



# Dimethylarginines correlate to common carotid artery wall layer dimensions and cardiovascular risk factors in pregnant women with/without preeclampsia: A group comparative study

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## ABSTRACT

**Objectives:** Asymmetric- and symmetric dimethylarginines (ADMA, SDMA) are elevated in cardiovascular disease (CVD). Preeclampsia is a pregnancy-specific syndrome and is an independent risk factor for subsequent CVD. Aims were to investigate whether ADMA, SDMA levels and L-arginine/ADMA and L-arginine/SDMA ratios during pregnancy and their changes from pregnancy to postpartum are associated to arterial wall layer dimensions and cardiovascular risk factors in women with and without preeclampsia.

**Study design:** Dimethylarginines were analyzed by LC–MS, and the common-carotid-artery (CCA) intima and media thicknesses were estimated using 22-MHz non-invasive ultrasonography in women with preeclampsia (cases = 48) and normal pregnancies (controls = 58) in similar gestational age, with reassessment one-year postpartum. A thick intima, thin media and high intima/media ratio (I/M) indicates a less healthy arterial wall.

**Results:** The median age of cases and controls was 30 years. During pregnancy, women with preeclampsia had higher plasma ADMA, SDMA and lower L-arginine/ADMA and L-arginine/SDMA (all  $p < 0.01$ ) than women with normal pregnancies. Further, ADMA, SDMA, L-arginine/ADMA and L-arginine/SDMA correlated to intima thickness ( $r_s = 0.33/0.33/-0.33/-0.35$  and  $p < 0.01$ ), I/M ( $r_s = 0.26/0.28/-0.22/-0.26$  and  $p < 0.05$ ) and mean arterial pressure (MAP) ( $r_s = 0.43/0.42/-0.39/-0.40$  and  $p < 0.0001$ ). Changes in ADMA, SDMA and L-arginine/SDMA from pregnancy to postpartum correlated to changes in intima thickness ( $r_s = 0.22/0.32/-0.21$  and  $p < 0.05/<0.01/<0.05$ ), I/M ( $r_s = 0.22/0.31/0.08$  and  $p < 0.05/<0.01/=0.43$ ) and MAP ( $r_s = 0.31/0.53/-0.25$  and  $p < 0.01/<0.001/<0.05$ ). No correlations were found for conventional CCA intima-media-thickness.

**Conclusions:** Dimethylarginines were associated to signs of adverse effects on arterial wall layer dimensions and cardiovascular risk factors in women with and without preeclampsia, during pregnancy and to their changes from pregnancy up to one-year postpartum.

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## Introduction

Dimethylarginines, asymmetric (ADMA) and symmetric (SDMA) are naturally occurring amino acids in plasma and generated by degradation of methylated protein as a result of

protein arginine methyltransferase [1]. Dimethylarginines is a direct endogenous inhibitor of nitric oxide synthase (NOS), which leads to decreased nitric oxide (NO) synthesis. Dimethylarginines may also reduce NO synthesis indirectly by inhibiting the cellular uptake of the NO precursor L-arginine [1]. NO is a potent vasodilator, generated in the vessel endothelium from L-arginine by the NOS enzyme [1]. Thus, elevated dimethylarginines levels are associated with decreased NO levels, which leads to endothelial dysfunction and development of atherosclerosis [2,3]. Plasma levels of dimethylarginines are increased in patients with cardiovascular disease (CVD) [4,5].

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Preeclampsia (PE) complicates 3–5 % of all pregnancies [6]. Risk of subsequent CVD is higher in women with previous PE [7–9]. Preeclampsia is associated with higher levels of dimethylarginines compared to normal pregnancy (NP) [10,11], whereas NP, especially in the beginning, is associated with lower levels of dimethylarginines compared to nonpregnant women, to facilitate NO's activities like hemodynamic adaptation to pregnancy and uterine relaxation [12]. Elevated dimethylarginines [13] together with exaggerated inflammation in PE [14] lead to endothelial dysfunction, underlying most of the symptoms of PE. Studies have shown that L-arginine/ADMA ratio, rather than only ADMA levels is the key determinant of NOS activity [15], and is a useful index for interpretation of effects of ADMA [16].

