Carotid artery stiffening does not explain baroreflex impairment in pre-eclampsia

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ABSTRACT

Stiffening of the barosensory vessel wall in hypertension has been suggested to play a role in the associated baroreflex impairment. The carotid distensibility–BRS (baroreflex sensitivity) relationship, however, has not been studied in pre-eclampsia, a condition where hypertension is spontaneously reversible. Twelve normotensive pregnant women and 12 patients with pre-eclampsia matched for maternal age and week of gestation were studied in the third trimester and 3 months postpartum. Carotid artery diastolic diameter and pulsatile distension was measured by echo-wall tracking and carotid pulse pressure by applanation tonometry, and the carotid distensibility coefficient was calculated. Spontaneous BRS was determined by the sequence and spectral methods from 10 min continuous recording of ECG and finger arterial blood pressure. In the third trimester, carotid distensibility was lower in patients with pre-eclampsia than in normotensive pregnant women (2.47 + 0.17 compared with 4.08 + 0.16 × 10⁻³/mmHg); postpartum, it increased moderately in patients, but remained below normotensive values (3.25 + 0.12 compared with 4.25 + 0.19 × 10⁻³/mmHg). In the third trimester, both patients and healthy pregnant women had equally low BRS values; postpartum, the various BRS indices increased markedly (by 60–190 %) and to the same level in both groups. No correlation was found between changes in carotid artery distensibility and those in BRS from the third trimester to postpartum period in patients and healthy pregnant women. The lack of association between changes in carotid distensibility and BRS suggest that stiffening of the carotid artery in pre-eclampsia is not responsible for baroreflex dysfunction.

INTRODUCTION

Hypertension is associated with impaired arterial baroreflex regulation of heart rate [1,2]. The precise mechanism underlying this abnormality is not known, but stiffening of the barosensory vessel wall has been suggested to play a role by reducing baroreceptor responsiveness to changes in pressure [3,4]. Indeed, a number of studies have indicated that the carotid artery is stiffer in patients with arterial hypertension [5,6].

Pre-eclampsia is a unique form of hypertension in the sense that the rapidly developing hypertension in T3 (third trimester) is spontaneously reversible after delivery. Also, the impairment in baroreflex control of heart
rate, which has been reported in patients with pre-eclampsia, was found to be reversible after delivery to healthy non-pregnant levels [7]. The mechanism of baroreflex changes in pre-eclampsia is not clear, and the possible role of arterial stiffening has not been studied. Stiffening of the carotid artery due to hypertension in pre-eclampsia and normalization of blood pressure after delivery might contribute to changes in baroreflex function.

The aim of the present investigation was to gain an insight into the baroreflex mechanism by studying the carotid distensibility–BRS (baroreflex sensitivity) relationship within the same subjects in two different conditions: the hypertensive state before delivery and in the normotensive state after delivery. To this end, we measured spontaneous time and frequency domain cardiovagal BRS indices and carotid artery elastic parameters (compliance, DC [distensibility coefficient], stiffness index $\beta$ and $E_{\text{inc}}$ [incremental elastic modulus]) in patients with pre-eclampsia and in healthy pregnant women at T3 and 3 months PP (postpartum).

**METHODS**

**Subjects**

We studied 12 patients with pre-eclampsia and 12 healthy normotensive pregnant women matched for age and week of gestation. All subjects were primigravides. Each subject was studied twice: in T3 and 3 months PP. Pre-eclampsia was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy [8]. None had a history of hypertension, cardiovascular or renal disease before the 20th week of pregnancy. All had blood pressure values exceeding 140/90 mmHg on two occasions at least 6 h apart and this reverted to normal within 2 months after delivery. All of them had proteinuria of $>300$ mg/day. Normotensive pregnant controls remained normotensive throughout their pregnancies, and were taking no medications other than iron or vitamins.

All subjects gave written informed consent to participate in the study, which was approved by the Ethical Committee of the Semmelweis University, Budapest, Hungary.

