Test-retest reliability of clitoral blood flow measurements using color Doppler ultrasonography at rest and after a pelvic floor contraction task in healthy adult women

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Abstract

Aims: Test-retest reliability assessment of the dorsal clitoral artery's blood flow at rest and after muscle activation has never been documented. If this outcome measure is to be used in conditions impeding vascularity, it requires a psychometric evaluation. The aim of this study was to assess the inter-session test-retest reliability of clitoral blood flow in healthy women using color Doppler ultrasonography at rest and after a pelvic floor muscle (PFM) contraction task.

Methods: Two assessment sessions were conducted using a clinical ultrasound system. Clitoral blood flow measurements were repeated at rest and after a PFM contraction task. Measurements of the peak systolic velocity (PSV), time-averaged maximum velocity (TAMX), end-diastolic velocity (EDV), pulsatility index (PI), and resistance index (RI) were taken. The test-retest reliability was assessed using paired t-test, intraclass correlation coefficient (ICC), and Bland-Altman plots.

Results: For reliability at rest, ICC values were 0.95 for PSV, 0.87 for TAMX, and 0.67 for both PI and RI. The variability between measurements, as per Bland-Altman plots, was small for PSV, TAMX, and RI and acceptable for PI. For reliability after the PFM contractions task, ICC values were 0.85 for PSV, 0.77 for TAMX, 0.79 for PI, and 0.81 for RI. The variability between measurements was small for PSV and RI and acceptable for TAMX and PI. EDV parameter did not perform as well in both conditions.

Conclusions: Assessment of the clitoral blood flow with color Doppler ultrasound is reliable at rest and after a PFM contraction task.

1 | INTRODUCTION

Color Doppler ultrasound is a non-invasive, inexpensive method to assess the clitoral blood flow. It has been used in previous studies to provide baseline data in healthy women,[1,2] to assess different populations (eg, athletes, aging women[3,4]) or different conditions (eg, sexual arousal disorders, polycystic ovary syndrome, systemic sclerosis women[5–7]) and to explore the impact of different treatments (eg, gynecological surgery and medication for sexual dysfunctions[8,9]). The clitoral blood flow assessments were performed mainly with participants in a rest condition or after sexual stimulation.

Impairments in the blood flow of skeletal muscle arteries have been studied with color Doppler ultrasonography in two conditions, namely at rest and after a muscle contraction task.[10,11] This second condition increases blood flow in arteries, alters vascular tone in vessels of active muscles within seconds after the onset of muscle work allowing a rapid improvement of muscle perfusion.[12] Therefore, evaluating blood flow at rest and after a muscle contraction task provides important information about the artery's vascular function at rest and its hemodynamic response to muscle activation. Different interventions or conditions such as perineal radio- therapy, surgery, aging, genitourinary syndrome of menopause, lactational atrophic vaginitis and diabetes are known to be linked to lower or challenged perineal vascularity.[13–16]

Assessment of the vascular function of perineal arteries and their hemodynamic response in those women could provide further understanding of the global impact of such interventions or conditions on the perineal blood flow.

The dorsal clitoral artery is one of the terminal branches of the internal pudendal artery, the main artery providing blood flow to the pelvic floor muscles (PFM), the perineum, the labia, the vagina, the clitoris and the rectum.[17] Seeing that PFM contractions should increase blood flow in the arteries related to the muscles activated, it is anticipated that a repeated PFM contraction task could change the clitoral artery blood flow. Assessment of the vascularization of the dorsal clitoral artery and its hemodynamic response to muscle activation is important to determine the level of clitoral artery blood flow variability and eventually differentiate normal from pathological changes in blood flow.

Color Doppler ultrasound of the clitoral blood flow has been shown reliable at rest in an inter-evaluator intra-session assessment, although with a limited number of vascular parameters.[18] Furthermore, its reliability has never been assessed in an inter-session assessment nor has it been studied after a PFM contraction task. Inter-session reliability as well as the hemodynamic response after a PFM contraction task are important to ensure the stability of the measurements over time.

