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Low-flow mediated constriction as a marker of endothelial function in healthy pregnancy and preeclampsia: A pilot study

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ABSTRACT

Objectives: Overwhelming clinical evidence exists on disturbed vascular and endothelial function in the pathophysiology of preeclampsia (PE). In a non-pregnant (NP) population, L-FMC (low-flow mediated constriction) provides insight in the 'resting' endothelial capacity in contrast to the gold standard of flow mediated dilatation (FMD), reflecting endothelial nitric oxide bioavailability.

Study design: Longitudinal follow-up of 100 healthy pregnant (HP) women, 33 PE women and 16 NP controls with non-invasive vascular assessments. HP women were evaluated at 12 and 35 weeks of gestation and at 6 months postpartum. PE patients were assessed at diagnosis (mean 30 weeks) and 6 months postpartum.

Main outcome measures.

Endothelial function (L-FMC, FMD, peripheral arterial tonometry (PAT)) and arterial stiffness (pulse wave velocity (PWV) and analysis (PWA)) were measured at the different visits and compared between groups.

Results: Overall endothelial dysfunction is present in PE (FMD HP 9.09 ± 4.20 vs PE 5.21 ± 4.47, $p = 0.0004$; L-FMC HP −1.90 ± 2.66 vs PE −0.40 ± 2.09, $p = 0.03$). L-FMC gradually elevates during the course of a HP (1st trim −0.31 ± 1.75 vs 3rd trim −1.97 ± 3.02, $p < 0.0001$) and is present in 85% of women in the third trimester. In NP, only 27% of women has L-FMC. In PE, L-FMC is present in 50% of cases. Arterial stiffness is increased in PE (all $p < 0.0001$). There is no correlation between L-FMC and other markers of vascular function ($p > 0.05$).

Conclusion: PE is characterized by dysfunction of both resting and recruitable endothelial capacity. This study offers new insights in different aspects of endothelial function in pregnancy, since L-FMC reflects an adaptation in HP that is absent in PE.

1. Introduction

The pathophysiology of preeclampsia (PE) has not been completely unravelled, yet evidence on endothelial dysfunction (ED) and arterial stiffness is abundant [7,15,16,23,29]. The endothelium plays a critical role in controlling vasomotor tone. Lowering the vasomotor tone is essential to allow haemodynamic adaptation to pregnancy, therefore quantitative measures of endothelial function have gained increasing attention over the last decade [1,7,16,21,29]. The gold standard for non-invasive assessment of endothelial function is flow mediated vasodilatation (FMD), measuring nitric oxide (NO)-dependent vasodilatation of the brachial artery in response to reactive hyperaemia and

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increased shear stress [33]. While FMD reflects the 'recruitable' capacity of the endothelium, it does not provide information concerning basal endothelial function [12,13]. In order to fill this gap, low-flow mediated vasoconstriction (L-FMC), mainly an endothelin-1 dependent phenomenon, has been recently introduced as an additional measure, however its relationship with changes in blood flow, cardiovascular risk factors and FMD has been less well demonstrated [12,13]. Research on L-FMC is scarce and discrepancies between studied populations have been described [34,38]. Controversy exist whether the presence of L-FMC is a beneficial condition or rather an inability of the endothelium to maintain tone during low-flow conditions [32]. The evolution of L-FMC during the course of a healthy pregnancy (HP) is unknown, and L-FMC has never been assessed in early PE.

Applanation tonometry (pulse wave velocity (PWV) and pulse wave analysis (PWA)) represents a reliable measure of vascular wall stiffness associated with endothelial function [9,39]. Arterial stiffness is an independent predictor of cardiovascular mortality and morbidity [20,24] and may play a role in the prediction of PE [25]. Recently, normograms for gestational changes in arterial stiffness have been described [27].

When discussing vascular function in pregnancy and PE, it is critical to apprehend its different aspects (endothelial function and arterial stiffness). In this study, a comprehensive assessment of non-invasive vascular function was performed in a longitudinal follow-up study of healthy pregnancies and PE, with a special focus on the value of L-FMC as potential marker of PE.

