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OBJECTIVE: Normal pregnancy is characterized by early and pronounced changes in the maternal cardiovascular system, to provide the optimal environment for sufficient fetal growth and development.[1] While the precise mechanisms for these hemodynamic changes are not yet fully clear, recent evidence suggests that changes in adaptive endothelial function are a contributory factor.[2,3] However, much of the evidence regarding the microvascular adaptation has been based on circulating endothelial markers or observations of the behavior of large blood vessels.[1,2,4–6]

Laser speckle contrast imaging (LSCI) is a novel noninvasive tool for the in vivo assessment of skin microvascular perfusion in real-time, offering very high spatial and temporal resolution.[7] Due to its high accessibility, the skin microcirculation can serve as a surrogate marker for systemic endothelial function in both physiological and pathological conditions.[8,9]

The main purpose of this study was to explore the physiological course of endothelial adaptation through pregnancy by assessing changes in skin microvascular reactivity across the 3 trimesters using LSCI combined with postocclusive reactive hyperemia (PORH). Additionally, the study aimed to compare the results in pregnant patients with those obtained from nonpregnant healthy females.

STUDY DESIGN: This prospective longitudinal study was conducted at Careggi University Hospital in Florence Italy between November 2022 and January 2024. Women carrying a singleton gestation were prospectively enrolled in the first trimester of pregnancy. Exclusion criteria were preexisting hypertension, cardiovascular diseases, type I or II diabetes mellitus, current smoking habit, II or III class obesity, and maternal age over 45 years.

Skin microvascular reactivity was evaluated by LSCI (PeriCam PSI system, Perimed, Järfälla, Sweden). This technique involves scanning the skin's surface with a 2-mW helium-neon laser. The light backscattered from moving erythrocytes undergoes a shift in frequency proportional to their velocity, according to the Doppler principle. The resulting color-coded image represents skin microvascular perfusion (flux) of the scanned area in real-time.[10] The median laser Doppler flux is calculated using a dedicated image-processing software and is expressed in perfusion units (PUs).

The microvascular flux at the dorsal face of the hand was recorded (Supplemental Figure 1, A). A PORH protocol was performed with a pneumatic cuff attached to the bicep. Following a baseline-measuring period of 2 minutes, the inflated pneumatic cuff applied a suprasystolic pressure of at least 40 mmHg above the systolic blood pressure. Following immediate cuff release, changes in microcirculation flux were recorded for 4 minutes (Supplemental Figure 1, B). The following parameters were recorded and calculated: (i) baseline flux (PU), (ii) occlusion flux (PU), (iii) peak flux, as the highest flux value at the postocclusive period (PU), (iv) base-to-peak flux, as the percentage increase of flux from baseline to the peak flux ([(peak − baseline)/baseline] × 100), (v) peak time (seconds), and (vi) time to half recovery (seconds).

Each LSCI study was performed in a temperature-controlled environment (24°C) with the woman resting in a semi-recumbent position. Caffeine-containing drinks were not allowed for 2 hours before the test. Pregnant women repeated the LSCI assessment 3 times longitudinally through the pregnancy, one at each trimester (T1, T2, and T3). To evaluate the change in continuous variables between time points, the paired *t* test or the Wilcoxon's signed rank test were used according to Shapiro-Wilk test for normality of the change distribution. Recordings from T1, T2, and T3 were then compared with parameters obtained in nonpregnant controls. As controls, nonpregnant females in reproductive age were enrolled, with no history of cardiovascular or metabolic diseases, smoking habit, or recent pregnancy within the last 12 months. To ensure comparability between the 2 groups, we matched cases and controls for age allowing a maximum difference of 1 year. To assess the difference between groups the unpaired *t* test, Satterthwaite test or Mann-Whitney test were used according to the Shapiro-Wilk test for normality distribution and the F-test for homoscedasticity.