Hence, the purpose of this prospective study was to assess the inter-session test-retest reliability of clitoral blood flow measurements in healthy women using color Doppler ultrasonography in two conditions: 1) at rest; and 2) after a PFM contraction task.

2 | MATERIALS AND METHODS

A convenience sample of healthy adult women was recruited for this prospective test-retest cohort study using email advertisements among a group of women interested in pelvic floor rehabilitation. The inclusion criteria were: age of 18 years and older and were not actively participating in a PFM training program during the study. Women were excluded if they had vulvovaginal atrophy or dermatological disease of the vulva, had previously received radiotherapy for gynecological cancer, were taking anti-estrogenic medication or, were pregnant. In addition, any reported hormonal therapy or arterial hypertension medication dosage had to be stable for 6 months to ensure vulvovaginal blood flow stability.

The institutional Ethics Committee approved the study (CER IUGM 12-13-002) and each volunteer gave her written consent prior to her participation.

One evaluator, a PhD fellow in physiotherapy, performed two repeated measurement sessions of the clitoral blood flow (time 1 [T1] and time 2 [T2]). The evaluator had 6 h training with a gynecologist and 25 h of practice prior the study. In order to control for the influence of circadian and hormonal rhythm on the pelvic blood flow, these assessments were made 1 month apart, at the same time of the day (±2 h) and during the same phase of a woman's menstrual cycle.[1] As caffeine, tobacco, sexual activity, and physical activity have been shown to influence blood flow parameters, they were controlled for a period of 24 h before the assessment.[19–22] To confirm compliance with these recommendations, the evaluator questioned the participant about each of them before the measurements sessions. Furthermore, to avoid potential changes in blood flow between assessment sessions, participants were asked not to perform any PFM training between T1 and T2.

Before each measurement session, participants were taught, using vaginal digital palpation, how to do a maximal pelvic floor muscle contraction without compensation (gluteal, adductor, and abdominal muscles contraction) in the supine lying position with the knees bent and feet flat on a conventional gynecology table.[23] Participants were asked to rest in the same position for 15 min in a noiseless room with constant heat and light to ensure standardized conditions. A clinical ultrasound system (Voluson E8, GE Healthcare, Salzburg, Austria) was then used to place a 4- 13 MHz linear probe on the pubis according to Khalifé's procedure[18] (Figure 1). Clitoral blood flow measurements were repeated: (1) three times at rest and (2) three times after a PFM contraction task (five 10-s maximal contractions followed by ten 1-s maximal contractions). Despite the conflicting results in studies examining the acute effect of exercise on local blood flow, local muscle endurance exercises task, and muscle power exercise tasks seem to cause the greatest changes in the arterial blood flow.[24]

Therefore, our PFM contraction task was chosen to target these parameters (endurance and high-intensity interval exercises). Five blood flow parameters per measurement session were collected for the two conditions (rest and PFM contraction) using the pulsed Doppler mode: peak systolic velocity, time-averaged maximum velocity, end-diastolic velocity, pulsatility index, and resistance index. Peak systolic velocity value refers to maximum velocity of blood flow during contraction of the heart ventricle (Figure 1). End-diastolic velocity value refers to the blood flow velocity at the end of the ventricular relaxation (Figure 1). Time-averaged maximum velocity value refers to the mean of maximum velocities averaged over a complete cardiac cycle. Pulsatility index and resistance index are calculated ratio reflecting peripheral resistance. The pulsatility index is defined as the difference between the peak systolic and minimum diastolic velocities divided by the mean velocity during the cardiac cycle. The resistance index is defined as the difference between the peak systolic velocity and end-diastolic velocity divided by the peak systolic velocity. The clearest waveform in a pulsed-wave Doppler recording was selected for analysis.