**Carotid ultrasonography**

Diameter of the right common carotid artery, its pulsatile distension and the IMT (intima-media thickness) of the posterior wall were measured with ultrasonography. The ultrasound device consisted of a vessel wall echotracking system (Wall Track System; Pie Medical, Maastricht, The Netherlands) combined with a conventional ultrasound scanner (Scanner 200; Pie Medical) and has been described in detail previously [9,10]. Briefly, arteries were visualized in the two-dimensional mode, then the ultrasound system was switched to M mode and ultrasound was emitted and received along a selected M line on sight. The Wall Track System is based on a data acquisition system capable of capturing the received and amplified radio frequency signals. After completion of data acquisition, the data were transferred to a personal computer. The first line acquired was then graphically presented on a display, allowing manual identification of the anterior and posterior boundaries by placing two markers representing the sample windows for data processing. To extract the change in position of either the anterior or the posterior wall, the approach based on the cross-correlation model for corresponding segments of subsequent radio frequency lines was applied. To ensure that the signals returned by the same structure were always considered, the position of the sample windows was adjusted according to the observed displacements (tracking window). The difference between the displacements of the signals of the anterior and posterior walls yielded the change in diameter as a function of time, i.e., the distension waveform.

**Carotid artery pulse pressure**

Carotid artery systolic and pulse pressure were measured by applanation tonometry (SPT-301; Millar Instruments, Houston, TX, U.S.A.), and the carotid pulse wave recording was calibrated by using diastolic and mean brachial pressure values measured by sphygmomanometry on the left brachial artery. Diastolic brachial pressure was assigned to the minimum value of the carotid pressure pulse wave and the mean pressure to its electrically averaged value. This calibration of the tonometric signal was based on the assumption that mean pressure did not change in large conduit arteries and that diastolic pressure was not substantially different between the brachial and carotid arteries [11]. The carotid tonometric pressure was used to calculate carotid artery elastic parameters. The cut-off frequency of the recording devices were above 25 kHz and, therefore, no distortion was induced into the frequency content of pulsatile signals.

**Carotid artery elastic variables**

Strain was calculated as the relative change in D (end-diastolic diameter) during pulsatile distension as $100 \times \Delta D/D$, where $\Delta D$ is the change in diameter from end diastole to peak systole. Carotid compliance was calculated as $\Delta D/\Delta P$, where $\Delta P$ is carotid pulse pressure. DC was calculated as $2 \times \Delta D/(D \times \Delta P)$. Stiffness index $\beta$, a pressure-independent measure of arterial elasticity, was expressed as $\ln(SBP/DBP) \times D/\Delta D$, where SBP and DBP are systolic and diastolic carotid pressure respectively. Carotid artery LCSA (lumen cross-sectional area) and IMCSA (intima-media cross-sectional area) were calculated as $\text{LCSA} = \pi \times D^2/4$, and $\text{IMCSA} = \pi \times (D/2 + \text{IMT})^2 - \pi \times (D/2)^2$. $E_{\text{inc}}$ was determined as $[3 \times (1 + \text{LCSA}/\text{IMCSA})]/\Delta D$.
BRS

We determined cardiovagal BRS from spontaneous fluctuations in RRI (RR interval) and systolic blood pressure. RRI was measured from continuously recorded ECG, and systolic pressure was recorded by photoplethysmography from the right middle finger (Finapres; Ohmeda 2300; Ohmeda, Inglewood, CO, U.S.A.). During data collection, the servo-reset mechanism of the Finapres was turned off to permit continuous data acquisition. Recordings of 10 min duration were digitized and analysed with the WinCPRS program (Absolute Aliens, Oy, Finland). To determine the time domain indices, we used the sequence method, calculating spontaneous BRS up-up (sBRS+) and down-down (sBRS-) sequences, as the slope of the regression line between SBP and RRI. Only sequences with a correlation coefficient > 0.85 were considered. To determine spectral indices, the signals were interpolated, resampled and their power spectra were determined using Fast-Fourier-transformation-based methods. The mean value of the α function (the square root of the ratio of the spectral powers of RRI and SBP), considering only those frequency components in the LF (low frequency) band (0.05–0.15 Hz), where the coherence was > 0.5, was taken as the α coefficient (LFα). We also determined LFgain (LF transfer function gain), which expresses RRI and SBP cross-spectral magnitude in the frequency range of 0.05–0.15 Hz, where coherence is > 0.5.

Protocol

Subjects reported to the laboratory in the early afternoon 2–3 h after a light meal. During the day of the study, they refrained from consuming coffee or alcohol. The subjects’ ECG and arterial blood pressure were recorded and they then rested for 15 min. The rest period was followed by 10 min continuous recording of ECG and finger arterial pressure for determination of BRS. Following that, ultrasonography of the right carotid artery with simultaneous tonometric pressure measurement on the left carotid artery was performed. Measurements were done by the same two well-trained examiners, who have many years of experience in ultrasound and tonometric investigation. Each subject was studied twice: during T3 of pregnancy and 3 months PP. During most of the study sessions, subjects were lying in the lateral position; only for the simultaneous tonometric and ultrasonographic carotid artery measurements were the subjects shifted to the supine position. One subject who felt uncomfortable in the supine position, had to be excluded from the study. In the remainder of the subjects, lying supine for 10–15 min did not cause discomfort and, based on the continuous heart rate and arterial pressure recordings, no cardiovascular disturbance was evident.

