The effect of oral contraceptives on the nitric oxide system and renal function

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Cherney DZ, Scholey JW, Cattran DC, Kang AK, Zimpelmann J, Kennedy C, Lai V, Burns KD, Miller JA. The effect of oral contraceptives on the nitric oxide system and renal function. Am J Physiol Renal Physiol 293:F1539–F1544, 2007. First published August 22, 2007; doi:10.1152/ajprenal.00351.2007.—We have demonstrated that oral contraceptive (OC) users exhibit elevated angiotensin II levels and angiotensin II type 1 receptor expression, indicative of renin-angiotensin system (RAS) activation, yet the renal and systemic consequences are minimal, suggesting that there is increased vasodilatory activity, counteracting the effect of RAS activation. We hypothesized that the nitric oxide (NO) system would be upregulated in OC users and that this would be reflected by a blunted hemodynamic response to L-arginine infusion. All subjects were studied after a 7-day controlled sodium and protein diet. Inulin and para-aminohippurate clearance techniques were used to assess renal function. L-Arginine was infused at 100, 250, and 500 mg/kg, each over 30 min. Skin endothelial NO synthase mRNA expression was assessed by real-time PCR. While OC nonusers exhibited significant increases in effective renal plasma flow (670.8 ± 35.6 to 816.2 ± 59.7 ml·min⁻¹·1.73 m⁻²) and glomerular filtration rate (133.4 ± 4.3 to 151.0 ± 5.7 ml·min⁻¹·1.73 m⁻², P = 0.04) and declines in renal vascular resistance (81.1 ± 6.1 to 63.5 ± 6.2 mmHg·ml⁻¹·min⁻¹, P = 0.001) at the lower L-arginine infusion rates, the responses in OC users were blunted. While L-arginine reduced mean arterial pressure at the 250 and 500 mg/kg doses in OC nonusers, OC users only exhibited a decrease in mean arterial pressure at the highest infusion rate. In contrast, tissue endothelial NO synthase mRNA levels were higher in OC users and that this would be reflected by a blunted hemodynamic response to ANG II. Serum AT1R homologous mRNA levels were blunted. While L-arginine reduced mean arterial pressure at 100, 250, and 500 mg/kg, each over 30 min. Skin endothelial NO synthase mRNA expression was assessed by real-time PCR. While OC nonusers exhibited significant increases in effective renal plasma flow (670.8 ± 35.6 to 816.2 ± 59.7 ml·min⁻¹·1.73 m⁻²) and glomerular filtration rate (133.4 ± 4.3 to 151.0 ± 5.7 ml·min⁻¹·1.73 m⁻², P = 0.04) and declines in renal vascular resistance (81.1 ± 6.1 to 63.5 ± 6.2 mmHg·ml⁻¹·min⁻¹, P = 0.001) at the lower L-arginine infusion rates, the responses in OC users were blunted. While L-arginine reduced mean arterial pressure at the 250 and 500 mg/kg doses in OC nonusers, OC users only exhibited a decrease in mean arterial pressure at the highest infusion rate. In contrast, tissue endothelial NO synthase mRNA levels were higher in OC users (P = 0.04). In summary, these findings suggest that the NO system is upregulated by OC use in young, healthy women. Increased activity of the NO pathway may modulate the hemodynamic effects of RAS activation in OC users.

Experimental evidence suggests that estrogens alters vascular function by enhancing NO production by the vascular endothelium (29). Physiological levels of estrogen cause a rapid release of NO in cultured bovine and human endothelial cells (2, 6) and enhance endothelial-dependent vasodilatation (7). These effects are mediated by increased transcription of endothelial NO synthase (eNOS) (25, 44) and by Akt-dependent activity of eNOS (12). Estrogen may also increase the vascular responsiveness to NO donors by enhancing NO bioavailability, possibly through antioxidative mechanisms (3, 15, 54). Considering these well-known genomic and nongenomic effects of estrogen on the NO pathway (7, 18), it is conceivable that NO is an important modulator of the hemodynamic effects of OC-induced RAS activation.

The overall objective of this set of experiments was to examine the mechanism whereby women who are OC users maintain normal renal and systemic hemodynamic function in the face of RAS activation. We hypothesized that the hemodynamic response to a graded L-arginine infusion would differ in normal, healthy women who were users and nonusers of OCs, that in users would exhibit a blunted renal and systemic hemodynamic response to L-arginine infusion, compared with OC nonusers. OC nonusers were studied during the follicular phase of the menstrual cycle when estrogen levels are low to amplify the hormonal differences between the two groups, similar to previous studies from this laboratory (22).