2.1 | Statistical analysis

Descriptive statistics served to provide the demographic parameters of the study population while a paired t-test was used to detect statistical differences in clitoral blood flow measurements between T1 and T2 for each condition. To assess the test-retest reliability of each blood flow parameter between T1 and T2, intraclass correlation coefficients (ICC) and their 95% confidence intervals (CI) were then calculated by using a two-way mixed model. Any ICC value lower than 0.4 was considered poor, 0.40-0.75 fair to good and 0.75-1.00 excellent.[25] Lastly, the degree of variability of the parameters between T1 and T2 at rest and after a PFM contraction task was analyzed by Bland-Altman plots. The mean difference and 95% limits of agreement between parameters were calculated.

3 | RESULTS

Twenty women aged 22-53 years old (mean of 33.6 ± 9.9 years old) were recruited. Among the data set, 19/20 measures were adequately visualized for the analysis as the quality was not sufficient for 1/20 image. Therefore, 19 study subjects were kept for analysis. Of these, 11 women were nulliparas, 8 multiparas; 17 women were pre- menopausal, 2 women were post-menopausal; 12 were taking hormonal contraception and 1 was taking systemic hormonal therapy. Table 1 summarizes the study subjects' demographics.

3.1 | Rest condition

Table 2 summarizes the results of blood flow measurements at rest, including mean, mean difference, pairwise comparison, ICC and Bland-Altman plots' limits of agreement. At rest, there was no significant difference between T1 and T2 for all parameters ($P > 0.05$). Based on the ICC results, excellent reliability was obtained for peak systolic velocity (0.95, 95% CI: 0.87-0.98, $P < 0.001$), time-averaged maximum velocity (0.87, 95%CI: 0.65-0.95, $P < 0.001$) and end-diastolic velocity (0.75, 95%CI: 0.36-0.9, $P = 0.002$) parameters. The reliability was fair to good for the pulsatility index (0.67, 95%CI: 0.14-0.87, $P = 0.012$) and the resistance index (0.67, 95%CI: 0.08-0.88, $P = 0.018$).

Bland-Altman plots (Figure 2) demonstrated minimal bias with the mean difference close to zero for all parameters. The 95% limits agreement range was narrow considering the mean values for peak systolic velocity, time-averaged maximum velocity, and resistance index parameters, indicating a small variability in the measurement between T1 and T2.

The 95% limits of agreement range for the pulsatility index parameter was larger considering the mean value, indicating a still acceptable measurement variability between T1 and T2. However, it was wide for the end-diastolic parameter considering its mean values, indicating larger measurement variability between T1 and T2.

3.2 | After a PFM contraction task condition

Table 3 summarizes the results of blood flow measurements at rest, including mean, mean difference, pairwise comparison, ICC and Bland-Altman plots' limits of agreement. There was no significant difference between T1 and T2 for all parameters ($P > 0.05$). Based on the ICC results, excellent reliability was obtained for peak systolic velocity (0.85, 95% CI: 0.61-0.94), $P < 0.001$), time-averaged maximum velocity (0.77, 95%CI: 0.40-0.91, $P = 0.002$) pulsatility index (0.79 (95%CI 0.45-0.92, $P = 0.001$) and resistance index parameters (0.81, 95%CI: 0.51-0.92, $P < 0.001$). End-diastolic velocity parameter showed fair to good reliability (0.73, 95%CI: 0.31-0.90, $P = 0.004$).

Bland-Altman plots (Figure 3) demonstrated minimal bias with the mean difference close to zero for all parameters. The 95% limits agreement range was narrow considering its mean values for peak systolic velocity and resistance index measurements after PFM contractions, indicating a small variability in measurements between T1 and T2. The 95% limits of agreement range for the time- averaged maximum velocity and pulsatility index parameters were larger considering the mean value, indicating a still acceptable measurement variability between T1 and T2. However, it was wide for the end-diastolic parameter considering its mean values, indicating larger measurement variability between T1 and T2.

