Effects of Oral Contraceptive Use on the Renal and Systemic Vascular Response to Angiotensin II Infusion

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Abstract. We have previously shown that users of oral contraceptive (OC) medications exhibit increased plasma levels of angiotensin II (Ang II) with only modest hemodynamic consequences, suggesting estrogen-mediated Ang II type 1 (AT1) receptor downregulation. Accordingly, in 10 women who were OC users and 10 women who, as OC nonusers, served as controls, all mean age 26 ± 1 yr, we examined the renal and peripheral hemodynamic response to graded Ang II infusion, plasma and urine cyclic guanosine monophosphate (cGMP) levels as a surrogate marker for AT1 and/or AT2 receptor-mediated activation of the nitric oxide pathway, and AT1 receptor expression in skin biopsies. The OC nonusers were studied during the follicular and luteal phases of the menstrual cycle, whereas OC users were studied once during the 21-d medication phase. Subjects ingested a controlled sodium diet for 7 d before each study. Renal hemodynamic function was assessed using standard inulin and p-aminohippurate clearance techniques. AT1 receptor mRNA levels in skin biopsy samples were assessed using a real-time PCR protocol. In response to graded Ang II infusion, OC users exhibited renal and peripheral hemodynamic responses that were augmented compared with those of OC nonusers, in conjunction with evidence of increased tissue AT1 receptor expression. Plasma cGMP levels and 24-h urinary cGMP excretion did not differ. These data suggest that, contrary to our original hypothesis, OC use does not appear to be associated with AT1 receptor downregulation. The factor protecting OC users from the hemodynamic impact of increased Ang II levels remains elusive.

Gender and estrogen status are important determinants of renin angiotensin system (RAS) function and the renal and systemic response to angiotensin II (Ang II) (1–3). This laboratory has previously shown that ethinyl estradiol use in the form of the oral contraceptive (OC) pill results in dramatic increases of circulating RAS components, including Ang II; yet, in normal women, the hemodynamic consequences are modest, consisting of a small increase in BP and filtration fraction (1). Ang II, the primary effector of the RAS, is a multifunctional peptide hormone, the actions of which are primarily mediated by two receptors, Ang II type 1 (AT1) and type 2 (AT2). The majority of the physiological and pathophysiological effects of Ang II occur via the AT1 receptor. The function of the AT2 receptor is controversial, but it may act in opposition to AT1 receptors (4). It has been reported in some (5–8) but not all (1–3) studies that high estrogen decreases circulating renin and Ang II levels, downregulates AT1 receptor expression in vascular smooth muscle cells, adrenal cortex, and hypothalamus (9–11), and upregulates renal AT2 receptor expression (4). It has also been demonstrated that the hemodynamic response to Ang II infusion is blunted in high-estrogen states, such as the luteal phase of the normal menstrual cycle (3), and in normal pregnancy (12–14). Therefore, we hypothesized that women ingesting OCs are protected from the hemodynamic effects of increased circulating Ang II levels by AT1 receptor downregulation, either directly by increased ethinyl estradiol levels and/or increases in circulating Ang II, resulting in homologous down-regulation, or indirectly by counter-regulatory mechanisms, including AT1 and/or AT2 receptor-mediated increases in the bioavailability of nitric oxide (NO) (4,15–17). Accordingly, we examined renal and hemodynamic function, both at baseline and in response to graded Ang II infusions, in 20 young, healthy women segregated into two groups of 10 subjects on the basis of OC use. We examined plasma and urine cyclic guanosine monophosphate (cGMP) levels as surrogate markers of the NO/cGMP signaling pathway (18). We also obtained skin biopsies from each subject for examination of tissue AT1 receptor expression (19).

