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Transdermal contraception and the renin-angiotensin-aldosterone system in premenopausal women

Ayodele Odutayo,1 David Cherney,2,5 Judith Miller,2 Sofia B. Ahmed,3 Vestal Lai,2 Sheila Dunn,4 Nicole Pun,2 Rahim Moineddin,5 and Michelle A. Hladunewich1,2*

1Division of Nephrology, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada; 2University Health Network, University of Toronto, Toronto, Ontario, Canada; 3University of Calgary, Calgary, Alberta, Canada; 4Department of Family and Community Medicine, Women’s College Hospital, University of Toronto, Toronto, Ontario, Canada; and 5The Institute for Clinical Evaluative Sciences, University of Toronto, Toronto, Ontario, Canada

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Transdermal contraception and the renin-angiotensin-aldosterone system in premenopausal women. Am J Physiol Renal Physiol 308: F535–F540, 2015. First published January 13, 2015; doi:10.1152/ajprenal.00602.2014.—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 (EVARA) does not undergo first-pass hepatic metabolism, we hypothesized that the RAAS response would be different from that of OCP subjects. Thirty-five nonsmoking, premenopausal women (15 control subjects, 10 OCP subjects, 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, and −40 mmHg). Baseline levels of angiotensinogen, angiotensin II, and plasma renin activity were significantly higher in OCP subjects compared with normotensive control and contraceptive patch subjects (P < 0.05), whereas aldosterone was significantly higher in OCP versus control subjects only (P < 0.05). Plasma renin levels were significantly lower at baseline in contraceptive patch subjects compared with normotensive control 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. control and OCP subjects). The contraceptive patch in healthy premenopausal 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.

oral contraception; contraceptive patch; renin-angiotensin-aldosterone system

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 μg 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 (EVARA) is distinct because of the avoidance of first-pass metabolism. Theoretically, the contraceptive patch should not stimulate hepatic production of angiotensinogen (AGT) (11), the precursor of ANG II and the effector hormone of the RAAS. Studies using transdermal postmenopausal hormone therapy have also suggested that circulating RAAS components are not increased by the transdermal route (6, 16, 28). Given that upregulation 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 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 with OCP subjects.

METHODS

Study population. Fifteen normotensive control subjects, 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 (blood pressure > 130/80 mmHg), kidney disease, heart disease, or diabetes. Smokers were excluded from the study. All participants were ≤40 yr old and were either already using the study contraceptive patch or OCP or were willing to initiate them for contraceptive purposes.

* D. Cherney and M. A. Hladunewich contributed equally to this work. Address for reprint requests and other correspondence: M. A. Hladunewich, Divisions of Nephrology, Critical Care, and Obstetric Medicine, Dept. of Medicine, Sunnybrook Health Sciences Center, A139, 2075 Bayview Ave., Toronto, ON, Canada M4N 3M5 (e-mail: michelle.hladunewich@sunnybrook.ca).
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, ON, 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 of the University of Toronto. All subjects gave written informed consent.

Preparation. Except for normotensive control subjects, all subjects had taken hormonal contraception for at least 3 mo before participating in the study. Pregnancy was ruled out by a negative serum β-human chorionic gonadotropin test before enrollment, and experiments were performed after 7 days on a controlled diet. Each subject was prescribed a diet to target 150 mmol/day of Na⁺ and 1.5 g·kg⁻¹·day⁻¹ of protein. Compliance was ascertained by measurements of Na⁺ and urea excretion in a 24-h urine collection. Healthy control subjects were studied during the follicular phase of their menstrual cycle as confirmed by a detailed menstrual history as well as measurements of 17β-estradiol. The average estradiol among normotensive control subjects was 89.1 ± 37.7 pmol/l, which is consistent with the follicular phase (normal range: 77–921 pmol/l).

Experimental procedures. All experiments commenced at 8 AM after an 8-h fast. Before 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 min throughout the study protocol. Baseline laboratory assays included AGT, active renin concentration, plasma renin activity (PRA), 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 chamber was connected to a vacuum source controlled by a rheostat. Serial increments of LBNP (−15, −25, and −40 mmHg) were applied to activate the RAAS by unloading baroreceptors. Each level of LBNP was applied for 15 min. LBNP was stopped if subjects were unable to continue due to the severe symptoms of orthostatic intolerance (light-headedness, nausea, or vomiting). ANG II, renin, and aldosterone were sampled at the end of each 15-min period as well as after a 15-min recovery period.

