a focus on initial and final baseline measurements, peak responses, strength, and duration times.

Keywords: Pregnancy-specific urinary incontinence, Ex vivo myo-mechanical assay, Rectus abdominis muscle, Long-term urinary incontinence, Qualitative analysis

Background

Pregnancy is a significant risk factor for long-term urinary incontinence (UI), regardless of delivery mode, whether Cesarean (C-section) or vaginal [1–5]. The onset of this condition is associated with disruptions in the skeletal muscle structure essential for maintaining urinary continence [6]. Pregnancy-specific UI (PSUI) refers to UI that a pregnant woman experiences for the first time during pregnancy [3], and studies have indicated an increased probability of developing long-term UI in this population [3, 4].

PSUI, a condition frequently underestimated in prenatal care, is notably absent from traditional obstetric textbooks [7, 8]. Conceived by Hvidman et al. in 2002 [3], this term is tied to hormonal factors associated with pregnancy. In previous research, we established a strong link between gestational diabetes mellitus (GDM) and pelvic floor muscle (PFM) dysfunction and UI up to 2 years postpartum [2], with rectus abdominis muscle (RAM) or PFM hyperglycemic-related myopathy potentially acting as a mediator [9]. However, it remains unknown whether RAM myopathy manifests similarly in mothers with normal glucose tolerance who experience PSUI [9]. Consequently, ex vivo myo-mechanical assessment (MMA) for these muscle disorders remains relatively unexplored in healthy pregnant women with PSUI.

PSUI and its correlation with the long-term UI

The incidence rates of PSUI vary from 26% [10] to 41.8% [11]. Addressing the question of how to mitigate long-term UI has encouraged interest in understanding the impact of potential risk factors for this condition. The processes underlying PSUI play a pivotal role in predicting future episodes of UI during a woman's adult life (long-term UI) [3]. Notably, PSUI dramatically increases the prevalence of UI in subsequent pregnancies, reaching up to 67% [12–16], highlighting the necessity of investigating this often neglected signal in prenatal care. Prior clinical findings [2] revealed that the PSUI and UI rates two years postpartum were 31.6% and 18.4%, respectively, in primiparous women who had undergone C-sections. Stress UI emerged as the most prevalent pattern during the first year postpartum, in line with previous investigations [17, 18]. Factors such as obesity [19], hormonal status [20], the risk of PSUI [17], and postpartum PFM dysfunction are associated with a history of PSUI [21].

Ex vivo myo-mechanical assessments

The ex vivo MMA serves as a crucial tool for evaluating the myo-mechanical properties of skeletal muscles linked to PSUI, providing insights into both endogenous and exogenous factors. This quantitative method measures force generation and fatigue in freshly isolated muscles. Ex vivo MMA, which is primarily conducted via a strip myograph and electrodes, allows the assessment of initial and final baseline measurements, peak responses, strength, duration times, and fatigue (loss of viability) [22].

This technique has demonstrated potential in diagnosing vascular damage [23–26] and has shown significant potential for assessing the electrical activity response of skeletal muscle [27].

Feasibility of ex vivo MMA as a diagnostic tool

The RAM, a striated muscle extending along the abdomen to the pubic symphysis, undergoes physiological adaptations during pregnancy [28–32]. The synergistic contractile activity of RAM with pelvic floor muscles is integral to labor and urinary continence [29, 33, 34]. Certain complications during pregnancy, such as GDM, can induce severe morphological changes in the RAM [35–37], leading to atrophy and, consequently, long-term UI [2, 9, 30, 35, 38]. Hence, evaluating the functionality of RAM is crucial. Furthermore, it is unclear whether isolated RAM in PSUI displays different myo-mechanical patterns than that in continent pregnant women with normal glucose tolerance does, potentially shedding light on the myo-mechanical properties in the process. As a result, our hypothesis suggests that an investigation into the contractile muscle response will shed light on the pathophysiology of PSUI and offer insights into long-term UI pathways. This study aims to comprehensively understand the association between PSUI and continent pregnancy in women with normal glucose tolerance. The study addresses three pivotal questions: 1. How do the ex vivo MMA functional profiles of isolated RAMs during pregnancy correlate with those of healthy pregnant women without GDM who are experiencing PSUI? 2. Does the functional profile of the RAM vary between the start and end of each electrical stimulus during ex vivo MMA in pregnant women with and without PSUI? 3. Does the functional profile of ex vivo MMA trials in pregnant women with and without PSUI exhibit a decline in force? Until the final experiment? Through this study, we aimed to explore the intricate connection between the PSUI and isolated RAM ex vivo MMA functional profile and investigate their potential in advancing our comprehension of this condition.

Results

Study participants and groups

Among 315 mothers with normal glucose tolerance recruited for the Diamater Study Group, 145 met the eligibility criteria. Moreover, 87 pregnant women with normal glucose tolerance and RAM biopsy samples obtained during C-section were included for ex vivo myo-mechanical analysis: 39 in the control group (without PSUI) and 48 in the PSUI group (with PSUI) (Fig. 1). The demographic characteristics according to PSUI status are presented in Table 1.

Impact of demographic characteristics on muscle samples

The effects of maternal and perinatal demographic characteristics on muscle samples following ex vivo MMA were analyzed through the factors detailed in Tables 1 and 2. Both groups presented similar demographic profiles. Pregnant women with PSUI had lower weight gain during pregnancy than did the control group ($p > 0.015$).