Materials and Methods

Subjects
Twenty normal healthy women (10 OC users and 10 OC nonusers) were recruited to participate in the study. Their mean age was 26 ± 1 yr. Each subject underwent a detailed history and physical examination by a qualified internist. All were normotensive, nonobese, and nonsmokers. No subject was ingesting any regular medication other than OCs. The OC users had been on therapy for at least three cycles before the study. The study was performed with the approval of the
University Health Network Research Ethics Board and with the informed written consent of each subject. All subjects were counseled to adhere to a diet that maintained their normal caloric intake, their sodium intake to greater than 2 mmol/kg per 24 h, and their protein intake to 1 to 1.5 g/kg per 24 h for 7 d before the study. A 24-h urine sample was collected to determine compliance with the diet, and data from subjects were used only if the excretion rate of sodium was greater than 2 mmol/kg per d and urea excretion rate was between 3 and 6 mmol/kg per d. No data were excluded on this basis. Protein intake was estimated from the urine urea concentration using standard equations and was corrected for body weight. An aliquot from each 24-h urine sample was collected for measurement of urine cGMP. The subjects were advised not to ingest any caffeine for 12 h before the study. All OC nonusers were studied in both the luteal and follicular phases of their menstrual cycle, determined by counting days and confirmed by estrogen and progesterone measurements. OC users were studied once during the medication phase, between days 7 and 21 of their cycle, during which they were ingesting 30 μg of ethinyl estradiol plus 150 μg of levonorgestrel. On the day of testing, the volunteer subjects reported to the Renal Physiology Laboratory at Toronto General Hospital. All studies were conducted at 8:30 AM with the subjects lying supine in a cubital vein for infusions of inulin, p-aminohippurate (PAH), and Ang II, and another cannula was placed in the opposite arm for blood sampling. Each subject voided spontaneously before starting the study. Hemodynamic parameters [mean arterial pressure (MAP), heart rate] were measured throughout the study with an automated sphygmomanometer (Dinamap) and were recorded once in each half-hour of the protocol. Renal hemodynamics were measured using inulin and PAH clearance techniques. After collecting blood for inulin and PAH blank, hematocrit (HCT), plasma cGMP, progesterone, and 17β-estradiol (in OC nonusers only), a priming infusion containing 25% inulin (60 mg/kg) and 20% PAH (8 mg/kg) was administered. Thereafter, inulin and PAH were infused continuously at a rate calculated to maintain the respective plasma concentrations constant at 20 and 1.5 mg/dl. Subjects remained supine at all times. After a 90-min equilibration period, and in each half-hour for 90 min, blood was collected for inulin, PAH, and HCT. GFR and effective renal plasma flow (ERPF) were estimated by steady-state infusion of inulin and PAH according to the calculation method described by Schnurr et al. (20).

Blood was also collected for measurement of aldosterone, norepinephrine, Ang II, angiotensinogen, plasma renin activity (PRA), and plasma renin concentration (PRC).

A solution of Ang II (51.2 μg/vial) (Clinalfa, Switzerland) was prepared by dissolving the diluent in 1 ml of normal saline to produce a concentration of 50 μg/ml. Ninety-nine milliliters of normal saline was then added to 1 ml of Ang II to produce a concentration of 500 ng/ml. Ang II was infused at two doses, 3 and 6 ng/kg per min, each dose for 30 min. Subjects remained supine. Blood was collected once at the end of each Ang II infusion period for HCT, inulin, and PAH. A further collection of blood for HCT, inulin, and PAH was obtained at the end of the second Ang II infusion, after a 30-min recovery period.

At the end of the study, a skin biopsy was obtained from each subject under sterile conditions after subcutaneous infusion of local anesthetic with Xylocaine. In OC nonusers, the biopsy was obtained during the follicular phase.

Sample Collection and Analytic Methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3000 rpm for 15 min at 4°C. Plasma was separated, placed on ice, and then stored at −70°C before the assay. Inulin concentrations were measured by a modified method of Walser et al. (21), and PAH concentrations were measured by a spectrophotometric method according to Brun (22). The mean of the two pre-Ang II clearance periods represented baseline GFR and ERPF, expressed per 1.73 m². Filtration fraction (FF) represented the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by (1 − HCT). Renal vascular resistance (RVR) was derived by dividing MAP by the RBF.