We hypothesize that during the course of a HP, vascular function improves to meet the higher hemodynamic needs of pregnancy. Resting endothelial function is important in this cardiovascular adaptation. We presume that during PE these vascular adaptations are impaired, resulting in lower FMD, higher arterial stiffness and less L-FMC, since increased arterial stiffness will inhibit the vessels to further constrict to a low-flow stimulus.

2. Methods

2.1. Study population

One hundred women with a healthy pregnancy (HP), 33 PE women (gestational age (GA) 25 + 0 weeks–36 + 5 weeks (mean 30 weeks)) admitted to the maternal intensive care unit and 16 non-pregnant controls (NP) were included between January 2016 and December 2017. We defined PE according to the revised ISSHP definition [36]. Exclusion criteria were (gestational) diabetes, multiple pregnancies, foetal malformations, hypercholesterolemia, kidney disease, auto-immune disorders, connective tissue diseases or use of acetylsalicylic acid. Since the Antwerp University Hospital serves as a tertiary referral centre, most women were already initiated on anti-hypertensive medication, low molecular weight heparin (LMWH) and MgSO4 at the moment of referral and inclusion (Table 1). HP were included in the study during their first trimester and were longitudinally followed throughout the whole pregnancy. They were free from medication and did not have a history of PE, pregnancy-induced hypertension, hypertension, cardiovascular disease or other chronic conditions. NP subjects (n = 16) matched for age, BMI and parity, served as an additional control group. At 6 months PP, 17 HP women and 19 PE women were re-assessed.

Table 1
Antihypertensive medication and doses given to the PE women.

Medication	n	Duration
No antihypertensive medication	5 women	/
Labetalol 100 mg 3x/d	7 women	1–5 days
Labetalol 200 mg 3x/d	5 women	1–5 days
Labetolol (IV) 6–10 ml/h	9 women	< 24 h
Labetalol 100 mg 3x/d + Felodipine 10 mg 2x/d	3 women	1–3 days
Felodipine 5 mg 2x/d	2 women	1–5 days
Methyldopa 500 mg 3x/d + Nifedipine 30 mg 3x/d	2 women	1–2 days
MgSO4 (1 gr/h)	27 women	< 24 h
Low Molecular Weight Heparin (Enoxaparin 4000 IE) 1x/d	33 women	< 24 h

The Research and Ethics committee of the Antwerp University Hospital approved the study protocol of the ENDOPREG study (Belgian number: B300201524783), and written informed consent was obtained from all subjects.

2.2. Vascular function measurements

Women were asked not to eat high-fat substances nor drink caffeine or alcohol 24 h prior to examination and to refrain from smoking at least 6 h prior to examination [5]. Fingernails had to be short and no nail polish applied. Women were studied in a quiet, temperature-controlled room (21–24 °C) and stressful situations were avoided (people entering the room unexpectedly, telephone ringtones, etc.) The examinations were performed in a supine lying position with the arm in a comfortable position for imaging the brachial artery. In all subjects, one blood pressure (BP) measurement was taken after 5 min of rest using an automated BP device (OMRON® Intellisense, Healthcare Japan) in a supine position. The systolic BP was used to determine occlusion pressure for the FMD/L-FMC/peripheral arterial tonometry (PAT) measurements. FMD/L-FMC and PAT measurements were performed simultaneously. After the endothelial function measurements, arterial stiffness was recorded. Repeat measurements in HP and PE groups were performed at the same arm and at approximately the same time of day [21]. All recordings were performed by two experienced investigators (IG, TS).

2.2.1. Brachial artery low-flow mediated constriction and flow-mediated dilatation

L-FMC/FMD were assessed by measuring changes in brachial artery diameter in response to a respectively decrease and increase in blood flow and endothelial shear stress, elicited by inflating a cuff at the forearm for 5 min [12,33]. An ultrasound diagnostic instrument (Prosound alfa6, Hitachi Aloka Medical®) equipped with vascular software for 2D-imaging, colour Doppler imaging and ECG-triggering, was used with a high frequency linear array transducer (UST-5413, 5–13 MHz, Hitachi Aloka Medical ®) to perform the FMD/L-FMC measurements as previously described [21]. L-FMC was calculated as the decrease in brachial arterial diameter in the last 30 s of cuff occlusion as compared to the resting diameter [12]. FMD was expressed as % increase in brachial arterial diameter after cuff release (post-occlusion maximal diastolic diameter – baseline diastolic diameter)/baseline diastolic diameter [14,33]. Modified FMD (mFMD) is calculated as maximum percentage change in vessel diameter from end-occlusion diameter following cuff release [14]. Fig. 1 illustrates the principles for the L-FMC and FMD measurements.

