Transdermal Contraception and the Renin Angiotensin Aldosterone System (RAAS) in Pre-Menopausal Women

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The oral contraceptive pill (OCP) activates the renin-angiotensin-aldosterone system (RAAS) through first-pass hepatic metabolism. Although usually benign, RAAS activation may have detrimental effects on renal and hemodynamic function in some women. Since combined hormonal contraception with the transdermal patch (EVRA) does not undergo first-pass hepatic metabolism, we hypothesized that the RAAS response would be different from that of OCP subjects. Thirty-five non-smoking, pre-menopausal women (15 controls, 10 OCP and 10 contraceptive patch subjects) without evidence of cardiovascular disease, renal disease or diabetes were studied. Baseline angiotensinogen, renin, angiotensin II, aldosterone and plasma renin activity were assessed along with hormonal and hemodynamic responses to simulated orthostatic stress using incremental lower body negative pressure (LBNP; -15, -25, -40 mmHg). Baseline levels of angiotensinogen, angiotensin II and plasma renin activity were significantly higher in OCP subjects compared to normotensive controls and contraceptive patch subjects (p<0.05) while aldosterone was significantly higher in OCP subjects versus controls only (p<0.05). Plasma renin levels were significantly lower at baseline in contraceptive patch subjects compared to normotensive controls and OCP subjects (p<0.05). In response to LBNP, increases in renin, angiotensin II and aldosterone were attenuated in contraceptive patch subjects in conjunction with an exaggerated decline in mean arterial pressure (p<0.05 vs. controls and OCP subjects). The contraceptive patch in healthy pre-menopausal women is associated with an impaired ability to maintain blood pressure in response to LBNP, possibly due to insensitivity of the endogenous RAAS. Further evaluation may be beneficial in women with kidney disease.
INTRODUCTION

As a potent stimulus of the renin angiotensin aldosterone system (RAAS), the combined oral contraceptive pill (OCP) produces hemodynamic and physiological alterations that increase the risk for cardiovascular and renal disease.(1, 3, 4, 14) Perhaps as a consequence of these effects, a subset of women develop significant hypertension with OCP use (4) and face an increased risk of both cardiac and vascular events even with second or third generation combined OCPs (<50 ug of ethinyl estradiol).(3) OCP use is also associated with increased renal vascular resistance and filtration fraction,(14) which has been linked to a greater risk of diabetic nephropathy in young diabetic women.(1) More severe, but rare adverse events such as malignant hypertension and acute renal failure have also been reported.(10, 24, 30)

In contrast to the OCP, combined hormonal contraception with the transdermal patch (EVRA) is distinct because of the avoidance of first-pass metabolism. Theoretically, the contraceptive patch should not stimulate hepatic production of angiotensinogen,(11) the precursor of angiotensin II and the effector hormone of the RAAS. Studies using transdermal postmenopausal hormone therapy also suggest that circulating RAAS components are not increased by the transdermal route.(6, 16, 28) Given that up-regulation of the RAAS is detrimental to cardiorenal function, a contraceptive patch may be physiologically advantageous to the OCP in premenopausal women with underlying kidney disease.

Accordingly, we systematically examined healthy, normotensive, premenopausal women and compared the effects of the contraceptive patch and the OCP on circulating RAAS mediators and systemic blood pressure. We also measured incremental responses in these physiological parameters to lower body negative pressure (LBNP), a known stimulus of the endogenous RAAS,(12) to examine dynamic changes in circulating RAAS mediators and systemic blood pressure. We also included a healthy control group for comparison. We hypothesized that RAAS contraceptive patch users would exhibit blunted RAAS responses compared to OCP subjects.
METHODS

Study Population

Fifteen normotensive controls, 10 OCP subjects and 10 contraceptive patch subjects were studied. Participants were interviewed and examined by a qualified internist and all participants were healthy without evidence of hypertension (BP>130/80 mmHg), kidney disease, heart disease or diabetes. Smokers were excluded from the study. All participants were ≤40 years old and were either already using the study contraceptive patch or OCP or were willing to initiate them for contraceptive purposes. Recruitment was performed through the family medicine clinics at Sunnybrook Health Sciences Centre, Women’s College Hospital, University Health Network and via public advertisements in Toronto, Canada. The study was conducted in the Renal Physiology Laboratory at the University Health Network with the approval of the Human Subjects Research Ethics Board at the University of Toronto. All subjects gave written informed consent.