METHODS

Subjects. The study was performed with the approval of the University Health Network Research Ethics Board and with the informed, written consent of each subject. Recruitment was in accordance with the policies of the Human Subjects’ Review Committee of the University of Toronto. We studied age-matched, otherwise healthy women, aged 18–40 yr. All subjects had normal values for body mass index, arterial pressure, renal function, liver function, and electrocardiogram. Exclusion criteria included a history of renal, cardiac, or lung disease, or current smoking. Except for OCs, no subjects ingested any regular medications. Pregnancy was excluded with a negative serum β-human chorionic gonadotropin test before enrollment. All subjects were interviewed and examined by a qualified internist. Preparations. Each subject was studied on one occasion. OC users were studied during the first 21 days of the menstrual cycle, and OC nonusers were studied during the first 7 days of the menstrual cycle. As in previous protocols from these investigators (8, 9, 22, 40), each study was performed after 7 days on a controlled diet consisting of 150 mmol/day sodium and 1.5 g·kg⁻¹·day⁻¹ protein. Compliance was ascertained by measurement of 24-h urine sodium, potassium, and urea excretion on the 7th day. Data were analyzed if urine sodium excretion were >150 mmol/day and urea excretion were 3–6
mmol·kg⁻¹·day⁻¹. No subjects were excluded. Each subject presented to the Renal Physiology Laboratory at the Toronto General Hospital at 0800 after an overnight fast.

**Experimental procedures.** Each subject had an 18-gauge peripheral venous cannula inserted into an antecubital vein for sampling and a second cannula in the contralateral arm for infusions. Each OC nonuser had a blood sample collected for estradiol levels, to delineate phases of the menstrual cycle. Data were not used if levels were inappropriate to the follicular phase. No subjects were excluded based on these criteria. Blood samples were collected for inulin blank and for baseline values for renin, plasma renin activity (PRA), ANG II, aldosterone, and cGMP. Hemodynamic parameters (arterial pressure, heart rate) were measured by an automated sphygmomanometer (Dinamapp, Critikon,) at 15-min intervals throughout the study. Renal hemodynamic function was assessed using inulin and para-aminohippurate (PAH) clearance techniques, as previously described in this investigator’s studies (8, 9, 22, 40). Skin biopsies were stored in liquid nitrogen at −70°C. Skin biopsies were analyzed for eNOS expression.

**RESULTS**

**OC users vs. nonusers.** At baseline, OC users and nonusers exhibited similar systemic arterial pressure parameters and similar dietary intake of sodium and protein (Table 1). Renal hemodynamic testing revealed that the GFR, ERPF, RBF, FF, RVR, ANG II, renin, PRA, aldosterone, cGMP, and MAP were similar between the two groups. No significant between-group baseline differences were assessed using nonparametric tests.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OC User Group</th>
<th>OC Nonuser Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26±5</td>
<td>25±4</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>60±10</td>
<td>60±11</td>
</tr>
<tr>
<td>Estrogen, pmol/l</td>
<td>N/A</td>
<td>67±11</td>
</tr>
<tr>
<td>Sodium excretion, mmol/24 h</td>
<td>214±29</td>
<td>185±20</td>
</tr>
<tr>
<td>Protein intake, g·kg⁻¹·day⁻¹</td>
<td>1.2±0.06</td>
<td>1.1±0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. OC, oral contraceptive.
were that increased activity of the NO system. Our principle findings
Our aim was to determine whether OC use was associated with young women: OC users and OC nonusers. In addition, we exogenous estrogen administration raises plasma, hepatic, and the angiotensinogen gene is responsive to estrogen (13), and baseline tissue expression of eNOS was also augmented in infusion in both groups but remained higher in the OC users; DISCUSSION

In this study, we examined the renal and systemic hemodynamic responses to L-arginine in OC users vs. follicular phase subjects