2.2.2. Peripheral arterial tonometry (PAT)

PAT was recorded using the Endo-PAT2000® (Itamar Medical, software version 3.2.4) using disposable fingertip probes (Itamar Medical) in accordance with the manufacturer's recommendations and as previously described [21]. PAT is a less operator-dependent and more reproducible technique and measures microvascular endothelial function. The system uses pneumatic finger probes which assess digital volume changes as a response to reactive hyperaemia. The result is expressed as the reactive hyperaemia index (RHI).

2.2.3. Arterial stiffness

Systemic arterial stiffness was evaluated using pulse wave analysis (PWA) and pulse wave velocity (PWV) using the Sphygmocor system® (Atcor Medical, West Ryde, Australia) as previously described [8,19,21]. For PWA, a tonometer was placed at the radial artery from

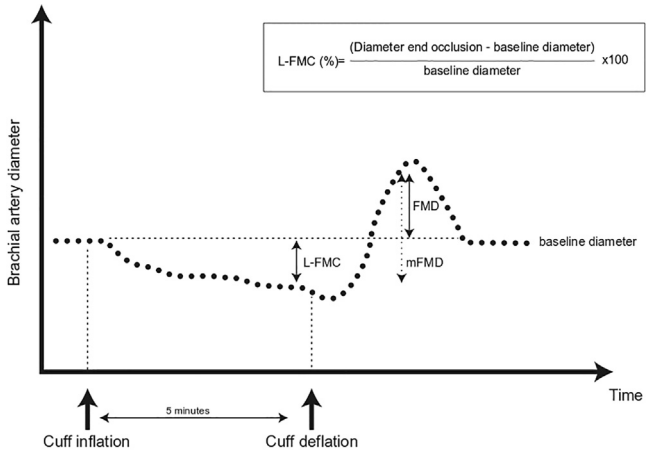

Fig. 1. Principles for the L-FMC and FMD measurements. The brachial artery is visualized with ultrasound for 11 min (1 min rest, 5 min cuff inflation and 5 min cuff deflation). L-FMC is acquired during the last 30 s of cuff inflation. FMD is calculated as maximum percentage change in vessel diameter from baseline following cuff release. mFMD is calculated as maximum percentage change in vessel diameter from end-occlusion diameter following cuff release. (L-FMC: low-flow mediated constriction; FMD: flow mediated dilatation; mFMD: modified FMD).

which the aortic pressure waveform was derived [19]. From this aortic pressure waveform, the augmentation pressure (AP) and augmentation index (AIx) were calculated. The AP is defined as the height of the late systolic peak above the inflection point on the waveform. The AIx is defined as AP expressed as a percentage of the aortic pulse pressure. As AIx is affected by heart rate, it was standardized to a heart rate of 75 bpm (AIx-75) [8]. For PWV three measurements at the level of the carotid artery and subsequently the femoral artery were obtained. As the arterial wall stiffens, the velocity of the travelling waves in the lumen increase. The aortic PWV (measured by carotid-femoral pulse wave velocity (CF-PWV)) is the gold standard method for evaluating arterial stiffness [3,30,37]. CF-PWV was multiplied by 0.8, a correction factor for body surface distance measurements [30]. All recordings were performed by the same two experienced investigators (DM, EF).