Drug pharmacokinetics and laboratory determinations. Women taking the OCP received 30 μg ethinyl estradiol and 150 μg levonorgestrel daily, whereas women taking the contraceptive patch received 20 μg ethinyl estradiol and 150 μg 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 norgesterel. The active compound in norgesterel 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). AGT was measured in conjunction with PRA using the GammaCoat Plasma Renin Activity 125-I RIA kit (DiaSorin, Stillwater, MN). In the immunoassay, ANG I in the sample and radiolabeled ANG I compete for binding to rabbit anti-ANG I serum on the coated tubes. Total ANG I is determined by prolonged incubation (18 h) at 37°C in the presence of excess renin to convert AGT into ANG I. The conversion of ANG I to ANG II during incubation is inhibited by the addition of EDTA and phenylmethylsulfonyl fluoride (PMSF). The amount of endogenous ANG I is determined in a separate assay by incubation at 4°C, which is subtracted from total ANG I in the previous step to obtain AGT. PRA determination involves an initial incubation of plasma to generate ANG I followed by the quantitation of ANG 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 with standards, samples, and 125I-labeled ANG I in the GammaCoat tube, the reaction mixture is removed by aspiration, and the bound tracer counted in a γ-counter. A standard curve is constructed, and the concentration of ANG I of the unknown sample obtained by interpolation. Plasma renin concentration was measured by a two-site immunoradiometric assay where two monoclonal antibodies to human active renin were used (catalog no. 79986, Bio-Rad). One antibody was coupled to biotin, whereas the second antibody 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 ANG II were collected into prechilled tubes containing EDTA and angiotensinase inhibitor (0.1 ml bestatin solution, Buhlmann Laboratories). Angiotensin was measured by radioimmunoassay (Coat-A-Count system, Siemens).

Statistical analysis. Results are presented as either means ± SE or medians (interquartile ranges). One-way ANOVA, a Kruskal-Wallis test, or a χ²-test was used to assess for significant differences among normotensive control subjects, 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 used to compare proportions. Due to small but statistically significant baseline differences in age, analysis of covariance was used to adjust for age when baseline differences were compared in the 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 used.

RESULTS

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

Baseline AGT, PRA, and ANG II levels were significantly higher in OCP subjects compared with control and contraceptive patch subjects (P < 0.05). Levels of these mediators were, however, similar between control and contraceptive patch subjects. Plasma aldosterone was also numerically highest in the OCP group, but between-group differences only reached
Our major observations were as follows: 1) contraceptive patch subjects had significantly lower baseline levels of AGT, ANG II, and PRA compared with OCP subjects and 2) contraceptive patch subjects displayed blunted circulating renin, ANG II, and aldosterone responses along with an exaggerated decline in MAP to LBNP compared with control and OCP subjects.

Fig. 1. Renin-angiotensin-aldosterone system (RAAS) response to lower body negative pressure (LBNP). Serial increments of LBNP resulted in significant stimulation of renin and ANG II in normotensive control subjects and oral contraceptive pill (OCP) subjects compared with contraceptive patch subjects. Results for each stage of LBNP were limited to participants who tolerated the maneuver. □, Control subjects; ▲, OCP subjects; ○, contraceptive patch subjects. *P < 0.05 compared with control subjects; †P < 0.05 compared with OCP subjects; ‡P < 0.05 compared with baseline.

Table 2. Baseline circulating components of the renin-angiotensin-aldosterone system

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control Subjects</th>
<th>OCP Subjects</th>
<th>Contraceptive Patch Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensinogen, ng/ml</td>
<td>2.55 ± 500</td>
<td>6.144 ± 460*</td>
<td>2.305 ± 186†</td>
</tr>
<tr>
<td>Renin, ng/l</td>
<td>14.2 ± 2.7</td>
<td>11.9 ± 2.1</td>
<td>4.7 ± 0.9†</td>
</tr>
<tr>
<td>ANG II, pmol/ml</td>
<td>8.8 ± 1.4</td>
<td>15.4 ± 3.5*</td>
<td>5.5 ± 1.2†</td>
</tr>
<tr>
<td>Aldosterone, pmol/liter</td>
<td>143 ± 21</td>
<td>266 ± 44*</td>
<td>238 ± 48</td>
</tr>
<tr>
<td>Plasma renin activity, ng/L</td>
<td>1.2 ± 0.4</td>
<td>2.3 ± 0.2*</td>
<td>1.2 ± 0.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with control subjects; †P < 0.05 compared with OCP subjects.

significance versus control subjects (P < 0.05). For plasma renin concentration, baseline levels were lowest in contraceptive patch subjects compared with control and OCP subjects (P < 0.05).

