From preeclampsia to renal disease
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Chapter 7

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

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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 be a potential mechanism behind this increased risk. This study aimed to assess arterial stiffness under low sodium (LS) and high sodium (HS) conditions in a healthy well-characterized group of formerly early-onset preeclamptic (fPE) women compared to formerly healthy pregnant (fHP) women.

Methods
18 fHP and 18 fPE women were studied in balance on one week of LS (50 mmol Na+/day) and one week of HS (200 mmol Na+/day) intake. Arterial stiffness was measured by augmentation index (AIx), AIx corrected for heart rate (AIx@75) and pulse wave velocity (PWV). Circulating parameters of renin-angiotensin aldosterone system (RAAS) activity, extracellular volume (ECV), nitrate, nitrosated species (RxNO) and cyclic GMP (cGMP) were measured to identify pathways underlying adaptation of arterial stiffness.

Results
As compared to HS diet, AIx and AIx@75 upon LS diet were significantly decreased in fHP women while there was no effect of LS consumption on AIx and AIx@75 in fPE women. PWV was similar in both groups and between diets. In both groups, comparable sodium dependent changes in RAAS, ECV and nitrate and were observed.

Conclusions
fPE women have an impaired ability to adapt their arterial stiffness (AIx/AIx@75) upon low sodium. The non-adaptation of arterial stiffness occurred independent of blood pressure, RAAS, ECV, and nitrates. The pathways involved in impaired adaptation, and its possible contribution to the increased long term risk for cardiovascular diseases in fPE women remains to be investigated.
INTRODUCTION

Preeclampsia complicates approximately 1-5% of all pregnancies and is characterized by new-onset hypertension and proteinuria during the second half of pregnancy. Delivery of the placenta is the only therapeutic solution and results in rapid normalization of the maternal manifestations. 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.

Unravelling the underlying mechanisms for the increased cardiovascular and renal risk after preeclampsia has been subject of recent studies. The increased risk could be the result of pre-existing cardiovascular risk factors as well as long-term effects caused by preeclampsia itself. Since preeclampsia affects the maternal vascular bed, persistent vascular alternations in the postpartum period might be a potential mechanism for the increased cardiovascular risk in formerly preeclamptic women.

Recent studies in formerly preeclamptic women have reported subtle vascular alternations such as arterial stiffness as measured by pulse wave analysis (PWA) and pulse wave velocity (PWV). 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 these 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 volume reduction and reducing oxidative stress. High sodium intake affects arterial stiffness by the induction of endothelial dysfunction, stimulation of vascular smooth muscle tone and hypertrophy of vascular wall. To our knowledge, adaptation or non-adaptation of arterial stiffness by short term changes in dietary sodium has not been studied in healthy subjects at risk for development of premature vascular diseases.

In this study, we aimed to explore arterial stiffness in the 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). 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 diet on arterial stiffness. To identify pathways involved in the adaptation of arterial stiffness in response to salt we measured circulating components of the renin-angiotensin aldosterone system (RAAS), extracellular volume (ECV; $^{125}$I-iothalamate distribution volume) and the endothelial function markers nitrate, nitrosated species (RxNO) and cyclic guanosine cyclic monophosphate (cGMP).
MATERIALS AND METHODS

Study population
Our study population consisted of 18 formerly early-onset preeclamptic women and 18 previously healthy pregnant controls who participated in the Response to Angiotensin II in Formerly Preeclamptic women (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) 46. In short, this study showed no differences in GFR (measured by $^{125}$I-iothalamate (IOT)) 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.

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/m² at screening) and women using any antihypertensive medication. Additional exclusion criteria were pregnancy, current lactation and post-menopausal status. None of the included women were using oral contraceptives. The formerly preeclamptic women were matched for age and year of index pregnancy (within one year) with a parous control whose pregnancy had been uncomplicated and normotensive. All subjects were non-smokers and normotensive, having a sitting systolic blood pressure <140mmHg and diastolic blood pressure <90mmHg measured by Dinamap (the average of three measurements was taken). All patients underwent physical examination and electrocardiography at intake of the study, which did not reveal any abnormalities. The study was approved by the local medical ethical committee (Medical Ethical Committee UMCG Groningen, the Netherlands; number 2010/294) and all subjects gave written informed consent.