We previously showed that the imaging of CCA (common carotid artery) by 20–25 MHz ultrasound and the use of intima thickness and intima/media thickness ratio (I/M), instead of CCA-IMT (intima-media thickness) better image vascular effects in those with prevalent CVD, diabetes mellitus, hypertension and hyperlipidemia [17–19], as well as more subclinical atherosclerosis in women with PE, at diagnosis, at one-year postpartum and at seven-year follow-up [20,21].

Association between dimethylarginines and CVD has been studied extensively using CCA-IMT with varying results [22–24]. We aimed to investigate whether dimethylarginines during pregnancy and their changes from pregnancy up to about one-year postpartum are associated to CCA wall layer dimensions and cardiovascular risk factors in women with and without PE, because such studies are lacking.

## Methods

### Study population

The study was designed as a longitudinal, group comparative study. The material has been used in our previous publication, regarding artery wall layer dimensions [20], and later also regarding association of pentraxin-3 [25] and angiogenic factors [26] to artery wall layer dimensions, in women with PE. Participants were recruited between 2007 and 2010 at the Uppsala University hospital and the method of recruitment used has been extensively described previously [20,25,26]. Cases were pregnant women with PE and they were included only when a diagnosis of PE was confirmed. At that time, PE was defined as new-onset hypertension ( $\geq 140$  and/or  $\geq 90$  mm Hg, observed on at least two separate measurements  $\geq 6$  h apart), combined with proteinuria ( $\geq 2$  on a dipstick or a 24 h urine sample showing leakage of  $\geq 300$  mg albumin/24 h),  $>20$  gestational week [27]. Preeclampsia was defined as early-onset PE (EOPE) if diagnosis was confirmed  $<34$  gestational week, otherwise it was as late-onset PE (LOPE).

Women in the control group were recruited in a longitudinal study of the CCA wall layers in women with NP [28]. If pregnancy was normotensive and resulted in delivery in gestational week  $\geq 37$  of a normal weight infant, this was defined as NP. Controls were matched to cases for  $\pm 2$  gestational weeks. For both cases and controls, exclusion criteria were chronic hypertension, renal disease, pre-gestational or gestational diabetes mellitus.

### Assessment at inclusion and postpartum

Data on maternal demographics were recorded at inclusion (during pregnancy) and about one-year postpartum. High blood pressure (BP) and obesity are two modifiable risk factors with regard to the development of CVD [29]. Maternal height, weight and BP were monitored during both assessments. Blood pressure was measured (Umedico apparatus) according to standard procedure, with an appropriate calf-size for the arm

circumference. Accuracy of mean arterial pressure (MAP) is better than systolic BP (SBP) or diastolic BP (DBP) in predicting PE [30] and was calculated as  $DBP + \frac{1}{3}(SBP - DBP)$ . Data about possible pregnancy-related complications and pregnancy outcomes were collected through reviewing the medical records. Infants born with birth weights  $>2$  SDs below or above the mean birth weight for gestational age were defined as small- or large- for gestational age infants respectively [31].

### Bioanalytical method

Blood samples were taken in a heparinized tube at both assessments. None of the participants were in active labor, nor did they have rupture of membranes or signs of infection at the time of blood sampling. The samples were centrifuged for 10 min at 2000g and then the plasma samples were separated and stored at  $-70^\circ\text{C}$  until levels of dimethylarginines were analysed.

The determination of plasma levels of ADMA, SDMA and L-arginine was performed with liquid chromatography – tandem mass spectrometry (LC-MS/MS) at the National Veterinary Institute in Uppsala, Sweden. The calibration of all three analytes was performed using the peak area ratio of analyte/internal standard, using a linear regression with the weight  $1/x^2$ . ADMA had a measurement interval of 0.09–3.4  $\mu\text{M}$  and a relative standard deviation (RSD%) in measured concentration of (precision) 5.3–7.3 %. SDMA had a measurement interval of 0.38–3.0  $\mu\text{M}$  with a precision of 5.8–9.3 %. L-arginine had a measurement interval of 4.5–150  $\mu\text{M}$  and a precision of 3.5–6.2 %. A detailed description of analytical procedures is available in a previous study [32] and in the supplementary material.