4 | DISCUSSION

To our knowledge, this inter-sessions study is the first to assess the test-retest reliability of clitoral blood flow measurements using color Doppler ultrasonography at rest and after a PFM contraction task. At rest, an excellent reliability with a small variability between sessions was found for peak systolic velocity and time-averaged maximum velocity parameters. Pulsatility index and resistance index parameters also obtained good results, with fair to good reliability and acceptable variability between sessions.

After a PFM contractions task, the results showed an excellent reliability with a small variability between sessions for peak systolic velocity and resistance index parameters. Time-averaged maximum velocity and pulsatility index also obtained excellent reliability but with a larger variability between sessions.

The results showed fair reliability for the pulsatility index and the resistance index in the rest condition. Unlike peak systolic velocity and end-diastolic velocity, which both consist in a single measurement at a single time point, resistance index and pulsatility index are ratios (with a numerator and denominator) that respectively depend on two or three measurements which are all acquired at different time points. Intuitively, parameters that depend on several measurements are more likely to have lower reliability, compounded by the imperfect reliability of individual measurements included in their equations. Further, if differ- ences in the numerator and denominator occur in opposite directions, this will further accentuate the differences in ratios and negatively affect reliability.

Khalifé et al[18] assessed the test-retest reliability of the clitoral blood flow at rest in an inter-evaluator intra-session study. As in our study, Khalifé obtained the highest correlations with no significant mean differences between repeated assessments for the peak systolic velocity parameter. Good reliability was also shown for the pulsatility index and resistance index parameters.

Our study aimed to assess the test-retest reliability of the clitoral blood flow in an intra-observer, inter-session study and has added psychometric information about new clitoral blood flow measurement parameters (time-averaged maxi- mum velocity and end-diastolic velocity). Also added is a new condition for investigating clitoral blood flow that is after a PFM contraction task.

Evaluation of the reliability of a measurement technique is indispensable in order to assess its stability and repeatability.[26] The results obtained in this study support the use of color Doppler ultrasonography by researchers and clinicians to assess the evolution over time or the changes following an intervention of clitoral blood flow parameters both at rest and after a contraction task. As the clitoral blood flow has reliable parameters after a PFM contraction task, it would be of interest to collect normative data among healthy women using this particular test condition. Assessment of the dorsal clitoral artery at rest and after a PFM contraction task could also be used to observe vascular changes in postpartum women, for example, or after menopause, with aging or in conditions leading to lower or challenged perineal vascularity. Dorsal clitoral artery blood flow could also be use prior and after treatments designed to improve perineal vascularity: hormonal therapy, laser therapy, and PFM training.

One of the limitations of this reliability study is that it is closely linked to the population in which the measurements were acquired, that is, healthy young and middle-aged women, aged between 22 and 53, mostly pre-menopausal. It would therefore be important to carry out a similar study with other populations (eg, older women, women with challenged vascular conditions). Another limitation is that to reach the same reliability results as this study, evaluator need to have a similar training with the measurement techniques. Lastly, an inter-evaluator reliability study should be undertaken in the condition following a PFM contractions task for research protocols involving more than one assessor.

5 | CONCLUSION

Our findings suggest that the peak systolic velocity, time- averaged maximum velocity, pulsatility index, and resistance index are reliable parameters of the clitoral blood flow at rest and after a PFM contraction task. These parameters can form the basis for further research on the clitoral artery's vascular properties in different conditions or before and after an intervention.

