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Hypoalbuminemia is related to endothelial dysfunction resulting from oxidative stress in parturients with preeclampsia

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ABSTRACT

Serum albumin levels are inversely related with oxidative stress, but positively related with endothelial function, in pregnant women. However, it is unclear whether hypoalbuminemia in pregnant women with preeclampsia (PE) increases the production of oxygen-derived free radicals and impacts endothelial function. The present study aimed to assess the relationship between serum albumin, oxidative stress, and endothelial dysfunction in pregnant women with PE. A total of 75 women with control pregnancy (Control group, n = 30), PE (PE group, n = 24), or gestational hypertension (GH) (GH group, n = 21) were enrolled. We assessed serum albumin levels, diacron-reactive oxygen metabolites (d-ROMs) as an oxygen-derived free radical marker, and flow-mediated dilation (FMD) as a readout for vascular endothelial function during the gestational period and at one month after delivery. During the gestational period, FMD was lower, but d-ROM levels were higher, in the PE and GH groups compared with the Control group. Serum albumin levels were lower in the PE group compared with the Control and GH groups. d-ROM levels were inversely correlated with serum albumin levels ($r = -0.54$, $p < 0.05$) and FMD ($r = -0.56$, $p < 0.05$) in the PE group, and negatively correlated with FMD, but not serum albumin levels, in the GH group. Serum levels of d-ROMs and albumin, as well as FMD, were similar between groups after delivery. Our findings suggest that reduced serum albumin levels enhance the production of oxygen-derived free radicals, resulting in impaired maternal vascular endothelial function in parturients with PE.

Keywords: FMD, gestational hypertension, human serum albumin, oxidative stress, preeclampsia

Abbreviations:
BAP: biological antioxidant potential
BMI: body mass index
BP: blood pressure
d-ROMs: diacron-reactive oxygen metabolites
FMD: flow-mediated dilation
GH: gestational hypertension
JSSHP: The Japan Society for the Study of Hypertension in Pregnancy
PE: preeclampsia
SD: standard deviation

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INTRODUCTION

Hypertensive disorders of pregnancy refer to a group of diseases involving hypertension in pregnancy and are a significant cause of maternal, fetal, and neonatal morbidity and mortality.[1] Vasospasm, which reflects a vasomotor imbalance in maternal blood vessels, is common to the disorders,[2] and maternal endothelial dysfunction is associated with a systemic increase in oxygen-derived free radicals, resulting in oxidative stress and maternal morbidity.[3] Hypertensive disorders of pregnancy are further classified into gestational hypertension (GH), preeclampsia (PE), superimposed preeclampsia, and chronic hypertension. GH is defined as persistent de novo hypertension that develops at or after 20 weeks gestation. PE is a specific type of GH which is accompanied by a combination of new-onset proteinuria, maternal organ dysfunction, or uteroplacental dysfunction at or after 20 weeks gestation.[4] Hypoalbuminemia is a comorbidity of PE, but not GH, and results from serum albumin leaking from the maternal body via urine. The relationship between hypoalbuminemia and the increased production of oxygen-derived free radicals is unclear, although some studies have reported a potential association.[5] We previously reported that serum albumin reduces oxidative stress by inhibiting NADPH oxidase in human vascular smooth muscle, and that serum albumin is inversely related to oxidative stress and positively related to endothelial function in pregnant women.[5] However, it remains unclear whether hypoalbuminemia in pregnant women with PE increases the production of oxygen-derived free radicals and impacts endothelial function. It is also unclear whether serum albumin levels, production of oxygen-derived free radicals, and endothelial function change after delivery.

Here we hypothesized that hypoalbuminemia increases oxygen-derived free radical levels, leading to vascular endothelial dysfunction in pregnant women with PE. To this end, the present study aimed to assess relationships between serum albumin, oxidative stress, and endothelial dysfunction in pregnant women with PE.