Preparation

Except for normotensive controls, all subjects were taking hormonal contraception for at least three months prior to participating in the study. Pregnancy was ruled out by a negative serum β-human chorionic gonadotropin test prior to enrollment and studies were performed after seven days on a controlled diet. Each subject was prescribed a diet to target 150 mmol/day of sodium and 1.5 g/kg/day of protein. Compliance was ascertained by the measurement of sodium and urea excretion in a 24-hour urine collection. Healthy controls were studied during the follicular phase of their menstrual cycle as confirmed by a detailed menstrual history as well as measurements of 17 β-estradiol. The average estradiol among normotensive controls was 89.1±37.7 pmol/L, which is consistent with the follicular phase (normal range: 77-921 pmol/L).

Experimental Procedures

All studies commenced at 8 am after an 8-hour fast. Prior to initiating the study, subjects
were instructed to void spontaneously. Subjects remained supine throughout the entire study.

Arterial pressure and heart rate were measured with an automated sphygmomanometer (Dinamap, Criticon, Tampa, FL) at baseline, and then every 15 minutes throughout the study protocol. Baseline laboratory assays included angiotensinogen, active renin concentration, plasma renin activity (PRA), Angiotensin II (Ang II), and aldosterone.

First, the subject was positioned in an LBNP chamber, which was sealed at the iliac crest encasing the body below the waist. The LBNP was connected to a vacuum source controlled by a rheostat. Serial increments of LBNP (-15, -25, -40 mmHg) were applied to activate the RAAS by unloading baroreceptors. Each level of LBNP was applied for 15 minutes. LBNP was stopped if subjects were unable to continue due to the severe symptoms of orthostatic intolerance (lightheadedness, nausea or vomiting). Ang II, renin and aldosterone were sampled at the end of each 15-minute period as well as following a 15-minute recovery period.

**Drug Pharmacokinetics and Laboratory Determinations**

Women taking the OCP were receiving 30μg of ethinyl estradiol and 150μg of levonorgesterol daily while women taking the contraceptive patch were receiving 20μg of ethinyl estradiol and 150μg of norelgestromin daily. Although the contraceptive patch contained less ethinyl estradiol than the OCP, a pharmacokinetic study using the same drug doses demonstrated that the average serum ethinyl estradiol concentration is 1.6 times higher with the contraceptive patch versus the OCP. (29) With respect to the progesterone components, norelgestromin in the contraceptive patch is metabolized to norgestrel. The active compound in norgestrel is levonorgestrel, the same compound in the OCP.

All laboratory measurements were completed at the Toronto General Hospital, University Health Network, using validated assays. Estradiol measurements were performed by chemiluminescent immunoassay on the Abbott Architect analyzer using the manufacturer's reagents (Abbott Diagnostics, IL, USA). Angiotensinogen (AGT) is measured in conjunction with
plasma renin activity (PRA) using the GammaCoat® Plasma Renin Activity 125-I RIA kit (DiaSorin Inc., Stillwater, MN, USA). In the immunoassay, angiotensin I (ATI) in the sample and radiolabelled ATI compete for binding to rabbit anti-ATI serum on the coated tubes. Total ATI is determined by prolonged incubation (18h) at 37 °C in the presence of excess renin to convert AGT into ATI. Conversion of ATI to ATII during incubation is inhibited by the addition of EDTA and phenylmethylsulfonyl fluoride (PMSF). The amount of endogenous ATI is determined in a separate assay by incubation at 4 °C, which is subtracted from the total ATI in the previous step to obtain AGT. PRA determination involves an initial incubation of plasma to generate angiotensin I, followed by quantitation of angiotensin I by radioimmunoassay. In the GammaCoat® PRA 125I RIA Kit, the antibody is immobilized onto the lower inner wall of the GammaCoat tube. After incubation of standards, samples and 125I angiotensin I in the GammaCoat tube, the reaction mixture is removed by aspiration and the bound tracer counted in a gamma counter. A standard curve is constructed and the concentration of angiotensin I of the unknown sample obtained by interpolation. Plasma renin concentration was measured by two-site immunoradiometric assay where two monoclonal antibodies to human active renin were used (Cat #79986, Bio-Rad, CA, USA). One antibody was coupled to biotin while the second was radiolabeled for detection. The sample containing active renin was incubated simultaneously with both antibodies to form a complex. The radioactivity of this complex was directly proportional to the amount of immunoreactive renin present in the sample.(5) Blood samples for angiotensin II were collected into pre-chilled tubes containing EDTA and angiotensinase inhibitor (0.1 ml Bestatin Solution, Buhlmann Laboratories, Switzerland). After centrifugation, plasma was stored at -70°C until analysis. On the day of analysis, plasma samples were extracted on phenylsilysilica columns followed by a competitive angiotensin II radioimmunoassay kit supplied by Buhlmann Laboratories AG (Switzerland). Aldosterone was measured by radioimmunoassay (Coat-A-Count system, Siemens).
Statistical Analysis

