

## The Role of Vasodilator Receptors of Renin–angiotensin System on Nitric Oxide Formation and Kidney Circulation after Angiotensin II Infusion in Renal Ischemia/Reperfusion Rats

### Abstract

**Background:** Nitric oxide (NO) as a vasodilator factor has renoprotective effect against renal ischemia. The balance between angiotensin II (Ang II) and NO can affect kidney homeostasis. The aim of this study was to determine NO alteration in response to renin–Ang system vasodilator receptors antagonists (PD123319; Ang II type 2 receptor antagonist and A779; Mas receptor antagonist) in renal ischemia/reperfusion injury (IRI) in rats. **Materials and Methods:** Sixty-three Wistar male and female rats were used. Animals from each gender were divided into four groups received saline, Ang II, PD123319 + Ang II, and A779 + Ang II after renal IRI. Renal IRI induced with an adjustable hook. Blood pressure and renal blood flow (RBF) measured continuously. The nitrite levels were measured in serum, kidney, and urine samples. **Results:** In female rats, the serum and kidney nitrite levels increased significantly by Ang II ( $P < 0.05$ ) and decreased significantly ( $P < 0.05$ ) when PD123319 was accompanied with Ang II. Such observation was not seen in male. Ang II decreased RBF significantly in all groups ( $P < 0.05$ ), while PD + Ang II group showed significant decrease in RBF in comparison with the other groups in female rats ( $P < 0.05$ ). **Conclusion:** Males show more sensibility to Ang II infusion; in fact, it is suggested that there is gender dimorphism in the Ang II and NO production associated with vasodilator receptors.

**Keywords:** Nitric oxide, ischemia/reperfusion injury, renin–angiotensin system, Ang II type 2, Mas receptor

### Introduction

Nitric oxide (NO) is a vasodilator factor produced by NO synthase (NOS). It is known as renoprotective factor due to its anti-inflammatory, vasodilatory, and antioxidant properties against of renal ischemia/reperfusion injury (IRI).<sup>[1,2]</sup> Renal IRI is largely considered a reversible phenomenon,<sup>[3]</sup> and it enhanced and activated the expression of NOS proteins.<sup>[4]</sup> Studies have shown that NO and nitrite have cytoprotection properties in IRI models<sup>[5,6]</sup> and endothelium-derived NO has a reciprocate interaction with angiotensin II (Ang II).<sup>[7,8]</sup>

NO formation are affected through Ang II type 2 ( $AT_2R$ ) and Ang 1–7 (Mas receptor [MasR]) receptors which they antagonize the effects of Ang II type 1 ( $AT_1R$ ) receptor.<sup>[9,10]</sup>  $AT_1R$  antagonists can increase the renal NO, and this increase can be attenuated by  $AT_2R$  blockade.<sup>[11,12]</sup> Ang 1–7 through MasR

causes vasodilation through excitation of endothelium-dependent NO release.<sup>[10]</sup> It can stimulate the production of the NO and prostaglandin,<sup>[13]</sup> thereby be a physiological antagonist of Ang II.<sup>[14]</sup> It is important that integrity of kidney system can be maintained with balance between NO and Ang II.<sup>[15]</sup>

Furthermore, it is known that sex differences exist in kidney IRI.<sup>[16]</sup> It has been made clear that males are more susceptible to renal IRI than females,<sup>[16,17]</sup> and renal function disturbance is gender related.<sup>[18,19]</sup> Accordingly, we hypothesized that renal blood flow (RBF) response to Ang II is associated with NO production from one side and it is related to interaction between receptors activity by the other side. Therefore, the aim of the study was to determine RBF and NO response to Ang II when vasodilator receptors were blocked in male and female rats subjected to IRI.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: [reprints@medknow.com](mailto:reprints@medknow.com)

**How to cite this article:** Maleki M, Hasanshahi J, Moslemi F. The Role of Vasodilator Receptors of Renin–angiotensin System on Nitric Oxide Formation and Kidney Circulation after Angiotensin II Infusion in Renal Ischemia/Reperfusion Rats. *Adv Biomed Res* 2018;7:25.