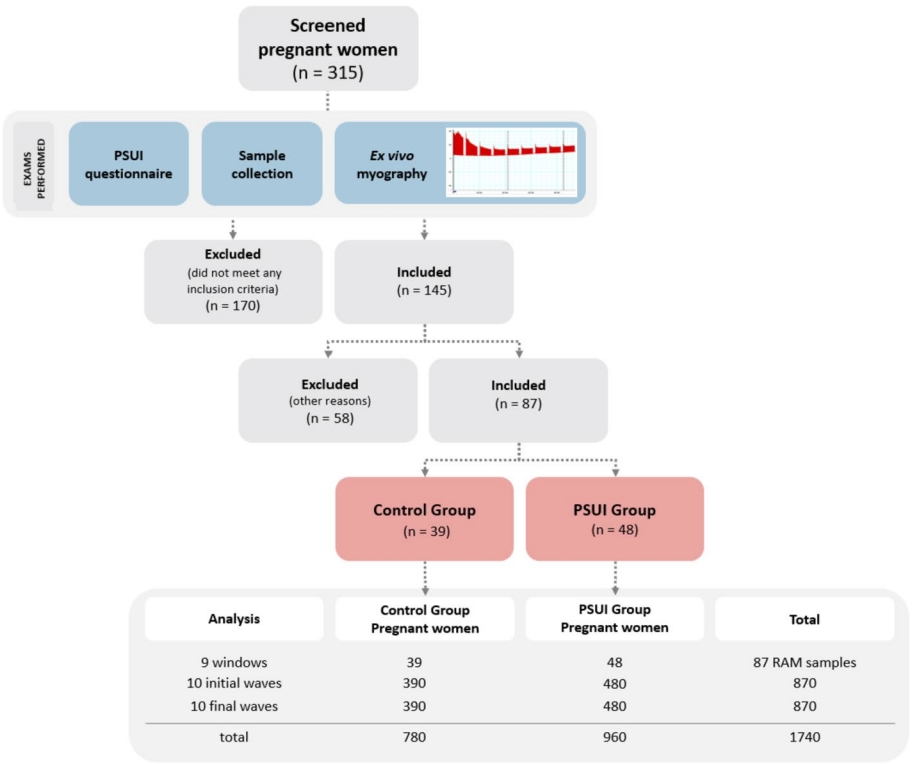

Fig. 1 Flowchart illustrating participant distribution in each group: control and PSUI

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

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

Data are expressed as the means ± standard deviations or number of subjects and percentages. $p < 0.05$ denotes a significant difference between two groups according to the t test for continuous variables and the Chi-square test** for weight gain. *BMI* body mass index, *kg* kilograms, *g* grams, *PSUI* pregnancy-specific urinary incontinence group

Table 2 RAM sample weight, width, and length in the PSUI and control groups

Variables	Control ($n=39$)	PSUI ($n=48$)	*p* value
Sample weight (g)	0.3 ± 0.2	0.3 ± 0.2	0.505
Sample width (cm)	0.4 ± 0.2	0.5 ± 0.2	0.099
Sample length (cm)	1.2 ± 0.3	1.3 ± 0.3	0.132

n sample, *g* grams, *cm* centimeters; independent t test and Chi-square test**. *PSUI* pregnancy-specific urinary incontinence group; $p < 0.05$, significant difference between the two groups

RAM characteristics after ex vivo myography

The weight, width, and length of the RAM samples at the end of the ex vivo MMA protocol were not significantly different between the PSUI and control groups (Table 2).

RAM ex vivo MMA quantitative analysis

A total of 1740 waves were analyzed for quantitative parameters, including initial baseline, final baseline, peak, strength, and duration time. The parameters were investigated to identify differences between control and PSUI groups within each time window (Figure S1C).

Patterns of behavior in the initial and final windows

Both groups displayed a progressive decline in peak and strength from the 1st to the 9th window. The control group exhibited a gradual decline in duration time, whereas PSUI group experienced a slow decline followed by stabilization.

Comparison of initial waves between groups

An analysis of initial wave measurements revealed that the average initial baseline significantly decreased from 1st window (8.19 ± 4.22) to other windows for the control group; from the 2nd window to the 6th window (mean $\pm$ sd 6.89 ± 4.64 to 6.49 ± 5.86, respectively), there was no difference in the average values, and from the 7th to 9th window (5.05 ± 8.09 to 4.84 ± 9.07, respectively), there was also no difference in the average values (Table 3). For the PSUI group, there was no difference between the means for these windows. Between groups, there was a difference in means for the 2nd and 3rd windows associated with opposite results. For the other windows, there was no difference in the means between the groups.

Final baseline

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

Peak

The peak tension values gradually and continuously decreased from the 1st to 8th windows in control group (14.06 ± 22.96 and 1.75 ± 3.79, respectively). In PSUI group, this progressive reduction in peak tension of twitch tension curve occurred from 1st to 6th window and remained constant until ninth window (11.97 ± 16.76 and 1.83 ± 3.36, respectively).

Strength

During ex vivo MMA**,** the initial strength progressively decreased from 1st to 3rd window and remained constant until 9th window in control group. (6.58 ± 8.89 and

Table 3 Quantitative comparison of wave characteristics in the PSUI and control groups