MATERIALS AND METHODS

Participants were 24 pregnant women with PE (PE group), 21 with GH (GH group), and 30 with uncomplicated pregnancies (Control group) who were recruited between April 1, 2010, and March 31, 2018, at Aichi Medical University (Aichi, Japan). Blood pressure (BP) measurements were performed in the sitting position after a 10-minute resting period during pregnancy and at one month after delivery. GH was defined according to the Japan Society for the Study of Hypertension in Pregnancy (JSSHP) criteria, as follows: systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg after 20 weeks' gestation and hypertension normalize by 12 weeks' postpartum. PE was defined according to criteria of JSSHP, as follows: PE is gestational hypertension accompanied by one or more of the following new-onset conditions at or after 20 weeks' gestation, but all symptoms are normalized by 12 weeks' postpartum. Proteinuria (≥300 mg protein/24 hours), other maternal organ dysfunction, or uteroplacental dysfunction.[4] None of the participants smoked, consumed alcohol or caffeine, or had a history of thyroid disease, hypertension, diabetes mellitus, hyperlipidemia, or liver disease, and were not taking any medications known to influence lipoprotein metabolism. All control group participants underwent cesarean section due to previous cesarean section, breech presentation, or surgery involving the uterine myometrium. Written informed consent was obtained from each participant and the study was conducted in

accordance with the principles of the Declaration of Helsinki. The Ethics Committee of Aichi Medical University approved this study on November 26, 2018 (Registration No. 2018-H295).

We collected blood sample and measured FMD at the time of onset in the PE and GH groups, and examination around 34weeks of pregnancy before cesarean section in the control group.

Blood samples were processed as previously described,[6-8] and serum was stored at –80ºC until use. Serum diacron-reactive oxygen metabolites (d-ROMs) and serum biological antioxidant potential (BAP) were analyzed using the Free Radical Analytical System 4 (FRAS, Diacron, Grosseto, Italy).[9,10]

Flow-mediated dilation (FMD) of the brachial artery was measured as a proxy for maternal endothelial function, using a high-resolution Doppler ultrasound (Voluson E8, GE Healthcare, Zipf, Austria) with a 10-MHz transducer, as previously described.[11,12] We collected blood samples and measured FMD during pregnancy and at one month after delivery.

Statistical analyses were performed using JMP Statistical Software version 12 for Windows (SAS Institute Inc., Cary, NC). Data were expressed as mean ± standard deviation (SD). G*Power software 3.1.9.3 for Windows (Heinrich-Heine-Universität, Düsseldorf, Germany) was used for power calculations. According to the power calculations, a sample size of 24 would provide 91% power to detect a d-ROM difference between the three groups at a significance level of 0.05 (SD = 163.0), 99% power to detect a serum albumin difference between the three groups at a significance level of 0.05 (SD = 0.4), and 100% power to detect a FMD difference between the three groups at a significance level of 0.05 (SD = 2.6). Patient characteristics, albumin levels, serum d-ROM levels, and FMD before and after delivery were compared using one-way analysis of variance followed by Fisher's multiple comparison test. Correlations between d-ROM levels and FMD, and between albumin and d-ROM levels, in the PE and GH groups were assessed using single regression analysis. $P<0.05$ was considered statistically significant. Statistical power was calculated using a post hoc power analysis among the Control, PE, and GH groups (significance level, $\alpha=0.05$).

RESULTS

We analyzed 75 pregnant women (Control group, n = 30; PE group, n = 24; GH group, n = 21) during the study period. Maternal age, body mass index (BMI), parity, and Gestational age at time of study did not differ among the three groups (Table 1). Gestational age at delivery was significantly shorter in the PE group compared to GH and Control groups (*p<0.01* vs. GH, *p<0.0001* vs. Control), but did not differ between the GH and Control groups (Table 1). Neonatal weight was significantly lower in the PE group compared to GH and Control groups (*p<0.0*1 vs. GH, *p<0.0001* vs. Control), also significantly lower in the GH group compared to Control group (*p<0.0001*) (Table 1). Systolic and diastolic BP values were higher in the PE (*p<0.0001*) and GH (*p<0.0001*) groups compared with the Control group, but did not differ between the PE and GH groups (Table 1). FMD values were lower in the PE (*p<0.0001*) and GH (*p<0.0001*) groups compared with the Control group, but did not differ between the PE and GH groups (Table 1).