Plasma cGMP samples were deproteinized with ethanol. cGMP was measured using the acetylation method with an assay kit purchased from Cayman Chemical Company (Ann Arbor, Michigan). Ang II was measured by RIA. Blood was collected into prechilled tubes containing EDTA and angiotensinase inhibitor (0.1 ml of Bestatin Solution; Buhlmann Laboratories, Schonembuch, Switzerland). After centrifugation, plasma samples were stored at −70°C until analysis. On the day of analysis, plasma samples were extracted on phenylisilsilica columns. A competitive RIA kit supplied by Buhlmann Laboratories AG (Schonembuch, Switzerland) was used to measure the extracted Ang II. The detection limit of the assay was approximately 2.0 pg/ml, with a precision of 8.3% intra-assay variation and 9.0% interassay variation.

PRA was determined by the quantification of angiotensin I generation by RIA using the New England Nuclear kit. Aldosterone was measured by RIA using the Coat-A-Count system. Angiotensinogen was measured indirectly by converting endogenous angiotensinogen to angiotensin I and then quantitating the amount of angiotensin I by RIA. Conversion was done by incubating the plasma with an excess amount of exogenous renin at 37°C for 18 h. After measuring the produced angiotensin I, the endogenous angiotensin I obtained before incubation was subtracted (23). PRC was measured by two-site immunoradiometric assay in which two monoclonal antibodies to human active renin were used. One antibody was coupled to biotin and 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 (24).

Plasma 17β-estradiol was determined by RIA using the DPC kit (Drug Products Corporation, Los Angeles, CA).

Skin biopsies were stored in liquid nitrogen at −70°C before processing. AT1 mRNA levels were assessed by a PCR protocol. RNA was isolated using an RNeasy kit (Qiagen, Mississauga, Canada), and 25 to 50 ng was used per reaction. Reverse transcription-PCR was performed using the One-Step RT-PCR kit (Applied Biosystems, Foster City, CA). One-step PCR was performed under the following conditions: 48°C for 30 min, 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 60 s, using a real-time PCR protocol and the ABI PRISM 7000 Sequence Detection System. The human AT1 receptor forward primer was 5'-AAATGCTGGGTTT-TATCTGAAT-3' and the reverse primer was 5'-TTTGTACACCT-GGGTCAATT-3', corresponding to bp 67 to 90 and 152 to 173, respectively, of the human AT1 receptor cDNA sequence. The final concentration of AT1 primers was 0.5 μmol/L. The human AT1 probe was 5'-ACTCACTGTGCCATCCAGAAAGTGC-3', corresponding to bp 92 to 119 of the human cDNA sequence. The AT1 receptor probe used a 5-carboxyfluorescin (FAM) dye with a 6-carboxytetramethylrhodamine (TAMRA) quencher at a final concentration of 0.2 μmol/L. For internal control, human glyceraldehyde phosphate dehydrogenase (GAPDH) primers were used as supplied by the manufac-
turer (TaqMan GAPDH Control reagents; Applied Biosystems) at a final concentration of 0.1 μmol/L. The final concentration of human GAPDH probe was 0.05 μmol/L. The GAPDH probe used a 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (JOE) dye and a TAMRA quencher according to the manufacturer. Standard curves were developed with RNA obtained from human kidney cortex (0.4 to 50 ng). Experiments were performed in duplicate or triplicate and repeated at least once. Data are presented as the ratio of human AT₁ receptor mRNA/GAPDH mRNA in arbitrary units.

**Statistical Analyses**

Results are presented as means ± SEM. Between-group baseline differences were determined using nonparametric methods (Wilcoxon rank sums). Within-subject and between-group differences in the responses to graded Ang II infusion were determined by repeated-measures ANOVA and Bonferroni correction. All statistical analyses were performed using the statistical package SAS (SAS Institute, Inc., Cary, NC).

**Results**

**Baseline Characteristics**

The baseline characteristics of the two groups are shown in Tables 1 and 2. There were no significant differences between groups in terms of age, body mass index, 24-h sodium excretion corrected for body weight, and calculated protein intake. The OC user group exhibited significantly higher MAP, PRA, and renin although the hemodynamic effects are modest, exhibiting values of 3.8 ± 0.6 pmol/ml and control subjects exhibiting values of 4.3 ± 0.5 pmol/ml in the follicular phase and 3.9 ± 0.5 pmol/ml in the luteal phase (P = NS).