### Ultrasound imaging of the CCA

Separate estimates of left CCA intima and media layers were made with a broadband probe with 22 MHz center-frequency (Collagenoson, Minhorst Company, Meudt, Germany) (Figure S1). A detailed description of the method has been given in our previous reports [17,18]. Ultrasound evaluation was not adjusted to the cardiac cycle. About 20 images were saved on a PC by one researcher Marita Larsson (ML) and analysed off-line by another researcher Tansim Akhter (TA), the latter being blinded with regards to the identities within the study group and time of assessment. Means of about 10 technically acceptable measurements were calculated and used in the analysis. The coefficient of variation in our laboratory was 3.9 % for intima thickness and 3.4 % for media thickness [17].

### Outcomes

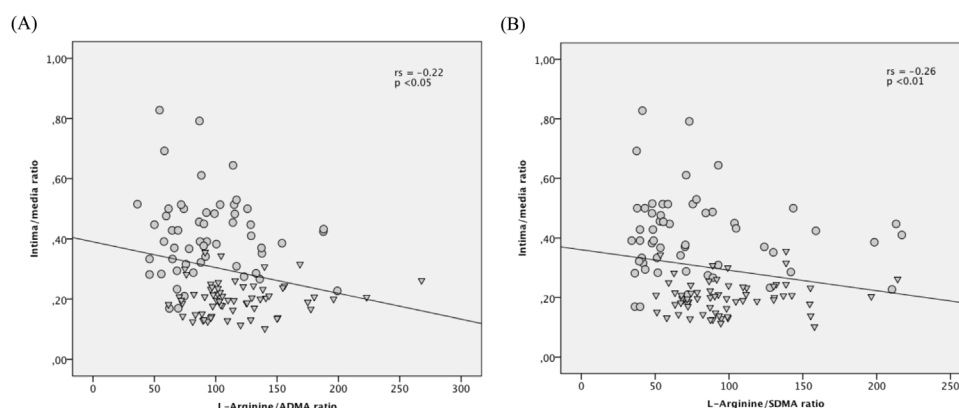
To investigate whether dimethylarginines during pregnancy and their changes from pregnancy up to about one-year postpartum are associated to arterial wall layer dimensions and cardiovascular risk factors in women with and without PE.

### Ethical considerations

The study protocol was approved by the local Ethics Committee of the Medical Faculty of Uppsala University, Dnr 2006/259. Informed written consent was obtained from each woman before their inclusion.

### Statistical analysis

The results are presented as median with interquartile ranges. Differences in distributions were tested by Chi-square tests. Between-group differences were tested using Mann-Whitney U



**Fig. 1.** Correlations between common carotid artery intima/media ratio vs. plasma levels of (A) L-arginine/ADMA and (B) L-arginine/SDMA ratios during pregnancy in women with preeclampsia (circles) and in women with normal pregnancies (triangles), assessed using the Spearman Rank Correlation test. *rs*, correlation coefficient.

**Table 1**

Plasma levels of dimethylarginines during pregnancy and at postpartum in the combined groups.

