OBSTETRICS

Preeclampsia in healthy women and endothelial dysfunction 10 years later

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OBJECTIVE: Recent studies have shown that women with a history of preeclampsia have an increased risk of cardiovascular disease. The present study investigated cardiovascular risk factors 10 years after preeclampsia in previously healthy women.

STUDY DESIGN: Based on data from the Medical Birth Registry in Norway, we selected 182 women with and 180 women without preeclampsia in their first pregnancy 9-11 years earlier, excluding women with cardiovascular or renal disease before pregnancy. Flow-mediated dilation of the brachial artery (FMD) and intima-media thickness (IMT) of the carotid artery were measured and blood samples were drawn. Blood samples were analyzed for cardiovascular risk markers and for circulating markers of endothelial function.

RESULTS: A total of 89 women with previous preeclampsia and 69 women without preeclampsia participated, an overall attendance rate of 44%. FMD and IMT were similar between groups. Women with previous preeclampsia more often had urate and soluble fms-like tyrosine kinase values above the 75th percentile (odds ratio [OR], 2.4; $P = .03$, and OR, 2.4; $P = .04$, respectively) and high-density lipoprotein cholesterol values below the 25th percentile (OR, 2.3; $P = .04$). Women with preeclampsia with low birthweight offspring were associated with asymmetric dimethylarginine, L-arginine, and homoarginine above the 75th percentile, whereas the women with preeclampsia with normal-weight offspring were associated with urate and soluble fms-like tyrosine kinase above the 75th percentile.

CONCLUSION: Preeclampsia was not associated with impaired FMD or increased IMT 10 years after pregnancy in previously healthy women, but preeclampsia was associated with changes in circulating markers that might represent early endothelial dysfunction.

Key words: asymmetric dimethylarginine, endothelial dysfunction, flow-mediated dilation of the brachial artery intima-media thickness, preeclampsia, urate

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Several studies have shown that preeclampsia is associated with an increased risk of later cardiovascular disease[1-5] and that women with previous preeclampsia have increased blood pressure, body mass index, insulin resistance, and endothelial dysfunction several years after their preeclamptic pregnancy, as compared with women without preeclampsia.[6-12]

Endothelial dysfunction is regarded as a central factor in the pathophysiology of preeclampsia,[13,14] and recent discoveries have elucidated the process of maternal endothelial damage, in which antiangiogenic factors produced by the placenta seem to play an important role.[15-17] Soluble fms-like tyrosine kinase (sFlt-1) and placental growth factor (PlGF) have been shown to be important, and probably causal, factors in the widespread endothelial dysfunction that accompany preeclampsia.[15,16] Other important factors involved may be urate, shown to be strongly associated with the development of preeclampsia and also a marker of cardiovascular risk,[18-20] and asymmetric dimethylarginine (ADMA),

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Preliminary findings were presented at the combined 47th Congress of the European Renal Association and the European Dialysis and Transplant Association and the 2nd Congress of the German Nephrology Society, Munich, Germany, June 25-28, 2010.

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an endogenous inhibitor of the nitric oxide pathway and a promising novel cardiovascular risk marker.[21-23]

Endothelial dysfunction is also believed to be involved in the development and progression of atherosclerosis and kidney disease,[24,25] and endothelial dysfunction may be the unifying link between preeclampsia and later cardiovascular and renal disease.[26] Women with previous preeclampsia have been found to have manifestations of endothelial dysfunction several years after their preeclamptic pregnancy,[7,9,27] but several of the previous studies have investigated women with known underlying predisposing conditions and/or severe preeclampsia.[28-30]

In the present population-based study, we wanted to investigate whether preeclampsia in previously healthy women was associated with signs of endothelial dysfunction 10 years after the preeclamptic pregnancy. We chose a variety of markers of endothelial dysfunction, both functional (flow-mediated dilatation [FMD]) and structural measurements (intima-media thickness [IMT]) and different serum biomarkers related to preeclampsia, endothelial function, and inflammation. We also analyzed preeclampsia with and without low birthweight offspring separately because women with severe preeclampsia with low birthweight offspring may have a different pathogenesis than women with preeclampsia with normal-birthweight offspring. Our hypothesis was that preeclampsia would be associated with impaired FMD and increased IMT and with circulating markers of endothelial dysfunction.