Results are presented as either mean ± standard error of measurement or median (interquartile range). A one-way ANOVA, the Kruskal-Wallis test or Chi-square test was utilized to assess for significant differences among normotensive controls, OCP subjects, and contraceptive patch subjects, where appropriate. Within-subject and between-group differences were determined by mixed models ANOVA with a Bonferroni correction for multiple comparisons. The Fisher Exact Test was utilized to compare proportions. Due to small, but statistically significant baseline differences in age, an ANCOVA was used to adjust for age when comparing baseline differences in RAAS component. Age was also included as a variable in the mixed models ANOVA. As unadjusted and adjusted results were similar, only the unadjusted findings were reported. SAS 9.3 statistical software was utilized.

RESULTS

Baseline Study Subject Characteristics

Baseline demographic characteristics and circulating RAAS mediators for the three groups are displayed in Tables 1 and 2. No statistically significant differences were noted among the groups in baseline body mass index (BMI), pulse and mean arterial pressure (MAP) or 24-hour urine collections for both sodium and urea excretion. A small, but statistically significant difference was, however, noted between the age of OCP and contraceptive patch subjects.

Baseline angiotensinogen, PRA and Ang II levels were significantly higher in OCP subjects compared to controls and contraceptive patch subjects (p<0.05). Levels of these mediators were, however, similar between controls and contraceptive patch subjects. Plasma aldosterone was also numerically highest in the OCP group, but between-group differences only reached significance versus controls (p<0.05). For plasma renin concentration, baseline levels were lowest in contraceptive patch subjects compared to controls and OCP subjects (p<0.05).
Renin and Angiotensin II Response to Lower Body Negative Pressure (LBNP)

The renin, Ang II and aldosterone response to LBNP stimulation is displayed in Figure 1. Serial increments of LBNP resulted in significant stimulation of the renin and Ang II in normotensive controls and OCP subjects, and these groups demonstrated a significant rise in circulating renin compared to baseline (p<0.05). In contrast, plasma renin levels were not influenced by LBNP in contraceptive patch subjects, and remained suppressed at all stages compared to normotensive controls and OCP subjects, with a statistically significant difference noted at -40 mmHg (Figure 1). Normotensive controls and OCP subjects also exhibited a significant increase in plasma Ang II at -40 mmHg relative to baseline (p<0.05). In contrast, plasma levels of Ang II remained suppressed at all stages of LBNP for the contraceptive patch group (Figure 1). Similar to the renin response to LBNP, contraceptive patch subjects had significantly lower Ang II levels at -40 mmHg compared to normotensive controls and OCP subjects (p<0.05). Finally, normotensive controls and OCP subjects had a significant increase in aldosterone at -40 mmHg relative to baseline (p<0.05) whereas aldosterone levels were similar at all stages of LBNP for contraceptive patch subjects. With respect to between group differences, aldosterone was significantly higher in OCP subjects compared to controls at all stages of LBNP (p<0.05). There was also a statistically significant difference in aldosterone levels between contraceptive patch subjects and normotensive controls (-40 mmHg only, p<0.05) and OCP subjects (-25 mmHg and -40 mmHg, p<0.05).