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 22.0, SAS 9.4 and GraphPad Prism version 7. Data are expressed as mean ± standard deviation (SD). Normality of continuous variables was evaluated using Kolmogorov-Smirnov test. Unpaired data were compared using analysis of variance (ANOVA) with Tukey's multiple comparisons and Kruskal-Wallis with Dunn's multiple comparisons post-hoc tests as appropriate. Correlation between GA and vascular measurements was studied in the case-control study using Pearson and Spearman correlation analysis as appropriate. In the presence of correlation, analysis of covariance (ANCOVA) was used to correct for the influence of differences in GA. As CF-PWV is influenced by BP and heartrate [26,30], correlation was investigated and if present, corrected for by ANCOVA in the case-control group and linear mixed models in the longitudinal study. Fisher-exact test was used for comparison of categorical variables. A two-tailed $p < 0.05$ was considered significant.

3. Results

3.1. Patients characteristics

Characteristics of the three groups (HP, PE and NP) are summarized in Table 2. Pregnancy groups were comparable regarding age, BMI and cardiovascular risk. BP and birthweight were significantly different between groups. The differences in birthweight were due to differences in GA at birth. Women with co-existing IUGR were excluded from the study, since most of them were started on transdermal nitro-glycerine before measurements. In our PE population, 82% suffered from "early" PE (diagnosis < 34 weeks), while the other 18% suffered from severe "late" (34–37 weeks) PE, characterized by severe hypertension and/or disturbed laboratory parameters.

Table 2
Patients characteristics.

	PE pregnancy (n = 33)	Healthy pregnancy (n = 100)	Non-pregnant controls (n = 16)	P		
				PE vs HP	PE vs NP	HP vs NP
Age (years)	29.6 ± 4.1	30.4 ± 4.2	28.8 ± 3.4	0.24**		
BMI 3rd trimester (kg/m^2)	28.9 ± 4.3	28.0 ± 4.1	22.9 ± 2.7	< 0.0001*		
				0.99	< 0.0001	< 0.0001°
SBP 3rd trimester (mmHg)	159.0 ± 15.7	125.9 ± 11.9	123.8 ± 9.3	< 0.0001*		
				< 0.0001	< 0.0001	> 0.99
DBP 3rd trimester (mmHg)	95.9 ± 11.4	74.0 ± 8.1	75.3 ± 8.0	< 0.0001*		
				< 0.0001	< 0.0001	> 0.99
MAP 3rd trimester	116.9 ± 11.8	91.3 ± 8.4	90.2 ± 7.2	< 0.0001**		
				< 0.0001	< 0.0001	0.92
Heartrate (bpm)	77.2 ± 11.7	82.3 ± 13.3	73.1 ± 10.8	0.01*		
				0.34	0.53	0.02
Nulliparous (n)	26	52	15	0.004*		
				0.02	> 0.99	0.07
Gestation at measurements 3rd trimester (weeks)	30.6 ± 3.4	34.8 ± 0.9	na	< 0.0001	na	na
Gestation at delivery (weeks)	32.6 ± 4.1	38.8 ± 1.8	na	< 0.0001	na	na
Birthweight (g)	1462 ± 580.7	3389 ± 539.0	na	< 0.0001	na	na
Smoking (n)	2	3	0	0.33	0.54	0.99

Data are expressed as mean ± SD, as median (range) or as number of total (n). Not applicable (na). BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure. ° There was no significant difference in BMI between NP and HP at 12 weeks (p = 0.49). * Statistical analysis was performed using Kruskal-Wallis ** Statistical analysis was performed using ANOVA.

Table 3
Vascular function in preeclamptic (PE) pregnancy, healthy pregnancy (HP) and non-pregnant (NP) controls.