**Received:** October, 2016, **Accepted:** November, 2016.

Maryam Maleki,  
Jalal Hasanshahi,  
Fatemeh Moslemi

From the Water and Electrolytes Research Center/Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran

### Address for correspondence:

Dr. Maryam Maleki,  
Department of Physiology,  
Isfahan University of Medical Sciences, Isfahan, Iran.  
E-mail: [maryam\\_maleki@med.mui.ac.ir](mailto:maryam_maleki@med.mui.ac.ir)

### Access this article online

Website: [www.advbiores.net](http://www.advbiores.net)

DOI: 10.4103/2277-9175.225596

### Quick Response Code:



## Materials and Methods

### Animals

A total of 63 male and female Wistar rats (respectively weighed  $211.8 \pm 0.9$  g,  $n = 29$  and  $185.6 \pm 0.6$  g,  $n = 34$ ) were used in this experimental study.

### Renal ischemia reperfusion and catheterization

Rats were anesthetized with 1.7 g/kg urethan (Sigma, St. Louis, MO, USA), trachea was isolated to insert air ventilation tube, and also catheters were implanted into the left carotid and femoral arteries and jugular vein. An adjustable hook (as clamp) was placed around the abdominal aorta (above renal arteries) to induce renal IRI and also adjust renal perfusion pressure (RPP) in base levels during infusion of Ang II. Blood pressure was monitored through carotid artery, and following surgical process, RPP was set at 25 mmHg through tightening the abdominal aortic clamp to induce renal IRI for 30 min and then allowed to reperfusion by loosening it. The left kidney was exposed and fixed in adjustable cup. Renal artery was separated from the renal vein. The ultrasound flow probe interfaced with a compatible flowmeter (T108; Transonic Systems) was hooked around the renal artery to measure RBF directly.

### Group design

Each male or female group was divided into four subgroups (total eight subgroups). In summary, the designed groups were as following:

1. Group 1: Male or female rats treated with vehicle; saline (as solvent for antagonist and Ang II)
2. Group 2: Male or female rats treated with vehicle for antagonist, and then Ang II was infused
3. Group 3: Male or female rats treated with AT<sub>2</sub>R antagonist; PD123319 (Sigma, St. Louis, MO, USA), and then Ang II was infused
4. Group 4: Male or female rats treated with MasR antagonist; A779 (Bachem, King of Prussia, MO, USA) and then Ang II was infused.

### Experimental protocol

At the beginning of reperfusion, the antagonist was administered as bolus dose of 50  $\mu$ g/kg followed by continuous infusions at 50  $\mu$ g/kg/h for A779 and bolus doses of 1 mg/kg followed by continuous infusions at 1 mg/kg/h for PD123319 using microsyringe pumps (New Era Pump Systems Inc., Farmingdale, NY, USA) and jugular vein tube. The antagonists' infusions were continued during the experiment to the end. After 30 min commencing vehicle or antagonist treatments, intravenous Ang II infusion (500 ng/kg/h) started for 45 min. At the end of experiment, the blood and urine samples were obtained after the vehicle or Ang II infusion for nitrite concentration determination using a colorimetric assay kit (Promega Corporation, USA) that involves the Griess reaction.

## Statistical analysis

Data are expressed as mean and standard error mean. SPSS software version 20 was used to analyze the data. The serum, tissue, and urine levels of nitrite, RBF, RPP were compared through ANOVA between the groups, and LSD was used as a posttest to find the significant difference between each two groups.  $P \leq 0.05$  was considered to be statistically significant.

## Results

### Effect of angiotensin II on serum, kidney, and urine nitrite levels in the presence of A779 or PD123319 or both

In female rats, the serum and kidney tissue levels of nitrite increased significantly by Ang II infusion alone ( $P < 0.05$ ), but Ang II-induced NO production decreased when PD123319 or A779 was accompanied with [Figure 1] so that there is significant difference between Group 2 and 3. Such observation was not seen in male [Figure 1].