Characteristics	Preeclampsia		Normal Pregnancies	
	During pregnancy (n = 48)	One-year postpartum (n = 48)	During pregnancy (n = 58)	One-year postpartum (n = 58)
ADMA (micm/L)	0.50 (0.41, 0.61) *	0.47 (0.38, 0.57) †	0.40 (0.36, 0.44) ‡	0.52 (0.43, 0.62)
SDMA (micm/L)	0.61 (0.52, 0.76) §, ¶	0.46 (0.41, 0.52)	0.48 (0.40, 0.58) ¶	0.53 (0.47, 0.68)
L-Arginine (micm/L)	39 (32, 54) ‡	71 (53, 83) †	44 (38, 50) ‡	87 (64, 107)
L-Arginine/ADMA	87 (66, 117) *, ¶	143 (119, 187)	104 (93, 139) ‡	161 (127, 186)
L-Arginine/SDMA	67 (43, 89) #, ¶	153 (120, 178)	92 (73, 122) ‡	151 (123, 184)

Medians (interquartile ranges). ADMA, asymmetric dimethylarginine; micm/L, micromole/liter; SDMA, symmetric dimethylarginine.

\* $p < 0.01$ , † $p < 0.05$ , § $p < 0.0001$  and # $p < 0.001$  compared to corresponding period in women with normal pregnancy, after adjustment for age, BMI and gestational length at inclusion except ‡ and ||.

‡ $p < 0.01$  and ¶ $p < 0.0001$  compared to postpartum values within the group.

test (adjusted for age, BMI and gestational length at inclusion, not for BP as the BP is a criterion for diagnosis of PE) and within-group differences using the Wilcoxon signed rank test. Spearman rank correlation test was used for test of correlation on the combined group (PE and NP), justified by substantial overlapping between study groups with regard to dimethylarginine levels (Fig. 1). The 95 % confidence interval was calculated using percentile Bootstrap confidence interval based on 1000 samples. The level of significance was set at  $p$  value  $< 0.05$ . Statistical analysis was performed using the SPSS, version 22.0 (SPSS Inc. PASW statistics) for Windows software package.

## Results

As extensively described in our previous study [20], Table S1 shows the demographic data of the study population. At baseline, 55 women with PE and 64 women with NP were included. At the postpartum examination, 48 women in the PE group and 58 women in NP group remained for evaluation, thereby enabling our aim to evaluate the changes from pregnancy to postpartum.

At inclusion, 46/48 women had antihypertensive medication, however no one needed to continue the treatment six weeks after delivery. As we showed in our previous publication [20], cardiovascular risk factors, *i.e.*, BMI, SBP, DBP and MAP were significantly higher in women with PE than in women with NP, both during pregnancy and at about one-year postpartum (Table S2). Further, women with PE had significantly thicker intima, thinner media and higher I/M than women with NP, but no difference in CCA-IMT (Table S2) [20].

During pregnancy, after adjustment for age, BMI and gestational length, women with PE had significantly higher plasma ADMA ( $p < 0.01$ ), SDMA ( $p < 0.0001$ ), and lower L-arginine/ADMA

( $p < 0.01$ ) and L-arginine/SDMA ( $p < 0.001$ ), than women with NP. In women with PE, there was a reduction of ADMA, and especially SDMA levels from pregnancy to postpartum, whereas these levels had increased in women with NP. Levels of L-arginine and L-arginine/ADMA and L-arginine/SDMA had increased from pregnancy to postpartum for both PE and NP groups. However, at postpartum, the levels of L-arginine were higher in women with NP than in women with PE (Table 1). In sub-analysis, after adjustment for gestational length at inclusion, there was no differences in dimethylarginines during pregnancy between EOPE and LOPE (data not shown).

During pregnancy, for the combined groups, there was a positive correlation between ADMA and SDMA levels vs. intima thickness ( $rs = 0.29/0.33$  and  $p < 0.01/<0.001$  respectively); I/M ( $rs = 0.24$  and  $0.30$  respectively and  $p < 0.01$  for both); BMI ( $rs = 0.19$  and  $p < 0.05$  for both); SBP ( $rs = 0.38/0.39$  respectively and  $p < 0.0001$  for both); DBP ( $rs = 0.41/0.38$  respectively and  $p < 0.0001$  for both) and MAP ( $rs = 0.42/0.40$  respectively and  $p < 0.0001$  for both) (Table 2). Consequently, L-arginine/ADMA and L-arginine/SDMA showed a negative correlation vs. intima thickness ( $rs = -0.33/-0.35$  and  $p < 0.001/<0.0001$  respectively); I/M ( $rs = -0.22/-0.26$  and  $p < 0.05/<0.01$  respectively) (Fig. 1) (Table 2); BMI ( $rs = -0.23/-0.20$  respectively and  $p < 0.05$  for both); SBP ( $rs = -0.37/-0.39$  respectively and  $p < 0.0001$  for both); DBP ( $rs = -0.39$  and  $p < 0.0001$  for both) and MAP ( $rs = -0.40$  and  $p < 0.0001$  for both) (Table 2).