**Renal and Systemic Hemodynamic Responses to Ang II**

As shown in Table 3 and in Figure 1, in response to Ang II infusion, the OC users exhibited responses for GFR, ERPF, and RBF that were equivalent to those of OC nonusers and values for MAP, FF, and RVR that were significantly augmented compared with those of OC nonusers in both the luteal and follicular phases. Baseline MAP in OC users was 82 ± 2 mmHg, increased to 96 ± 3 mmHg and then to 112 ± 4 mmHg, and returned to 82 ± 2 mmHg on recovery (P = 0.002 versus baseline). In OC nonusers during the luteal phase, the baseline MAP was 76 ± 1 mmHg (P = 0.02 versus baseline for OC users), increased to 91 ± 5 mmHg and then to 98 ± 2 mmHg (P = 0.01 versus baseline, and P = 0.03 versus response of OC users), subsequently returning to baseline during the recovery period. There was no significant difference in the MAP response to Ang II between the follicular and luteal phases.

**Skin Biopsy AT₁ Receptor Expression**

Data are presented as the ratio of human AT₁ receptor mRNA/GAPDH mRNA in arbitrary units. OC users exhibited mRNA corrected for GAPDH signal significantly higher than that of nonusers (23 ± 3 versus 6 ± 4; P = 0.006). There was a weak correlation between the Ang II-mediated change in MAP and tissue AT₁ receptor levels (r = 0.26, P = 0.05).

**Discussion**

In this study of healthy, young OC users and nonusers, we examined the baseline components of the RAS, the renal and peripheral responses to Ang II infusion, tissue AT₁ receptor expression, and plasma and urine cGMP levels. The rationale for this study was based on the observation that OC users exhibit significant increases in circulating levels of Ang II, PRA, and renin although the hemodynamic effects are modest, suggesting that the AT₁ receptor may be downregulated by high ambient Ang II levels, by ethinyl estradiol itself, or by AT₁- or AT₂-mediated activation of the NO/cGMP pathway. We hypothesized that OC users would display a blunted response to Ang II infusion, together with evidence of diminished tissue AT₁ receptor expression, and augmented plasma cGMP levels, indicative of activation of the NO system.

The key findings in this study are as follows: (1) the renal and peripheral hemodynamic responses to graded Ang II infusion were at least equal to, and in the case of MAP, FF, and RVR, significantly augmented, in the OC users compared with the control subjects; (2) the mean values for tissue AT₁ receptor mRNA corrected for GAPDH signal were significantly increased in OC users, suggesting upregulation of AT₁ receptor expression; and (3) baseline plasma and urine cGMP did not differ between OC users and control subjects.

We have previously demonstrated that OC users exhibit increased circulating Ang II levels and modest hemodynamic evidence of RAS activation that can be abolished by Ang II

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**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OC Users</th>
<th>Nonusers</th>
<th>Follicular Phase</th>
<th>Luteal Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26 ± 1</td>
<td>26 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20 ± 0.5</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>81 ± 2</td>
<td>75 ± 1</td>
<td>76 ± 1</td>
<td></td>
</tr>
<tr>
<td>UNa (mmol/kg per d)</td>
<td>3.6 ± 0.4</td>
<td>4.5 ± 0.6</td>
<td>3.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Protein intake (g/kg per d)</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Progesterone (nmol/L)</td>
<td>–</td>
<td>63 ± 5</td>
<td>477 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

* Protein intake is calculated from 24-h urine urea excretion. 
BMI, body mass index; MAP, mean arterial pressure; OC, oral contraceptive; UNa, 24-h urine sodium excretion corrected for body weight. 

* P < 0.05 versus OC users. 