Subjects and Methods

Registries

Medical data on all births in Norway are forwarded to the Medical Birth Registry of Norway by compulsory notification.[31] The notification form includes extensive data on the mother and the newborn and is completed by the attending midwife and doctor. The regional ethics committee approved the study.

Study design

The study design has been described in more detail in another paper.[32] We identified women living in the Bergen (Norway) area (population count approximately 325,000) with their first pregnancy in the years 1998-2000. Those diagnosed with diabetes, rheumatic disease, essential hypertension, or renal disease before first pregnancy and those with later preeclamptic pregnancies were excluded. From these, we invited women with and without preeclampsia in their first pregnancy, the latter matched on age, year of first birth, and municipality but otherwise randomly selected as a control group. The number of eligible women for inclusion was 182 in the preeclampsia group and 180 in the control group.

The women who agreed to participate were examined between December 2009 and October 2010. The participants were instructed to be overnight fasting and to abstain from hard exercise and high-fat foods 24 hours before examination, smoking, and intake of caffeine or any medication on the examination day. Women who were menstruating or had acute illness were rescheduled to a later appointment. On the examination day, a questionnaire was completed, body size was measured, and resting blood pressure was measured manually according to European Society of Hypertension—European Society of Cardiology guidelines.[33]

Vascular measurements

The women were examined in a quiet, temperature-controlled room, between 9:00 AM and 5:00 PM. Measurements of endothelial function by postocclusive FMD of the right brachial artery were done according to guidelines by the International Brachial Artery Reactivity Task Force.[34] The blood pressure cuff was placed on the forearm, and the brachial artery was imaged above the antecubital fossa in the longitudinal plane (identical sites of measurements were ensured using anatomical landmarks and pen marks). Images of the artery were recorded continuously for 5 minutes after cuff deflation. After 10 minutes of rest, a single dose (0.4 mg) of nitroglycerin spray was administered sublingually to determine endothelial-independent vasodilation. Measurements were done at the time of maximum dilation. All images were recorded in end diastole. For imaging, we used a GE Vingmed system (GE Vingmed, Vivid 7; GE, Horten, Norway) with a multiple linear array transducer (6-13 MHz).

The same ultrasound equipment was used for IMT measurements. The distal segment of the common carotid artery was identified, and 5 images were recorded on both sides. For the analysis of IMT, Vivid 7 Dimension '05 semi-automatic software for IMT analysis (GE Vingmed, Vivid 7) was used.

The FMD and IMT measurements were obtained by one trained physician (M.K.S.), and all image measurements and analyses were done in a blinded manner after data collection was concluded. Another trained physician (E.L.) also analyzed a random selection of subject measurements ($n = 20$), and the interobserver variability expressed as 1-way random intraclass correlation coefficient (ICC [1,1]) was 0.96 for baseline measurements and 0.78 for FMD. For IMT, the ICC (1,1) was 0.95.

Blood samples and biomarkers

Serum cholesterols (total/high-density lipoprotein [HDL]/low-density lipoprotein [LDL]), serum triglycerides, serum C-reactive protein (CRP), serum glucose, serum insulin, serum urate, blood hemoglobin A1C (HbA1C), and plasma fibrinogen were analyzed at Haukeland University Hospital laboratory. A semi—high-sensitive assay was used for quantification of CRP, with values specified between 1 and 5. Serum samples were frozen immediately after centrifugation (3000 rpm, 15 minutes) and aliquotation and stored at −80°C. These were later analyzed for soluble vascular cell adhesion molecule-1 (VCAM-1), sFlt-1, PlGF, vascular endothelial growth factor (VEGF), and tumor necrosis factor alpha (TNF-α) (high sensitive) using enzyme-linked immunosorbent assay kits (quantikine human immunoassays) from R&D Systems (Minneapolis, MN). ADMA, symmetric dimethylarginine, neopterin, L-arginine, and homoarginine were measured by BEVITAL AS (Bergen, Norway) in serum

TABLE 1
Characteristics and risk factors according to preeclampsia in first birth 10 years earlier