Study protocol
The selected participants underwent a randomized cross-over protocol consisting of two one-week periods with at least four weeks in between, a 7-day period on LS diet (aim: 50 mmol Na⁺/day) and a 7-day period on a HS diet (aim: 200 mmol Na⁺/day). For assessment of dietary compliance and the achievement of stable sodium balance, 24-hour urine was collected at day 3 and day 6 during each period. All women were studied at day 7 of each treatment after an overnight fasting period at day 7 ± 2 of the 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 semisupine 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. To obtain the augmentation index (Alx), recording of the radial pulse wave contour was performed by using applanation tonometry. In short, the artery of interest was pressed gently at the site of maximal pulsation with the tip of the tonometer containing a micromanometer that accurately records the pressure within the artery (Millar Instruments, Houston, TX). First a successive recording of the pressure waveform at the right brachial artery was assessed. These values were entered in the program (SphygmoCor; AtCor Medical, Sydney; version 8.2) and subsequently three successive recordings were performed on the right radial artery. The SphygmoCor software incorporates a quality control feature (operator index) which is displayed on the screen. An operator index above 80 was called a successive reading. Peripheral Alx is defined as the ratio of late systolic pressure (P2) to early systolic pressure (P1). Alx and Alx corrected for heart rate (Alx@75) were automatically calculated by the SphygmoCor. The average of the three successive readings was used in the 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 computed with that of the R-wave on the simultaneously recorded ECG. To reduce 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 IOT. Assessment of ECV by the constant infusion method with IOT was validated and the method was demonstrated to be reproducible. 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 1.5-h equilibration and was followed by a 2-h period of clearance. ECV was calculated as follows: 

\[
\text{ECV = } \frac{(I \times V + B \times V) - (U \times V)}{P},
\]

where \(I \times V\) is the infusion rate of the tracer, \(B \times V\) the bolus infusion of the tracer and \(U \times V\) the urinary excretion of the tracer. This formula equals the amount infused IOT minus the amount excreted IOT. ECV was indexed for body surface area (BSA) by dividing the crude values by BSA multiplying it by 1.73 m². BSA was calculated according to the DuBois-DuBois formula. Data of uncorrected ECV in this population were published earlier in the RETAP-renal manuscript.

**Blood and urine sampling and analysis**

Fasting blood samples were drawn for analysis of renin-angiotensin aldosterone system (RAAS) activity and endothelial function markers. Aldosterone was measured with a commercially available radioimmunoassay kit (Coat-A-count RIA, Siemens). PRA was measured with a radioimmunoassay that detects the amount of angiotensin I produced per hour in the presence of excess endogenous angiotensinogen (nanograms of angiotensin I produced per liter of plasma per hour; CisBio International, France). Plasma cGMP levels were assessed using a competitive enzyme immunoassay.
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(ELISA) according to the manufacturer’s instructions (KGE003; R&D Systems, Minneapolis, MN). The concentration of the total pool of nitrosated species (RxNO) and nitrate were measured in the laboratory of M. Feelisch, University of Southampton. RxNO was assessed using group-specific reductive denitrosation by iodine-iodide in glacial acetic acid, with subsequent detection of liberated NO into the gas phase by its chemiluminescent reaction with ozon $^{12}$. Plasma nitrates were quantified by ion chromatography with reduction of nitrate to nitrite and post-column Griess diazotization (ENO20 Analyser; Eicom, Kyoto, Japan) $^{34}$. 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. PWV values were log transformed. Generalized Estimated Equations (GEE) analysis was performed for AIx, AIx@75, PWV, 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 nitrate, RxNO, and GMP. To determine whether age is a determinant of arterial stiffness we used linear regression, which was performed for both LS and to HS diet. Differences were considered significant if p<0.05.