	PE pregnancy (n = 33)	Healthy pregnancy (n = 100)	Non-pregnant controls (n = 16)	P		
				PE vs HP	PE vs NP	HP vs NP
Endothelial function						
FMD (%)	5.21 ± 4.47	9.09 ± 4.20	8.95 ± 3.76	0.0005**		
				0.0004	0.02	> 0.99
mFMD (%)	7.90 ± 6.34	11.41 ± 4.85	8.25 ± 4.40	0.002*		
				0.005	> 0.99	0.08
L-FMC (%)	−0.40 ± 2.09	−1.90 ± 2.66	0.60 ± 1.22	0.0005**		
				0.03	0.42	0.001
Time to peak diameter (s)	55.1 ± 35.4	52.6 ± 23.5	39.4 ± 13.1	0.1*		
Baseline diameter (mm)	3.72 ± 0.66	3.31 ± 0.34	2.98 ± 0.31	< 0.0001**		
				0.0003	< 0.0001	0.02
Minimal diameter prior to cuff release (mm)	3.68 ± 0.67	3.25 ± 0.34	2.99 ± 0.32	< 0.0001**		
				0.0002	< 0.0001	0.11
Maximal diameter post-occlusion (mm)	3.95 ± 0.65	3.60 ± 0.33	3.23 ± 0.32	< 0.0001*		
				0.003	< 0.0001	0.01
Brachial artery flow, rest (cm/s)	70.9 ± 12.3	72.1 ± 17.0	/	0.441	/	/
Endopat RHI	2.1 ± 0.43	1.49 ± 0.31	1.71 ± 0.41	< 0.0001*		
				< 0.0001	0.03	0.12
Arterial stiffness						
CF-PWV (m/s)	7.58 ± 0.91	6.01 ± 0.97	6.36 ± 1.11	< 0.0001*		
				< 0.0001	0.002	0.64
AIx75 (%)	23.77 ± 9.37	3.61 ± 11.36	5.77 ± 10.06	< 0.0001**		
				< 0.0001	< 0.0001	0.74
AP (mmHg)	11.51 ± 7.54	−0.04 ± 4	3.23 ± 3.74	< 0.0001**		
				< 0.0001	< 0.0001	0.048

Vascular function in PE pregnancy vs healthy pregnancy (35 weeks) vs non-pregnant controls. Values are mean ± SD. CF-PWV, carotid femoral pulse wave velocity; AP, augmentation pressure; AIx75, augmentation index; FMD, flow-mediated dilation; mFMD, modified FMD; L-FMC, low-flow mediated constriction; RHI, reactive hyperaemia index. * Statistical analysis was performed using Kruskal-Wallis ** Statistical analysis was performed using ANOVA.

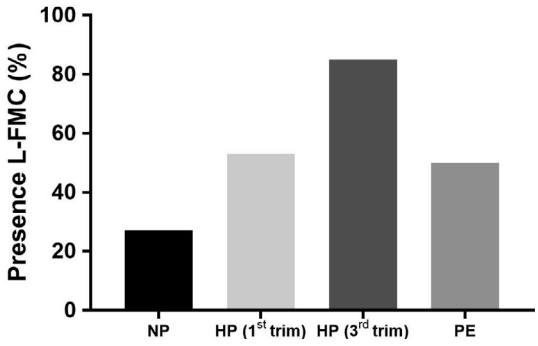

Fig. 2. Presence of low-flow mediated constriction (L-FMC) in the non-pregnant (NP), healthy pregnant (HP, first pregnancy trimester), healthy pregnant (HP, third pregnancy trimester) preeclamptic (PE) and preeclamptic (PE) population.

3.2. Vascular function in preeclamptic versus healthy pregnancy

Results of the vascular measurements of 33 PE women and 100 HP controls are shown in Table 3. FMD and mFMD are significantly decreased in PE compared to HP, confirming ED. L-FMC is significantly attenuated in PE compared to HP. In PE we found vasoconstriction during the occlusion phase (L-FMC) in 50% of patients, while in the third trimester of HP L-FMC was present in 85% of women. (Fig. 2) Regarding microvascular endothelial function, RHI was increased in PE women ($p < 0.0001$). Arterial stiffness is significantly higher in PE women, as seen by increased CF-PWV, AP and AIx75.

CF-PWV, AIx75 and FMD all correlated with GA. After adjustment for GA, all three remained significantly increased in PE (respectively $p < 0.0001$, $p < 0.0001$ and $p = 0.013$). CF-PWV was correlated with MAP, but not with heart rate. After correction for MAP, CF-PWV remained significantly increased in PE ($p = 0.025$).