A power analysis was performed to determine the sample size of both groups (cases and controls), based on the base-to-peak flux value reported in healthy subjects using PORH coupled with LSCI in the study by Gkaliagkousi et al.[8] To detect a difference of 20% with a power of 80% at a significance level of 5%, at least 21 women had to be included in each group.

This study was performed according to the principles of the Declaration of Helsinki and was approved by the Institutional ethics committee (reference number 22853_oss, approved on October 4th, 2022). All women who accepted to participate in the study signed an informed consent at the time of enrollment.

RESULTS: Thirty-six pregnant women were initially enrolled in the study. Two patients failed to attend the second and third trimester registration and were excluded. Two pregnancies were complicated by late fetal growth restriction (FGR) and gestational hypertension and were excluded from the analysis. Therefore, the remaining 32 patients were

included in the analysis. Thirty-two age-matched nonpregnant women were enrolled as a control group.

Maternal and pregnancy characteristics are illustrated in Supplemental Table 1, along with the characteristics of the control group. Results of the longitudinal assessment in the 3 trimesters of pregnancy and in controls are reported in Supplemental Table 2, in Figure and Supplemental Figure 2. The baseline flux increased gradually from T1 to T3, with statistically significant differences between T1 and T3 (P=.03), and between T2 and T3 (P=.04) (Supplemental Table 2). This increase is consistent with the well-established peripheral vasodilation that occurs in normal pregnancy.

The peak flux, whether expressed as the absolute value or in relation to baseline, represents the most used parameter of the PORH test.[9] A lower peak flux indicates impaired microvascular endothelial response and has been associated with cardiovascular diseases.[8,11,12] In the longitudinal cohort, both the peak flux and the base-to-peak flux significantly increased from T1 to T3 (Figure, A and B and Supplemental Table 2). The median change in peak flux from T1 to T3 was 29.06 PU (95% confidence Interval 19.48−40.1, P<.0001) and the mean increase in base-to-peak flux was 40.8% (95% confidence Interval 13.34−68.2, P<.01). The peak flux was also significantly higher at T2 compared to T1 (P=.02) and at T3 compared to T2 (P<.01). Both the peak flux and the base-to-peak flux were higher in T3 compared to nonpregnant controls (P<.001 and <.0001, respectively). The base-to-peak flux was also higher at T1 and T2 compared to nonpregnant controls (P<.01 and <.0001 respectively) (Figure, A and B and Supplemental Table 2).

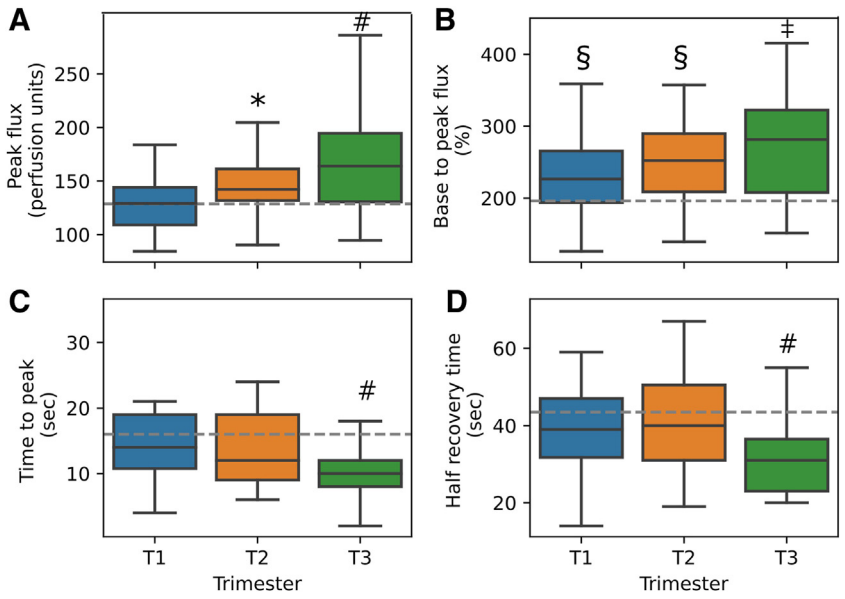
The time to reach peak perfusion since cuff release decreased progressively from T1 to T3 with mean change −3.56 (P=.001) (Figure, C and Supplemental Table 2). It was also significantly lower at T3 compared to T2 (P=.001), and in T3 compared to nonpregnant controls (P<.001). Similarly, the half recovery time decreased progressively from T1 to T3 with mean change −7.63 seconds (P<.01) (Figure, D and Supplemental Table 2). The half recovery time was also significantly shorter in T3 compared to T2 (P<.01), and in T3 compared to nonpregnant controls (P<.0001).