One-year postpartum, no significant correlation remained between dimethylarginines vs. arterial wall layer dimensions or cardiovascular risk factors (data not shown). However, with regard to changes in dimethylarginines and artery wall layer dimensions from pregnancy to postpartum, ADMA and SDMA levels were positively associated vs. intima thickness ( $rs = 0.22/0.32$  and

**Table 2**

Associations of dimethylarginines to common carotid artery wall layer dimensions and cardiovascular risk factors during pregnancy in the combined groups.

	ADMA $r_s$ (95% CI)	SDMA $r_s$ (95% CI)	L-arginine $r_s$ (95% CI)	L-Arginine/ADMA $r_s$ (95% CI)	L-Arginine/SDMA $r_s$ (95% CI)
<b>CCA wall layers</b>					
Intima thickness	0.29 (0.12, 0.46) *	0.33 (0.17, 0.48) †	−0.20 (−0.37, −0.03) ‡	−0.33 (−0.49, −0.17) ‡	−0.35 (−0.49, −0.19) §
Media thickness	−0.05 (−0.23, 0.13)	−0.09 (−0.26, 0.09)	−0.02 (−0.21, 0.17)	−0.01 (−0.19, 0.19)	0.04 (−0.16, 0.22)
Intima/media ratio	0.24 (0.06, 0.41) *	0.30 (0.13, 0.44) *	−0.12 (−0.30, 0.06)	−0.22 (−0.39, −0.04) ‡	−0.26 (−0.42, −0.07) *
Intima-media thickness	0.04 (−0.13, 0.22)	0.02 (−0.16, 0.20)	−0.09 (−0.28, 0.10)	−0.12 (−0.31, 0.08)	−0.09 (−0.29, 0.11)
<b>Cardiovascular risk factors</b>					
Body mass index, kg/m <sup>2</sup>	0.19 (0.00, 0.36) ‡	0.19 (0.01, 0.35) ‡	−0.16 (−0.32, 0.03)	−0.23 (−0.40, −0.04) ‡	−0.20 (−0.38, −0.02) ‡
Systolic BP, mmHg	0.38 (0.20, 0.53) §	0.39 (0.24, 0.53) §	−0.18 (−0.35, 0.01)	−0.37 (−0.52, −0.19) §	−0.39 (−0.54, −0.23) §
Diastolic BP, mmHg	0.41 (0.25, 0.53) §	0.38 (0.22, 0.52) §	−0.19 (−0.36, −0.01) ‡	−0.39 (−0.55, −0.22) §	−0.39 (−0.54, −0.23) §
MAP, mmHg	0.42 (0.26, 0.54) §	0.40 (0.23, 0.54) §	−0.19 (−0.36, −0.01) ‡	−0.40 (−0.55, −0.22) §	−0.40 (−0.54, −0.24) §

Spearman rank correlation test.  $r_s$ , correlation coefficient (95 % CI). ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; CCA, common carotid artery; BP, blood pressure and MAP, mean arterial pressure.\*  $p < 0.01$ ; †  $p < 0.001$ ; ‡  $p < 0.05$  and §  $p < 0.0001$ .

Data on CCA wall layers reused with permission from the American Heart Association [20].

**Table 3**

Associations of changes (from pregnancy to about one-year postpartum) in dimethylarginines to changes in artery wall layers and cardiovascular risk factors in the combined groups.

