Articles in PresS. Am J Physiol Heart Circ Physiol (April 8, 2016). doi:10.1152/ajpheart.00010.2016

Impaired sodium dependent adaptation of arterial stiffness in formerly preeclamptic women: The RETAP – vascular study

Anne Marijn van der Graaf[1], Nina D. Paauw[2], Tsjitske J. Toering[1], Martin Feelisch[3], Marijke M. Faas[1,4], Thomas R. Sutton[3], Magdalena Minnion[3], Joop. D. Lefrandt[5], Sicco A. Scherjon[4], Arie Franx[2], Gerjan Navis[6], A. Titia Lely[2]

[1] Department of Pathology and Medical Biology, Division of Medical Biology, University of Groningen, University Medical Center Groningen, the Netherlands; [2] Department of Obstetrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, The Netherlands; [3] Clinical and Experimental Sciences, Faculty of Medicine, NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, United Kingdom; [4] Department of Obstetrics and Gynaecology, University Medical Center Groningen, the Netherlands; [5] Department of Vascular Medicine, University of Groningen, University Medical Center Groningen, the Netherlands; [6] Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, the Netherlands

Running head: Vascular health after preeclampsia

Correspondence address:

A.T. Lely, MD, PhD

Department of Obstetrics and Gynaecology

Lundlaan 6, 3508 AB Utrecht, The Netherlands

e-mail: a.t.lely@umcutrecht.nl

ABSTRACT

Objectives: Women with a history of preeclampsia have an increased risk for cardiovascular diseases later in life. Persistent vascular alterations in the postpartum period might contribute to this increased risk. The current study assessed arterial stiffness under low sodium (LS) and high sodium (HS) conditions in a well-characterized group of formerly early-onset preeclamptic (fPE) women and formerly pregnant (fHP) women.

Methods: 18 fHP and 18 fPE women were studied at an average of 5 years after pregnancy on one week of LS (50 mmol Na^+/day) and one week of HS (200 mmol Na^+/day) intake. Arterial stiffness was measured by pulse wave analysis (aortic augmentation index, AIx) and carotid-femoral pulse wave velocity (PWV). Circulating markers of the renin-angiotensin aldosterone system (RAAS), extracellular volume (ECV), nitric oxide (NO) and hydrogen sulfide (H_2S) were measured in an effort to identify potential mechanistic elements underlying adaptation of arterial stiffness.

Results: AIx was significantly lower in fHP women on LS compared to HS while no difference in AIx was apparent in fPE women. PWV remained unchanged upon different sodium loads in either group. Comparable sodium dependent changes in RAAS, ECV and NO/H_2S were observed in fHP and fPE women.

Conclusions: fPE women have an impaired ability to adapt their arterial stiffness in response to changes in sodium intake, independently of blood pressure, RAAS, ECV, and NO/H_2S status. The pathways involved in impaired adaptation of arterial stiffness, and its possible contribution to the increased long-term risk for cardiovascular diseases in fPE women remain to be investigated.

News & Noteworthy: This study assessed arterial stiffness in healthy formerly preeclamptic (fPE) women under standardized sodium intake and shows that fPE women have a blunted response to adapt their arterial stiffness in response to low sodium intake. This phenomenon might be a first indication of an unfavorable vascular profile in fPE women.

KEYWORDS

Preeclampsia, formerly preeclamptic women, cardiovascular risk, arterial stiffness, augmentation index, sodium intake.

INTRODUCTION

Preeclampsia complicates approximately 1-5% of the all pregnancies (19) and is characterized by new-onset hypertension and proteinuria or other findings such as new-onset thrombocytopenia, renal insufficiency, neurological complications, liver involvement and fetal growth restriction during the second half of pregnancy (1). Delivery of the placenta is the only therapeutic solution and results in rapid normalization of the maternal manifestations (46). Despite normalization of hypertension and proteinuria after termination of pregnancy, a history of preeclampsia entails long-term vascular consequences. Over the years, observational cohort studies have shown that women with a history of preeclampsia experience an increased risk to develop premature cardiovascular and renal diseases in later life (3, 51, 55).

Unraveling the underlying mechanisms accounting for the increased cardiovascular and renal risk after preeclampsia has been the subject of recent studies. The enhanced risk could be the result of pre-existing cardiovascular risk factors or long-term effects caused by preeclampsia itself (38, 42, 52). Since preeclampsia affects the maternal vascular bed, persistent vascular alterations in the postpartum period might potentially contribute to the increased cardiovascular risk of formerly preeclamptic women.

Recent studies in fPE women have reported subtle vascular alterations such as arterial stiffness, as measured by pulse wave analysis (PWA) and pulse wave velocity (PWV) (10, 11, 25, 27, 33, 37, 39, 47, 57). However, these studies show some inconsistencies; differences in study design, heterogeneity of the preeclamptic phenotype, and the presence or absence of comorbidities (i.e. hypertension and increased BMI) may explain the contradictory findings. Moreover, none of these studies were performed under standardized sodium intake. Sodium restriction has been reported to be an important extrinsic factor in the reduction of blood pressure and arterial stiffness by reduction in extracellular volume and oxidative stress (15, 26, 44). High sodium intake affects arterial stiffness by the induction of endothelial dysfunction, enhancement of vascular smooth muscle tone and

hypertrophy of the vascular wall (9, 17). To our knowledge, adaptation or non-adaptation of arterial stiffness by short-term changes in dietary sodium load has not been studied in healthy subjects at risk for development of premature vascular disease.

In this study, we therefore aimed to explore arterial stiffness in healthy formerly early-onset preeclamptic (fPE) women and formerly healthy pregnant (fHP) women in the absence of comorbidity. For this purpose, we measured arterial stiffness in the population of the REsponse To Angiotensin II in formerly Preeclamptic women (RETAP) study of which we previously reported on renal function (RETAP-renal) (48). All women were studied after one week of low sodium (LS) and after one week of high sodium (HS) intake to assess the influences of short-term changes in sodium load on measures of arterial stiffness. To identify potential pathways involved in the adaptation of arterial stiffness in response to differences in sodium intake we measured circulating components of the renin-angiotensin aldosterone system (RAAS), extracellular volume (ECV) and nitric oxide (NO)/hydrogen sulfide (H_2S) related markers of endothelial function.