RESULTS

Baseline characteristics
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 both during LS and HS 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 during both LS and HS diet. No differences in PRA and aldosterone were found between the groups, and the change 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 and pulse pressure measured after LS diet and HS diet week $^{46}$. Both fHP and fPE women demonstrated a significant increase in blood pressure in response to HS as compared to LS, but there were no differences in blood pressure responses to diet (salt sensitivity) between the fHP and fPE women.
<table>
<thead>
<tr>
<th></th>
<th>History of normotensive pregnancy (n = 18)</th>
<th>History of preeclamptic pregnancy (n = 18)</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>36 ± 5</td>
<td>36 ± 5</td>
<td>0.95</td>
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<tr>
<td>Gravidity</td>
<td>2.5 ± 1.3</td>
<td>2.6 ± 1.1</td>
<td>0.95</td>
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<tr>
<td>Parity</td>
<td>2.0 ± 0.7</td>
<td>2.2 ± 1.0</td>
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<tr>
<td>Elapsed time since index pregnancy (years)</td>
<td>4.2 ± 2.6</td>
<td>5.3 ± 3.0</td>
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</tr>
<tr>
<td>Waist/Hip ratio</td>
<td>0.83 ± 0.04</td>
<td>0.84 ± 0.06</td>
<td>0.44</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>81 ± 7</td>
<td>83 ± 8</td>
<td>0.38</td>
</tr>
<tr>
<td>HS</td>
<td>85 ± 8</td>
<td>86 ± 9</td>
<td>0.71</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LS</td>
<td>43 ± 6</td>
<td>42 ± 5</td>
<td>0.72</td>
</tr>
<tr>
<td>HS</td>
<td>44 ± 5</td>
<td>43 ± 5</td>
<td>0.62</td>
</tr>
<tr>
<td>HR (beats/min)</td>
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<td></td>
<td></td>
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<tr>
<td>LS</td>
<td>67 ± 8</td>
<td>67 ± 9</td>
<td>0.95</td>
</tr>
<tr>
<td>HS</td>
<td>67 ± 8</td>
<td>66 ± 10</td>
<td>0.64</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
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<td></td>
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</tr>
<tr>
<td>LS</td>
<td>22.6 ± 2.6</td>
<td>25.3 ± 3.3</td>
<td>0.01</td>
</tr>
<tr>
<td>HS</td>
<td>23.2 ± 2.7</td>
<td>25.9 ± 3.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Urinary sodium (mmol/24h)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LS</td>
<td>39 ± 14</td>
<td>45 ± 23</td>
<td>0.33</td>
</tr>
<tr>
<td>HS</td>
<td>221 ± 64</td>
<td>258 ± 86</td>
<td>0.15</td>
</tr>
<tr>
<td>Urinary potassium (mmol/24h)</td>
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<td></td>
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<tr>
<td>LS</td>
<td>66 ± 21</td>
<td>76 ± 25</td>
<td>0.20</td>
</tr>
<tr>
<td>HS</td>
<td>80 ± 34</td>
<td>73 ± 15</td>
<td>0.46</td>
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<tr>
<td>Urinary urea (mmol/24h)</td>
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<tr>
<td>LS</td>
<td>264 ± 91</td>
<td>306 ± 63</td>
<td>0.12</td>
</tr>
<tr>
<td>HS</td>
<td>339 ± 89</td>
<td>340 ± 65</td>
<td>0.97</td>
</tr>
<tr>
<td>Serum sodium (mmol/l)</td>
<td></td>
<td></td>
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<tr>
<td>LS</td>
<td>140 ± 1.6</td>
<td>140 ± 1.9</td>
<td>0.36</td>
</tr>
<tr>
<td>HS</td>
<td>142 ± 1.8</td>
<td>141 ± 2.4</td>
<td>0.31</td>
</tr>
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</table>
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<table>
<thead>
<tr>
<th></th>
<th>History of normotensive pregnancy (n = 18)</th>
<th>History of preeclamptic pregnancy (n = 18)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRA (nmol ANG I·l⁻¹·h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>0.80 (0.50-1.20)</td>
<td>0.85 (0.70-1.50)</td>
<td>0.50</td>
</tr>
<tr>
<td>HS</td>
<td>0.20 (0.10-0.50)</td>
<td>0.20 (0.09-0.30)</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Aldosterone (pmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>255 (204-395)</td>
<td>341 (214-477)</td>
<td>0.16</td>
</tr>
<tr>
<td>HS</td>
<td>71 (29-93)</td>
<td>59 (35-96)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Aldosterone:PRA ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>331 (201-450)</td>
<td>456 (250-494)</td>
<td>0.38</td>
</tr>
<tr>
<td>HS</td>
<td>224 (151-499)</td>
<td>316 (181-517)</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Change in PRA HS to LS (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>225 (100-350)</td>
<td>325 (160-700)</td>
<td>0.18</td>
</tr>
<tr>
<td>HS</td>
<td>320 (187-462)</td>
<td>436 (90-700)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