Statistical analysis

Data are given as means ± S.E.M. Subject characteristics were compared by unpaired Student’s t test. Two-way repeated measure ANOVA with the Duncan post-hoc test for multiple comparisons was used to test for differences between groups (normotensive compared with hypertensive) and conditions (T3 compared with PP). Relationships between changes in blood pressure, carotid artery elastic parameters and BRS were evaluated by linear regression analysis. Differences were considered significant at P < 0.05. Statistical analyses were performed by the StatSoft, STATISTICA for Windows, Release 5.0 program package.

RESULTS

Subject characteristics are summarized in Table 1. Carotid artery dimensions and various elastic parameters obtained in T3 and PP are given in Table 2. BRS data are shown in Table 3. Figure 1 shows the changes in MBP (mean arterial blood pressure), carotid artery DC and BRS (LFgain) from T3 to PP.

In normotensive pregnant women, blood pressure was not different in T3 and PP (93 ± 1 compared with 94 ± 1 mmHg for mean brachial pressure, and 40 ± 1 compared with 41 ± 1 mmHg for ΔP). Heart rate decreased from T3 to PP (89 ± 2 compared with 72 ± 2 beats/min). Carotid artery elastic parameters did not change from T3 to PP, whereas all BRS indices increased considerably. The sBRS+ and LFgain indices increased by more than 50 %, and the sBRS− and LFα indices more than doubled.

Patients with pre-eclampsia had elevated blood pressure in T3 compared with normotensives (120 ± 2 and 64 ± 4 mmHg for mean brachial pressure and ΔP respectively). At PP, both mean brachial pressure and ΔP decreased in patients (96 ± 2 and 49 ± 4 mmHg), and returned to normotensive control levels. Heart rate decreased from T3 to PP to a similar extent as in the normotensive subjects (85 ± 2 compared with 74 ± 2 beats/min).

Carotid artery elastic parameters in patients in T3 indicated significant stiffening compared with healthy pregnant women. After delivery, carotid elastic parameters

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of normotensive pregnant subjects and patients with pre-eclampsia</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normotensive subjects</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.2 ± 0.8</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>35 ± 0.6</td>
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<tr>
<td>Pregnant weight (kg)</td>
<td>85 ± 3.1</td>
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<tr>
<td>Pregnant BMI (kg/m²)</td>
<td>31.5 ± 1.6</td>
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<tr>
<td>PP time (weeks)</td>
<td>12 ± 0.4</td>
</tr>
<tr>
<td>PP weight (kg)</td>
<td>75 ± 3.8</td>
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<tr>
<td>PP BMI (kg/m²)</td>
<td>27.8 ± 1.2</td>
</tr>
</tbody>
</table>

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Table 2  Carotid artery elastic parameters in pregnant normotensive subjects and patients with pre-eclampsia at T3 and 3 months PP

Results are expressed as means ± S.E.M. Groups were compared for differences by two-way repeated measure ANOVA and the post-hoc Duncan test. *P < 0.05 compared with T3; † P < 0.05 when patients with pre-eclampsia are compared with pregnant normotensive subjects.

<table>
<thead>
<tr>
<th></th>
<th>Normotensive subjects</th>
<th>Patients with pre-eclampsia</th>
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<tr>
<td></td>
<td>T3</td>
<td>PP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3</td>
</tr>
<tr>
<td>D (µm)</td>
<td>6273 ± 120</td>
<td>5859 ± 88*</td>
</tr>
<tr>
<td>∆D (µm)</td>
<td>500 ± 15</td>
<td>503 ± 24</td>
</tr>
<tr>
<td>Carotid wall IMT (µm)</td>
<td>440 ± 20</td>
<td>486 ± 15</td>
</tr>
<tr>
<td>Strain (%)</td>
<td>8.09 ± 0.22</td>
<td>8.49 ± 0.29</td>
</tr>
<tr>
<td>Compliance (µm/mmHg)</td>
<td>12.73 ± 0.42</td>
<td>12.42 ± 0.52</td>
</tr>
<tr>
<td>DC (x 10⁻¹/mmHg)</td>
<td>4.08 ± 0.16</td>
<td>4.25 ± 0.19</td>
</tr>
<tr>
<td>Stiffness index β</td>
<td>5.69 ± 0.50</td>
<td>5.04 ± 0.44</td>
</tr>
<tr>
<td>Eac (mmHg)</td>
<td>2.94 ± 0.16</td>
<td>2.55 ± 0.08</td>
</tr>
</tbody>
</table>

Table 3  BRS indices in pregnant normotensive subjects and patients with pre-eclampsia at T3 and 3 months PP

Values are expressed as means ± S.E.M. Groups were compared for differences by two-way repeated measure ANOVA and the post-hoc Duncan test. *P < 0.05 compared with T3; † P < 0.05 when patients with pre-eclampsia are compared with pregnant normotensive subjects.