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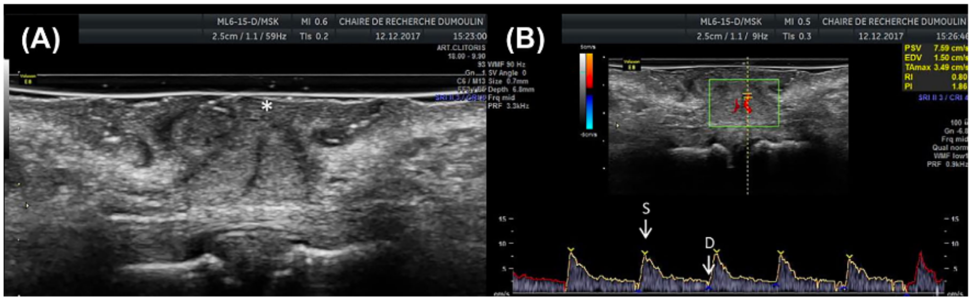

FIGURE 1 A, B-mode image acquired in the transverse plane at the level of the symphysis pubis (*). B, Color Doppler (top half of image) acquired at the same level and pulsed Doppler waveform (bottom half of image) of the dorsal clitoral artery shows peak systolic velocity (S) and end-diastolic velocity (D)

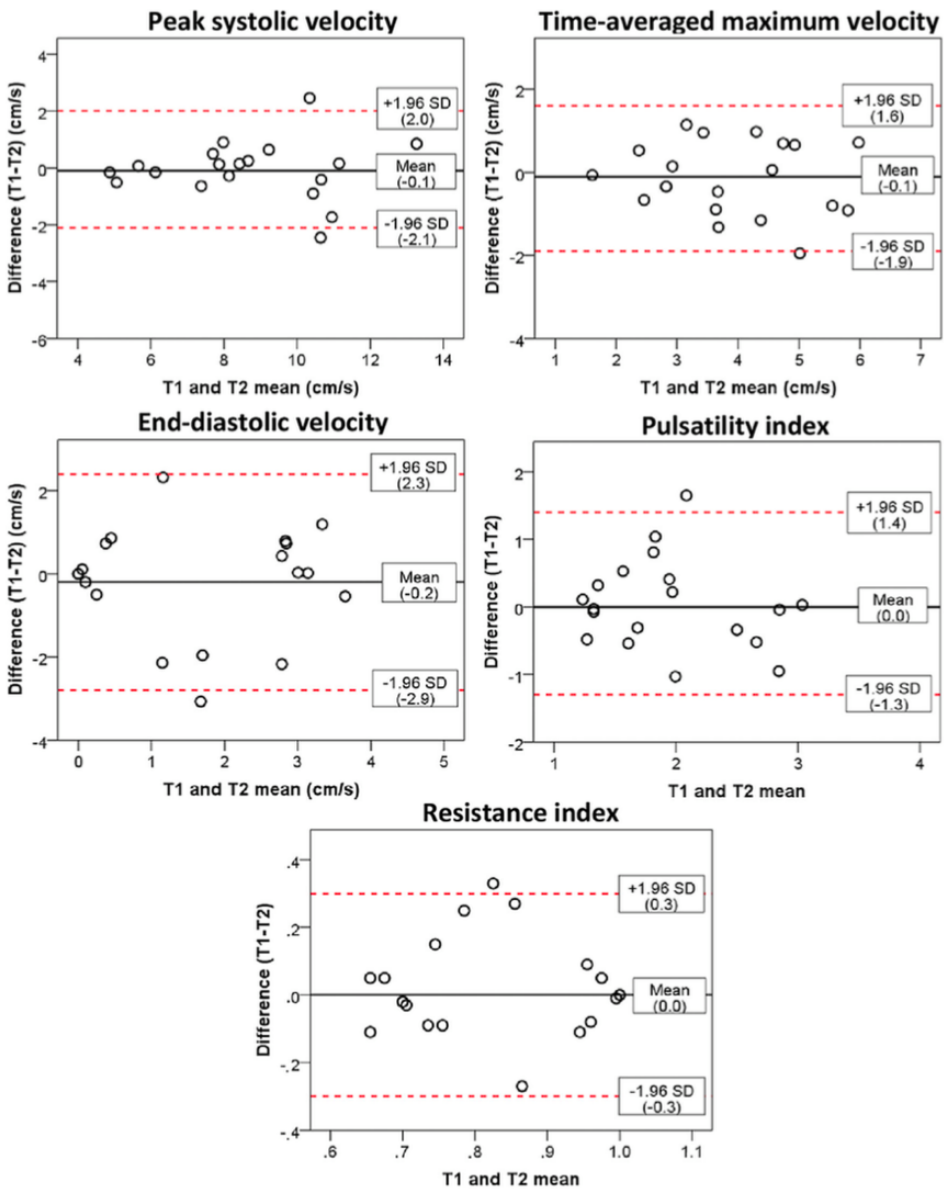

FIGURE 2 Measurements at rest. T1, First measurement session; T2, Second measurement session