Window	Initial									Final								Initial x final	Initial x final
	Variable	Control	SD			PSUI	SD			*p* value*	Variable	Control	SD			PSUI	SD			*p* value*	Control	PSUI
		Mean ($n=390$)				Mean ($n=480$)						Mean ($n=390$)				Mean ($n=480$)					*p* value**	*p* value**
1	Initial baseline	8.19	4.22	a	A	10.01	5.98	a	A	0.2029	Initial baseline	7.78	4.49	a	A	10.04	7.80	b	A	0.9126	0.4145	0.942
2	Initial baseline	6.89	4.64	a	B	10.02	8.02	b	A		Initial baseline	6.83	5.44	a	A	10.80	10.66	b	A		0.9204	0.2696
3	Initial baseline	6.49	5.86	a	B	10.43	10.98	b	A		Initial baseline	6.25	7.12	a	A	10.87	12.58	b	A		0.7229	0.6307
4	Initial baseline	5.46	7.17	a	B	8.79	11.78	a	A		Initial baseline	5.75	8.16	a	A	9.14	12.64	a	A		0.6677	0.6985
5	Initial baseline	5.68	8.21	a	B	7.63	10.12	a	A		Initial baseline	5.53	7.47	a	A	8.22	11.93	a	A		0.8287	0.501
6	Initial baseline	6.24	9.51	a	B	7.54	12.23	a	A		Initial baseline	5.70	8.44	a	A	8.07	13.91	a	A		0.4814	0.5628
7	Initial baseline	5.05	8.09	a	C	7.79	13.79	a	A		Initial baseline	5.42	9.05	a	A	8.45	15.32	a	A		0.60	0.502
8	Initial baseline	5.23	9.09	a	C	8.74	15.36	a	A		Initial baseline	5.18	9.13	a	A	8.71	16.37	a	A		0.946	0.9731
9	Initial baseline	4.84	9.07	a	C	8.31	15.88	a	A		Initial baseline	4.72	9.31	a	A	8.12	16.42	a	A		0.8566	0.8541
1	Final baseline	8.43	4.31	a	A	10.21	6.06	a	A	0.1865	Final baseline	7.95	4.47	a	A	10.19	7.83	b	A	0.548	0.3464	0.957
2	Final baseline	7.06	4.68	a	B	10.22	8.05	b	A		Final baseline	6.88	5.45	a	A	10.90	10.68	b	A		0.7612	0.3453
3	Final baseline	6.60	5.95	a	B	10.55	11.04	b	A		Final baseline	6.33	7.17	a	A	10.96	12.62	b	A		0.6798	0.6549
4	Final baseline	5.51	7.13	a	B	8.93	11.88	a	A		Final baseline	5.78	8.17	a	A	9.25	12.73	a	A		0.6909	0.7342

Table 3 (continued)

Window	Initial									Final								Initial x final	Initial x final
	Variable	Control Mean ($n=390$)	SD			PSUI Mean ($n=480$)	SD			p value*	Variable	Control Mean ($n=390$)	SD			PSUI Mean ($n=480$)	SD			p value*	Control p value**	PSUI p value**
5	Final baseline	5.71	8.23	a	B	7.68	10.19	a	A		Final baseline	5.55	7.49	a	A	8.28	11.97	a	A		0.8183	0.4992
6	Final baseline	6.27	9.51	a	B	7.58	12.27	a	A		Final baseline	5.75	8.54	a	A	8.07	13.88	a	A		0.5012	0.5988
7	Final baseline	5.06	8.12	a	B	7.82	13.81	a	A		Final baseline	5.44	9.08	a	A	8.46	15.33	a	A		0.5922	0.5188
8	Final baseline	5.23	9.08	a	B	8.77	15.38	a	A		Final baseline	5.15	9.10	a	A	8.73	16.38	a	A		0.9195	0.9754
9	Final baseline	4.87	9.12	a	B	8.33	15.88	a	A		Final baseline	4.73	9.33	a	A	8.14	16.45	a	A		0.8382	0.8569
1	Peak	14.06	22.96	a	A	11.97	16.76	a	A	0.0903	Peak	6.00	7.94	a	A	6.04	7.19	a	A	0.1156	< 0.0001	< 0.0001
2	Peak	6.80	10.11	a	B	7.24	9.22	a	B		Peak	3.88	5.23	a	B	3.64	4.24	a	B		< 0.0001	< 0.0001
3	Peak	4.80	7.62	a	C	4.55	5.65	a	C		Peak	2.68	4.21	a	C	2.59	3.23	a	C		< 0.0001	< 0.0001
4	Peak	3.22	5.81	a	D	3.20	4.53	a	D		Peak	2.29	3.87	a	D	2.22	3.02	a	D		0.0037	< 0.0001
5	Peak	2.89	5.38	a	E	2.52	3.81	a	E		Peak	1.95	3.42	a	E	1.59	2.56	a	E		0.001	< 0.0001
6	Peak	2.47	4.56	a	F	1.83	3.36	a	G		Peak	1.61	2.95	a	F	1.23	2.37	a	F		0.0003	< 0.0001
7	Peak	2.03	4.20	a	G	1.63	3.21	a	G		Peak	1.36	2.70	a	G	1.15	2.40	a	F		0.0011	0.0006
8	Peak	1.75	3.79	a	H	1.49	3.23	a	G		Peak	1.19	2.41	a	H	1.04	2.43	a	F		0.0019	0.0004
9	Peak	1.41	3.30	a	H	1.40	3.29	a	G		Peak	1.02	2.27	a	H	0.99	2.17	a	F		0.008	0.0009
1	Strength	6.58	8.89	a	A	5.02	6.24	a	A	0.2416	Strength	3.64	4.12	a	A	3.59	5.18	a	A	0.8155	< 0.0001	< 0.0001
2	Strength	3.73	4.74	a	B	4.05	5.72	a	A		Strength	2.62	3.49	a	B	2.99	5.69	a	A		0.0002	0.0002
3	Strength	2.81	3.99	a	C	3.44	6.02	a	A		Strength	2.20	4.01	a	B	3.04	6.78	a	A		0.0179	0.1685
4	Strength	2.29	4.45	a	C	2.48	5.42	a	A		Strength	1.98	4.41	a	B	2.31	6.79	a	A		0.2102	0.4716
5	Strength	2.09	4.46	a	C	2.51	7.23	a	A		Strength	1.77	4.16	a	B	2.47	8.81	a	A		0.1671	0.9017
6	Strength	1.82	4.35	a	C	2.42	9.01	a	A		Strength	1.62	4.38	a	B	2.37	10.38	a	A		0.3376	0.8617