3.3. Evolution of vascular function during normal pregnancy

One hundred HP women were included and underwent an extensive vascular assessment at the first (12.0 ± 0.6w, n = 100) and third (34.8 ± 0.9w, n = 82) trimester of pregnancy and 6 months PP (n = 17). At the third trimester, only 82 patients were measured due to drop-outs (n = 3), development of pregnancy complications (gestational diabetes (n = 3) and of PE at 38–39 weeks (n = 2)) and inferior vena cava syndrome during FMD/L-FMC/PAT measurements (n = 10). Due to logistic reasons, only 85% of all HP women underwent FMD/L-FMC measurements.

FMD was comparable between the first and third trimester (Fig. 3). L-FMC on the other hand became more prominent with advancing pregnancy. In the third trimester, 85% of HP had a decrease in brachial artery diameter during occlusion, compared to 53% in the first trimester ($p = 0.0003$) (Fig. 2). Six months after HP, FMD/L-FMC return to NP levels (Fig. 3), except for microvascular endothelial function which seems to be improved after a HP compared no NP controls. In contrast to FMD, RHI was significantly lower at the third trimester compared to the first trimester. Fig. 3 illustrates that with progression of pregnancy, there is a decrease in aortic stiffness (CF-PWV, corrected for MAP) and AP, but no difference in overall arterial resistance (AIx75). Six months after HP, CF-PWV was again normalized, but arterial resistance (AIx75) was increased compared to 1st trimester values, however, not different from NP.

A negative correlation was seen between baseline diameter of the brachial artery and FMD (1st trim $p = 0.03$, 3rd trim $p = 0.001$) in HP, but this relation was absent in PE ($p = 0.19$). No correlation was found between baseline diameter and L-FMC (1st trim $p = 0.84$, 3rd trim $p = 0.44$, PE $p = 0.15$).

3.4. Evolution of vascular function in preeclampsia (Fig. 3)

L-FMC values during PE were comparable to the values of a NP population ($p = 0.42$). While FMD improved again 6 months after a PE