**Table 2. Renal and systemic hemodynamic responses to l-arginine in OC users vs. follicular phase subjects**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>100 mg/kg</th>
<th>250 mg/kg</th>
<th>500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OCP nonusers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>79±2</td>
<td>76±3</td>
<td>74±2*</td>
<td>73±3†</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·1.73 m⁻²</td>
<td>133±4</td>
<td>136±5</td>
<td>142±7</td>
<td>151±6§</td>
</tr>
<tr>
<td>ERPF, ml·min⁻¹·1.73 m⁻²</td>
<td>671±36</td>
<td>684±40</td>
<td>739±50§</td>
<td>816±60§</td>
</tr>
<tr>
<td>FF</td>
<td>0.20±0.01</td>
<td>0.20±0.01</td>
<td>0.20±0.01</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·1.73 m⁻²</td>
<td>1,104±93</td>
<td>1,058±62</td>
<td>1,132±77</td>
<td>1,227±90</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min⁻¹·1.73 m⁻²</td>
<td>81±6</td>
<td>77±6</td>
<td>71±7§</td>
<td>65±6§</td>
</tr>
<tr>
<td><strong>OCP users</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>80±2</td>
<td>78±2</td>
<td>77±2</td>
<td>75±2‡</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·1.73 m⁻²</td>
<td>124±4</td>
<td>124±7</td>
<td>125±6</td>
<td>131±6</td>
</tr>
<tr>
<td>ERPF, ml·min⁻¹·1.73 m⁻²</td>
<td>645±21</td>
<td>641±22</td>
<td>678±25</td>
<td>720±39</td>
</tr>
<tr>
<td>FF</td>
<td>0.20±0.02</td>
<td>0.19±0.01</td>
<td>0.19±0.01</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·1.73 m⁻²</td>
<td>1,002±36</td>
<td>1,002±42</td>
<td>1,047±47</td>
<td>1,081±60</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min⁻¹·1.73 m⁻²</td>
<td>85±4</td>
<td>84±4</td>
<td>80±4</td>
<td>76±6</td>
</tr>
</tbody>
</table>

Values are means ± SE. OCP, OC phase; MAP, mean arterial pressure; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; RBF, renal blood flow; RVR, renal vascular resistance. In OC nonusers, *P = 0.005 at 250 mg/kg and †P = 0.046 at 500 mg/kg vs. baseline MAP values. In OC users, ‡P = 0.006 at 500 mg/kg vs. baseline MAP values. In OC nonusers, §P < 0.05 vs. baseline for renal hemodynamic parameters.

expression corrected for GAPDH signal in skin biopsy specimens (4.9 ± 0.9 vs. 1.5 ± 0.4, P = 0.04) (Fig. 4).

DISCUSSION

In this study, we examined the renal and systemic hemodynamic responses to L-arginine infusion in two groups of healthy young women: OC users and OC nonusers. In addition, we related these responses to changes in circulating RAS components, plasma levels of cGMP, and tissue expression of eNOS. Our aim was to determine whether OC use was associated with increased activity of the NO system. Our principle findings were that 1) the systemic and renal hemodynamic responses to L-arginine infusion were blunted in OC users compared with OC nonusers; 2) circulating levels of the effectors of the RAS system, ANG II and aldosterone, declined during L-arginine infusion in both groups but remained higher in the OC users; 3) baseline tissue expression of eNOS was also augmented in OC users.

Studies have suggested both activation (11, 19, 48) and suppression (47) of the RAS in OC users. A promoter region in the angiotensinogen gene is responsive to estrogen (13), and exogenous estrogen administration raises plasma, hepatic, and renal angiotensinogen levels and has the potential to raise plasma concentrations of ANG II. For example, the ingestion of ethinyl estradiol as part of a combined OC leads to an increase in plasma angiotensinogen that is only slightly less than that seen during pregnancy (38). In previous studies, our laboratory has observed increases in ANG II, angiotensinogen, and aldosterone levels in OC users, with only minimal hemodynamic consequences (22), despite increased tissue AT1R expression (1).