Table 3 (continued)

Window	Initial									Final								Initial x final	Initial x final
	Variable	Control Mean ($n=390$)	SD			PSUI Mean ($n=480$)	SD			Variable	Control Mean ($n=390$)	SD			PSUI Mean ($n=480$)	SD			Control p value**	PSUI p value**
7	Strength	1.70	4.56	a	C	2.70	10.83	a	A	Strength	1.63	4.97	a	B	2.69	11.90	a	A	0.7444	0.9872
8	Strength	1.76	5.14	a	C	2.87	11.99	a	A	Strength	1.64	5.24	a	B	2.75	12.59	a	A	0.606	0.7272
9	Strength	1.29	4.59	a	C	3.02	12.86	a	A	Strength	1.17	4.81	a	C	2.94	13.24	a	A	0.4584	0.8287
1	Duration time	0.40	0.44	a	A	0.40	0.12	a	A	Duration time	0.34	0.18	a	A	0.35	0.14	a	A	0.0011	< 0.0001
2	Duration time	0.32	0.18	a	B	0.38	0.16	a	A	Duration time	0.28	0.18	a	B	0.32	0.16	a	B	0.0067	< 0.0001
3	Duration time	0.28	0.20	a	C	0.34	0.18	a	B	Duration time	0.22	0.21	a	C	0.27	0.18	a	C	0.0015	< 0.0001
4	Duration time	0.20	0.21	a	D	0.27	0.28	a	B	Duration time	0.18	0.21	a	D	0.22	0.22	a	D	0.1627	0.0071
5	Duration time	0.18	0.21	a	D	0.22	0.22	a	B	Duration time	0.16	0.19	a	E	0.18	0.19	a	C	0.0784	0.0018
6	Duration time	0.17	0.21	a	D	0.17	0.21	a	C	Duration time	0.14	0.19	a	E	0.15	0.20	a	C	0.0151	0.1544
7	Duration time	0.15	0.35	a	D	0.16	0.20	a	C	Duration time	0.11	0.18	a	F	0.15	0.20	a	C	0.0055	0.3437
8	Duration time	0.12	0.19	a	E	0.16	0.20	a	C	Duration time	0.12	0.45	a	F	0.15	0.30	a	C	0.759	0.2601
9	Duration time	0.10	0.17	a	E	0.15	0.21	a	C	Duration time	0.09	0.16	a	F	0.13	0.19	a	C	0.3279	0.0613

Quantitative comparison of wave characteristics (initial baseline, final baseline, peak, strength, and duration time) according to initial and final 5 s of nine windows among two pregnant groups: those with and without the PSUI. Comparison of the initial and final waves of each window within each group: control and PSUI. Model adjustment for repeated measures evaluating group versus wave interactions

* p value refers to the group versus wave interaction test; **p value refers to the comparison between the initial and final measurements for each group

Means followed by the same lowercase letter (fixing waves and testing groups) do not differ at the 5% level according to the Wald test

Means followed by the same lowercase letter (fixing groups and testing waves) do not differ at the 5% level according to the Wald test

2.81 ± 3.99, respectively). In PSUI group, all initial strength values remained similar from 1st window until the 9th window (5.02 ± 6.24 and 3.02 ± 12.86, respectively).

Duration time

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

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

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

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

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

Initial baseline

According to Table S1, for initial measurements, initial baseline variable means decrease but do not differ until wave 5 (6.90 ± 8.24 to 6.96 ± 8.24) for the control group. From waves 6 to 9 (5.38 ± 6.93 to 4.96 ± 6.83, respectively), there was no difference between the means, and there was also no difference between waves 9 and 10 (4.96 ± 6.83 to 4.85 ± 6.77). For the PSUI group, the profile was similar. Furthermore, no significant difference was observed between the group means for each wave.

Final baseline

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

Peak

For peak, there is an oscillation of averages (increases and decreases for each wave), presenting significant differences in several averages for the control group. For the PSUI group, the profile is also variable, but differences appear in 1st wave (5.60 ± 9.84) for the other groups, with no significant difference from the 2nd to 5th wave (4.23 ± 7.56 to 4.19 ± 8.38, respectively) and from 6 to 9th wave (3.53 ± 7.19 to 3.61 ± 8.29, respectively). The 10th percentile (3.18 ± 6.76) differed from the others. Despite the differences between the profiles, there were no differences between the groups for each wave.

Strength

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

Duration time

The average duration of the waves did not differ from 1st to 5th (0.25 ± 0.23 to 0.23 ± 0.21) and from 6 to 10th (0.19 ± 0.21 to 0.20 ± 0.49) for the control group. In the PSUI group, there was a difference from 1st group (0.31 ± 0.30) to the other groups, but there was no difference between the averages from 2nd to 5th groups (0.27 ± 0.21 to. 0.28 ± 0.21, respectively) and from 6 to 10th (0.22 ± 0.21 to 0.21 ± 0.20, respectively). When the waves were fixed, there was no difference between the groups. The same was observed in the comparisons of the final waves of the nine windows between the control and PSUI groups.

Comparison between the initial and final windows characteristics inside each of two groups: the control and PSUI groups

At initial baseline, control and PSUI groups did not differ on average for each wave from 1st to 10th. Regarding the waves, it is noted that both from 1st to 5th and from 6 to 10th there are no differences for both groups. But there is a difference between 1st to 5th and 6th to 10th wave for both groups. The others follow in a similar way. Groups did not differ with respect to waves for final baseline, peak, strength and duration time. But fixing groups, the differences appear in the waves.

For final baseline, there is a difference of 1st to 5th and 6th to 10th for both groups.

For peak, there is an oscillation between the wave averages for both groups. In strength, only 1st wave differs from 2nd to 9th wave, which differs from 10th wave, for control group. In PSUI group there is no difference.