	ADMA $r_s$ (95% CI)	SDMA $r_s$ (95% CI)	L-arginine $r_s$ (95% CI)	L-Arginine/ADMA $r_s$ (95% CI)	L-Arginine/SDMA $r_s$ (95% CI)
<b>CCA wall layers</b>					
Intima thickness	0.22 (0.12, 0.41) *	0.32 (0.14, 0.50) †	0.04 (−0.16, 0.24)	−0.19 (−0.39, 0.01)	−0.21 (−0.41, −0.01) *
Media thickness	−0.06 (−0.27, 0.15)	−0.08 (−0.27, 0.09)	−0.10 (−0.30, 0.11)	−0.07 (−0.25, 0.13)	−0.07 (−0.25, 0.12)
Intima/media ratio	0.22 (0.01, 0.40) *	0.31 (0.13, 0.49) †	0.11 (−0.09, 0.32)	−0.08 (−0.30, 0.12)	−0.08 (−0.29, 0.12)
Intima-media thickness	0.02 (−0.18, 0.24)	0.00 (−0.19, 0.18)	−0.05 (−0.24, 0.16)	−0.09 (−0.28, 0.10)	−0.09 (−0.27, 0.09)
<b>Cardiovascular risk factors</b>					
Body mass index	0.09 (−0.11, 0.27)	0.20 (−0.00, 0.38) *	0.15 (−0.05, 0.34)	0.03 (−0.18, 0.24)	−0.01 (−0.22, 0.18)
Systolic blood pressure	0.26 (0.08, 0.43) †	0.53 (0.37, 0.64) ‡	0.14 (−0.03, 0.33)	−0.06 (−0.26, 0.15)	−0.23 (−0.42, −0.02) *
Diastolic blood pressure	0.31 (0.12, 0.48) †	0.46 (0.28, 0.61) ‡	0.09 (−0.09, 0.27)	−0.19 (−0.38, 0.02)	−0.21 (−0.38, −0.03) *
Mean arterial pressure	0.31 (0.13, 0.47) †	0.53 (0.39, 0.64) ‡	0.12 (−0.07, 0.30)	−0.16 (−0.35, 0.05)	−0.25 (−0.41, −0.07) *

Spearman rank correlation test.  $r_s$ , correlation coefficient (95 % CI). ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine and CCA, common carotid artery.\*  $p < 0.05$ ; †  $p < 0.01$  and ‡  $p < 0.0001$ .

Data on CCA wall layers reused with permission from the American Heart Association [20].

$p < 0.05$ / $<0.01$  respectively); I/M ( $r_s = 0.22/0.31$  and  $p < 0.05$ / $<0.01$  respectively) and L-arginine/SDMA were negatively associated vs intima thickness ( $r_s = -0.21$  and  $p < 0.05$ ) (Table 3). Similarly, changes in ADMA and SDMA levels were positively associated and changes in L-arginine/SDMA was negatively associated vs. changes in SBP ( $r_s = 0.26/0.53$  and  $p < 0.01$ / $<0.0001$ / $<0.05$ , respectively), DBP ( $r_s = 0.26/0.46$  and  $p < 0.01$ / $<0.0001$ / $<0.05$ , respectively) and MAP ( $r_s = 0.31/0.53$  and  $p < 0.01$ / $<0.0001$ / $<0.05$ , respectively) (Table 3).

For analysis of CCA-IMT, again we found no significant correlations to analysed dimethylarginines, neither during pregnancy (Table 2) or one-year postpartum (data not shown) nor in their changes from pregnancy to postpartum (Table 3).

## Discussion

To our knowledge this is the first report showing dimethylarginines associate to signs of adverse effects on CCA wall layer dimensions in women during pregnancy, as well as in their changes up to one-year postpartum. Further, significant and logical correlations between carotid intima and I/M, BMI and BP with regard to analysed dimethylarginines were found. In contrast, for conventional CCA-IMT, none of these tests were found to be significant. Further, correlations for intima thickness were often stronger than for I/M, which is expected considering the short time period during pregnancy and that changes in intima thickness is the first morphologic sign in the atherosclerosis process [33,34]. Test of correlation in the combined study groups aimed to focus on

vascular effects of the dynamic in plasma dimethylarginines per se. Correlations were significant despite artery wall layer dimensions are temporarily adversely affected during NP [28], whereas dimethylarginines are lower (improved) during NP, compared to non-pregnancy.