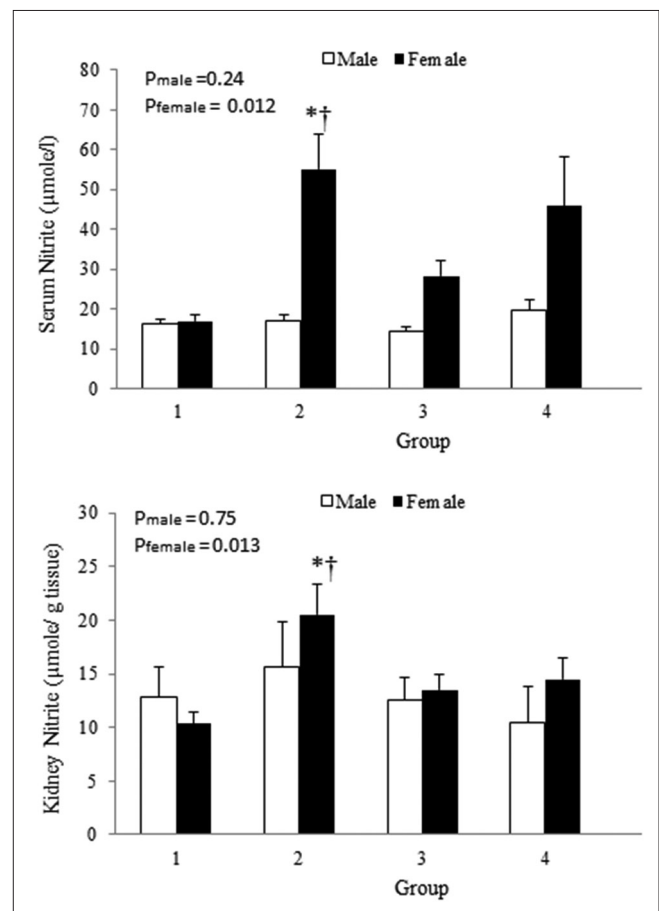


Figure 1: Serum and kidney nitrite levels in male and female rats. \*Significant difference from control group ( $P < 0.05$ ); †Significant differences from Group 3 in same gender ( $P < 0.05$ ). Group 1 (control): Rats treated with vehicle; saline. Group 2: Rats treated with vehicle for antagonist, and then angiotensin II was infused. Group 3: Rats treated with angiotensin II type 2 antagonist; PD123319 and then angiotensin II was infused. Group 4: Rats treated with Mas receptor antagonist; A779 and then angiotensin II was infused

There is no difference in urine nitrite concentration between male or female groups [Table 1].

### Effect of angiotensin II on renal blood flow in the presence of A779 or PD123319 or both

Ang II infusion decreased RBF in female and male rats significantly at constant RPP ( $P < 0.05$ ) [Figure 2]. In addition, PD + Ang II group showed significant decrease in RBF was observed when PD123319 was accompanied with Ang II [Figure 2].

Groups	Female	Male	P
1	9.47 $\pm$ 1.36	8.37 $\pm$ 1.58	0.43
2	11.32 $\pm$ 1.47	9.13 $\pm$ 1.40	0.31
3	9.36 $\pm$ 0.62	9.14 $\pm$ 0.73	0.90
4	11.25 $\pm$ 1.94	10.40 $\pm$ 1.12	0.83
ANOVA (P)	0.51	0.27	-