Characteristic	Women with preeclampsia (n = 89)	Women without preeclampsia (n = 69)	*P* value
Traditional cardiovascular risk factors, mean ± SD			
Age	37.9 ± 4.2	39.0 ± 5.3	.15
Weight, kg	73.9 ± 15.9	73.7 ± 14.8	.95
BMI, kg/m^2	26.7 ± 5.7	26.0 ± 5.1	.44
Waist/hip ratio	0.87 ± 0.05	0.86 ± 0.05	.25
Systolic blood pressure, mm Hg	118 ± 16	114 ± 11	.10
Diastolic blood pressure, mm Hg	73 ± 12	71 ± 9	.18
Physical exercise less than 3 hours per week, n (%)	55 (62)	40 (58)	.63
Current smoking, n (%)	11 (12)	10 (15)	.70
Use of antihypertensive medication, n (%)	4 (5)	2 (3)	.60
Close relative with cardiovascular disease, n (%)[a]	22 (25)	19 (28)	.69
Close relative with hypertension, n (%)[a]	50 (56)	30 (44)	.11
Standard blood tests, mean ± SD			
Insulin, mIE/L	3.70 (<2.00 to 5.50)[b]	2.60 (<2.00 to 5.20)[b]	.17
HOMA-IR (insuline resistance)[b]	0.79 (0.42-1.25)	0.58 (0.42-1.05)	.29
Glucose, mmol/L	4.98 ± 1.02	4.85 ± 0.38	.30
HbA1C, %	5.32 ± 0.43	5.28 ± 0.29	.54
Urate, μmol/L	255.67 ± 56.76	239.35 ± 41.24	.046
CRP, mg/L	1.87 ± 3.01	1.71 ± 3.25	.76
Fibrinogen, g/L	2.96 ± 0.53	3.04 ± 0.53	.38
Creatinine, μmol/L	60.84 ± 8.28	62.71 ± 8.60	.17
Cholesterol total, mmol/L	4.84 ± 0.89	4.87 ± 0.80	.82
HDL, mmol/L	1.51 ± 0.36	1.63 ± 0.39	.056
LDL, mmol/L	3.00 ± 0.79	2.92 ± 0.67	.49
Triglycerides, mmol/L	0.98 ± 0.54	0.91 ± 0.51	.40
Vascular measurements, mean ± SD			
FMD, %	8.28 ± 3.68	8.21 ± 4.02	.90
Nitro-mediated arterial dilation, %	22.47 ± 5.89	22.60 ± 7.57	.91
Mean IMT left/right, mm[a]	0.49 ± 0.07	0.50 ± 0.06	.67
Biomarkers, mean ± SD			
ADMA, μmol/L	0.51 ± 0.08	0.50 ± 0.07	.37
SDMA, μmol/L	0.60 ± 0.11	0.59 ± 0.10	.63
L-arginine, μmol/L	109.54 ± 19.31	109.56 ± 18.96	1.00
Homoarginine, μmol/L	2.10 ± 1.00	1.82 ± 0.65	.050
VCAM-1, ng/mL	656.42 ± 216.52	678.78 ± 195.29	.53
VEGF, pg/mL	328.03 ± 237.83	300.26 ± 212.24	.46
PlGF, pg/mL	5.70 ± 2.69	5.52 ± 2.66	.72
sFlt-1, pg/mL	65.48 ± 19.73	60.93 ± 18.24	.16

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TABLE 1
Characteristics and risk factors according to preeclampsia in first birth 10 years earlier *(continued)*

Characteristic	Women with preeclampsia (n = 89)	Women without preeclampsia (n = 69)	*P* value
sFlt-1/PlGF ratio	15.54 ± 12.81	16.34 ± 14.39	.76
Neopterin, nmol/L	15.18 ± 4.66	14.55 ± 3.70	.36
TNF-α, pg/mL	1.44 ± 1.34	1.52 ± 2.28	.81

ADMA, asymmetric dimethylarginine; *BMI*, body mass index; *CRP*, C-reactive protein; *FMD*, flow-mediated dilatation; *HbA1C*, blood hemoglobin A1C; *HDL*, high-density lipoprotein; *HOMA-IR*, homeostasis model assessment insulin resistance index; *IMT*, intima-media thickness; *LDL*, low-density lipoprotein; *PlGF*, placental growth factor; *SDMA*, symmetric dimethylarginine; *sFlt-1*, soluble fms-like tyrosine kinase; *TNF-α*, tumor necrosis factor alpha; *VCAM-1*, vascular cell adhesion molecule-1; *VEGF*, vascular endothelial growth factor.