The increase in the peak flux and in the base-to-peak flux, as well as the more rapid reach of the peak, observed in pregnant patients from T1 to T3 and compared to nonpregnant controls, are expression of the physiological improvement of the microvascular function that occurs during pregnancy.[2,3,5,6]

In both patients that were excluded due to gestational hypertension and late FGR, after an initial increase in the peak flux and base-to-peak flux from T1 to T2, both parameters were markedly decreased in the T3 measurement. Since they were only 2 cases, we could not perform a comparison with the group of physiological pregnancies.

Very few studies have investigated the changes in microvascular reactivity throughout normal pregnancy. To our knowledge this is the first longitudinal study using LSCI

FIGURE
The parameters of postreactive hyperemia measured with LSCI across the 3 trimesters of gestation (boxplots) and compared to controls (dashed lines representing the mean value)



A, Peak flux; **B,** base-to-peak flux increase; **C,** time to peak; and **D,** half recovery time. Blue: first trimester; orange: second trimester; green: third trimester. *P<.05 vs T1; #P<.05 vs T2, T1 and controls; §P<.05 vs controls; ‡P<.05 vs T1 and controls.

coupled with PORH for this purpose. Only 1 previous study by Iacobeaus et al[3] demonstrated that skin microvascular reactivity to acetylcholine increased during pregnancy, using LSCI coupled with iontophoresis. Other studies have shown enhanced endothelial function during pregnancy by assessing the flow-mediated dilatation of the brachial artery diameter.[2,3,5,6]

The enhanced endothelial response throughout pregnancy is traditionally attributed to the increased activity and availability of nitric oxide (NO).[5] Nevertheless, NO does not seem to be involved in either the development or the maintenance of the hyperemic response.[13] Thereby, our results may not be explained by the increase in NO availability.

LSCI coupled with PORH provides an overall assessment of microvascular function.[9] The reactive hyperemia is believed to be endothelium-dependent and seems to be mediated by calcium-activated potassium channels, by sensory nerves, and by endothelium-derived hyperpolarizing factors.[14] However, the exact underlying mechanism is yet to be fully discovered.

CONCLUSION: Microvascular reactivity improves from the first to the third trimester of pregnancy and in pregnant compared to nonpregnant women. The enhanced microvascular reactivity is evident as early as the first trimester of pregnancy, compared to nonpregnant controls.

Using LSCI coupled with PORH, we have presented objective measures of microvascular function in each trimester in uncomplicated pregnancies. These measurements can be used to investigate deviation from normal response in pathological conditions, such as preeclampsia, a disorder involving widespread endothelial dysfunction. The LSCI technique may also improve our comprehension of the increased risk for future cardiovascular diseases in women who had preeclampsia.[15] ■

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An abstract of the results was presented as a poster at the fifth international congress on maternal hemodynamics in Florence, Italy (March 7–9, 2024).