Systemic Vascular Response to Lower Body Negative Pressure (LBNP)

The systemic vascular response to incremental LBNP stimulation is displayed in Figure 2. Normotensive controls and OCP subjects did not exhibit any statistically significant change in MAP at any stage of LBNP compared to baseline. In keeping with the blunted rise in circulating renin and Ang II, contraceptive patch subjects were unable to maintain baseline blood pressure values at -25 and -40 mmHg compared to baseline. The decline in MAP at -25 mmHg was
statistically significant in comparison to controls and OCP subjects, and only 50% of contraceptive patch subjects remained in the study until -40 mmHg. In contrast, 70% and 86% of OCP and control subjects remained in the study until the end of LBNP stimulation at -40 mmHg. As such, the within and between group changes at -40 mmHg did not meet statistical significance likely due to the small numbers of subjects on the contraceptive patch that were able to tolerate this level of LBNP.

**DISCUSSION**

Our study provides the first examination of the RAAS response to transdermal estrogen in premenopausal women. Our major observations were: 1) Contraceptive patch subjects had significantly lower baseline levels of angiotensinogen, Ang II and PRA compared to OCP subjects; and 2) Contraceptive patch subjects displayed blunted circulating renin and Ang II responses along with an exaggerated decline in MAP to LBNP compared to control and OCP subjects. Together, these results suggest that the contraceptive patch is associated with less renin and Ang II activation as well as blunting of the renin and Ang II response to a simulated orthostatic stress.

Although the RAAS response to the contraceptive patch requires further studies to elucidate the mechanism of action, it is already known that transdermal estrogen acts at multiple points along the RAAS activation cascade. First, the transdermal route is known for the absence of first pass hepatic metabolism and limited production of angiotensinogen. Our study noted a similar finding, particularly highlighting that contraceptive patch subjects exhibited significantly lower angiotensinogen levels in comparison to OCP subjects.

Downstream to angiotensinogen, estrogen administration also produces important alterations in the RAAS cascade. Using data from a prospective cohort study, other investigators have demonstrated that plasma renin levels were suppressed in post-menopausal women receiving either transdermal or oral estrogen compared to those without estrogen
replacement. These findings mirror the results of our study, wherein renin suppression occurred at all stages of LBNP in contraceptive patch subjects and at -40 mmHg in OCP subjects compared to controls. Other investigators have also shown that prorenin and total renin levels were increased among post-menopausal subjects receiving transdermal 17β estradiol, but there was no concomitant rise in plasma renin activity. These findings suggest that estrogen inhibits the conversion of prorenin to active renin, thereby providing more proximal inhibition of the RAAS. This is consistent with our study wherein PRA was lower in contraceptive patch subjects compared to OCP subjects and similar between contraceptive patch subjects and controls. Further work is needed to better understand these observations.

Downstream to renin, contraceptive patch users had significantly lower plasma levels of Ang II in comparison to OCP and control subjects (at -40 mmHg only). This finding likely reflects the lower renin levels in contraceptive patch subjects, but we cannot exclude a possible impact on angiotensin converting enzyme (ACE) activity. Tissue expression of the angiotensin II receptor, type 1 (AT1) may also be altered in contraceptive patch subjects, and our group has previously reported that tissue AT1 receptor mRNA is markedly increased in OCP users. Although beyond the scope of this study, lower AT1 receptor mRNA in contraceptive patch subjects may produce differences in tissue sensitivity to circulating Ang II, accounting for the differential MAP responses noted. Finally, the blunting of Ang II in contraceptive patch subjects was not associated with a significantly lower plasma aldosterone compared to OCP and control subjects at baseline, but a statistically significant difference was evident at -40 mmHg. This finding is in agreement with upstream blunting of renin in contraceptive patch subjects.

Progesterone may also impact the RAAS. A previous study demonstrated that the administration of 300 mg/day of micronized intravaginal progesterone to women already receiving transdermal 17β estradiol resulted in increased circulating levels of renin, PRA and
aldosterone compared to the placebo group. This is in contrast to our study, wherein a much lower dose of progesterone (150μg of levonorgesterol daily) was administered. Further, the OCP and contraceptive patch contained similar doses of progesterone, suggesting that the dose of progesterone included in the contraceptive patch and OCP may have had a limited RAAS augmenting effect compared to the differences associated with the ethinyl estradiol component.