[a] Mean of left and right values; [b] Insulin values <2 μU/mL could not be measured with the assay that was used. Sixty-three women (40%) had values <2 μU/mL. Median values (25% percentile to 75% percentile) are given.

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using liquid chromatography–tandem mass spectrometry, whereas homocysteine was measured in serum using gas chromatography–tandem mass spectrometry.[35]

Exposure variables

Criteria for preeclampsia have been in accordance with recommendations by the American College of Obstetricians and Gynecologists[36] (ie, increased blood pressure after gestational week 20 [blood pressure ≥140/90 mm Hg] associated with proteinuria [≥0.3 g in a 24 hour urine specimen or +1 or greater in a random urinary dipstick]). Birthweight is measured shortly after birth; a weight below 2.5 kg was categorized as low birthweight. The estimation of gestational age was based on routine ultrasonographic examination between gestational weeks 17 and 20. Birth at a gestational age below 37 weeks was defined as preterm.

Statistical analyses

Unpaired Student *t* tests were used to compare mean values for women with and without preeclampsia; values are given as mean (SD). For skewed parameters, Mann-Whitney *U* tests were used to compare median values, and values were given as median (25th percentile, 75th percentile). χ^2 tests were used to compare proportions. Pearson's correlation coefficients were calculated for correlation analyses. Logistic regression analyses were used to obtain unadjusted and adjusted odds ratios for risks of cardiovascular risk factors or markers of endothelial dysfunction above/below the reported thresholds. The analyses were performed using the statistical package SPSS 20.0 for Macintosh (SPSS Inc, Cary, NC).

Results

A total of 89 women with previous preeclampsia and 69 women with a normal first pregnancy participated in the study, resulting in an overall participation rate of 44%, 49% for women with previous preeclampsia and 38% for women without preeclampsia. The remaining women declined the invitation or did not respond. Mean duration from end of first pregnancy to follow-up was 10.9 ± 1.0 years.

Comparison of study participants with population average

We compared characteristics of our participants with the total population of women with their first pregnancy registered in the Medical Birth Registry of Norway and with the same inclusion/exclusion criteria as the participants. Study participants without preeclampsia were older (28.0 vs 26.6 years; $P < .001$) and had slightly more often low birthweight offspring (7.2% vs 3.1%; $P = .05$) and preterm birth (11% vs. 5.2%; $P = .07$) but similar frequency of being single or having a cesarean section as compared with the population average. There were no differences between study participants with preeclampsia, and all women with preeclampsia except that the participants were somewhat older (27.1 vs 26.3 years; $P < .001$).

Using data from Statistics Norway, we examined self-reported educational level between the participating women aged 30-49 years and compared these with the expected number from official statistics. These analyses showed that a higher percentage of our participants had completed a higher education above high school level than in the general population (68% vs 53%; $P < .001$).

Traditional cardiovascular risk factors

There were no differences in body weight, body mass index, or waist/hip ratio between women with or without preeclampsia (Table 1). There was a nonsignificant trend toward higher blood pressure in women with previous preeclampsia as compared with women without preeclampsia (Table 1). Eight women with preeclampsia and 3 women without preeclampsia ($P = .26$) had hypertension defined as blood pressure of 140/90 mm Hg[33] or greater and/or use of antihypertensive medications and/or current hypertension diagnosis (self-report). Further analyses showed that women with preeclampsia more often had systolic blood pressure above the 75th percentile, significant after adjustments (Table 2).

There was a near significantly lower level of HDL and a significantly higher level of urate in women with preeclampsia than in women without preeclampsia (Table 1). Further analyses

TABLE 2
OR for risk factors at examination, according to preeclampsia 10 years earlier