NO is synthesized from the amino acid L-arginine by the enzyme NO synthase (NOS) (41). In the vascular endothelium, NO regulates vasodilator tone (16) by activating soluble guanylate cyclase and increasing intracellular production of cGMP (20). Although all three types of NOS (eNOS, neuronal NOS, and inducible NOS) are present in the kidney, only eNOS and inducible NOS are directly involved in the regulation of ERPF and GFR under normal physiological conditions (26). The investigation of the physiological role of NO in regulation of arterial pressure, ERPF, and GFR has relied on the use of nonspecific NOS inhibitors, such as Nω-nitro-L-arginine, and infusions of the physiological precursor of NO, L-arginine. Previous work has suggested that NO acts as a tonic

![Fig. 1. Effective renal plasma flow (ERPF) in oral contraceptive (OC) users and nonusers. The ERPF response to L-arginine is shown. OCP, OC phase. *P = 0.011, bP = 0.002, cP = 0.001, and dP = 0.001 vs. baseline ERPF.](http://ajprenal.physiology.org/)

![Fig. 2. Glomerular filtration rate (GFR) in OC users and nonusers. The GFR response to L-arginine is shown. *P = 0.04 vs. baseline GFR.](http://ajprenal.physiology.org/)
vasodilator regulating basal renal and systemic vascular hemodynamics and is important in arterial pressure regulation (16). Infusion of L-arginine results in a rise in ERPF and GFR in control animals (24) and in normal humans (28, 45).

Our first major observation was that L-arginine infusion led to a rise in ERPF and a decline in RVR in OC nonusers. We also observed a rise in GFR, which, in the context of a decline in RVR and a rise in ERPF, suggests that the predominant effect of L-arginine infusion is to cause relatively more afferent than efferent vasodilatation. In OC users, these responses were significantly blunted. We believe that the blunted response to L-arginine infusion supports the hypothesis that there is increased activity of the NO system in OC users, and that further delivery of the substrate for eNOS cannot overcome the hemodynamic effects of ongoing OC-induced RAS activation. Augmented tissue mRNA levels of eNOS supports our assertion that the NO system is activated in the OC users.

OC use increases basal production and release of NO in the brachial artery (5) in some studies (21). For example, John and coworkers (21) studied changes in forearm blood flow in OC users compared with OC nonusers (who were studied in the follicular phase of the menstrual cycle, as in the present study) in response to NOS inhibition using intra-arterial infusion of N\textsuperscript{G}-monomethyl-L-arginine. Most OC users do not develop hypertension, despite AT\textsubscript{1}R upregulation and increased ANG II and aldosterone levels. The observation that OC users did not exhibit a significant change in MAP until the highest 500 mg/kg dose of L-arginine is interesting, since this response may reflect baseline NO system activation in the systemic circulation of OC users.

Similar to previous observations (1, 22), OC users exhibited augmented circulating RAS mediators compared with OC nonusers, and our second major finding was that L-arginine infusion reduced circulating ANG II levels in both groups of subjects. De Nicola and coworkers (10) have demonstrated important ANG II-NO glomerular interactions in rats, suggesting that NO synthesis is activated by, and then functions as a physiological antagonist of, ANG II (10, 17, 39). ANG II-NO interactions have been suggested by others in in vitro and in vivo experiments (49, 51), and NO may abolish ANG II-dependent vascular and mesangial contraction through the intracellular actions of cGMP (49). Our findings are consistent with a NO-mediated negative feedback loop on ANG II, since ANG II levels decreased in a stepwise fashion in response to a graded infusion of L-arginine and then returned toward normal when the L-arginine was discontinued. Although the mechanism(s) responsible for this feedback loop was not elucidated in the present study, it may be hemodynamically mediated (10, 17, 39, 49, 51).

The OC users also displayed elevated baseline aldosterone levels, without evidence of systemic hypertension or renal vasoconstriction. NO activation is thought to be an important factor that protects against the development of hypertension during states of chronic mineralocorticoid excess (14, 43), such as during OC use (36–38, 55). It does so through both hemo
dynamic and diuretic mechanisms in dogs (14), thereby blunting the long-term hypertensive effects of mineralocorticoids in most settings. The blunted effect of L-arginine infusion in the OC group supports the concept that the NO system is upregulated and serves to modulate hemodynamic function in the setting of high-circulating aldosterone and ANG II levels.