Table 1 Clinical characteristics of the study population

	Control (n=30)	PE (n=24)	GH (n=21)
Maternal age (year)	33.9 ± 4.2	32.8 ± 4.1	32.7 ± 4.3
Body mass index (kg/m^2)	23.6 ± 2.2	26.3 ± 4.0	26.6 ± 4.1
Parity	0.8 ± 0.7	0.7 ± 0.7	0.6 ± 0.7
Gestational age at time of study (week)	34.6 ± 1.2	34.5 ± 3.0	35.6 ± 1.9
Gestational age at delivery (week)	38.2 ± 1.1	35.8 ± 2.6 [a,b]	37.4 ± 2.6
Neonatal weight (g)	3001 ± 286	2017 ± 601 [a,b]	2469 ± 494 [a]
Systolic BP (mmHg)	114.6 ± 14.4	159.0 ± 15.7 [a]	151.8 ± 7.2 [a]
Diastolic BP (mmHg)	71.3 ± 11.3	96.4 ± 12.1 [a]	93.6 ± 10.7 [a]
FMD (%)	9.8 ± 2.6	3.1 ± 2.6 [a]	3.7 ± 2.5 [a]

BP: blood pressure
PE: preeclampsia
GH: gestational hypertension
FMD: flow mediated dilation
Data are expressed as mean ± SD.
[a] p<0.0001 vs. Control
[b] p<0.01 vs. GH
Statistical power calculated with post-hoc power analysis among the Control, PE and GH groups. (significance level α=0.05)

Table 2 summarizes biochemical data in the Control, PE, and GH groups. Serum total protein (*p<0.05* vs. Control, *p<0.05* vs. GH) and albumin levels (*p<0.001* vs. Control, *p<0.001* vs. GH) were lower in the PE group compared with the Control and GH groups, but did not differ between the Control and GH groups (Table 2). Uric acid levels were higher in the PE (*p<0.001*) and GH (*p<0.05*) groups compared with the Control group, and higher in the PE group compared with the GH group (*p<0.05*) (Table 2). Creatinine (*p<0.001* vs. Control, *p<0.05* vs. GH) and triglyceride (*p<0.001* vs. Control, *p<0.05* vs. GH) levels were higher in the PE group compared with the Control and GH groups, but did not differ between the Control and GH groups (Table 2). High density lipoprotein cholesterol levels were lower in the GH group compared with the Control and PE groups (*p<0.05* vs. Control, *p<0.05* vs. PE), but did not differ between the Control and PE groups. d-ROM levels were higher in the PE (*p<0.05*) and GH (*p<0.05*) groups compared with the Control group, but did not differ between the PE and GH groups (Table 2).

Table 2 Biochemical data before the delivery in the study population

	Control (n=30)	PE (n=24)	GH (n=21)
Total protein (g/dL)	6.1 ± 0.2	5.8 ± 0.3 [a,c]	6.1 ± 0.2
Albumin (mg/dL)	3.4 ± 0.4	2.9 ± 0.3 [b,d]	3.5 ± 0.4
Uric acid (mg/dL)	3.6 ± 0.8	6.6 ± 1.8 [b,c]	5.0 ± 1.7 [a]
Creatinine (mg/dL)	0.5 ± 0.1	0.6 ± 0.2 [b,c]	0.5 ± 0.1
Total cholesterol (mg/dL)	238 ± 53	261 ± 60	234 ± 43
HDL cholesterol (mg/dL)	71 ± 13	74 ± 17 [c]	60 ± 16 [a]

LDL cholesterol (mg/dL)	115 ± 48	145 ± 38	122 ± 35
Triglyceride (mg/dL)	133 ± 54	295 ± 195 [b,c]	197 ± 105
Fasting blood sugar (mg/dL)	82 ± 11	81 ± 12	77 ± 22
d-ROMs (CARR U)	594 ± 163	724 ± 124 [a]	725 ± 137 [a]

BP: blood pressure
GH: gestational hypertension
PE: preeclampsia
HDL: high density lipoprotein
LDL: low density lipoprotein
d-ROMs: diacron-reactive oxygen metabolites
Data are expressed as mean ± SD.
[a] $p<0.05$ vs Control
[b] $p<0.001$ vs Control
[c] $p<0.05$ d $p<0.001$ vs GH
Statistical power calculated with post-hoc power analysis among the Control, PE and GH groups. (significance level $\alpha=0.05$)

At one month after delivery, serum total protein, albumin, uric acid, creatinine, and triglyceride levels in the PE group were normalized, and values of blood pressure, d-ROM, and FMD in the PE and GH groups were restored to levels seen in the Control group (Table 3).