Variable	Total, n	Preeclampsia, n[a]	OR, unadjusted (95% CI)	*P* value	OR, adjusted (95% CI)[b]	*P* value
BMI ≥75th percentile (29.2 kg/m^2)						
No	119	66	1.0 (referent)		1.0 (referent)	
Yes	39	23	1.15 (0.55–2.40)	.70	1.37 (0.64–2.96)	.42
Systolic BP ≥75th percentile (123 mm Hg)						
No	116	61	1.0 (referent)		1.0 (referent)	
Yes	41	27	1.74 (0.83–3.65)	.14	2.34 (0.99–5.54)	.053
HOMA-IR ≥75th percentile (1.2)						
No	119	65	1.0 (referent)		1.0 (referent)	
Yes	39	24	1.33 (0.64–2.78)	.45	1.11 (0.48–2.57)	.81
Urate ≥75th percentile (278 μmol/L)						
No	119	61	1.0 (referent)		1.0 (referent)	
Yes	39	28	2.42 (1.10–5.30)	.027	3.00 (1.16–7.77)	.024
LDL ≥75th percentile (3.5 mmol/L)						
No	117	64	1.0 (referent)		1.0 (referent)	
Yes	41	25	1.29 (0.63–2.67)	.49	1.35 (0.62–2.94)	.46
HDL <25th percentile (1.3 mmol/L)						
No	120	62	1.0 (referent)		1.0 (referent)	
Yes	38	27	2.30 (1.05–5.05)	.038	3.04 (1.08–8.59)	.036
FMD <5%						
No	126	74	1.0 (referent)		1.0 (referent)	
Yes	30	15	0.70 (0.32–1.56)	.39	0.68 (0.29–1.63)	.39
IMT ≥75th percentile (0.54 mm)						
No	115	66	1.0 (referent)		1.0 (referent)	
Yes	40	22	0.91 (0.44–1.87)	.79	1.05 (0.48–2.32)	.90
ADMA ≥75th percentile (0.55 μmol/L)						
No	119	63	1.0 (referent)		1.0 (referent)	
Yes	39	26	1.78 (0.83–3.79)	.14	1.67 (0.74–3.73)	.22
L-arginine ≥75th percentile (123 μmol/L)						
No	119	64	1.0 (referent)		1.0 (referent)	
Yes	39	25	1.54 (0.73–3.24)	.26	1.74 (0.79–3.85)	.17
Homoarginine ≥75th percentile (2.31 μmol/L)						
No	119	63	1.0 (referent)		1.0 (referent)	
Yes	39	26	1.78 (0.83–3.79)	.14	1.76 (0.80–3.89)	.16
sFlt-1 ≥75th percentile (75.1 pg/mL)						
No	107	56	1.0 (referent)		1.0 (referent)	
Yes	36	26	2.37 (1.04–5.39)	.040	2.09 (0.87–5.04)	.099

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(continued)

TABLE 2
OR for risk factors at examination, according to preeclampsia 10 years earlier *(continued)*

Variable	Total, n	Preeclampsia, n[a]	OR, unadjusted (95% CI)	*P* value	OR, adjusted (95% CI)[b]	*P* value
sFlt-1/PlGF ratio ≥75th percentile (19.4)						
No	82	47	1.0 (referent)		1.0 (referent)	
Yes	27	15	0.93 (0.39–2.24)	.87	1.16 (0.44–3.10)	.76

ADMA, asymmetric dimethylarginine; *BMI*, body mass index; *BP*, blood pressure; *CI*, confidence interval; *FMD*, flow-mediated dilatation; *HDL*, high-density lipoprotein; *HOMA-IR*, homeostasis model assessment insulin resistance index; *LDL*, low-density lipoprotein; *OR*, odds ratio; *PlGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase.

[a] Number of women with previous preeclampsia; [b] Adjusted for age, BMI, marital status, annual household income and highest education level.

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showed that women with previous preeclampsia more often had urate values above the 75th percentile and HDL values below the 25th percentile (Table 2). There was a stronger association between high values of urate and preeclampsia with normal-birthweight offspring than preeclampsia with low-birthweight offspring (Table 3).

Vascular measurements

There was no difference in mean FMD between women with or without previous preeclampsia (Table 1). Impaired FMD (<5%) were present in 19% of the women, and preeclampsia was not associated with decreased FMD (Table 2). Nitro-mediated arterial dilation was also not significantly different between the 2 groups. Mean IMT of the carotid artery was similar in women with and without previous preeclampsia (Table 1), and preeclampsia was not associated with an IMT at the 75th percentile or greater (Table 2).