MATERIALS AND METHODS

Study population

Our study population consisted of 18 fPE women and 18 fHP controls who participated in the RETAP study (The Netherlands National Trial Register www.trialregister.nl; trial registration number: 2635). Baseline characteristics of this study group and data on renal function and renal response to angiotensin II infusion within this group were published before (RETAP-renal) (48). In short, this study showed no differences in glomerular filtration rate (GFR; measured by ^{125}-I-iothalamate) and no differences in renal hemodynamic response to angiotensin II infusion between groups, but the study did reveal a higher filtration fraction (FF) in the fPE group on both LS and HS diet (48). The study population was selected from the electronic delivery database of the department of Obstetrics and Gynecology at the University Medical Center Groningen. Preeclampsia was defined according to the definition of the International Society for the Study of Hypertension in Pregnancy (2), and early-onset preeclampsia was defined as developing preeclampsia before 34 weeks of gestation. Participants without comorbidity were selected by the exclusion of women with renal disease, diabetes or a history of gestational diabetes, obesity (BMI>30 kg/m^2 at screening) and women using antihypertensive medication. Additional exclusion criteria were pregnancy, current lactation and post-menopausal status. None of the women included were using oral contraceptives. A control for each preeclamptic women was selected using the following selection criteria: same parity, age (within one year) and year of index pregnancy (within one year). All subjects were non-smokers and normotensive, having a sitting systolic blood pressure <140mmHg and diastolic blood pressure <90mmHg measured by Dinamap (an average of three measurements was taken). All patients underwent physical examination and electrocardiography at enrolment into the study, which did not reveal any abnormalities. The study was approved by the local ethics committee (Medical Ethical Committee UMCG Groningen, the Netherlands; number 2010/294), and all subjects gave written informed consent. For this cross-over study a multivariate power calculation with three factors and

two confounders was performed for three renohemodynamic endpoints (48). The regular power calculation demanded 25 women/group. After inclusion of 18 women/group the interim analysis showed significant difference between both groups and the inclusions for the study were stopped.

Study protocol

The selected participants underwent a cross-over protocol consisting of two one-week periods with at least four weeks wash-out in between, a 7-day period on LS diet (target: 50 mmol Na^+/day) and a 7-day period on a HS diet (target: 200 mmol Na^+/day). For assessment of dietary compliance and the achievement of a stable sodium balance, 24-hour urines were collected at day 3 and day 6 of each period. All women were studied at day 7 of each treatment after an overnight fasting period at day 7 ± 2 of their menstrual cycle.

Blood pressure and arterial stiffness measurements

Blood pressure was assessed at the end of each dietary period. After a two-hour rest in semi-supine position in a quiet room, blood pressure and heart rate were measured by the use of an automated sphygmomanometer (Dinamap; GE Medical Systems, Milwaukee, Wisconsin, USA) at 15-min intervals for two hours (10am till 12am). Arterial stiffness was measured using the Sphygmocor System (AtCor Medical; Sydney, Australia) by experienced researchers. To obtain the augmentation index (AIx), the radial pulse wave contour was recorded by means of applanation tonometry and converted to the central arterial waveform using a generalized transfer function. In short, the radial artery of the right arm was pressed gently at the site of maximal pulsation with the tip of the tonometer containing a micromanometer (Millar Instruments, Houston, TX) that accurately records the pressure pulsations within the artery. First, the mean of the last 3 successive recordings of the systolic and diastolic blood pressure measured at the right brachial artery was calculated and entered in the program

(SphygmoCor; version 8.2) and subsequently three successive tonometry recordings were obtained from the right radial artery. Only recordings with an operator index > 80 were used for averaging. Central AIx is defined as the ratio of late systolic pressure (P2) to early systolic pressure (P1) and varies as a function of heart rate. Central aortic AIx and AIx adjusted for heart rate (AIx@75; AIx at 75 beats per minute), pulse pressure (PP), augmentation pressure (AP), time to first peak (t1), time to second peak (t2), time to reflection (tr), subendocardial variability ratio (SEVR) and ejection duration (ED) were automatically calculated by the software. The average of three successive readings was used for analysis. The SphygmoCor system was subsequently used to assess carotid-femoral pulse wave velocity (PWV). The PWV was determined by sequential acquisition of pressure waveforms from the carotid and the femoral arteries. The timing of these waveforms was synchronized with that of the R-wave on the simultaneously recorded ECG. To minimize the influence of body contour, the proximal distance was measured from the sternal notch to the sampling site on the carotid artery and the distal distance was measured from the acromial angle to the sampling site on the femoral artery. The average of more than 8 successive measurements was used in the analysis to cover a complete respiratory cycle.

Extra-cellular volume measurements

ECV was estimated from the distribution volume of ^{125}-I-iothalamate (IOT). Assessment of ECV by the constant infusion method with IOT was validated previously and the method was demonstrated to be reproducible (53). A priming solution containing 20 ml infusion solution (0.04 MBq) plus an extra amount of 0.6 MBq IOT was given at a constant infusion of 12 ml/h. Plasma concentrations of IOT were stabilized during a 1.5-h equilibration, followed by a 2-h period of clearance. ECV was calculated as follows: $[(I \times V + B \times V) - (U \times V)]/P$, where $I \times V$ is the infusion rate of the tracer, $B \times V$ the bolus infusion of the tracer, $U \times V$ the urinary excretion of the tracer and P the plasma levels of IOT (53). This formula equals the amount of infused IOT minus the amount of excreted IOT. ECV was

indexed for body surface area (BSA) crude values of ECV were divided by BSA, calculated according to the DuBois-DuBois formula (8), and multiplied by 1.73 m^2. Data of uncorrected ECV in this population have been reported earlier (48).