Table 3 Biochemical date at one month after the delivery

	Control (n=30)	**PE (n=24)**	**GH (n=21)**
Systolic BP (mmHg)	116.8 ± 7.7	123.9 ± 8.8	124.1 ± 10.1
Diastolic BP (mmHg)	66.9 ± 9.1	73.0 ± 5.4	72.5 ± 8.3
Total protein (g/dL)	6.4 ± 0.2	6.4 ± 0.2	6.5 ± 0.2
Albumin (mg/dL)	4.2 ± 0.2	4.1 ± 0.4	4.0 ± 0.4
Uric acid (mg/dL)	4.7 ± 1.1	5.3 ± 1.0	5.4 ± 0.8
Creatinine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Total cholesterol (mg/dL)	220 ± 35	233 ± 39	211 ± 32
HDL cholesterol (mg/dL)	71 ± 13	66 ± 14	62 ± 17
LDL cholesterol (mg/dL)	117 ± 33	138 ± 25	135 ± 20
Triglyceride (mg/dL)	79 ± 34	114 ± 51	96 ± 44
Fasting blood sugar (mg/dL)	81 ± 7	81 ± 12	83 ± 6
d-ROMs (CARR U)	422 ± 105	357 ± 59	455 ± 223
FMD (%)	9.6 ± 1.6	8.3 ± 3.2	8.0 ± 2.1

BP: blood pressure
GH: gestational hypertension
PE: preeclampsia
FMD: flow mediated dilation
d-ROMs: diacron-reactive oxygen metabolites
Data are expressed as mean ± SD.
Statistical power calculated with post-hoc power analysis among the Control, PE and GH groups. (significance level $\alpha=0.05$)

In the PE group, d-ROM levels were negatively correlated with serum albumin levels (*r=–0.54*, *p<0.05*; Figure 1A) and FMD (*r=–0.56*, *p<0.05*; Figure 1B) during pregnancy. In the GH group, d-ROM levels were negatively correlated with FMD (*r=–0.60*, *p<0.01*; Figure 1D), but no correlation was observed between albumin and d-ROM levels during pregnancy (Figure 1C).

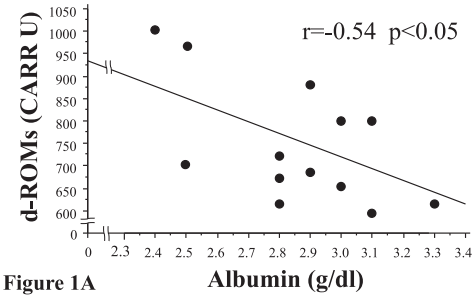

Figure 1A

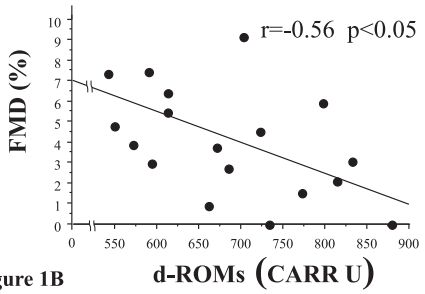

Figure 1B

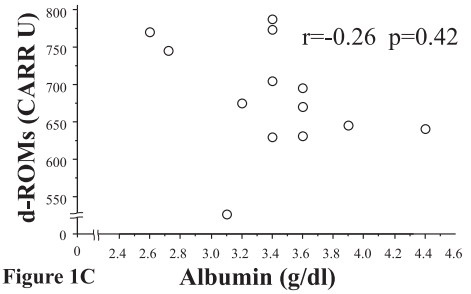

Figure 1C

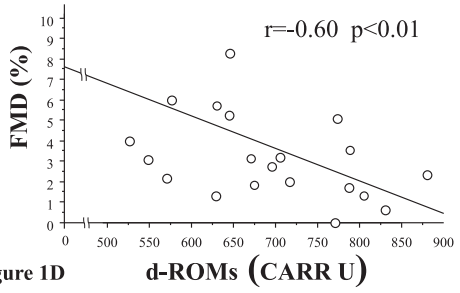

Figure 1D

Fig. 1 Relationship between human serum albumin, concentrations of diacron reactive oxygen metabolites (d-ROMs) and flow-mediated vasodilation (FMD) in pregnant women with preeclampsia and gestational hypertension