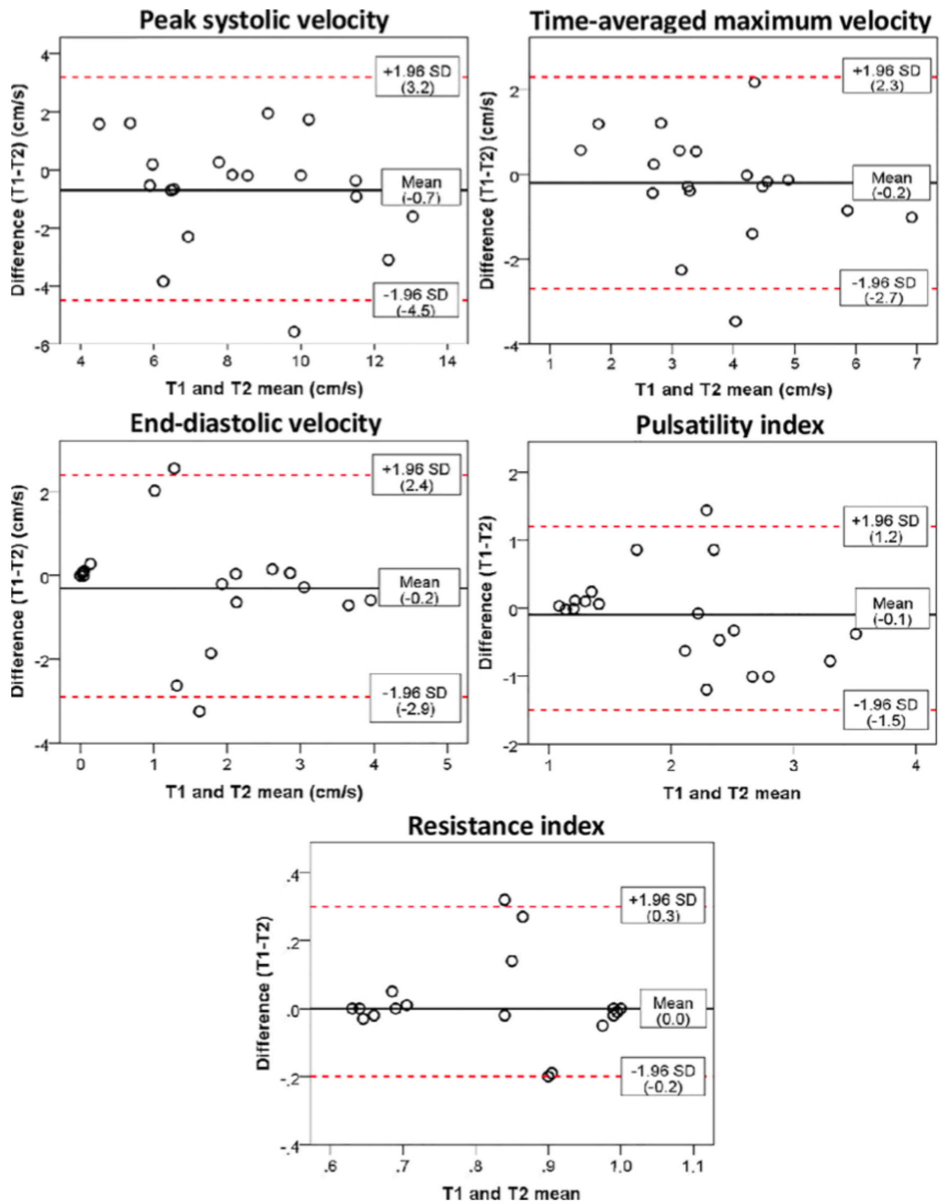

FIGURE 3 Measurements after a PFM contraction task.T1: First measurement session; T2: Second measurement session