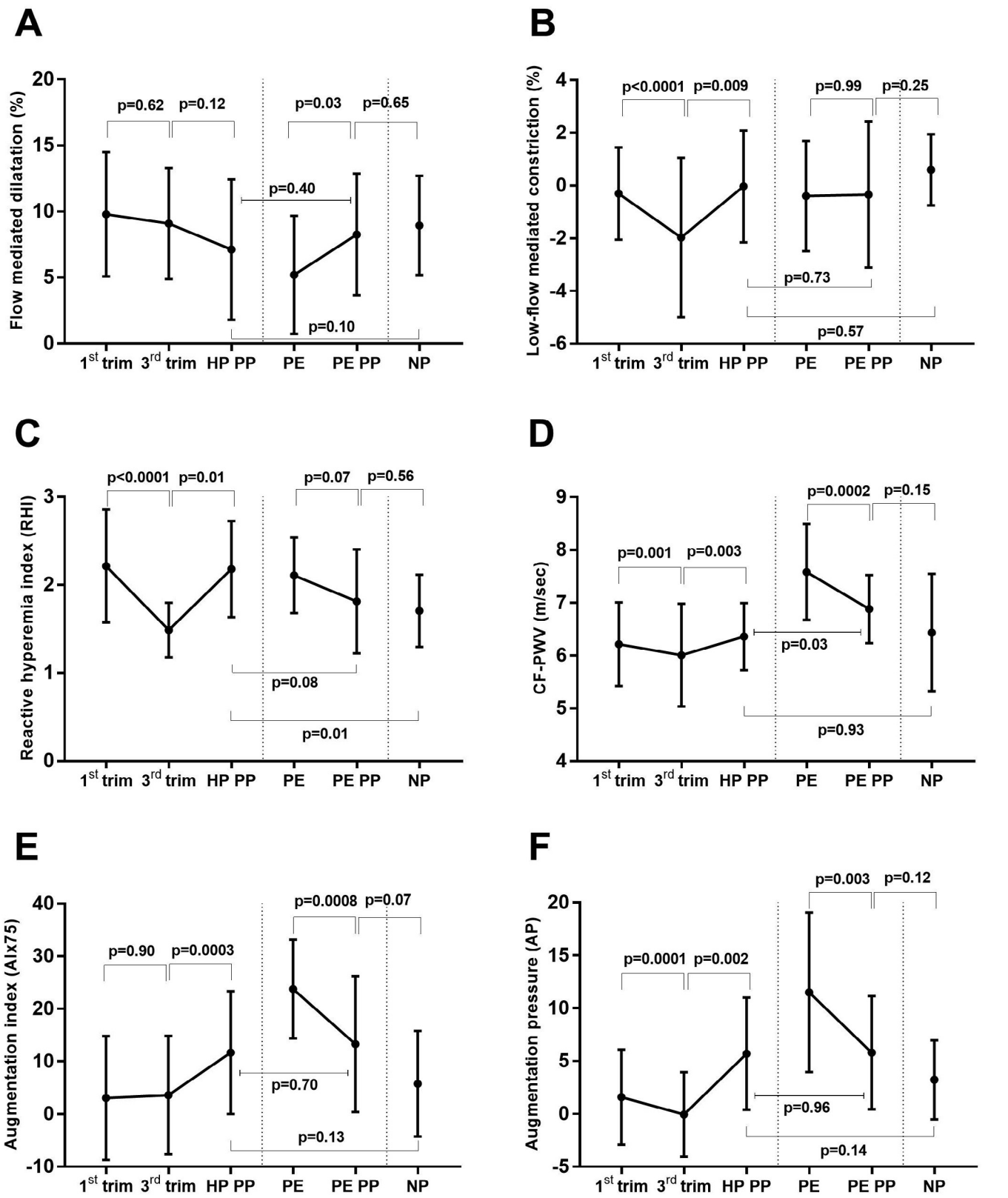

Fig. 3. Vascular function in healthy pregnancy (HP) (n = 100; first trimester, third trimester and post-partum (PP)), preeclampsia (PE) (n = 33, at diagnosis and post-partum (PP)) versus non-pregnant (NP) controls (n = 16). (Endothelial function (Flow mediated dilatation (FMD, Fig. 3A), Low-flow mediated constriction (L-FMC, 3B), Reactive hyperaemia index (RHI, Fig. 3C)) and arterial stiffness (Carotid-femoral pulse wave velocity (CF-PWV, 3D), Augmentation index (AIx75, Fig. 3E), Augmentation pressure (AP, Fig. 3F)).

pregnancy, L-FMC, on the other hand, did not significantly change PP. (Fig. 3) Regarding arterial stiffness, CF-PWV, AIx75 and AP improved 6 months PP. When comparing vascular measurements after a HP versus after a PE pregnancy, one single parameter remained significantly higher (CF-PWV PE PP 6.88 ± 0.64, HP PP 6.36 ± 0.64, $p = 0.03$), however, after correction for MAP, this was no longer significant ($p = 0.12$). (Fig. 3) Microvascular endothelial function (PAT) is not different after a PE pregnancy compared to HP. Although groups were very small, we did not find a difference in vascular function between early and late PE.

3.5. Relation between markers of vascular function

We found no significant correlation between FMD and L-FMC in neither of the groups, nor between FMD and RHI, nor between L-FMC and RHI (all $p > 0.05$). No correlation was found between L-FMC and markers of arterial stiffness (all $p > 0.05$). In the case-control group, FMD was significantly correlated with CF-PWV ($p = 0.03$, $r = -0.3$). Throughout the whole study, CF-PWV was correlated with MAP ($p < 0.0001$, $r = 0.6$) and was corrected for as appropriate.

4. Discussion

In this study, an innovative aspect of endothelial function was objectified in early PE and longitudinally during the course of a HP. L-FMC was compared to other markers of endothelial function and arterial stiffness. The key findings presented here are that the extent of L-FMC increases during HP healthy pregnancy, without a concurrent change in FMD, but that in PE pregnancies, both L-FMC and FMD are attenuated versus HP and arterial stiffness is increased. Six months PP, overall vascular function in PE women recovered to levels comparable to HP and NP.