Systemic markers of endothelial function

There were no differences in mean values of ADMA, symmetric dimethylarginine, or L-arginine (Table 1), but mean values of homoarginine were higher in women without preeclampsia. Preeclampsia was, however, associated with having sFlt-1 values above the 75th percentile, and there were similar trends seen for ADMA, L-arginine, and homoarginine above the 75th percentile (Table 2). For values of ADMA and L-arginine above the 75th percentile, the associations were significantly stronger for women with preeclampsia with low-birthweight offspring than for women with preeclampsia with normal-weight offspring. sFlt-1 values were, on the other hand, associated with women with normal birthweight preeclampsia and not with women with preeclampsia with low-birthweight offspring. All associations remained significant after adjustments. No associations were seen between preeclampsia and arginine or homoarginine levels below the 25th percentile.

There was a significant positive correlation between ADMA and urate, ADMA and L-arginine, ADMA and body mass index (BMI), urate and BMI, and urate and homeostasis model assessment insulin resistance index (HOMA-IR) and a negative relationship between ADMA and HDL. There was no significant correlation between ADMA and FMD. Investigating separately women with and without previous preeclampsia, correlations between ADMA and urate, ADMA and BMI, and ADMA and HDL were only significant for women with previous preeclampsia (Figure).

Comment

The present study appears to be one of the larger long-term follow-up studies that investigate a broad range of cardiovascular risk factors in otherwise healthy women with a history of preeclampsia only in their first pregnancy. The most important finding in our study is that previous preeclampsia was not associated with several important cardiovascular risk factors, including BMI, HOMA-IR, FMD, and IMT. Previous preeclampsia was, however, associated with subtle markers of cardiovascular risk and endothelial dysfunction such as HDL cholesterol, urate, ADMA, and sFlt-1. Interestingly, women with preeclampsia with low-birthweight offspring were associated with higher values of ADMA, L-arginine, and homoarginine, whereas women with preeclampsia with normal-weight offspring were associated with higher values of sFlt-1 and urate.

The relatively low participation rate of 44% represents a weakness in this study, but it is comparable with other studies.[8,28] Our participants were more educated than the general population, and compared with other European studies on previous preeclampsia, our women had somewhat lower blood pressure but similar BMI.[10,27,30,37] Furthermore, the participants with previous preeclampsia had a similar prevalence of preterm birth, low-birthweight offspring, and other pregnancy complications compared with the population average, indicating that our findings of similar FMD and IMT could not be explained by our participants having a less severe preeclampsia than average. A potential selection bias cannot be ruled out, but this possibility seems to be fairly moderate.

The present study is mainly negative because previously reported differences between women with and without previous preeclampsia could not be demonstrated. The sample sizes were, however, chosen to be able to detect a difference in prevalence of microalbuminuria[32] and exceeded those required to detect previously reported differences in markers of endothelial dysfunction.

TABLE 3
OR for cardiovascular risk factors according to PE with or without LBW offspring at first birth

Variable	Total, n	PE, n[a]	OR, unadjusted (95% CI)	*P* value for trend	OR, adjusted (95% CI)[b]	*P* value for trend
Urate ≥75th percentile						
No PE	69	11	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	71	25	2.87 (1.28–6.43)	.21	3.67 (1.36–9.92)	.16
PE with LBW offspring	18	3	1.06 (0.26–4.26)		1.23 (0.24–6.47)	
HDL <25th percentile						
No PE	69	11	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	71	21	2.22 (0.97–5.04)	.046	2.58 (0.86–7.72)	.018
PE with LBW offspring	18	6	2.64 (0.82–8.52)		5.44 (1.22–24.21)	
FMD <5%						
No PE	67	15	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	71	11	0.64 (0.27–1.51)	.63	0.61 (0.24–1.55)	.67
PE with LBW offspring	18	4	0.99 (0.28–3.46)		0.99 (0.27–3.57)	
ADMA ≥75th percentile						
No PE	69	13	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	71	18	1.46 (0.65–3.28)	.039	1.30 (0.55–3.10)	.049
PE with LBW offspring	18	8	3.45 (1.14–10.44)		3.70 (1.15–11.87)	
L-arginine ≥75th percentile						
No PE	69	14	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	71	16	1.14 (0.51–2.57)	.039	1.28 (0.54–3.01)	.022
PE with LBW offspring	18	9	3.93 (1.32–11.74)		4.48 (1.43–14.00)	
Homoarginine ≥75th percentile						
No PE	69	13	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	71	19	1.57 (0.71–3.50)	.072	1.54 (0.67–3.56)	.080
PE with LBW offspring	18	7	2.74 (0.89–8.43)		2.77 (0.88–8.66)	
sFlt-1 ≥75th percentile						
No PE	61	10	1.0 (referent)		1.0 (referent)	
PE without LBW offspring	65	23	2.79 (1.20–6.52)	.24	2.45 (0.99–6.07)	.40
PE with LBW offspring	17	3	1.09 (0.26–4.52)		1.04 (0.24–4.50)	