<table>
<thead>
<tr>
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<th>Normotensive controls</th>
<th>Patients with pre-eclampsia</th>
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<td></td>
<td>T3</td>
<td>PP</td>
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<tr>
<td></td>
<td></td>
<td>T3</td>
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<tr>
<td>sBRS+ (ms/mmHg)</td>
<td>6.42 ± 0.35</td>
<td>10.13 ± 0.59*</td>
</tr>
<tr>
<td>sBRS− (ms/mmHg)</td>
<td>5.22 ± 0.38</td>
<td>11.80 ± 0.76*</td>
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<tr>
<td>LFa (ms/mmHg)</td>
<td>5.07 ± 0.25</td>
<td>11.56 ± 0.86*</td>
</tr>
<tr>
<td>LFgain (ms/mmHg)</td>
<td>4.59 ± 0.37</td>
<td>8.37 ± 0.69*</td>
</tr>
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improved, but remained lower than the values in the healthy controls. BRS indices of patients in T3 were not different from those of normotensives, except for sBRS+, which was reduced moderately (Table 3). After delivery, all BRS indices increased in patients to the same extent as in controls.

Correlations between changes from T3 to PP in MBP, carotid DC and BRS (LFgain) in patients and healthy pregnant women are shown in Figure 2. There was a negative correlation between changes in MBP and carotid DC (r = -0.58, P < 0.01), but no relationship was found between changes in MBP and LFgain, or between changes in carotid DC and LFgain. Similarly, no significant relationship was found when the other baroreflex indices (sBRS+, sBRS− and LFa) were substituted for LFgain.

**DISCUSSION**

In the present study, we investigated the carotid artery distensibility–BRS relationship in patients with pre-eclampsia. We found that the reversal of pregnancy-related impairment of baroreflex function after delivery was not related to changes in carotid artery distensibility.

**Carotid artery**

In contrast with increases in systemic arterial compliance, the carotid artery has been found to stiffen in healthy human pregnancy [12]. In pre-eclampsia, the increase in systemic arterial compliance is absent [13]; however, carotid artery elastic properties have not been studied. In the present study, we demonstrated that carotid artery stiffens to a greater extent in pre-eclampsia than in normotensive pregnancy. Carotid compliance was reduced in women with pre-eclampsia at T3, because increased ΔP was associated with approximately the same vessel distension as in normal pregnancy, indicating a less steep pressure–diameter relationship, i.e. reduced compliance. Pulsatile strain, the relative change in diameter, was also lower in women with pre-eclampsia at T3 than in normal pregnancy, due to significant increase in D. The reduction in distensibility (strain/ΔP) was, therefore, the result of both reduced strain and increased pulse pressure.

Changes in MBP were found to be related to changes in carotid artery distensibility from T3 to PP. This may be a causative relationship, as elevated blood pressure shifts the set point in the non-linear pressure–diameter relationship to the right. However, elevation in arterial pressure may not be fully responsible for decreased carotid distensibility, because stiffness index β, a pressure-independent measure of arterial elasticity, was also found to increase. The following pressure-independent mechanisms may be considered as contributing factors to stiffening of the carotid artery. (i) Oedema develops in the carotid artery wall due to endothelial damage and increased capillary permeability, reducing its distensibility; this possibility may be supported by our findings that IMT and Eac, an index of wall material stiffness, were increased in patients compared with controls. (ii) The pregnancy-related increase in sympathetic nervous activity is accentuated in pre-eclampsia [7,14]; plasma levels of vasoconstrictor agents are elevated [15] and shear-stress-mediated NO (nitric oxide)-release is
impaired [16], all of which result in increased vascular smooth muscle tone, leading to stiffer carotid artery wall. As a result of its functional character, carotid wall stiffening in pre-eclampsia is probably reversible as, at 3 months PP, all carotid elastic parameters improved, although did not reach healthy non-pregnant levels.