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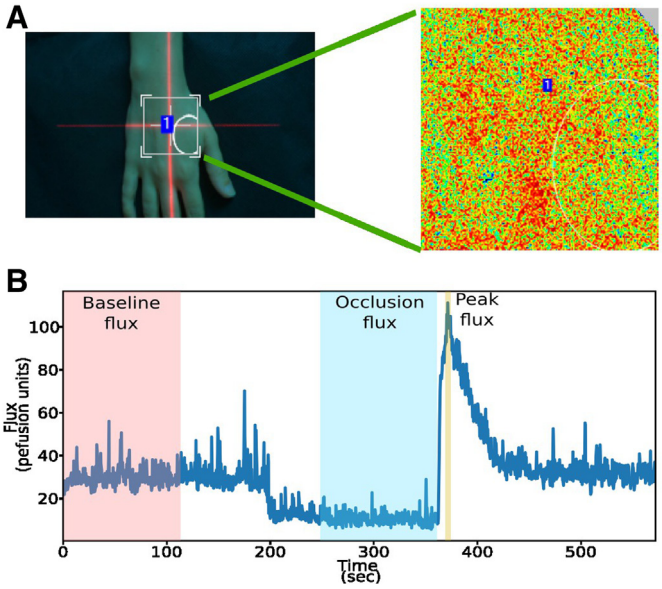
SUPPLEMENTAL FIGURE 1