ADMA, asymmetric dimethylarginine; *BMI*, body mass index; *CI*, confidence interval; *Flt-1*, fms-like tyrosine kinase; *FMD*, flow-mediated dilatation; *LBW*, low-birthweight offspring; *OR*, odds ratio; *PE*, preeclampsia.

[a] Number of women with previous preeclampsia; [b] Adjusted for age, marital status, BMI, annual household income, and highest education level.

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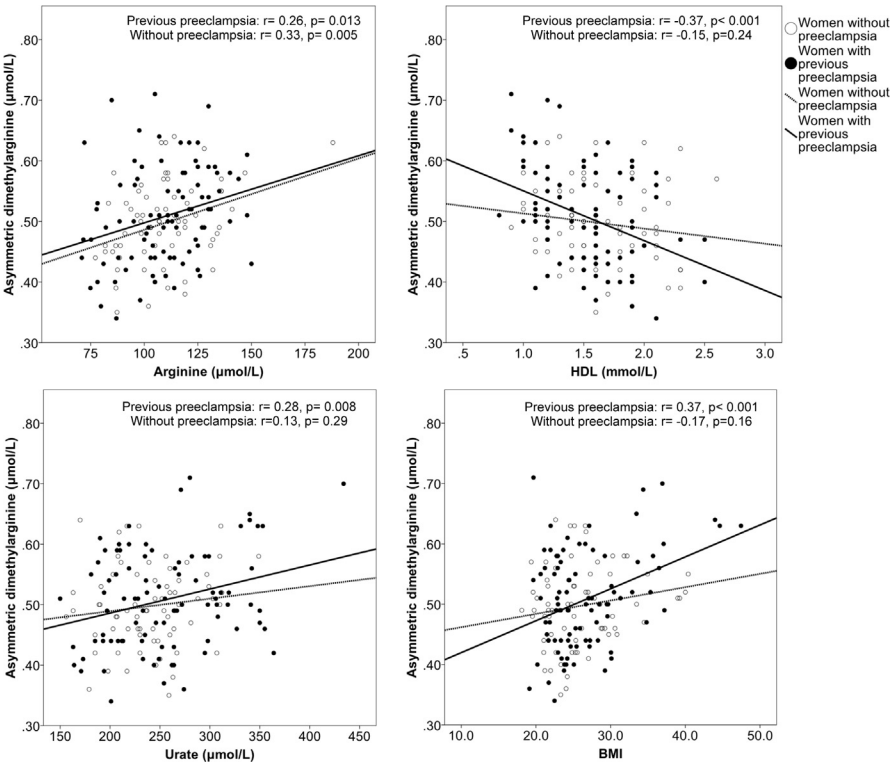
Strengths of the present study include the large number of participants, considerably larger than in most previous studies,[26,28,38] a population-based selection, and the long follow-up time. The main outcomes, endothelial dysfunction and IMT, were measured using well-documented methods; one operator did all examinations and measurements, and control analyses by another operator showed an interobserver variability similar to that reported in other studies,[39] with ICC values indicating excellent interrater agreement.[40] Our main exposure variable, preeclampsia, was well documented, only women with preeclampsia in first pregnancy were included, and the severity of preeclampsia in our participants was similar to the population average. Only Norwegian women without diabetes, hypertension, renal disease, or rheumatic

FIGURE
Relationship between L-arginine, HDL, BMI and urate, and ADMA



Scatterplots and correlations showing the relationship between L-arginine, HDL, BMI and urate, and ADMA.

ADMA, asymmetric dimethylarginine; *BMI*, body mass index; *HDL*, high-density lipoprotein.