* P < 0.05 versus follicular phase.
receptor blockade (1). Others have noted similar findings (25–27). Hollenberg and colleagues (27) noted dramatic increases in Ang II and PRA in 19 OC users, and in a subset of 8 OC users, RBF was 75% of that predicted for age and sodium diet. Our RBF results were consistent, but less dramatic, averaging 85% of the values found in nonusers. Because both estrogen (28) and progesterone (29) have been shown to relax renal arteries and increase renal blood flow in animal models, probably mediated by increased NO activity, we cannot implicate a direct genomic or nongenomic effect of either hormone in this phenomenon. Rather, both Hollenberg and colleagues (27) and Kang and colleagues (1) suggest RAS activation as a cause of these renal hemodynamic effects. Interestingly, and contrary to our findings, the RVR response to Ang II (Hypertensin; Ciba-Geigy) infused into the renal artery was reduced rather than augmented in the study by Hollenberg et al. (27). We are unable to determine why such disparities exist and can only attribute them to the decreased ethinyl estradiol concentrations in modern OC preparations and to differences in the Ang II preparation used in the two studies. Kono and colleagues (30) demonstrated that the pressor and steroidogenic responses to Hypertensin differed considerably from the responses to Ile5-angiotensin II (human Ang II), which was the preparation used in the present study, and recommended that human Ang II should always be used when assessing the Ang II response in human subjects.

A promoter region in the angiotensinogen gene is responsive to estrogen (31). Ingestion of ethinyl estradiol as part of an OC results in increases in plasma angiotensinogen to levels only marginally less than those seen in pregnancy (32). In the present study, we observed that a clear difference exists between OC users and control subjects in the systemic and renal hemodynamic response to Ang II infusion, with OC users exhibiting increased responsiveness to Ang II infusion. Bowyer et al. (33) demonstrated similar findings when measuring the forearm blood flow response to Ang II infusion in OC users, who exhibited an exaggerated vasoconstrictor response to Ang II during the medication phase. Blunting of the response to Ang II infusion has been demonstrated in other high-estrogen states, including the luteal phase of the menstrual cycle (3), and in normal pregnancy (12–14). Magnness and colleagues (14) have shown that in normal pregnancy, baseline plasma Ang II levels are increased and the pressor response to infused Ang II is blunted, with no evidence of increased activity of counter-regulatory prostaglandins. The fact that, contrary to our initial hypothesis, we found no evidence of blunting of the Ang II hemodynamic response is intriguing in that several physiological processes should act to cause this phenomenon, including the estrogen-mediated increased bioavailability of NO (15–17), the upregulation of AT2 receptors by estrogen (4), which also mediates NO production through cGMP (18,34), and increased circulating plasma Ang II, resulting in homologous downregulation. The mechanism underlying the maintenance of normal BP in OC users is not immediately clear, but we sought to relate this finding to either AT1 receptor expression or the production of cGMP.

The fact that OC users showed markedly increased tissue AT1 receptor mRNA levels compared with controls was a surprising finding. A number of factors influence AT1 receptor levels. Previous studies have suggested that Ang II may regulate the expression of its own receptor (35), with tissue-specific differences. Downregulation of AT1 receptor mRNA expression with administration of Ang II has been reported in cultured vascular smooth muscle cells (36) and mesangial cells (37). Infusion of Ang II increases expression in the adrenal gland but not that of the aorta or kidney (38). Nickenig and colleagues (19) demonstrated that cultured skin fibroblasts expressed AT1 receptors that, in response to Ang II, induced homologous downregulation of AT1 receptor mRNA, similar to vascular smooth muscle cells and renal mesangial cells (39). Estradiol causes the downregulation of AT1 receptor mRNA and density in vascular smooth muscle cells, and estrogen deficiency leads to the upregulation of vascular AT1 receptor expression accompanied by an increased effect of Ang II on tension in isolated rat aortic rings (40). Several studies (19,41,42) have demonstrated skin fibroblast behaviors in response to Ang II. We do not assume that skin tissue cells represent vascular smooth muscle cells or specific renal cells. But it is clear that tissue cell behaviors may reflect processes related to Ang II responsiveness, and in the present study, increased receptor expression is consistent with the acute hemodynamic Ang II response.