**Renin and ANG II responses to LBNP.** Renin, ANG II, and aldosterone responses to LBNP stimulation are shown in Fig. 1. Serial increments of LBNP resulted in significant stimulation of renin and ANG II in normotensive control and OCP subjects, and these groups demonstrated a significant rise in circulating renin compared with 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 with normotensive control and OCP subjects, with a statistically significant difference noted at −40 mmHg (Fig. 1). Normotensive control 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 (Fig. 1). Similar to the renin response to LBNP, contraceptive patch subjects had significantly lower ANG II levels at −40 mmHg compared with normotensive control and OCP subjects (P < 0.05). Finally, normotensive control subjects had a significant increase in aldosterone at −40 mmHg relative to baseline (P < 0.05). With respect to between-group differences, there was a statistically significant difference in aldosterone levels between contraceptive patch and OCP subjects at −40 mmHg (P < 0.05).

**Systemic vascular response to LBNP.** The systemic vascular response to incremental LBNP stimulation is shown in Fig. 2. Normotensive control and OCP subjects did not exhibit any statistically significant change in MAP at any stage of LBNP compared with 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 with baseline. The decline in MAP at −25 mmHg was statistically significant compared with control 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.
Together, these results suggest that the contraceptive patch is associated with less RAAS activation as well as blunting of the RAAS 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 AGT (6, 16). Our study noted a similar finding, particularly highlighting that contraceptive patch subjects exhibited significantly lower AGT levels compared with OCP subjects.

Downstream to AGT, estrogen administration also produces important alterations in the RAAS cascade. Using data from a prospective cohort study (27), other investigators have demonstrated that plasma renin levels were suppressed in postmenopausal women that received either transdermal or oral estrogen compared with those without estrogen replacement. Within the postmenopausal hormone therapy groups, the authors also reported that renin levels were most suppressed in women with higher serum estradiol levels (27). 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 with control subjects. Other investigators have also shown that prorenin and total renin levels were increased among postmenopausal subjects that received transdermal 17β-estradiol, but there was no concomitant rise in PRA (28). These findings suggest that estrogen inhibits the conversion of prorenin to active renin, thereby providing more proximal inhibition of the RAAS (28). This is consistent with our study, wherein PRA was lower in contraceptive patch subjects compared with OCP subjects and similar between contraceptive patch and control subjects. Further work is needed to better understand these observations.

Downstream to renin, contraceptive patch users had significantly lower plasma levels of ANG II compared with 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 (22). Tissue expression of the ANG II type 1 (AT1) receptor 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 (2). 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 significantly lower plasma aldosterone compared with OCP 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.

Progestosterone may also impact the RAAS. A previous study (28) 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 with the placebo group. This is in contrast to our study, wherein a much lower dose of progesterone (150 μg levonorgesteral daily) was administered. Furthermore, 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 with differences associated with the ethinyl estradiol component.

Our control subjects were only studied during the follicular phase of the menstrual cycle, since a previous study (7) has demonstrated that healthy premenopausal subjects failed to maintain MAP during LBNP in the luteal phase compared with 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 (7). This pattern mirrors our findings in contraceptive patch subjects, suggesting that contraceptive patch subjects may be more similar to control subjects 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 physiological 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 (14). Furthermore, OCP use increased albumin excretion (23), 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 odds ratio: 1.90, 95% confidence interval: 1.23–2.93) (17). Similar interactions have been published in a small prospective study, which reported an almost ninefold risk of conversion from microalbuminuria to macroalbuminuria among diabetic sub-

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**Fig. 2.** Hemodynamic response to LBNP. In response to incremental LBNP, normotensive control and OCP subjects did not exhibit any statistically significant change in mean arterial pressure at any stage of LBNP, whereas contraceptive patch subjects were unable to maintain baseline blood pressure at ~25 and ~40 mmHg. Numbers above the graph refer to the number of participants who managed to complete each stage of LBNP stimulation. ▲ Control subjects; □ OCP subjects; ● contraceptive patch subjects. *P < 0.05 compared with controls; †P < 0.05 compared with OCP subjects; ‡P < 0.05 compared with baseline.
Accordingly, the contribution of our study is to highlight the importance of 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 compared with 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 with 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 forms of contraception may be beneficial alternatives.

Our study has important limitations. It was nonrandomized 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 prestudy dietary preparation and using a repeated-measures study design. Finally, assessing the therapeutic application of the contraceptive patch was beyond the scope of our present 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 premenopausal 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 premenopausal women using the transdermal patch for contraception compared with 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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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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