TABLE 1 Subject demographics

Parameters	
Mean age ± SD (years)	33.5 ± 10.1
Parity	
Nulliparas	11 (58%)
Multiparas	8 (42%)
Menopausal status	
Pre-menopausal	17 (89%)
Post-menopausal	2 (11%)
Hormonal therapy	
None	6 (32%)
Hormonal contraception	12 (63%)
Systematic hormonal therapy	1 (5%)

TABLE 2 Blood flow measurements at rest

	Data				ICC		Bland-Altman plots
	Mean T1 ± SD	**Mean T2 ± SD**	**Mean difference (95% CI)**	***P*-value**	**ICC (95% CI)**	***P*-value**	**Limits of agreement**
PSV(cm/s)	8.6 ± 2.3	8.7 ± 2.3	−0.1 (−0.6 to 0.4)	0.806	0.95* (0.87 to 0.98)	<0.001	−2.1 to 2.0
TAMX(cm/s)	3.9 ± 1.2	4.0 ± 1.4	−0.1 (−0.6 to 0.3)	0.510	0.87* (0.65 to 0.95)	<0.001	−1.9 to 1.6
EDV(cm/s)	1.6 ± 1.5	1.7 ± 1.5	−0.2 (−0.8 to 0.5)	0.569	0.75* (0.36 to 0.90)	0.002	−2.8 to 2.4
PI	2.0 ± 0.6	1.9 ± 0.7	0.0 (−0.3 to 0.4)	0.784	0.67** (0.14 to 0.87)	0.012	−1.3 to 1.4
RI	0.8 ± 0.1	0.8 ± 0.1	0.0 (−0.0 to 0.1)	0.513	0.67** (0.08 to 0.88)	0.018	−0.3 to 0.3

ICC, intraclass correlation coefficients; PSV, peak systolic velocity; TAMX, timed-average maximum velocity; EDV, end-diastolic velocity; PI, pulsatility index; RI, resistance index.

*Excellent.

**Fair to good.

TABLE 3 Blood flow measurements after a PFM contractions task

	Data				ICC		Bland-Altman plots
	Mean T1 ± SD	Mean T2 ± SD	Mean difference (95% CI)	*P*-value	ICC (95% CI)	*P*-value	Limits of agreement
PSV (cm/s)	8.1 ± 2.5	8.8 ± 2.9	−0.7 (−1.6 to 0.3)	0.149	0.85* (0.61 to 0.94)	<0.001	−4.5 to 3.2
TAMX (cm/s)	3.6 ± 1.3	3.9 ± 1.6	−0.2 (−0.8 to 0.4)	0.454	0.77* (0.40 to 0.91)	0.002	−2.7 to 2.3
EDV (cm/s)	1.4 ± 1.3	1.7 ± 1.6	−0.3 (−0.9 to 0.4)	0.419	0.73** (0.31 to 0.90)	0.004	−2.9 to 2.4
PI	2.0 ± 0.9	2.1 ± 0.9	−0.1 (−0.4 to 0.2)	0.464	0.79* (0.45 to 0.92)	0.001	−1.4 to 1.2
RI	0.8 ± 0.1	0.8 ± 0.2	−0.00 (−0.0 to 0.1)	0.149	0.81* (0.51 to 0.92)	<0.001	−0.2 to 0.2

ICC, intraclass correlation coefficients; PSV, peak systolic velocity; TAMX, timed-average maximum velocity; EDV, end-diastolic velocity; PI, pulsatility index; RI, resistance index.

*Excellent.

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