Our last major observation was that tissue eNOS expression was augmented in OC users. It is surprising that, despite this

![Fig. 3. Renal vascular resistance (RVR) in OC users and nonusers. The RVR response to L-arginine is shown. *P = 0.028, \( \cdot P = 0.008 \), and \( \cdot P = 0.001 \) vs. baseline RVR.](https://example.com/fig3)

![Fig. 4. Endothelial nitric oxide synthase (NOS) expression in OC users and OC nonusers. *P = 0.04 for eNOS expression in OC users vs. nonusers.](https://example.com/fig4)

### Table 3. Humoral renin-angiotensin system and nitric oxide response to L-arginine in OC users vs. OC nonusers

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>100 mg/kg</th>
<th>250 mg/kg</th>
<th>500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OCP nonusers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>339±81</td>
<td>76±12</td>
<td>65±12</td>
<td>72±11</td>
</tr>
<tr>
<td>ANG II</td>
<td>9.9±2.2</td>
<td>5.8±1.5</td>
<td>4.1±0.7</td>
<td>2.6±0.5</td>
</tr>
<tr>
<td>PRA</td>
<td>1.1±0.3</td>
<td>0.6±0.2</td>
<td>0.4±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Renin</td>
<td>18.7±4.6</td>
<td>10.1±1.8</td>
<td>9.7±1.5</td>
<td>9.8±1.8</td>
</tr>
<tr>
<td>cGMP</td>
<td>4.9±0.4</td>
<td>4.9±0.5</td>
<td>5.2±0.6</td>
<td>6.9±0.6</td>
</tr>
<tr>
<td><strong>OCP users</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>546±88</td>
<td>104±19(\textsuperscript{de})</td>
<td>96±22(\textsuperscript{de})</td>
<td>124±25(\textsuperscript{de})</td>
</tr>
<tr>
<td>ANG II</td>
<td>17.3±2.8</td>
<td>9.7±1.2(\textsuperscript{de})</td>
<td>7.6±1.3(\textsuperscript{de})</td>
<td>5.3±0.9(\textsuperscript{de})</td>
</tr>
<tr>
<td>PRA</td>
<td>1.6±0.2</td>
<td>0.7±0.1(\textsuperscript{de})</td>
<td>0.6±0.1(\textsuperscript{de})</td>
<td>0.5±0.2(\textsuperscript{de})</td>
</tr>
<tr>
<td>Renin</td>
<td>16.8±2.6</td>
<td>9.3±1.3</td>
<td>9.2±1.3</td>
<td>7.6±1.3</td>
</tr>
<tr>
<td>cGMP</td>
<td>3.9±0.3</td>
<td>4.3±0.4</td>
<td>5.2±0.5</td>
<td>6.6±0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. PRA, plasma renin activity. Significant difference vs. baseline: \( \cdot P < 0.003 \), \( \cdot P = 0.001 \), \( \cdot P = 0.05 \), \( \cdot P < 0.008 \), \( \cdot P < 0.001 \), \( \cdot P = 0.04 \), \( \cdot P = 0.009 \), \( \cdot P = 0.0001 \), \( \cdot P < 0.03 \), \( \cdot P = 0.001 \), \( \cdot P = 0.002 \), \( \cdot P < 0.006 \), \( \cdot P = 0.03 \) change in aldosterone greater in users vs. nonusers.

\*P = 0.012 change in ANG II greater in users vs. nonusers.
apparent increase in eNOS expression, the systemic and renal hemodynamic response to l-arginine infusion was blunted, since one may have predicted an augmented hemodynamic effect based on increased delivery of substrate. Although speculative, one possible explanation is that NO bioavailability might have been limited in the setting of OC use, because ANG II increased the production of reactive oxygen species and the generation of peroxynitrite (27, 46, 53) by activating NADPH oxidase (42). Increased reactive oxygen species production would also favor a pattern of hemodynamic vasoconstriction that we observed in OC users, such as the higher RVR and lower ERPF. The response to l-arginine may also have been blunted in OC users due to higher levels of aldosterone and ANG II in that group at baseline and throughout the l-arginine infusion. The ongoing vasoconstrictive influence of RAS mediators may have limited the ability of NO to exert a vasodilatory effect. Whatever the cause, our results suggest that OC use is associated with RAS activation and upregulation of the NO system, and that renal and systemic hemodynamic function reflect the balance between these two effects.

This study has several limitations. We attempted to minimize the effect of the small sample size by utilizing homogeneous study groups and by careful pre-study preparation with a focus on known factors, such as sodium and protein intake and phase of the menstrual cycle, all of which are known to influence the RAS (30–35). Last, we decreased variability by using a study design that allowed each subject to act as the control.

In summary, our findings suggest that the NO system is a major factor that acts to modulate the hemodynamic effects of OC-mediated RAS activation in the majority of women who take them. Whether or not defective NO activity underlies the hypertensive effect of OC use seen in some women requires future study.

ACKNOWLEDGMENTS

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GRANTS

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REFERENCES