Fig. 1A: Relationship between human serum albumin and concentrations of diacron reactive oxygen metabolites (d-ROMs). Closed circles represent pregnant women with preeclampsia. (*r=–0.54*; *p<0.05*)

Fig. 1B: Relationship between concentrations of diacron reactive oxygen metabolites (d-ROMs), and flow-mediated vasodilation (FMD). Closed circles represent pregnant women with preeclampsia. (*r=–0.56*; *p<0.05*)

Fig. 1C: Relationship between human serum albumin and concentrations of diacron reactive oxygen metabolites (d-ROMs). Open circles represent pregnant women with gestational hypertension. (*r=–0.26*; *p=0.42*)

Fig. 1D: Relationship between concentrations of diacron reactive oxygen metabolites (d-ROMs), and flow-mediated vasodilation (FMD). Open circles represent pregnant women with gestational hypertension. (*r=–0.60*; *p<0.01*)

DISCUSSION

Albumin is the most abundant protein in plasma and generally considered to be a multifunctional transport protein. It is thought to be the predominant circulating antioxidant in plasma, and is continuously exposed to oxidative stress.[5] Serum albumin levels were lower in the PE group compared with the GH and Control groups, with no difference between the GH and Control groups, in the present study. All women in the PE group during pregnancy had proteinuria, likely reflecting protein (e.g., albumin) secretion from the body via urine.

Serum d-ROM levels in the PE and GH groups were higher during pregnancy, although the levels did not significantly differ between the two groups. There was also a negative correlation between levels of oxidative stress and FMD between the PE and GH groups. These results suggest that enhanced levels of serum oxidative stress may cause maternal endothelial dysfunction. This conclusion is supported by our previous finding that an increase in oxygen-derived free radicals during pregnancy impairs the endothelial function of patients with hypertensive disorders of pregnancy.[3] A negative correlation was also found between serum albumin and d-ROM levels in the PE group, but not in the GH group, suggesting that lower serum albumin levels may predict higher serum levels of oxygen-derived free radicals in pregnant women with PE, but not GH. In human vascular smooth muscle cells, albumin reportedly reduces superoxide levels by inhibiting membrane recruitment of the NADPH oxidase cytosolic subunit p47phox, a critical mediator of oxygen-derived free radical production in cardiovascular diseases.[5,13,14] Therefore, the reduced levels of serum albumin may, at least in part, play a role in augmenting oxidative stress in pregnant women with PE, but not GH. Levels of serum albumin, d-ROMs, and FMD in the PE group were restored to the normal range at one month after delivery. The recovery of serum albumin levels may have contributed to both normalizing FMD and reducing oxidative stress, although the underlying mechanism is unclear.

This study has some limitations. First, the sample population was small and came from a single medical institution. Second, there was no difference in FMD among the Control, PE, and GH groups at one month after delivery. These results are somewhat inconsistent with previous studies showing that women with a history of PE had a lower FMD at one year after delivery and thus are at an increased risk of developing cardiovascular diseases later in life.[15-21] Further large-scale multicenter clinical studies will be needed to address the above issues.

In conclusion, reduced serum albumin levels enhance the production of oxygen-derived free radicals, which in turn impairs maternal vascular endothelial function in parturients with PE.

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DISCLOSURE

HK is a consult of IMI Co. Ltd, Koshigaya, Saitama, Japan. Other authors declare no conflicts of interest in connection with this article.

REFERENCES

1 Mackay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet*

Gynecol. 2001;97(4):533–538.