*Data are presented as means ± SD or as medians (25th-75th percentiles). LS: low sodium diet (<50 mmol Na⁺/24h), HS: high sodium diet (>200 mmol Na⁺/24h), MAP: mean arterial pressure, PP: pulse pressure, HR: heart rate, PRA: plasma renin activity, ANG: angiotensin.*

### Arterial stiffness

Arterial stiffness in fHP and fPE on LS en HS expressed as AIX and PWV is showed in Figure 1. No overall difference in AIX was found between groups and between diets. However, the GEE-analysis showed that LS intake was associated with a significant decrease in AIX in fHP women (p<0.016) while no effect of LS intake on AIX was observed in fPE women. As for AIX, no significant differences in AIX@75 were found between group and diet, but in the fHP women again a significant decrease in AIX@75 in response to LS diet (pdiet*fHP = 0.024) was observed, while there was no effect of LS intake on AIX@75 in fPE women. No significant differences between groups was observed for PWV. Linear regression showed a positive relation between age and arterial stiffness under both low and high salt 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 low salt.

### Extracellular volume

ECV corrected for body surface area (ECV/BSA) in fHP and fPE on LS en HS is shown in Figure 2. ECV/BSA did no differ between groups, but we did observe an overall effect of diet (pdiet < 0.001). There was a significant decrease in ECV/BSA in response to LS diet within both groups (pdiet*fHP < 0.001 and pdiet*fPE = 0.01) without differences between the groups.
Figure 1. Arterial stiffness during low and high sodium diet.
Augmentation index (Alx, A), Augmentation index corrected for heart rate (Alx@75, B) and log pulse wave velocity (PWV, C) during low sodium (LS, white bars) and high sodium (HS, black bars) intake in women with a history of healthy pregnancy (fHP) and in formerly preeclamptic (fPE) women. Data are expressed as estimated marginal means ± standard error. *P < 0.05 by generalized estimating equation analysis.

Figure 2. Extra cellular volume during low and high sodium diet.
Extra cellular volume corrected for body surface area (ECV/BSA) during low sodium (LS, white bars) and high sodium (HS, black bars) intake in women with a history of healthy pregnancy (fHP) and in formerly preeclamptic (fPE) women. Data are expressed as estimated marginal means ± standard error. *P < 0.05; by generalized estimating equation analysis.

Endothelial function markers
As markers for endothelial function, we measured plasma nitrate, RxNO and cGMP in fHP and fPE women (Figure 3). GEE analysis for nitrates showed no significant differences between groups while diet did affect plasma nitrates (p_diet = 0.008). Low salt intake significantly increased the nitrate concentration in both groups (p_diet*fHP = 0.016, p_diet*fPE = 0.04). We did not find differences in RxNO and cGMP concentrations between diet and groups but there was a large inter-individual variation of these parameters within our data.
Figure 3. Endothelial function markers during low and high sodium diet.
Nitrate (A), total total nitrosated species (RxNO, B) and cyclic GMP (cGMP, C) during low sodium (LS, grey circle) and high sodium (HS, black square) intake in women with a history of healthy pregnancy (fHP) and in formerly preeclamptic (fPE) women. Data are expressed as medians with interquartile ranges. *P < 0.05; by generalized estimating equation analysis.

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 (Alx and Alx@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, extracellular volume expansion and plasma availability of nitrate, RxNO, and cGMP, or RAAS-activity. Increased arterial stiffness is known to be associated with the development of hypertension and cardiovascular diseases. An impaired ability to decrease Alx in response to low sodium in fPE women might be a first indication of an unfavorable vascular profile.

While both Alx and PWV were similar in fHP and fPE women on HS diet, LS diet reduced the Alx and Alx@75 in fHP-women but not in fPE-women. We are the first to show this effect of non-adaptation of arterial stiffness in a well-controlled setting, studying subjects at risk for premature vascular disease and healthy controls in absence of any comorbidity. LS diet normally reduces arterial stiffness; as was shown by a meta-analysis exploring the effect of dietary and nutritional interventions on arterial stiffness and by a study in (postmenopausal) female hypertensive subjects. Our finding 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 menstruation 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 Alx and PWV between groups. Under this assumption, our results are in line with two other studies that showed no differences in Alx in fPE women compared to controls. However, other studies have reported increased arterial stiffness as measured by Alx as well as by PWV in fPE women.
These conflicting findings probably result from differences in study design, the heterogeneity of the preeclamptic phenotype, and the presence of comorbidity (i.e. hypertension and increased BMI). The strength of our study is that we studied a well-characterized group of healthy fPE women under standardized dietary conditions matched to a control group. Therefore, we can conclude that fPE women without comorbidity do not differ in arterial stiffness compared to fHP women on a regular western diet.