Our controls were only studied during the follicular phase of the menstrual cycle, since previous studies have demonstrated that healthy premenopausal subjects failed to maintain MAP during LBNP in the luteal compared to the follicular phase of the menstrual cycle. Furthermore, study subjects in our previous work exhibited an augmented humoral response with higher levels of circulating RAAS components in the luteal phase, suggesting vascular insensitivity to the RAAS. This pattern mirrors our findings in the contraceptive patch subjects, suggesting that contraceptive patch subjects may be more similar to controls than OCP subjects.

From a clinical perspective, the differential in baseline RAAS components as well as the differential response to simulated orthostatic hypotension in the contraceptive patch and OCP subjects may have implications for patients at risk for the development and progression of kidney disease. To date, renal physiologic studies have demonstrated that OCP use was associated with renal and peripheral hemodynamic differences at baseline, namely higher systolic blood pressure, filtration fraction and renal vascular resistance. Furthermore, OCP use increased albumin excretion, and in a large administrative database study, OCP subjects exhibited a significantly higher risk for the development of microalbuminuria, even after adjustment for age, hypertension, diabetes, obesity, hyperlipidemia, and smoking (adjusted OR=1.90, 95% CI 1.23-2.93). Similar interactions were published in a small prospective study, which reported an almost 9-fold risk of conversion from microalbuminuria to macroalbuminuria among diabetic subjects using the OCP (adjusted OR=8.90, 95% CI 1.79-44.36). Interestingly, the risk of proteinuria with OCP use was reversible after 6 months of discontinuation, reinforcing a hemodynamic, non-structural
Given the use of ACE inhibitors and angiotensin receptor blockers in the management of renal disease, the contraceptive patch may be a beneficial form of contraception in these patients. In particular, estrogen replacement therapy has been shown to further suppress renin, even in the presence of ACE inhibitor use. (27) Accordingly, concomitant use of the contraceptive patch with ACE inhibitors and angiotensin receptor blockers (ARBs) may provide a safer form of additional RAAS blockade than the strategies examined in the ONTARGET, (20) VA NEPHRON D (9) and ALTITUDE clinical trials. (21) To our knowledge, there are no clinical outcome data available for women receiving transdermal contraception or postmenopausal hormone replacement therapy in conjunction with ACE inhibitors or ARBs, highlighting this combination as an important area for future research. Our work provides the rationale for further investigation into the potential role of hormonal therapies as modulators of the RAAS.

The exaggerated decline in MAP in contraceptive patch subjects may also be clinically important from the perspective of future risk for cardiovascular disease. Notably, individuals at increased risk for cardiovascular disease do not have this depressor response and instead maintain their MAP during LBNP. For instance, in the early postpartum phase, women with pregnancies complicated by previous preeclampsia maintained their MAP in response to -40 mmHg of LBNP in comparison to healthy previously pregnant control subjects and healthy, never pregnant female volunteers of reproductive age. (13) As contraceptive patch subjects had a greater responsiveness to LBNP compared to control and OCP subjects, this may also be suggestive of a more favorable vascular profile.

Finally, our findings have important implications with respect to strategies to prevent unplanned pregnancies in women with kidney disease, particularly given that these pregnancies are high risk to women and their children. (18, 19) Effective contraception is therefore needed in these premenopausal women to avoid the detrimental effect of ACE inhibitors and ARBs during pregnancy. (8, 25, 26) Since the OCP increases the risk for the development and progression of
kidney disease and cardiovascular disease, the contraceptive patch or other non-oral forms of contraception may be beneficial alternatives.

Our study has important limitations. It was non-randomized in design and the sample size was small. These factors resulted in baseline differences between study groups that may have limited our ability to detect some differences in hormonal and hemodynamic parameters during LBNP. We attempted to minimize this limitation by following careful pre-study dietary preparation and using a repeated measures study design. Finally, assessing the therapeutic application of the contraceptive patch was beyond the scope of our current study, but we do note that current formulations of transdermal contraceptives may increase the risk of venous thromboembolism and thrombotic stroke.(15) The clinical utility of transdermal contraception will, therefore, require careful prospective studies in women with increased risks for thromboembolic events. Accordingly, the contribution of our study is to highlight the physiological RAAS response to transdermal estrogen in pre-menopausal women.