Blood and urine analysis

Fasting blood samples were drawn for analysis of RAAS activity and endothelial/metabolic function markers. Aldosterone was measured with a commercially available radioimmunoassay kit (Coat-A-count RIA, Siemens). Plasma renin activity (PRA) was measured with a radioimmunoassay that detects the amount of angiotensin I produced in the presence of excess endogenous angiotensinogen (nanograms of angiotensin I produced per liter of plasma per hour; CisBio International, France). Plasma levels of cyclic 3',5'-guanosine monophosphate (cGMP) were assessed using a competitive enzyme immunoassay (ELISA) according to the manufacturer's instructions (KGE003; R&D Systems, Minneapolis, MN). The plasma concentrations of total nitrosated species (RxNO) were measured following group-specific reductive denitrosation by iodine-iodide in glacial acetic acid, with subsequent detection of liberated NO by its chemiluminescent reaction with ozone (12). Plasma nitrate (NO_3^-) was quantified by ion chromatography with online reduction of nitrate to nitrite and post-column Griess reaction (ENO20 Analyser; Eicom, Kyoto, Japan) (36). Plasma total sulfide (HS-) levels were measured using a novel UPLC- MS/MS technique based on the derivatization of sulfide by N-ethylmaleimide (NEM) **(31) .** Briefly, the disubstituted sulfide adduct (NEM-S-NEM) was separated from other alkylated thiols by C18 reversed-phase chromatography and the NEM-SH_2^+ fragment produced in the electrospray source operating in positive ionization mode was detected by a Xievo triple quadrupole (Waters, Waltham; m/z 285>160) (MM, TS, MF; manuscript in preparation). Plasma sulfide levels were semi-quantified using a standard curve produced from a dilution series of NEM derivatized Na_2S. The standard was used to verify the elution time and fragmentation pattern of the sulfide adduct. Urine samples were drawn from the 24-hour urine and the levels of sodium,

potassium and urea were assessed by the use of an automated clinical chemistry analyzer (Roche Modular Basel).

Data analysis

Statistical analysis was performed using SPSS for Windows (Version 21.0). Parametric data are presented as means ± standard deviation (SD) or Estimated Marginal Means (EMM) ± standard error (SE) and non-parametric data as medians with interquartile ranges such as stated in text, table and figures. Differences in baseline characteristics were tested with a Student *t*-test for parametric data and Mann-Whitney *U*-test for nonparametric data. Generalized Estimated Equations (GEE) analysis was performed for AIx, AIx@75, PWV, MAP and ECV to separately test the effects of history of preeclampsia (factor group) and sodium intake (factor diet). In addition, this analysis enabled us to separately study the changes in parameters in response to change in diet within the fHP and fPE group. The same GEE analysis was performed to analyze plasma nitrate, RxNO, cGMP, and total sulfide. To determine whether age is a determinant of arterial stiffness we used linear regression, which was performed for both LS and HS diet. Differences were considered significant if $p<0.05$.

RESULTS

Baseline characteristics and blood pressure response to changes in sodium intake

Baseline characteristics of our study population are presented in **Table 1**. No differences were found for age, gravidity, parity, and time since last pregnancy (index pregnancy) between the two groups. fPE women had a significantly higher BMI compared to fHP-women while on either diet. Hip-to-waist ratio did not significantly differ between groups. Urinary sodium concentrations showed that the dietary compliance during LS and HS diet was excellent in both groups. No statistically significant differences in potassium and urea excretion were found between the groups reflecting an equal intake of potassium and proteins. Serum sodium did not differ between fHP and fPE women on either LS or HS diet. No differences in PRA and aldosterone were found between groups, and the changes in PRA and aldosterone in response to low sodium intake was similar in both groups. All women were normotensive at the time of the screening visit, and there were no significant differences in mean baseline 2-hour blood pressure, aortic mean pressure, pulse pressure and ECV/BSA measured after LS diet and HS diet week (47). Both fHP and fPE women demonstrated a significant higher blood pressure and ECV/BSA on HS as compared to LS and the GEE-analyses showed that differences in blood pressure and ECV/BSA between HS and LS were similar between groups.

Changes in arterial stiffness as a function of sodium load

Arterial stiffness in fHP and fPE on LS and HS intake expressed as AIx and PWV is shown in **Figure 1A-C**. No differences for AIx between fHP and fPE were found on both LS and HS conditions. However the GEE-analysis showed that LS intake was associated with a significant lower AIx compared to HS intake in fHP women ($p_{diet*fHP}$ = 0.016) while no difference in AIx between LS and HS intake was observed in fPE women. As for AIx, there were no differences between the groups on LS and HS, but

the fHP women showed a significant lower AIx@75 on LS diet compared to HS diet ($p_{diet*fHP}$= 0.024), while there was no difference in AIx@75 between LS and HS diet in fPE women. The other parameters obtained during the PWA did not differ between groups (**Table 2**). No effect of group (fHP or fPE) and diet (LS or HS) within groups was observed for PWV. Linear regression showed a positive relation between age and arterial stiffness under both LS and HS conditions (P<0.05 for age*AIx, age*AIx@75 and age*PWV). GEE-analysis corrected for age showed that differences in age did not affect the adaptation of arterial stiffness in response to LS.

Changes in endothelial function markers

Both NO and H_2S are produced by endothelial cells to regulate vascular tone and arterial stiffness. We therefore measured plasma nitrate, RxNO and cGMP (NO related read-outs) and total sulfide (H_2S related metabolite) as markers of endothelial function in fHP and fPE women **(Figure 2A-D)**. GEE analysis for nitrate showed no significant differences between groups, buton LS intake nitrate concentrations were significantly higher compared to HS in both groups ($p_{diet*fHP}$= 0.016, $p_{diet*fPE}$= 0.04). We did not find differences in RxNO, cGMP and sulfide concentrations between diet and groups, in part presumably due to the rather large inter-individual variation in circulating biomarker levels observed.

DISCUSSION

This is the first study that assessed arterial stiffness in formerly early-onset preeclamptic women without any comorbidity, under standardized low and high sodium conditions. We demonstrated that fPE women have an impaired ability to adapt their arterial stiffness (AIx and AIx@75) upon low sodium diet compared to fHP women. The non-adaptation of arterial stiffness could not be explained by differential responses between fHP and fPE women in blood pressure, circulating RAAS components, extracellular volume expansion, and plasma concentrations of nitrate, RxNO, cGMP, and sulfide. Increased arterial stiffness is known to be associated with the development of hypertension and cardiovascular diseases (4, 21, 28, 32). An impaired ability to decrease AIx in response to sodium status in fPE women might be a first indication of an unfavorable vascular profile.