Our finding of non-adaptation of arterial stiffness upon LS diet was only reflected in AIx and not in the PWV measurements. This might be explained by the different (age-related) vascular responses that these measures represent. In general, the AIx is influenced by the resistance of the vessels and highly dependent on endothelial function. PWV is affected by structural changes such as narrowing and sclerosis of the vessels that occur during the late process of atherosclerosis. While PWV is the gold standard for measurement of arterial stiffness, AIx might be more accurate in detecting early stage vascular dysfunction based on sensitivity to detect functional instead of structural abnormalities. In our study all women were healthy and relatively young, and therefore one might not expect structural vascular abnormalities reflected by differences in PWV.

Increased arterial stiffness is a result of aging (changes in extracellular matrix composition) and is associated with hypertension, diabetes mellitus, atherosclerosis and renal failure. We carefully excluded these factors from our study design by matching our fPE group with a control group of the same age and by the exclusion of women with co-morbidity. To overcome the influences of differences in sodium intake on arterial stiffness we had an excellent standardization of the diets as observed in the urinary sodium values. On both LS and HS serum sodium did not significantly differ between fHP en fPE but interestingly there was a slightly different response of serum sodium to the change in diet within groups (fHP LS vs HS p<0.05, while fPE LS vs HS ns; p-values earlier not shown). This slight difference might have influenced the non-adaptation of arterial stiffness in response to LS in the fPE group.

Other important determinants of arterial stiffness are the RAAS, volume status and endothelial function. These determinants are also known to be affected by dietary sodium intake and could therefore be mechanisms underlying non-adaptation of arterial stiffness in response to sodium. However, we did not detect any differences in circulating RAAS components between groups and we found that the systemic RAAS was adequately modulated by sodium intake in both groups as observed by a similar increase in PRA and aldosterone in response to LS in our study. Our ECV data show that both the fHP and fPE group are reducing their ECV upon LS to a similar extent. As expected, the volume reduction resulted in reduction in arterial stiffness in fHP women, illustrating the ability of healthy vessels to shift stiffness along their compliance curve in response to volume reduction. Since non-adaptation of arterial stiffness was observed in fPE women, we hypothesize that fPE women have stiffer vessels that already work at the upper end of their compliance having less adaptability upon extrinsic factors.

In respect to endothelial function, plasma nitrate, RxNO and cGMP did also not differ between groups, while both groups showed the expected increase in plasma nitrate on LS. Plasma nitrate, RxNO and cGMP are part of the L-arginine-NO-synthase pathway (nitrate as precursor...
for NO \(^{26}\), and cGMP as activated product of the NO-pathway), which play an important role in regulation of endothelial function (relaxation). Based on our findings we cannot conclude with certainty that endothelial function is not involved in the non-adaptation of arterial stiffness. 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 \(^{6,16,18,31,41,53}\) 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 salt conditions the endothelial sodium channels are upregulated resulting in an increased sodium influx in the endothelial cells, which leads to increased stiffness \(^{23}\). Defects in the glycocalyx might result in increased arterial stiffening under LS condition by increased access of sodium to the sodium channels of the endothelium and subsequently reduced NO release causing contracted smooth muscle cells and vasoconstriction \(^{13,23}\). 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, this study is the first to show an effect of non-adaptation of arterial stiffness in healthy fPE women in response to LS. We could not explain the non-adaptation by differences in blood pressure, plasma RAAS parameters, ECV, plasma nitrate and plasma NO availability. The exact underlying pathways involved in the non-adaptation in this fPE group remain therefore to be elucidated. However, independent of the underlying mechanisms, impaired adaptation of the arterial stiffness in response to LS might be a marker of subclinical vascular damage in fPE women without comorbidity. We propose that non-adaptation in response to LS is a first sign of unfavorable vascular alterations in fPE women, which might put them at risk to develop hypertension and cardiovascular disease.
REFERENCES