For duration time there is a difference from 1st to 5th and from 6 to 10th wave for control group. For PSUI, 1st differs from 2nd to 5th, which differs from 6 to 10th. The same occurs for final baseline. At the initial and final baseline there is no difference between groups and waves. For peak, strength and duration time there are significant differences between waves but not between groups.

A detailed Table S1 with all the results can be found in the supplementary material (SM).

Qualitative analysis of ex vivo RAM myography of the study groups

From comprehensive statistical tests, including the Wald test, t test, and Chi-square test, it was initially observed that when quantitative statistics for the entire population were considered, there were no significant differences. However, Fig. 2A, B suggests varying individual profiles/trends within the overall population. This observation prompted us to perform a qualitative analysis of the ex vivo RAM MMA, where we

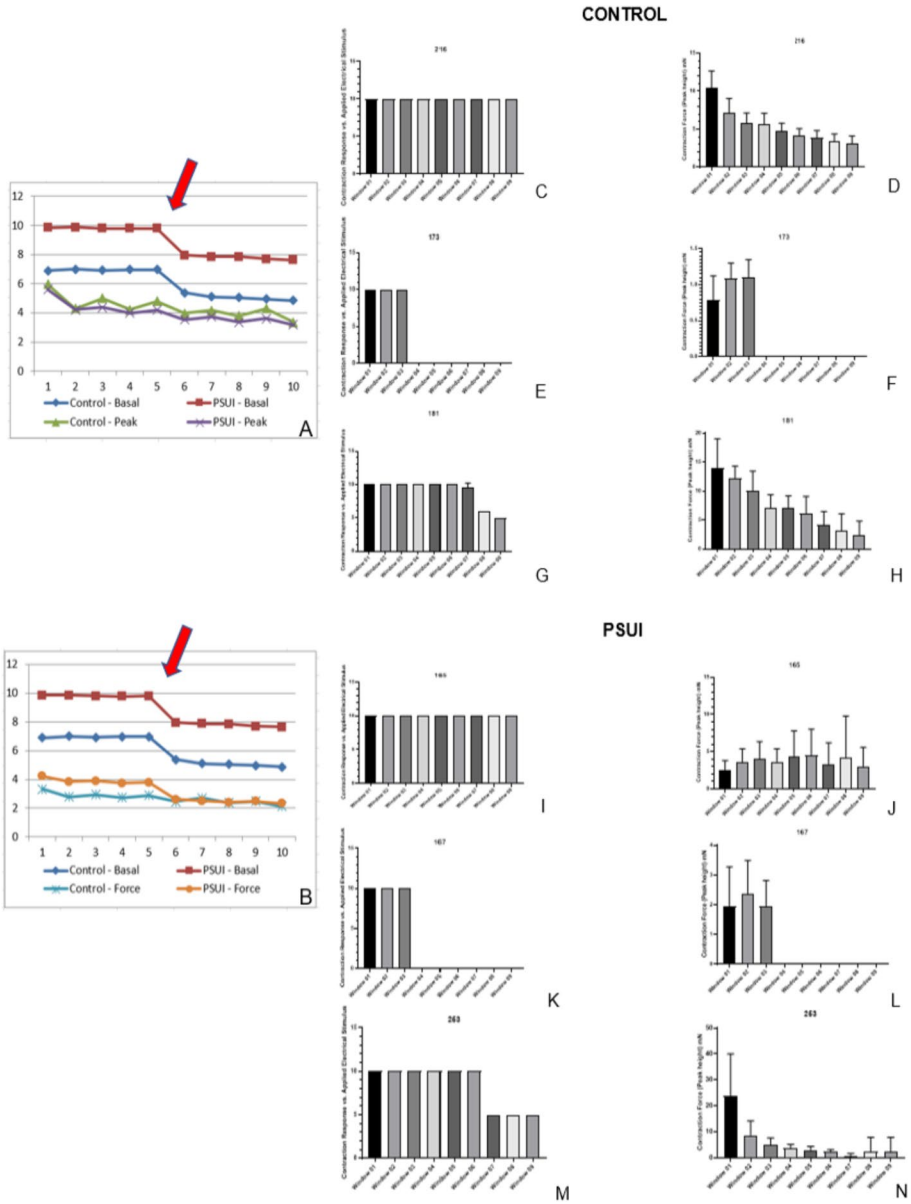

Fig. 2 **A** and **B** Qualitative statistical data of peak and force in the control and PSUI groups. The red arrows indicate regions where there is a change in behavior, although statistically significant differences were not found ($p < 0.05$); **C**, **E** and **G** represent contraction response vs. applied electrical stimulus in the control group; **D**, **F** and **H** represent contraction response vs. applied electrical stimulus in the PSUI group; **I**, **K** and **M** represent contraction force (peak height) in the control group; and **J**, **L** and **N** represent contraction force (peak height) in the PSUI group

identified and described three distinct trends within the control and PSUI groups: 1st pattern—progressive decrease in strength; 2nd pattern—sudden muscular arrest; and 3rd pattern—asynchronous registration with irregular fluctuations (Fig. 2).

Qualitative analyses were conducted for each patient via GraphPad Prism software, version 9 (Figure S2). Serial bar graphs were generated to analyze the average number of peaks within 10-s intervals across the nine analyzed windows (comprising 5-s

initial and final intervals). A similar approach was taken to analyze the contractile force (peak height).

Control group

1st pattern—Fig. 2C, D indicates that the participant demonstrated a 100% response to electrical stimuli (with 2 stimuli applied every 5 s, totaling 10 responses per period), though with a gradual decline in strength.