Given that the OC users in this study exhibited only modest hemodynamic effects of increased Ang II levels despite their

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OC Users</th>
<th>OC Nonusers</th>
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<tbody>
<tr>
<td>Renin (µU/ml)</td>
<td>11 ± 1</td>
<td>8 ± 1b</td>
</tr>
<tr>
<td>Ang II (pg/ml)</td>
<td>15 ± 1</td>
<td>6 ± 1b</td>
</tr>
<tr>
<td>Aldosterone (pmol/L)</td>
<td>160 ± 50</td>
<td>83 ± 14b</td>
</tr>
<tr>
<td>Angio (ng Ang I/ml)</td>
<td>3949 ± 256</td>
<td>961 ± 76b</td>
</tr>
<tr>
<td>PRA (ng Ang I/L per min)</td>
<td>2 ± 0.4</td>
<td>0.3 ± 0.04b</td>
</tr>
</tbody>
</table>

* Ang I, angiotensin I; Ang II, angiotensin II; Angio, angiotensinogen; PRA, plasma renin activity.

** P < 0.05 versus OC users.

† P < 0.05 versus follicular phase.
Table 3. Renal response to Ang II in groups segregated by OC use

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OC Nonusers</th>
<th>OC Users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Recovery</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>125 ± 4</td>
<td>115 ± 7³</td>
</tr>
<tr>
<td>ERPF (ml/min per 1.73 m²)</td>
<td>125 ± 4</td>
<td>115 ± 7³</td>
</tr>
<tr>
<td>RBF (ml/min per 1.73 m²)</td>
<td>125 ± 4</td>
<td>115 ± 7³</td>
</tr>
<tr>
<td>FF</td>
<td>0.20 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>RVR (mmHg/L per min)</td>
<td>73 ± 4</td>
<td>120 ± 6³</td>
</tr>
</tbody>
</table>

Increased tissue AT₁ receptor expression, and the fact that there was only a weak correlation between receptor expression and the Ang II-mediated MAP response, this suggests the existence of a counter-regulatory protective mechanism. Armando et al. (4) recently demonstrated that estrogen administration in ovariectomized mice upregulates AT₂ receptor expression in the kidney, significantly altering the AT₁/AT₂ receptor expression ratio. It has been demonstrated that AT₂ receptors may act in opposition to, and in balance with, AT₁ receptors. Stimulation of the AT₂ receptor dilates efferent arterioles (43), decreases mesangial cell hypertrophy (44), is natriuretic (45), and is proposed to stimulate the NO/cGMP signaling pathway, as shown by Gohlke and colleagues (34) using rat aortic endothelial cells. Therefore, the possibility exists that this represents the protective mechanism. Although we obtained insufficient RNA for the measurement of AT₂ receptor expression, we found no evidence of increased cGMP production, which mediates vasodilation. However, forearm blood flow responses to the NO synthase antagonist N⁴-monomethyl-L-arginine (L-NMMA) were uniform across the menstrual cycle, with no difference between users of OCs and women with ovulatory cycles, suggesting that there is no difference in basal NO activity as a result of OC use (33). Our finding of no significant differences in plasma cGMP levels and urine cGMP excretion tends to support this hypothesis. However, we cannot eliminate the possibility that alterations in NO production cause a chronic if not an acute blunting of the Ang II response. Not only were our numbers small, allowing us to detect only major differences, but we did not place subjects on a low-nitrate diet before the study. In addition, it has been suggested by Schmidt et al. (47) that blood and urine measurements of indices of NO activity are of limited utility in humans, even when measured under optimum conditions. Therefore, we cannot exclude the counter-regulatory effects of NO as a factor influencing our findings, and it is possible that a combination
of counter-regulatory mechanisms exist in OC users to limit the detrimental hemodynamic consequences of an activated RAS.

In summary, we have confirmed that young, healthy OC users exhibit an increase in circulating RAS components with only modest hemodynamic consequences. Contrary to our original hypothesis, however, there was no evidence of AT1 receptor downregulation, as is seen in other high-estrogen states; rather, the renal and peripheral hemodynamic response to Ang II was augmented and tissue AT1 mRNA was increased compared with OC nonusers. The deleterious hemodynamic and mitogenic effects of Ang II are well known. The modest baseline hemodynamic differences in OC users suggest the existence of a protective factor that does not appear to be stable inulin.

**References**


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