We did not adjust our results for BP based on the fact that BP is the main criterion for definition of PE.

We also confirmed that, during pregnancy, plasma levels of ADMA and SDMA were higher whereas L-arginine, L-arginine/ADMA and L-arginine/SDMA were lower in women with PE, compared to women with NP. In PE, ADMA and SDMA levels decreased from pregnancy to postpartum, whereas in NP the levels increased.

Lower levels of ADMA in NP, compared to postpartum levels, support the findings of Holden et al. [12] and are logical in facilitating the cardiovascular and uterine adaptation during pregnancy through decreased inhibitory effect on NOS [12]. Two possible explanations for the lower levels of ADMA in NP could be; increase in circulating plasma volume with physiological decrease in ADMA levels and increased degradation of ADMA in the renal tubules [35], whereas in PE, when blood vessels become less compliant, the blood flow becomes turbulent and oscillatory; a stimulus for release of superoxide, which in turn creates an environment more prone to develop atherogenic lesions [36]. Further, early in PE, an ischemic placenta, caused by impaired vasculogenesis leads to oxidative stress that could be responsible for elevated ADMA levels through the reduced dimethylarginine-dimethylaminohydrolase activity that is known to be very



sensitive to oxidative stress [13]. Later in PE, impaired renal function on the basis of endothelial damage leads to decreased renal excretion and/or degradation, which could be an additional mechanism to the elevated ADMA levels [37].

Highly significant and logical correlations of ADMA and SDMA and of L-arginine/ADMA and L-arginine/SDMA vs. artery wall layer dimensions, as well as in their changes, strongly support vascular effect of dimethylarginines in women with PE and are in accordance with the known increased risk of later CVD events in these women [8,38] and support and extend our previous findings of adverse vascular effects of PE on artery wall layer dimensions [20,39]. At postpartum, L-arginine/ADMA and L-arginine/SDMA increased significantly, both in PE and NP, mostly due to an increase in L-arginine, in accordance with a report by Pettersson et al. [40]. Lack of significant correlations between dimethylarginines and artery wall layer dimensions at postpartum might be explained by less volatility in the dimethylarginines system, being calmed down in the non-pregnant state at postpartum and that deviations in dimethylarginines more reflect current more acute situations/processes. The ADMA-system reacts quite rapidly; a recent study by Henrohn et al. showed that single dose phosphodiesterase, PDE5-inhibitor rapidly changed the pattern of dimethylarginines levels in patients with pulmonary hypertension [41].

The strength of our study is that we had data on dimethylarginines and CCA wall dimensions both during pregnancy and up to one-year postpartum in the same individuals with a very low dropout rate, which allowed analysis of postpartum changes.

One limitation is the relatively small sample size with the associated potential risk of type 2 errors; a problem for non-significant findings only.

## Conclusions

Our results indicate that plasma levels of dimethylarginines correlated significantly to CCA intima thickness and I/M and to cardiovascular risk factors both during pregnancy, as well as in their changes up to about one-year postpartum. Correlations in this study do not prove causality but the results suggest that dimethylarginines might be one mechanistic link between vascular effects in PE and later risk of CVD.

## Financial support

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## Authors contributions

TA and TN conceived the study. ML performed ultrasound examinations. Ulf Bondesson and Mikael Hedeland were responsible for biochemical analyses. TA and TN analyzed and interpreted the data. TA drafted the manuscript and TN, GW, ML, UB, and MH revised it.

## Declaration of Competing Interest

None, except that Tord Naessen (TN) is holder of the US Patent #8556817, 'non-invasive methods for determining the cardiovascular status of an individual' using the principle of intima thickness and I/M instead of conventional CCA-IMT.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ejogrb.2021.01.016>.

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