2nd pattern—Fig. 2E, F displays an initially strong response, observed in the first three windows, followed by a sudden cessation in the muscular response to electrical stimuli. Furthermore, there was a noticeable increase in the strength of contraction.

3rd pattern—Fig. 2G, H illustrates a pattern of continuous decline in response, accompanied by a progressive loss of strength. Additionally, the strength observed here is lower than what was observed in C and D (SM).

PSUI group

1st—Fig. 2I, J depicts the outcomes for the second study group (PSUI). Despite achieving a 100% response to electrical stimulation, there was no progressive strength loss observed in the control group.

2nd—Certain patients in the PSUI group exhibited responses only in the first three analyzed windows, mirroring the control group. However, a lack of coordination in strength was evident (Fig. 2K, L).

3rd—Fig. 2M, N show that there is an abrupt and gradual decrease in muscle response as well as a progressive but intense decrease in strength.

Discussion

This study marks a significant milestone in the field, as it explores the ex vivo MMA of RAM, a muscle without a tendon, in pregnant women, an area previously explored by The Diamater Study Group. To the best of our knowledge, this study represents the first published study in pregnant women with PSUI to combine both qualitative and quantitative analyses of ex vivo RAM myography. Our focus was on RAM myography, where it was found that it reacts to electrical activity with contraction force and fatigue [27] as well as other striated muscles, such as extensor digitorum longus muscle [39] and gastrocnemius muscle [40–42].

Studies show that pathological changes, as follow GDM, trigger significant changes in the RAM structure. GDM increases the abundance of slow fibers and decreases the number and area of fast fibers, as well as decreasing collagen distribution [30]. Furthermore, they reveal that GDM causes morphological, biochemical and physiological changes in the RAM [36], leading to atrophy and predisposing women to long-term UI [9, 38].

We introduced a novel approach involving the use of ex vivo MMA to assess the RAM in healthy pregnant women with PSUI. This methodology involved both qualitative and quantitative analyses, revealing several noteworthy findings. First, our quantitative analysis, which involved a comprehensive examination of the MMA profile of the RAM, revealed only subtle changes. These changes were insufficient to differentiate between pregnant women with and without PSUI, indicating that traditional

quantitative measures might not be sensitive enough to discern muscle alterations in this context. Second, our quantitative measurements of peak strength revealed a consistent and progressive reduction in response to electrical stimuli in both groups during ex vivo experiments. This decline in muscle response suggests a potential link between PSUI and alterations in muscle function, although further investigation is needed to elucidate the exact mechanisms involved. Finally, our qualitative analysis, which focused on wave analysis using peak and strength parameters, allowed us to demonstrate a distinctive trend within the PSUI RAM MMA. This trend was characterized by a less pronounced decline in peak responses than in the control group, suggesting a potential differentiating factor between these two pregnant cohorts.

By definition, PSUI is considered a transient condition in the prenatal period. However, data suggest that women with PSUI continue to be at increased risk for postpartum and long-term UI [2, 19]. PSUI in women with gestational hyperglycemia worsens the occurrence and severity of UI and the impact of UI on quality of life (QoL) over the first year postpartum [28]. Moreover, the underlying mechanisms responsible for this stressful situation in pregnant women with normal glucose tolerance are undervalued and underinvestigated in the current literature.

We found that women with PSUI had similar reduced wave peak (contractile response) values in ex vivo myography from the first to ninth window compared with women without PSUI. The other four parameters analyzed did not significantly differ. Previously, we demonstrated that this control group showed decreased collagen expression and area and a decreased slow and fast fiber area [38]. These findings suggest possible interference in muscle contraction that we are unable to demonstrate by quantitative ex vivo MMA. The quantitative and qualitative data revealed some discrepancies. These disparities may be a result of the inability of quantitative analysis to adjust properly for the presence of two separate categories of wave records, which our group has classified, Type 1 and Type 2, in the quantitative analysis [27]. The failure to consider these differences may have contributed to the notably high standard deviation values and could have had an impact on the comparisons of means. Other studies have shown altered muscle morphology in women with UI, including muscle atrophy caused by pudendal nerve denervation and muscle loss caused by impairment of insertion points for the puborectalis muscle [43]. These changes are related to damage to the pelvic nerves, pelvic muscles, and connective tissue attachments due to delivery [44, 45].

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

Regarding the characteristics of muscle, there were no differences between weight, width or length among muscle biopsies collected for ex vivo analysis. This means that the same amount of muscle fibers was used in both groups. Therefore, the muscle response accurately reflects muscle condition and not the number of muscle fibers

present in the biopsy.These findings indicate that RAM sample size did not have a notable effect on ex vivo MMA outcomes.

Qualitative analysis and peak assays by ex vivo myography (Fig. 2) differentiated the control and PSUI groups. After each electrical stimulus, the contractile performance of the control group progressively decreased, which seems to represent the physiological muscular response (Fig. 2C, D). However, some RAM samples from the control group presented a decrease in contractile performance and sudden cessation of the muscle response to electrical stimulation. This may be due to muscle death during analysis. The sudden stop probably followed muscle death. This premature death may be due to several factors, such as difficulties in obtaining the RAM sample at the C-section, transport conditions from maternity room to experimental laboratory and even the assembly of the muscle in the equipment or its own characteristics (Fig. 2E, F).

Some patients in the control group experienced a progressive decrease in the electrical stimulation response, which was followed by a gradual loss of strength (Fig. 2G, H). This could be due to the patient's physical condition, such as maternal hypotonia, which causes a greater loss of response to electrical stimulation than those who have stronger muscles (lifestyle).

In the PSUI group, there was a significant difference in contraction force. However, with a lesser force and a large force disparity, a response trend similar to that of the control group (Fig. 2I, J) was found. This finding was not able to confirm the decreasing strength trend of the control group. During the experiment, some patients in the PSUI group displayed abrupt cessation of muscular response due to tissue death (Fig. 2K, L). A progressive loss of responsiveness and reduced contraction force were also noted in certain cases (Fig. 2M, N). This outcome suggests disrupted coordination of the contraction force.