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disease before first pregnancy were included, and the results should not be transferred to other populations without caution.

Most similar studies have demonstrated impaired endothelial function in women with previous preeclampsia,[8,41] and there are also reports of an increased IMT.[29,42] There are several factors that might explain why our findings differ from what we expected. The most important is probably that we included only healthy women with preeclampsia severity at population level; for example, only 16% of women with preeclampsia had a preterm birth, whereas most previous studies included higher proportions with severe preeclampsia.[28,29,43]

An important factor may also be that we included women 10 years after their preeclamptic pregnancy. This might be a time when the endothelial function has fully recovered after the preeclamptic pregnancy but too early for our healthy, mostly premenopausal women to have developed detectable cardiovascular abnormalities. Furthermore, several different methods have been used to measure endothelial function in previous studies, and this makes it difficult to compare findings. Most previous studies have been small with somewhat equivocal results,[9,43,44] and some smaller negative studies have also been published.[38,44] Results from a recent large, population-based study on Norwegian women also suggest that previous preeclampsia might not be as strongly associated with cardiovascular risk and death as has been assumed.[45]

The observed differences in levels of HDL, urate, sFlt-1, ADMA, L-arginine, and homoarginine in women with previous preeclampsia might, however, reflect subtle vasculopathic processes. sFlt-1 is produced by invasive cytotrophoblasts during the first trimester, and increased expression and circulating levels of this factor seem to be central in the pathophysiological development of preeclampsia.[15,16] sFlt-1 is also produced by other tissues, like the glomerular endothelium, and could possibly induce endothelial dysfunction, even after pregnancy by inhibition of VEGF and PlGF signalling.[16,46,47] ADMA, being an endogenous inhibitor of endothelial nitric oxide by competing with L-arginine in the production of nitric oxide,[48] is an important marker of endothelial dysfunction and has been found to be increased in women with preeclampsia.[21-23]

That severe preeclampsia was associated with high levels of both ADMA and L-arginine was somewhat surprising but not unprecedented: similar findings have been made in women with present and previous preeclampsia.[21,22,26] We speculate that the concomitant high L-arginine and homoarginine levels in women with previous severe preeclampsia might reflect regulatory processes counteracting the effects of ADMA and may partly explain the similarities in FMD and IMT between women with and without a history of preeclampsia.

Low HDL and high urate are well-known cardiovascular risk markers and related to the metabolic syndrome.[49] Urate has been proposed to be involved in the development of,[50] and is increased in women with, preeclampsia.[51] During pregnancy, high urate levels seem to be associated with preterm birth and low-birthweight offspring in women with gestational hypertension.[52] We made the unexpected finding that preeclampsia with normal-birthweight offspring and not preeclampsia with low-birthweight offspring was associated with high urate levels. High urate levels 10 years after pregnancy were in our study associated with increased BMI and insulin resistance, which during pregnancy seem to be associated with increased offspring birthweight,[53] a trend that was observed but far from significant in our study.

Our findings of associations between preeclampsia and circulating biomarkers

are weakened by multiple testing and are of uncertain importance. It is, however, interesting to notice the relation to endothelial dysfunction, and it is biologically plausible that these markers could be associated with preeclampsia.

In the present study of otherwise healthy women, preeclampsia was not associated with impaired endothelial-dependent arterial dilation or increased IMT, but preeclampsia was associated with changes in circulating factors that might represent subtle endothelial dysfunction, such as higher levels of urate, and sFlt-1 and lower levels of HDL. Preeclampsia with low-birthweight offspring was associated with higher values of ADMA and L-arginine.

Our findings indicate that preeclampsia might not have such a damaging effect on future health as previously assumed, at least not in healthy women without other predisposing conditions, and might contribute to an adjustment of the strong focus on adverse health effects later in life for women with preeclampsia. If most women with preeclampsia have a good prognosis with little or no negative impact on their future health, the negative focus may be an unnecessary added stress factor.

Our study demonstrates the importance of more population-based studies on cardiovascular dysfunction several years after a preeclamptic pregnancy, especially in otherwise healthy women without obesity, diabetes, or hypertension. Future studies on biomarkers should evaluate the underlying mechanisms for the different associations with previous preeclampsia with and without low birthweight. ■

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