Brachial artery diameter is significantly higher at the end of pregnancy and a known limitation of FMD is its invers relation to the baseline diameter of the measured artery [4,9,34]. Normalising FMD values for baseline diameter (FMD%index) [2] does not seem to correct for this influence [21]. The proposed parameter, L-FMC, attempts at addressing this limitation [13]. Regarding this 'resting' endothelial function we found a substantial L-FMC increase with advancing pregnancy which has never been described. Literature on L-FMC in pregnancy is very scarce and in the NP population discussion remains whether an increase in L-FMC is a compensation rather than a physiologic improvement of endothelial function [12,13]. Most studies on L-FMC are performed on the radial artery and studies on the brachial artery suggest that the presence of brachial L-FMC varies considerably among a diverse population [34,38]. Brachial artery FMD, however, is the gold standard for non-invasive assessment of endothelial function and simultaneously measuring L-FMC has large advantages [33]. Weissgerber et al. described a significant L-FMC in a small subgroup of active pregnant women ($n = 15$). In non-active pregnant women and NP women, there was no L-FMC observed in the brachial artery. Similarly, in our small NP population, L-FMC was absent in 73% of cases, while this was only the case in 15% of third trimester HP.

This study adds to the existing studies of vascular function in PE by investigating L-FMC as a novel parameter of endothelial function in a substantial group of early and severe PE women. L-FMC has previously been investigated in 8 cases of mild and late PE, without finding significant vasoconstriction during the occlusion phase [35]. Our results indicate that L-FMC is significantly attenuated in PE, which renders an inability to vasoconstrict to a low-flow stimulus. While an enhancement in L-FMC seems to be a manifestation of HP, L-FMC values in PE are comparable to the NP population, evidencing the hypothesis that PE is a maladaptation to pregnancy. This hypothesis becomes more and more supported by recent research [17,23]. Another explanation might be that the absence of L-FMC is due to the increased vascular stiffness in PE and acts as a protection mechanism to avoid severe hypertension. Since correlation between FMD and L-FMC is clearly absent, L-FMC describes a completely different aspect of endothelial function, which might fill the missing gap in PE.

PE is undoubtedly associated with a deterioration in vascular function. Compared to the third trimester of HP, PE women express an overall decrease in vascular and endothelial function [6,15,18,21,31]. Surprisingly, a larger baseline brachial artery diameter was found in PE, probably due to anti-hypertensive medication. However, the correlation between baseline diameter and FMD is absent in this population, rejecting the hypothesis that FMD is worse due to this increase in baseline diameter. Literature on the effect of antihypertensive medication on FMD in NP population, claims that FMD is interpretable despite recent intake of anti-hypertensives [11]. The majority of PE women in this study were on beta-blockers at the time of measurements. While calcium channel blockers (felodipine, nifedipine) and third generation beta-blockers (labetalol) are known to improve endothelial function [10,28], most studies are performed after chronic (> 1 month) medication intake, whilst this was not the case in our PE population. In our group, we did not find a difference between PE women that were or were not on beta-blockers in terms of L-FMC, FMC and arterial stiffness. In literature, no information is available on the effect of MgSO4 on endothelial function. Concerning LMWH, a recent study describes an acute improvement in FMD three hours after intravenous plus subcutaneous administration of enoxaparin. Our PE population received a significant lower dose of enoxaparin daily, however an effect on our FMD results is likely to be present [22].

Few prior studies were able to implement PP follow-up. Interestingly, and in contrast with our study hypothesis, differences between healthy and PE PP women were small. Interestingly, while FMD improved 6 months after a PE pregnancy, L-FMC, on the other hand, did not significantly change PP. This finding questions the influence of anti-hypertensive medication on L-FMC in PE.

Despite these novel findings, our study has some limitations. First, there is a small but significant difference in parity between HP and PE. Second, only a subgroup of our study population was measured PP. Last, PE women were already on medication during their vascular assessment, possibly influencing our results.

The main strength of our study is the longitudinal design in HP. This way we were able to investigate and understand the physiological changes in resting and recruitable endothelial function related to pregnancy itself before comparing them to preeclamptic pregnancies. To our knowledge, L-FMC has not been evaluated previously longitudinally in normal pregnancy, nor in a large group of early PE women. Furthermore, we have studied the association between L-FMC and other vascular function tests, proving that L-FMC clearly reflects an adaptation in HP which is absent in PE.

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Competing interests

None of the authors have competing interests to declare.

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