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

Therefore, the observations verified in myography in this study are clearly justified by the complex architecture of skeletal muscles, delineated into five distinct hierarchical levels (skeletal muscle, fascicles, muscle fibers, myofibrils and sarcomere) [46, 47] and composed predominantly of contractile tissue, which explains why most research on skeletal muscle focuses on its contractile properties [48]. Skeletal muscles stand out for their extremely high elasticity and also for their enormous tensile strength. Furthermore, they have a high tolerance to damage caused by their high tenacity, which allows them to resist millimeter-long cracks while maintaining almost their strength [49].

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

Furthermore, this research provides the foundation for understanding how the RAM functions biomechanically in pregnancies complicated by GDM, a condition previously linked to myopathy. Additionally, it will be critical to include new research groups and increase the sample size for each group to validate the quantitative and qualitative statistical analyses. Further research should also focus on the significance of the minor

peaks that follow the larger contraction peaks to acquire a better understanding of this response pattern and its potential biological implications.

The current study represents a pioneering effort in the field of RAM ex vivo MMA, and the comparisons made are still in the developmental stages, making them challenging to execute. However, we recognize several limitations in our study that require attention in future studies. These limitations include sample issues resulting from low quality or the impossibility of collecting viable RAM fragments, a lack of standardization in the literature already in existence, and incidents of muscle death, factors that need special attention and management.

Conclusion

In conclusion, this study highlights a notable lack of coordination in contraction force within the PSUI group, as observed through our innovative ex vivo MMA assessment. Conversely, the control group exhibited a trend toward a "normal and physiological" contractile response typically expected in skeletal muscle. Another noteworthy discovery is the feasibility of this analysis, which has not been previously documented in the literature for prenatal care management.

We anticipate that through further investigations, these findings will provide insights into the diverse pathways underlying the pathophysiology of PSUI by offering a more precise assessment of contractile characteristics in pregnant women via the ex vivo MMA of the RAM. Future studies are crucial to confirm whether the observed qualitative and quantitative wave patterns in ex vivo MMA persist and worsen after childbirth, potentially placing women with PSUI at a heightened risk of long-term UI.

Method

Study design and location

This study is part of the Diamater cohort and focuses on gestational urinary incontinence. The project was developed at Sao Paulo State University (UNESP), Botucatu Medical School (FMB), Botucatu, SP, Brazil. The Perinatal Diabetes Research Center (CIDP) oversees the project's development. Obstetric care, monitoring, evaluations, and sample collection were conducted at the Obstetrics Service of Clinical Hospital from Botucatu Medical School (HCFMB), Unimed Botucatu Maternity and the Clinical Research Unit (UPECLIN).

Study subjects and groups

Eligibility, inclusion, and discontinuation criteria: invitations for voluntary participation were extended to women from the 36th week of pregnancy.

Inclusion criteria and eligibility

Women aged 18 years or older were considered eligible; primiparous (nulliparous) or secundiparous (primiparous) with prior C-section; single pregnancy; usual risk; subjected to hyperglycemia screening and diagnosis protocol between 24 and 28 weeks, yielding a negative result [50, 51]; and possessing full intellectual capacity and not requiring any further stages of the study. Recommendations included women aged 37 weeks with prenatal indications for C-section at 37 weeks.

Discontinuation criteria

The subjects were allowed to withdraw their consent to participate at any point without compromising standard obstetric care. Discontinuous cases included women who withdrew consent, underwent spontaneous vaginal or premature delivery, or experienced loss of clinical and laboratory data, including samples unfit for processing and analysis.

Sample size calculation

We identified that for this study 17 samples per group are required, considering the hypothesis that there is a difference of one standard deviation between the groups studied, significance of 5% and statistical power of 80% [52, 53]. We use the Sample Size Calculators online application to design clinical research [54].

Study groups

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

Independent variable

The independent variable centered on the presence or absence of PSUI.

PSUI diagnosis

PSUI was defined as new urinary leakage onset during pregnancy [3] initiated during the current pregnancy [55]. Positive responses were identified as UI cases following the International Continence Society's criteria [55].

Control variables

The study considered various pregnancy and delivery characteristics potentially associated with the independent variable, PSUI. These included maternal factors such as age, parity, nutritional status (particularly overweight and obesity classes), and glycemic status. Factors related to the fetus/newborn included gestational age at delivery, birth weight, and the weight/gestational age ratio. Detailed standardized data on these factors are available in the Diamater cohort [9]. Maternal age: < 20 years (adolescent), 20–35 years (adult), and ≥ 35 years (advanced age). Women's parity: zero (primiparous) and one (secundiparous).

Pregestational BMI: overweight (BMI ≥ 25 kg/m^2) and obese (BMI ≥ 30 kg/m^2).

Weight gain: the difference between the initial and final pregnancy weights. Gestational glycemic mean—categorized as adequate (MG < 120 mg/dL) or inadequate (MG ≥ 120 mg/dL) [50, 51, 56–59].

Glycated Hb at delivery: categorized as adequate (Hb ≤ 6.5%) or inadequate (Hb > 6.5%). Gestational age at delivery—defined in gestational weeks: term 37–41 completed weeks) and postterm (from 42 incomplete weeks onward); birth weight

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

Data collection and techniques

Clinical and laboratory data of pregnancy and delivery

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

Data collection at the PSUI

PSUI is defined as any onset of new urinary leakage during pregnancy. Participants are interviewed and asked whether, during the current pregnancy, they had lost urine or control of even a small leakage of urine with activities such as coughing, lifting weights, or exercising or if they felt the urge or pressure to urinate and were unable to visit the bathroom quickly enough [3, 38, 55]. Pregnant women who reported PSUI were asked to complete the Brazilian version of the International Consultation on Incontinence Questionnaire-Urinary Incontinence-Short Form (ICIQ-SF) [60, 61].