While both AIx and PWV were similar in fHP and fPE women on LS and HS diet, AIx and AIx@75 were lower on LS diet compared to HS diet in fHP-women but not in fPE-women. We are the first to show this non-adaptation of arterial stiffness in a well-controlled setting, studying subjects at risk for premature vascular disease and healthy controls in the absence of any comorbidity. LS diet normally reduces arterial stiffness; as shown earlier by a meta-analysis exploring the effect of dietary and nutritional interventions on arterial stiffness (34) and by a study in (postmenopausal) female hypertensive subjects (44). Our findings of non-adaptation of arterial stiffness in response to LS in fPE thus suggests that fPE women lost the capability to adjust arterial stiffness upon LS intake.

Previous studies on arterial stiffness in fPE women were not performed under standardized dietary conditions and did not standardize for phase of menstrual cycle. Assuming that sodium intake in the previous studies is in the range of average intake in the Western diet, these studies are comparable with our HS condition under which we observed no differences in AIx and PWV between groups. Under this assumption, our results are in line with two other studies that showed no differences in AIx in fPE women compared to controls (25, 39). However, other studies have reported

281 increased arterial stiffness as measured by AIx (10, 27, 37, 47, 57) as well as by PWV (33, 37, 47) in
282 fPE women. These conflicting findings are likely a result of differences in study design, heterogeneity
283 of the preeclamptic phenotype, and variable presence of comorbidities (e.g. hypertension and
284 increased BMI). The strength of our study is that we studied a well-characterized group of healthy
285 fPE women under standardized dietary conditions compared to a control group with the same parity,
286 age and year of index pregnancy. Therefore, we can conclude that fPE women without comorbidity
287 do not differ in arterial stiffness compared to fHP women on a regular Western diet.

288 Our finding of non-adaptation of arterial stiffness upon LS diet was only reflected in AIx and
289 not in the PWV measurements. This might be explained by the different (age-related) vascular
290 responses that these measures represent (29). In general, PWV is affected by structural vascular
291 changes such as narrowing and sclerosis of the vessels that occur during the later phase of
292 atherosclerosis. The central AIx is a measure of arterial wave reflection which depends both on the
293 PWV and intrinsic properties of the vessel wall. While PWV is the gold standard for measurement of
294 arterial stiffness(49), AIx was found to be correlated with diverse cardiovascular risk factors at a
295 young age, in the absence of structural vascular changes (6, 50). In addition, vasoactive drugs
296 influence AIx independent of PWV which shows that wave reflection is influenced by small-artery
297 tone/structure (22). According to this evidence, AIx might be more suitable compared to PWV in
298 detecting early stage vascular dysfunction based on sensitivity to detect functional vessel
299 abnormalities.

300 Increased arterial stiffness is a result of aging (changes in extracellular matrix composition)
301 (23) and is associated with hypertension, diabetes mellitus, atherosclerosis and renal failure (35, 40).
302 We carefully excluded these factors from our study design by comparing our fPE group with a control
303 group of the same age and by the exclusion of women with co-morbidity. To overcome the
304 influences of differences in sodium intake on arterial stiffness we had an excellent standardization of
305 the diets as observed in the urinary sodium values. On both LS and HS serum sodium did not
306 significantly differ between fHP and fPE but there was a slightly different response of serum sodium

307 to the change in diet within groups (fHP LS vs HS $p<0.05$, while fPE LS vs HS ns; p values earlier not
308 shown). This slight difference might have influenced the non-adaptation of arterial stiffness in
309 response to LS in the fPE group.

310 Other important non-structural determinants of arterial stiffness are activity of the RAAS (14,
311 45), fluid volume status (20) and endothelial function (41). These determinants are also known to be
312 affected by dietary sodium intake and could therefore be mechanisms underlying the observed non-
313 adaptation of arterial stiffness in response to sodium (9). However, we did not detect any differences
314 in circulating RAAS markers between groups; moreover, we found that the systemic RAAS was
315 adequately modulated by sodium intake in both groups as observed by a similar increase in PRA and
316 aldosterone in response to LS in our study. Our ECV data show that both the fHP and fPE group are
317 reducing their ECV upon LS to a similar extent. As expected, reducing extracellular volume resulted in
318 a reduction in arterial stiffness in fHP women, illustrating the ability of healthy vessels to shift
319 stiffness along their compliance curve in response to volume reduction. Since non-adaptation of
320 arterial stiffness was observed in fPE women, we hypothesize that fPE women have stiffer vessels
321 that already work at the upper end of their compliance having less adaptability upon extrinsic
322 factors.

323 With respect to endothelial function, the production of the 'gasotransmitters' NO and H_2S
324 plays an important role in the control of vascular tone (30, 56). While endothelial NO production is
325 also a key regulator of arterial stiffness (54), little is currently known about the biological significance
326 of H_2S in this context. Nevertheless, there is ample cross-talk between these mediators at the
327 functional and chemical level with sulfide affecting NO bioavailability (7). Plasma nitrate, RxNO,
328 cGMP and sulfide did not differ between groups, while both fHP and fPE women showed an increase
329 in plasma nitrate on LS. Plasma nitrate is the final end product of NO metabolism, thus suggesting
330 enhanced endogenous NO production upon LS intake. In this study we were limited to plasma
331 analysis and we analysed the circulating components in a relatively small number of patients with

large inter-individual variation which might explain the lack of trend observed in cGMP while we do observe a trend in nitrates. While the true levels of H_2S related metabolites in blood are currently a matter of lively debate, total plasma sulfide concentrations are, at least in part, likely of endothelial origin but do not seem to change upon changes in sodium load. Based on our findings we cannot conclude with certainty that endothelial function is not involved in the non-adaptation of arterial stiffness, as circulating levels do not necessarily reflect vascular tissue concentrations. A limitation of our study is that we did not assess flow-mediated dilatation (FMD), the gold standard to assess endothelial function. Previous work suggests that fPE women have an impaired FMD compared to controls (5, 16, 18, 33, 43, 58) and this might be a mechanism associated with non-adaptation of arterial stiffness under LS.

Future studies should investigate the mechanistic pathways behind the non-adaptation of arterial stiffness in fPE women and should include arterial stiffness measurements in combination with FMD. It would be of interest to characterize the structure and character of the vessel wall and endothelial surface layer (glycocalyx). The glycocalyx plays an important role in both sodium homeostasis and regulation of arterial stiffness. Under high sodium conditions, endothelial sodium channels are upregulated resulting in an increased sodium influx in the endothelial cells, which leads to increased stiffness (24). Defects in the glycocalyx might result in increased arterial stiffening under LS condition by increased access of sodium via endothelial sodium channels with subsequently reduced NO release causing reduced arterial stiffness and vasoconstriction (13, 24). In addition, the question whether inability to adapt arterial stiffness in response to LS was pre-existing or induced by preeclampsia remains to be answered.