Skin blood flow assessed by LSCI combined with PORH

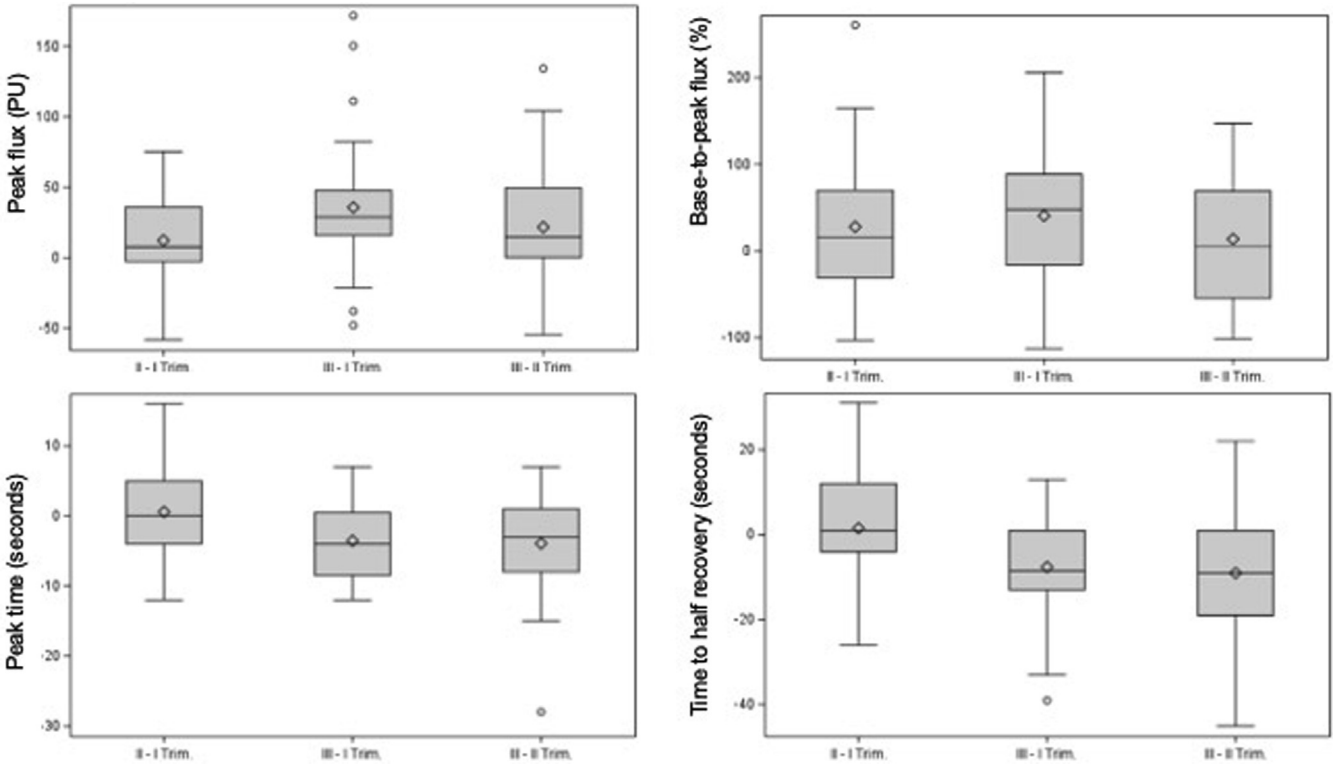


A, Microvascular skin blood flow at the dorsal face of the hand assessed with LSCI; **B,** example of a record of the skin blood flow using LSCI coupled with PORH.

LSCI, laser speckle contrast imaging; *PORH*, postocclusive reactive hyperemia.

SUPPLEMENTAL FIGURE 2

Changes in the parameter of postreactive hyperemia measured with LSCI between 2 time points: T2 vs T1, T3 vs T1, and T3 vs T2



LSCI, laser speckle contrast imaging; *PU*, perfusion unit.

SUPPLEMENTAL TABLE 1

Maternal and pregnancy characteristics

Characteristics	Longitudinal cohort	Control group
Age (y)	36.18±4.34	35.5 (33, 40)
Body mass index (kg/m^2)	22.74±2.67	20.95 (19.95, 23.45)
Nulliparity	20/32 (62.5%)	15/32 (46.9%)
Ethnicity		
White	32/32 (100%)	31/32 (96.9%)
Black	-	1/32 (3.1%)
Gestational age at delivery (wk)	39.44±1.12	-
Method of delivery		
Vaginal delivery	23 (71.9%)	-
Operative vaginal delivery	1 (3.1%)	-
Cesarean section	8 (25%)	-
Birthweight (g)	3292.81±379.73	-
Birthweight centile	47 (24.5, 70)	-
Fetal gender		
Male	16/32 (50%)	-
Female	16/32 (50%)	-

Data are expressed as n (%), mean±SD or median (25th, 75th IQR) based on their distribution.

IQR, interquartile range; *SD*, standard deviation.

SUPPLEMENTAL TABLE 2

Results of the longitudinal assessment of the parameters in the 3 trimesters of pregnancy and in controls

	Cases				*P* value Longitudinal assessment in cases			*P* value Cases vs controls		
Test parameters	**T1**	**T2**	**T3**	**Controls**	**T2 vs T1**	**T3 vs T2**	**T3 vs T1**	**T1 vs controls**	**T2 vs controls**	**T3 vs controls**
Basal flux (PU)	40.24±9.67	40.99±8.22	45.21±9.82	42.15 (36.8, 51.68)	.75	**.04**	**.03**	.08	.12	.92
Occlusion flux (PU)	15.41±4.71	14.38±4.25	15.66±3.87	15.99±3.68	.22	.06	.80	.58	.11	.73
Peak flux (PU)	131.37±32.53	143.71±28.14	167.17±45.04	130.75±29.71	**.02**	**<.01**	**<.0001**	.94	.08	**<.001**
Base-to-peak flux (%)	230.43±55.67	255.93±65.79	271.19±69.27	195.9±40.45	.06	.30	**<.01**	**<.01**	**<.0001**	**<.0001**
Peak time (s)	13.81±4.78	12 (9, 20)	10.25±4.12	16 (12, 21)	.63	**.001**	**.001**	.06	.21	**<.001**
Time to half recovery (s)	38.19±12.41	40.97±13.11	31 (23, 37)	44.63±13.27	.57	**<.01**	**<.01**	.09	.28	**<.0001**

Data are expressed as mean±SD or median (25th, 75th IQR) based on their distribution.

Bold values are statistically significant (*P*-value < .05).

PU, perfusion unit.