2 Goulopoulou S, Davidge ST. Molecular mechanisms of maternal vascular dysfunction in preeclampsia. *Trends Mol Med*. 2015;21(2):88–97.

3 Watanabe K, Mori T, Iwasaki A, et al. Increased oxygen free radical production during pregnancy may impair vascular reactivity in preeclamptic women. *Hypertens Res*. 2013;36(4):356–360.

4 Watanabe K, Matsubara K, Nakamoto O, et al. Outline of the new definition and classification of "Hypertensive Disorders of Pregnancy (HDP)"; a revised JSSHP statement of 2005. *Hypertens Res Pregnancy*. 2018;6(2):33–37.

5 Kinoshita H, Watanabe K, Azma T, et al. Human serum albumin and oxidative stress in preeclamptic women and the mechanism of albumin for stress reduction. *Heliyon*. 2017;3(8):e00369. doi:10.1016/j.heliyon.2017.e00369

6 Owaki Y, Watanabe K, Iwasaki A, Saitou T, Matsushita H, Wakatsuki A. Placental hypoplasia and maternal organic vascular disorder in pregnant women with gestational hypertension and preeclampsia. *J Matern Fetal Neonatal Med*. 2019;2:1–7.

7 Yoshida A, Watanabe K, Iwasaki A, Kimura C, Matsushita H, Wakatsuki A. Placental oxidative stress and maternal endothelial function in pregnant women with normotensive fetal growth restriction. *J Matern Fetal Neonatal Med*. 2018;31(8):1051–1057.

8 Kimura C, Watanabe K, Iwasaki A, et al. The severity of hypoxic changes andoxidative DNA damage in the placenta of early-onset preeclamptic women and fetal growth restriction. *J Maternal Fetal Neonatal Med*. 2013;26(5):491–496.

9 Trotti R, Carratelli M, Barbieri M. Performance and clinical application of a new, fast Method for the detection of hydroperoxides in serum. *Panminerva Med*. 2002;44(1):37–40.

10 Cesarone MR, Belcaro G, Carratelli M, et al. A simple test to monitor oxidative stress. *Int Angiol*. 1998;18(2):127–130.

11 Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol*. 1986;250:H1145–H1149.

12 Boutouyrie P, Laurent S, Girerd X. Common carotid artery stiffness and patterns of Left ventricular hypertrophy in hypertensive patients. *Hypertension*. 1995;25:651–659.

13 Rey FE, Cifuentes ME, Kiarash A, Quinn MT, Pagano PJ. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular $O2^{-}$ and systolic blood pressure in mice. *Circ. Re*s. 2001;89(5):408–414.

14 Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol Rev*. 2007;87(1):245–313.

15 Berks D, Hoedjes M, Raat H, Duvekot JJ, Steegers EA, Habbema JD. Risk of cardiovascular disease after preeclampsia and the effect of lifestyle interventions: a literature-based study. *BJOG*. 2013;12(8):924–31.

16 Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ*. 2001;323(7323):1213–1217.

17 Kurabayashi T, Mizunuma H, Kubota T, Nagai K, Hayashi K. Pregnancy-induced hypertension is associated with maternal history and a risk of cardiovascular disease in later life: Japanese cross-sectional study. *Maturitas*. 2013;75(3):227–231.

18 Watanabe K, Kimura C, Iwasaki A,et al. Pregnancy-induced hypertension is associated with an increase in the prevalence of cardiovascular disease risk factors in Japanese women. *Menopause*. 2015;22(6):656–659.

19 Mito A,Arata N,Sakamoto N, et al. Hypertensive disorders of pregnancy: a strong risk factor for subsequent hypertension 5 years after delivery. *Hypertension Res*. 2018; 41(2):141–146.

20 Oishi M, Iino K,Tanaka K, et al. Hypertensive disorders of pregnancy increase the risk for chronic kidney disease: A population-based retrospective study. *Clin Exp Hypertens*. 2017;39(4):361–365.

21 Hamad RR, Eriksson MJ, Silveira A, Hamsten A, Bremme K. Decreased flow-mediated dilation is present 1 year after a pre-eclamptic pregnancy. *J Hypertens*. 2007;25(11):2301–2307.