In conclusion, the current study is the first to show an effect of non-adaptation of arterial stiffness in fPE women in response to sodium intake. We could not explain the non-adaptation by differences in blood pressure, plasma RAAS parameters, ECV, circulating markers of NO production and availability or plasma sulfide as a read-out of H_2S activity. The exact underlying pathways

involved in the non-adaptation in fPE women therefore remains to be elucidated. However, independent of the underlying mechanisms, impaired adaptation of arterial stiffness in response to sodium intake might be a marker of subclinical vascular damage in fPE women without comorbidity. We propose that non-adaptation in response to sodium intake is a first sign of unfavorable vascular alterations in fPE women, which might put them at risk to develop hypertension and cardiovascular disease.

DISCLOSURES

None of the authors report any conflict of interests. The data in this manuscript has not been published and is not being considered for publication elsewhere.

References:

1. **American College of Obstetricians and Gynecologists and Task Force on Hypertension in Pregnancy.** Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet.Gynecol.* 122: 5: 1122-1131, 2013.

2. **Brown MA, Lindheimer MD, de Swiet M, Van Assche A and Moutquin JM.** The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens.Pregnancy* 20: 1: IX-XIV, 2001.

3. **Brown MC, Best KE, Pearce MS, Waugh J, Robson SC and Bell R.** Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis. *Eur.J.Epidemiol.* 28: 1: 1-19, 2013.

4. **Cavalcante JL, Lima JA, Redheuil A and Al-Mallah MH.** Aortic stiffness: current understanding and future directions. *J.Am.Coll.Cardiol.* 57: 14: 1511-1522, 2011.

5. **Chambers JC, Fusi L, Malik IS, Haskard DO, De Swiet M and Kooner JS.** Association of maternal endothelial dysfunction with preeclampsia. *JAMA* 285: 12: 1607-1612, 2001.

6. **Coppola G, Natale F, Torino A, Capasso R, D'Aniello A, Pironti E, Santoro E, Calabro R and Verrotti A.** The impact of the ketogenic diet on arterial morphology and endothelial function in children and young adults with epilepsy: a case-control study. *Seizure* 23: 4: 260-265, 2014.

7. **Cortese-Krott MM, Kuhnle GG, Dyson A, Fernandez BO, Grman M, DuMond JF, Barrow MP, McLeod G, Nakagawa H, Ondrias K, Nagy P, King SB, Saavedra JE, Keefer LK, Singer M, Kelm M, Butler AR and Feelisch M.** Key bioactive reaction products of the NO/H2S interaction are S/N-hybrid species, polysulfides, and nitroxyl. *Proc.Natl.Acad.Sci.U.S.A.* 112: 34: E4651-60, 2015.

8. **Du Bois D and Du Bois EF.** A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 5: 5: 303-11; discussion 312-3, 1989.

9. **Edwards DG and Farquhar WB.** Vascular effects of dietary salt. *Curr.Opin.Nephrol.Hypertens.* 24: 1: 8-13, 2015.

10. **Ehrenthal DB, Goldstein ND, Wu P, Rogers S, Townsend RR and Edwards DG.** Arterial stiffness and wave reflection 1 year after a pregnancy complicated by hypertension. *J.Clin.Hypertens.(Greenwich)* 16: 10: 695-699, 2014.

11. **Elvan-Taspinar A, Bots ML, Franx A, Bruinse HW and Engelbert RH.** Stiffness of the arterial wall, joints and skin in women with a history of pre-eclampsia. *J.Hypertens.* 23: 1: 147-151, 2005.

12. **Feelisch M, Rassaf T, Mnaimneh S, Singh N, Bryan NS, Jourd'Heuil D and Kelm M.** Concomitant S-, N-, and heme-nitros(yl)ation in biological tissues and fluids: implications for the fate of NO in vivo. *FASEB J.* 16: 13: 1775-1785, 2002.

13. **Fels J, Jeggle P, Liashkovich I, Peters W and Oberleithner H.** Nanomechanics of vascular endothelium. *Cell Tissue Res.* 355: 3: 727-737, 2014.

14. **Galmiche G, Pizard A, Gueret A, El Moghrabi S, Ouvrard-Pascaud A, Berger S, Challande P, Jaffe IZ, Labat C, Lacolley P and Jaisser F.** Smooth muscle cell mineralocorticoid receptors are mandatory for aldosterone-salt to induce vascular stiffness. *Hypertension* 63: 3: 520-526, 2014.

15. **Gates PE, Tanaka H, Hiatt WR and Seals DR.** Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. *Hypertension* 44: 1: 35-41, 2004.

16. **Goynumer G, Yucel N, Adali E, Tan T, Baskent E and Karadag C.** Vascular risk in women with a history of severe preeclampsia. *J.Clin.Ultrasound* 41: 3: 145-150, 2013.

17. **Gu JW, Anand V, Shek EW, Moore MC, Brady AL, Kelly WC and Adair TH.** Sodium induces hypertrophy of cultured myocardial myoblasts and vascular smooth muscle cells. *Hypertension* 31: 5: 1083-1087, 1998.

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

19. **Hernandez-Diaz S, Toh S and Cnattingius S.** Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. *BMJ* 338: b2255, 2009.

20. **Hung SC, Kuo KL, Peng CH, Wu CH, Lien YC, Wang YC and Tarng DC.** Volume overload correlates with cardiovascular risk factors in patients with chronic kidney disease. *Kidney Int.* 85: 3: 703-709, 2014.

21. **Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, Benjamin EJ, Vasan RS and Mitchell GF.** Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA* 308: 9: 875-881, 2012.

22. **Kelly RP, Millasseau SC, Ritter JM and Chowienczyk PJ.** Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. *Hypertension* 37: 6: 1429-1433, 2001.

23. **Kohn JC, Lampi MC and Reinhart-King CA.** Age-related vascular stiffening: causes and consequences. *Front.Genet.* 6: 112, 2015.

24. **Kusche-Vihrog K, Schmitz B and Brand E.** Salt controls endothelial and vascular phenotype. *Pflugers Arch.* 467: 3: 499-512, 2015.