In summary, inhibition of the RAAS is a key to the management of patients with kidney disease. Our study suggests that there are important differences in the RAAS in healthy pre-menopausal women using the transdermal patch for contraception as compared to the OCP. Further evaluation of the therapeutic implications of different modes of contraception is, therefore, warranted in young women with kidney disease.
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CONFLICTS: Nothing to declare.

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References


Serial increments of LBNP resulted in significant stimulation of renin and Ang II in normotensive controls and oral contraceptive pill (OCP) subjects compared to contraceptive patch subjects. Results for each stage of LBNP were limited to participants who tolerated the maneuver. Where the open square is controls; dark triangle is OCP and dark circle is contraceptive patch; δ p<0.05 compared to controls; † p<0.05 compared to OCP; ‡ p<0.05 compared to baseline. Note for the renin and Ang II graphs, there were no differences versus baseline or between groups at LBNP level -15 mmHg and -25 mmHg. Note for the aldosterone graph, the symbol † at -25 mmHg compares contraceptive patch subjects to OCP subjects.
In response to incremental LBNP, normotensive controls and OCP subjects did not exhibit any statistically significant change in the MAP at any stage of LBNP whereas contraceptive patch subjects were unable to maintain baseline blood pressure values at -25 and -40 mmHg. Numbers above the graph refer to the number of participants who managed to complete each stage of LBNP stimulation. Note that between group statistical significance at -25 mmHg is only for contraceptive patch subjects and OCP subjects. Where the open square is controls; dark triangle is OCP and dark circle is contraceptive patch; † $p<0.05$ compared to OCP; ‡ $p<0.05$ compared to baseline.
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<thead>
<tr>
<th>Table 1: Baseline Characteristics</th>
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<td><strong>Control</strong></td>
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<tr>
<td><strong>OCP</strong></td>
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<td><strong>Contraceptive Patch</strong></td>
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<tr>
<td><strong>Age (years)</strong></td>
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<tr>
<td><strong>BMI (kg/m²)</strong></td>
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<tr>
<td><strong>Pulse (beats/min)</strong></td>
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<td><strong>MAP (mmHg)</strong></td>
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<td><strong>Sodium Excretion (mmol/d)</strong></td>
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<tr>
<td><strong>Urea Excretion (mmol/d)</strong></td>
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BMI is body mass index, MAP is mean arterial pressure; † p<0.05 compared to OCP.
Table 2: Baseline Circulating Component of the RAAS

<table>
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<th>Controls</th>
<th>OCP</th>
<th>Contraceptive Patch</th>
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<tbody>
<tr>
<td>Angiotensinogen ng/ml</td>
<td>2555±500</td>
<td>6144±460 δ</td>
<td>2305±186 †</td>
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<tr>
<td>Renin ng/L</td>
<td>14.2±2.7</td>
<td>11.9±2.1</td>
<td>4.7±0.9 δ†</td>
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<tr>
<td>ANG II pmol/ml</td>
<td>8.8±1.4</td>
<td>15.4±3.5 δ</td>
<td>5.5±1.2 †</td>
</tr>
<tr>
<td>Aldosterone pmol/liter</td>
<td>143±21</td>
<td>266±44 δ</td>
<td>238±48</td>
</tr>
<tr>
<td>PRA ng ANG l•L⁻¹•min⁻¹</td>
<td>1.2±0.4</td>
<td>2.3±0.2 δ</td>
<td>1.2±0.2 †</td>
</tr>
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PRA is plasma renin activity; δ p<0.05 compared to controls; † p<0.05 compared to OCP. All comparisons adjusted for age. OCP is combined oral contraceptive pill.
Controls: 15 15 15 13
Contraceptive Patch: 10 10 9 5
OCP: 10 10 9 7

Mean Arterial Pressure (mmHg) vs. LBNP (mmHg)