Collection and preparation of rectus abdominis muscle samples

The collection of RAM samples was standardized in the Diamater cohort and was performed by medical staff from HC-FMB/UNESP and Unimed Botucatu Maternity at time of C-section with obstetric indications after fetal extraction (Figure S3). Immediately after resection, the samples were delivered to the researchers' team, which was previously trained, to be dissected and free of fat and connective tissue. After this procedure, the samples were prepared according to the technique to be performed [9].

Ex vivo myo-mechanical assessment (MMA) for RAM

Following immediate resection, a 1-cm^2 RAM sample was delicately excised and immersed in a Falcon tube with 5 mL of Krebs solution (118.5 μM NaCl, 30 μM KCl, 290 μM NaHCO3, 9 μM MgSO4, 9 μM KH2PO4, 20 μg CaCl2, 5.5 g D-glucose, and 300 μM L-arginine, maintaining a pH of 7.4), preoxygenated with carbogen gas (95% O2 and 5% CO2) and cooled to 4 °C. The samples were kept in Krebs solution until ex vivo MMA myography was initiated. The time window between sample collection and the commencement of ex vivo MMA myography was limited to a maximum of 30 min.

Myograph—instrument for ex vivo MMA

This study utilized the DMT-myograph system, specifically the model 820MS by DMT-Danish Myo Technology® (Ann Arbor, Michigan, USA), to assess the contractile responses (functional profile) of the RAM under electrical stimulation (Figure S4). The myograph chamber featured hooks designed to secure muscle samples along their edges. Within the chamber, a force transducer was positioned at one end, converting kinetic energy generated by the muscle's responses during electrical stimulation into an electrical signal. This signal was recorded and analyzed via the PowerLab data acquisition system (PowerLab Data Acquisition System, AD Instruments, São Paulo, Brazil) and analyzed via LabChart software (LabChart 8 for Windows, AD Instruments, São Paulo,

São Paulo, Brazil). Prior to use, the equipment was calibrated in accordance with the manufacturer's recommendations.

Muscle assembly method and myography performance

The settings adjusted on the stimulation equipment were based on previous literature [22, 62], which used other skeletal muscles with tendons and tissues [39, 63, 64]. This study was based on the standardization performed by the Diamater cohort group (data not disclosed) of the records of myographic activity, and they were carried out in five sequential steps [27] (Figure S5) and are described below.

1st step—sample recovery and chamber assembly: Isolated RAM samples were subjected to a 15-min recovery in Krebs solution at 37 °C infused with carbogenic gas. The RAM fragments were vertically positioned in the myograph chamber and oriented along the muscle fibers. The chamber was filled with 4 mL of Krebs solution with continuous gas flow to simulate the physiological environment.

2nd step—fiber stabilization: The Krebs solution was changed twice at 15-min intervals, with a constant tension force of 10 mN applied during each change. Electrical stimulation was initiated via platinum electrodes placed 0.8 cm from the muscle tissue and integrated into an electrical stimulator (Grass Model S48, Danish Myo Technology®, Michigan, USA). Predefined quadratic waveforms (e.g., gradient, ramp, sine, triangle) were configured via MyoD analog output software (Danish Myo Technology®, Michigan, USA).

3rd step—feasibility assessment: A feasibility assessment was conducted by applying three biphasic wave force stimuli with a voltage of 20 V and a pulse width of 25 ms, which lasted approximately 2 min.

4th step—programmed stimulation protocol: Continuous alternating current stimulation was applied via a fixed pulse train. The parameters included tension force: 10 mN, pulse voltage-controlled: 20 V, pulse width: 25 ms pulse interval: 60 ms, pulse frequency: 11.8 Hz, pause between pulse trains: 500 ms, pulse trains in group: 500 ms, repeat group: 10 times, pause between train groups: 60,000 ms, resulting in a total runtime of 45 min.

5th step—metric evaluation: After stimulation, the RAM sample parameters, including length, width, and weight, were measured. Measurements were taken via a digital caliper (STARFER®, Vargem Grande do Sul, SP, Brazil) for length and a digital analytical balance (model SHIMADZU® ATX224 Kern ABT 220-4NM, KYOTO, JAPAN) for weight. Muscle weight was recorded in grams.

Analysis of contractile response

The data collected were analyzed via LabChart software. The graphs were divided into 9 time windows of 5 min and further subdivided into 5-s periods (initial and final). The parameters analyzed included the initial and final baselines; peak force; strength; and duration time (Figure S1A).

Data analysis

The mean values and standard deviations (±SD) were calculated for each parameter and plotted via GraphPad Prism 9 software. Figures S1B and S1C depict the data analysis approach.

Statistical analysis

Data obtained from ex vivo MMA myography were evaluated according to a repeated measures model considering the interaction groups versus wave and groups versus windows via adjustment with a generalized linear model with a gamma distribution followed by the Wald multiple comparison test. The significance level was set at 5% or the corresponding p value was < 0.05. All analyses were performed via the program SAS for Windows, v.9.4.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12938-025-01366-9.

Supplementary material 1.

Supplementary material 2.

Supplementary material 3.

Supplementary material 4.

Supplementary material 5.

Supplementary material 6.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study adhered to the ethical guidelines and regulations outlined in the Declaration of Helsinki and was approved by the research ethics committee of Botucatu Medical School and the National Research Ethics Committee (CONEP) under the registration number CAAE: 82225617.0.0000.5411.

Competing interests
The authors declare no competing interests.

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