25. **Lampinen KH, Ronnback M, Kaaja RJ and Groop PH.** Impaired vascular dilatation in women with a history of pre-eclampsia. *J.Hypertens.* 24: 4: 751-756, 2006.

26. **Liu Z, Peng J, Lu F, Zhao Y, Wang S, Sun S, Zhang H and Diao Y.** Salt loading and potassium supplementation: effects on ambulatory arterial stiffness index and endothelin-1 levels in normotensive and mild hypertensive patients. *J.Clin.Hypertens.(Greenwich)* 15: 7: 485-496, 2013.

27. **Mahendru AA, Everett TR, McEniery CM, Wilkinson IB and Lees CC.** Cardiovascular function in women with recurrent miscarriage, pre-eclampsia and/or intrauterine growth restriction. *J.Matern.Fetal.Neonatal Med.* 26: 4: 351-356, 2013.

28. **McEniery CM, Spratt M, Munnery M, Yarnell J, Lowe GD, Rumley A, Gallacher J, Ben-Shlomo Y, Cockcroft JR and Wilkinson IB.** An analysis of prospective risk factors for aortic stiffness in men: 20-year follow-up from the Caerphilly prospective study. *Hypertension* 56: 1: 36-43, 2010.

29. **McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR and ACCT Investigators.** Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J.Am.Coll.Cardiol.* 46: 9: 1753-1760, 2005.

30. **Moncada S, Palmer RM and Higgs EA.** Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol.Rev.* 43: 2: 109-142, 1991.

31. **Nashef AS, Osuga DT and Feeney RE.** Determination of hydrogen sulfide with 5,5'-dithiobis-(2-nitrobenzoic acid), N-ethylmaleimide, and parachloromercuribenzoate. *Anal.Biochem.* 79: 1-2: 394-405, 1977.

32. **Nichols WW and Edwards DG.** Arterial elastance and wave reflection augmentation of systolic blood pressure: deleterious effects and implications for therapy. *J.Cardiovasc.Pharmacol.Ther.* 6: 1: 5-21, 2001.

33. **Paez O, Alfie J, Gorosito M, Puleio P, de Maria M, Prieto N and Majul C.** Parallel decrease in arterial distensibility and in endothelium-dependent dilatation in young women with a history of pre-eclampsia. *Clin.Exp.Hypertens.* 31: 7: 544-552, 2009.

34. **Pase MP, Grima NA and Sarris J.** The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *Am.J.Clin.Nutr.* 93: 2: 446-454, 2011.

35. **Prenner SB and Chirinos JA.** Arterial stiffness in diabetes mellitus. *Atherosclerosis* 238: 2: 370-379, 2015.

36. **Rassaf T, Bryan NS, Kelm M and Feelisch M.** Concomitant presence of N-nitroso and S-nitroso proteins in human plasma. *Free Radic.Biol.Med.* 33: 11: 1590-1596, 2002.

37. **Robb AO, Mills NL, Din JN, Smith IB, Paterson F, Newby DE and Denison FC.** Influence of the menstrual cycle, pregnancy, and preeclampsia on arterial stiffness. *Hypertension* 53: 6: 952-958, 2009.

38. **Romundstad PR, Magnussen EB, Smith GD and Vatten LJ.** Hypertension in pregnancy and later cardiovascular risk: common antecedents? *Circulation* 122: 6: 579-584, 2010.

39. **Ronnback M, Lampinen K, Groop PH and Kaaja R.** Pulse wave reflection in currently and previously preeclamptic women. *Hypertens.Pregnancy* 24: 2: 171-180, 2005.

40. **Safar ME, Plante GE and Mimran A.** Arterial stiffness, pulse pressure, and the kidney. *Am.J.Hypertens.* 28: 5: 561-569, 2015.

41. **Safar ME, Temmar M, Kakou A, Lacolley P and Thornton SN.** Sodium intake and vascular stiffness in hypertension. *Hypertension* 54: 2: 203-209, 2009.

42. **Sattar N and Greer IA.** Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? *BMJ* 325: 7356: 157-160, 2002.

43. **Scholten RR, Spaanderman ME, Green DJ, Hopman MT and Thijssen DH.** Retrograde shear rate in formerly preeclamptic and healthy women before and after exercise training: relationship with endothelial function. *Am.J.Physiol.Heart Circ.Physiol.* 307: 3: H418-25, 2014.

44. **Seals DR, Tanaka H, Clevenger CM, Monahan KD, Reiling MJ, Hiatt WR, Davy KP and DeSouza CA.** Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. *J.Am.Coll.Cardiol.* 38: 2: 506-513, 2001.

45. **Sohn SI and Kim CJ.** Modulation of renin-angiotensin system and arterial stiffness: evidence from clinical trials. *Curr.Hypertens.Rev.* 10: 1: 37-40, 2014.

46. **Steegers EA, von Dadelszen P, Duvekot JJ and Pijnenborg R.** Pre-eclampsia. *Lancet* 376: 9741: 631-644, 2010.

47. **Suzuki H, Watanabe Y, Arima H, Kobayashi K, Ohno Y and Kanno Y.** Short- and long-term prognosis of blood pressure and kidney disease in women with a past history of preeclampsia. *Clin.Exp.Nephrol.* 12: 2: 102-109, 2008.

48. **Toering TJ, van der Graaf AM, Visser FW, Groen H, Faas MM, Navis G and Lely AT.** Higher filtration fraction in formerly early-onset preeclamptic women without comorbidity. *Am.J.Physiol.Renal Physiol.* 308: 8: F824-31, 2015.

49. **Tomlinson LA.** Methods for assessing arterial stiffness: technical considerations. *Curr.Opin.Nephrol.Hypertens.* 21: 6: 655-660, 2012.

50. **van Trijp MJ, Bos WJ, Uiterwaal CS, Oren A, Vos LE, Grobbee DE and Bots ML.** Determinants of augmentation index in young men: the ARYA study. *Eur.J.Clin.Invest.* 34: 12: 825-830, 2004.

51. **Vikse BE.** Pre-eclampsia and the risk of kidney disease. *Lancet* 382: 9887: 104-106, 2013.

52. **Vikse BE, Irgens LM, Karumanchi SA, Thadhani R, Reisaeter AV and Skjaerven R.** Familial factors in the association between preeclampsia and later ESRD. *Clin.J.Am.Soc.Nephrol.* 7: 11: 1819-1826, 2012.