**BRS**

Our present data support earlier observations that cardiovagal BRS is reduced during normal human pregnancy and also in patients with pre-eclampsia, since the values obtained in the present study were considerably lower than of those observed in non-pregnant healthy women [7,17]. On the other hand, BRS indices at T3 were only slightly less in patients than in controls, with the difference being significant only for sBRS+. The degree of BRS reduction in pre-eclampsia is a controversial issue. Silver et al. [17] found reduced BRS in women with pre-eclampsia at T3 compared with healthy pregnant controls with the Valsalva manoeuvre and spontaneous heart rate variability measures, but not with the deep breathing test. Greenwood et al. [7] did not observe any difference in BRS between pregnancy-induced hypertensive patients and healthy pregnant controls using the Valsalva manoeuvre. In this latter study, similar to ours, the subjects were older and their mean BRS value was half of that in the study by Silver et al. [17]. In all studies, BRS values increased after delivery in both hypertensive patients and normotensive controls, findings that are in line with our present data [7,18].

In essential hypertension, BRS is reduced [1,2], and the reduction of BRS is partly explained by stiffening

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**Figure 1** MBP, carotid artery DC and BRS (LF\textsubscript{gain}) values in T3 and PP

○, Pregnant normotensive subjects; ○, patients with pre-eclampsia. Data are given as means ± S.E.M. Groups were compared for differences by two-way repeated measure ANOVA, and the post-hoc Duncan test. *\(P < 0.05\) compared with T3; †\(P < 0.05\) when patients with pre-eclampsia are compared with pregnant normotensive subjects.

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**Figure 2** Correlation between changes MBP (ΔMBP), carotid artery distensibility (ΔDC) and BRS (ΔLF\textsubscript{gain}) from T3 to PP in controls and patients

○, Pregnant normotensive subjects; ○, patients with pre-eclampsia.
of the arterial wall in which the baroreceptors are embedded [3,4], although a causative relationship has never been established experimentally. In the present study, we investigated the influence of carotid artery distensibility on baroreflex function in a design provided by pregnancy. BRS has been demonstrated to decrease in both normotensive and hypertensive pregnancy and to increase after delivery. We measured corresponding changes in carotid artery distensibility and BRS and found that carotid distensibility and BRS improved in parallel in patients with pre-eclampsia after delivery, but the same increase in BRS also occurred in the normotensive group without any change in carotid distensibility. In addition, PP BRS values in both patients and controls were similar to non-pregnant age-adjusted values reported in the literature [17], whereas carotid distensibility was lower than the appropriate reference values [19]. Furthermore, in the PP period, BRS was not significantly different in patients and controls, whereas carotid distensibility was lower in patients than in controls. All of these findings point towards a lack of relationship between changes in BRS and those in carotid distensibility, which are associated with pregnancy and pre-eclampsia.

Neurohumoral mechanisms may be responsible for the pregnancy-related changes in BRS. Hormonal changes are pronounced around term both before and after delivery, which might have an influence on baroreflex function. Oestrogenic hormones were found to facilitate, whereas a progestosterone metabolite, 3α-hydroxydihydroprogesterone was found to inhibit, baroreflex function centrally in healthy pregnancy [20,21]. The influences of oestrogen and progesterone often antagonize each other, the net effect probably depending on their relative concentrations and bioactivity. Pregnancy-associated decreases in the responsiveness of the sinus node to acetylcholine may be another factor in the reduction of BRS [22]. The mechanism of how neurohormonal changes around delivery affect baroreflex function remains to be clarified.

Limitations

This present study was slightly underpowered to detect small differences in blood pressure and stiffness indices; however, our primary aim was to investigate the relationship between changes in carotid distensibility and BRS and we found no correlation even with marked changes between these parameters.

To measure BRS we used spontaneous methods and not the pharmacological Oxford technique, which is considered to be the gold standard [23]. Although, results obtained with pharmacological and spontaneous methods are not identical, they are related [24]. Also, injecting phenylephrine into patients with pre-eclampsia may not be acceptable on ethical grounds. The sequence technique has been used in pregnant women before and was shown to be a highly reproducible one [17].

The use of peripheral systolic pressure to calculate BRS indices represents a limitation [25]. With higher heart rates, the peripheral pulse amplitude is larger for any given central (carotid or aortic) pulse pressure. The lower BRS values that we obtained at T3 compared with PP, therefore, may partly be accounted for by the higher heart rates at T3 compared with PP, considering that systolic pressure was measured at the periphery, whereas baroreceptors are located in the central arteries. We believe, however, that the error due to this limitation did not influence the major conclusions of the study.

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