53. **Visser FW, Muntinga JH, Dierckx RA and Navis G.** Feasibility and impact of the measurement of extracellular fluid volume simultaneous with GFR by 125I-iothalamate. *Clin.J.Am.Soc.Nephrol.* 3: 5: 1308-1315, 2008.

54. **Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP and Cockcroft JR.** Nitric oxide regulates local arterial distensibility in vivo. *Circulation* 105: 2: 213-217, 2002.

55. **Wu CC, Chen SH, Ho CH, Liang FW, Chu CC, Wang HY and Lu YH.** End-stage renal disease after hypertensive disorders in pregnancy. *Am.J.Obstet.Gynecol.* 210: 2: 147.e1-147.e8, 2014.

56. **Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH and Wang R.** H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 5901: 587-590, 2008.

57. **Yinon Y, Kingdom JC, Odutayo A, Moineddin R, Drewlo S, Lai V, Cherney DZ and Hladunewich MA.** Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. *Circulation* 122: 18: 1846-1853, 2010.

58. **Yinon Y, Kingdom JC, Odutayo A, Moineddin R, Drewlo S, Lai V, Cherney DZ and Hladunewich MA.** Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. *Circulation* 122: 18: 1846-1853, 2010.

547 **TABLES**

548 **Table 1** Baseline characteristics

		History of normotensive pregnancy (n = 18)	History of preeclamptic pregnancy (n = 18)	p-value
Age (years)		36 ± 5	36 ± 5	0.95
Gravidity		2.5 ± 1.3	2.6 ± 1.1	0.95
Parity		2.0 ± 0.7	2.2 ± 1.0	0.59
Elapsed time since index pregnancy (years)		4.2 ± 2.6	5.3 ± 3.0	0.24
Waist/Hip ratio		0.83 ± 0.04	0.84 ± 0.06	0.44
MAP (mmHg)	LS	81 ± 7	83 ± 8	0.38
	HS	85 ± 8	86 ± 9	0.71
HR (beats/min)	LS	67 ± 8	67 ± 9	0.95
	HS	67 ± 8	66 ± 10	0.64
BMI (kg/m2)	LS	22.6 ± 2.6	25.3 ± 3.3	0.01
	HS	23.2 ± 2.7	25.9 ± 3.5	0.02
ECV/BSA	LS	13.4 ± 1.9	13.5 ± 2.5	0.85
	HS	14.9 ± 2.0	14.9 ± 2.0	0.92
Urinary sodium (mmol/24h)	LS	39 ± 14	45 ± 23	0.33
	HS	221 ± 64	258 ± 86	0.15
Urinary potassium (mmol/24h)	LS	66 ± 21	76 ± 25	0.20
	HS	80 ± 34	73 ± 15	0.46
Urinary urea (mmol/24h)	LS	264 ± 91	306 ± 63	0.12
	HS	339 ± 89	340 ± 65	0.97
Serum sodium (mmol/l)	LS	140 ± 1.6	140 ± 1.9	0.36
	HS	142 ± 1.8	141 ± 2.4	0.31
PRA (nmol ANG I·l−1·h−1)	LS	0.80 (0.50-1.20)	0.85 (0.70-1.50)	0.50
	HS	0.20 (0.10-0.50)	0.20 (0.09-0.30)	0.58
Aldosterone (pmol/L)	LS	255 (204-395)	341 (214-477)	0.16
	HS	71 (29-93)	59 (35-96)	0.84
Aldosterone:PRA ratio	LS	331 (201-450)	456 (250-494)	0.38
	HS	224 (151-499	316 (181-517)	0.23

Change in PRA HS to LS (%)	225 (100-350)	325 (160-700)	0.18
Change in Aldosteron HS to LS (%)	320 (187-462)	436 (90-700)	0.48

Data are presented as means ± SD, as medians (25^{th}-75^{th} percentiles). LS: low sodium diet (<50mmol Na^+/24h), HS: high sodium diet (>200mmol Na^+/24h), MAP: mean arterial pressure HR: heart rate, BMI: body mass index, ECV/BSA: extracellular volume corrected for body surface area, PRA: plasma renin activity. *P < 0.05; by student t-test.

Table 2. Pulse wave analysis data

		History of normotensive pregnancy (n = 18)	History of preeclamptic pregnancy (n = 18)	p-value
PP (mmHg)	LS	43 ± 6	42 ± 5	0.72
	HS	44 ± 5	43 ± 5	0.62
AP (mmHg)	LS	3.4 ± 3.2	5.0 ± 3.2	0.13
	HS	5.2 ± 3.6	5.0 ± 3.8	0.91
P1 (mmHg)	LS	27 ± 5	26 ± 4	0.87
	HS	27 ± 5	26 ± 6	0.38
P2 (mmHg)	LS	30 ± 6	31 ± 6	0.47
	HS	32 ± 7	31 ± 7	0.46
T1 (ms)	LS	105 ± 10	107 ± 6	0.35
	HS	105 ±10	108 ± 7	0.28
T2 (ms)	LS	217 ± 16	223 ± 15	0.33
	HS	225 ± 20	229 ± 15	0.57
Tr (ms)	LS	147 ± 10	146 ±7	0.67
	HS	146 ± 14	148 ± 12	0.54
ED (ms)	LS	311 ± 16	312 ± 17	0.82
	HS	325 ± 20	324 ± 17	0.88
SEVR (%)	LS	150 ± 24	162 ± 36	0.22
	HS	144 ± 32	161 ± 34	0.15

Data are presented as means ± SD, LS: low sodium diet (<50mmol Na^+/24h), HS: high sodium diet (>200mmol Na^+/24h), PP: pulse pressure, AP: augmented pressure, P1: peak 1 first systolic inflection, P2: peak 2 systolic peak, T1: time to first peak, T2: time to second peak, Tr: time to reflection, ED: ejection duration, SEVR: subendocardial viability ratio.

560 **LEGENDS**

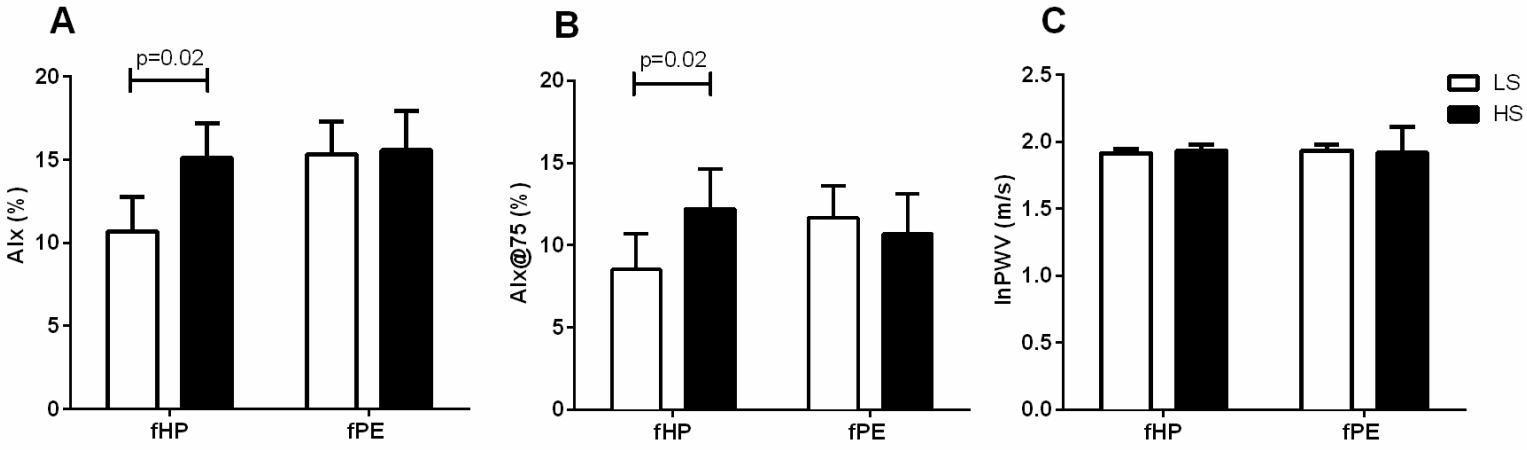
561

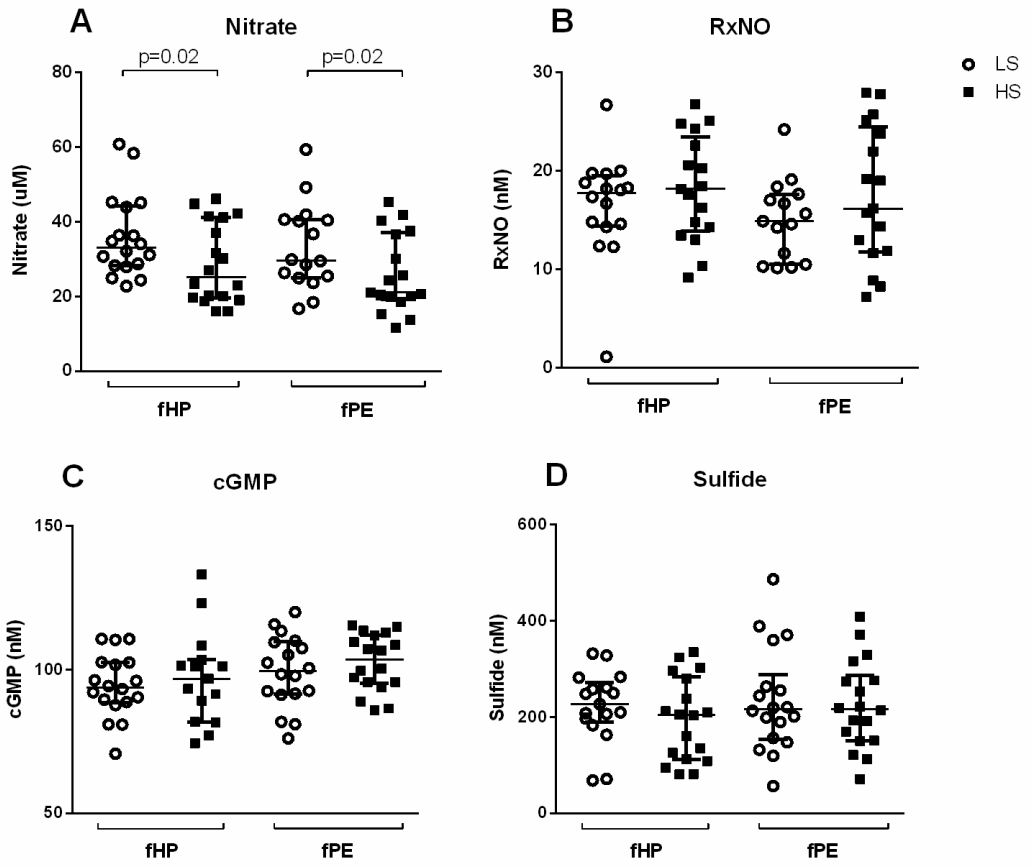
562 **Figure 1.** Augmentation index (AIx, **A**), Augmentation index corrected for heart rate (AIx@75, **B**) and
563 pulse wave velocity (PWV, **C**) during low sodium (LS, white bars) and high sodium (HS, black bars)
564 intake in women with a history of healthy pregnancy (fHP) and in formerly preeclamptic (fPE)
565 women. Data are expressed as estimated marginal means ± standard error.

566

567 **Figure 2.** Plasma concentrations of nitrate **(A)**, total nitrosated species (RxNO, **B**), cyclic GMP (cGMP,
568 **C**) and total sulfide **(D)** during low sodium (LS, grey circles) and high sodium (HS, black squares)
569 intake in women with a history of healthy pregnancy (fHP) and in formerly preeclamptic (fPE)
570 women. Data are expressed as medians with interquartile ranges and individual levels.

571


A
p=0.02
AIx (%)
20
15
10
5
0
fHP
fPE
B
p=0.02
AIx@75 (%)
20
15
10
5
0
fHP
fPE
C
LnPWV (m/s)
2.5
2.0
1.5
1.0
0.5
0.0
fHP
fPE
LS
HS


A
Nitrate
p=0.02
p=0.02
Nitrate (uM)
80
60
40
20
0
fHP
fPE
B
RxNO
RxNO (nM)
30
20
10
0
fHP
fPE
LS
HS
C
cGMP
cGMP (nM)
150
100
50
fHP
fPE
D
Sulfide
Sulfide (nM)
600
400
200
0
fHP
fPE