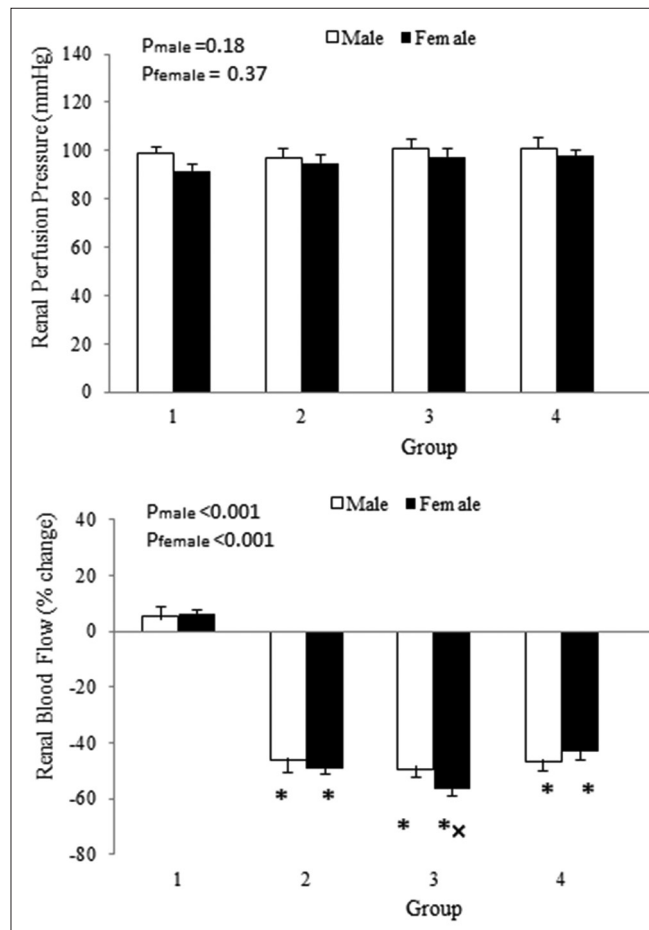


Figure 2: Renal perfusion pressure and the percentage change of renal blood flow in male and female rats. \* Significant difference from Group 1 ( $P < 0.05$ ) in similar gender; \*x Significant differences from Groups 2 and 4 ( $P < 0.05$ ). Group 1: Rats treated with vehicle; saline. Group 2: Rats treated with vehicle for antagonist, and then angiotensin II was infused. Group 3: Rats treated with Ang II type 2 antagonist; PD123319 and then angiotensin II was infused. Group 4: Rats treated with Mas receptor antagonist; A779 and then angiotensin II was infused

## Discussion

Increased glomerular NO synthesis in renal IRI seems to be a protective mechanism that counteracts vasoconstrictor and inflammatory phenomena occurring during the reperfusion period. These phenomena play a major role to impair the recovery of renal function after ischemia or after renal transplant.<sup>[20]</sup> In renal IRI, nitrite was not changed significantly after Ang II infusion in male rats; however, in age-matched female rats, it was increased significantly. However, when AT<sub>2</sub>R or MasR was blocked, nitrite level was reduced in serum. As we know, males show more sensibility to Ang II infusion; in fact, it is suggested that there is gender dimorphism in the Ang II activity and reported that males are more responsive to Ang II in isolated aorta rings and mesenteric microvessels than females.<sup>[21]</sup> Furthermore, it was reported a gender dimorphism in NO system including premenopausal women make more total NO than men<sup>[22]</sup> and there is a greater abundance of the constitutive NOS in young adult kidney of female than male rat.<sup>[23,24]</sup> It was showed that estrogen exerts marked stimulatory actions on endothelial NO levels, and in female rat, the active life of NO is prolonged probably due to sex-related antioxidant actions.<sup>[25]</sup> In accordance with us, it is demonstrated that testosterone induces inhibition of NO-dependent vasodilation,<sup>[25]</sup> and also, there is an upper ratio of AT<sub>2</sub>R/AT<sub>1</sub>R of Ang II in blood vessels and kidney in females compared with males.<sup>[21,26]</sup> Despite lesser findings about the endothelial Ang II signaling, some studies suggest that the endothelial Ang II signaling positively, as well as negatively, regulates NO signaling pathway and so that modulates endothelial dysfunction.<sup>[27]</sup> More evidence suggests the role of the AT<sub>1</sub>R in regulating the balance between NO and reactive oxygen species through endothelial signaling.<sup>[28,29]</sup> Endothelial dysfunction, characterized by less production of NO as well as NO bioavailability, leads to accelerated vasoconstriction.<sup>[28,30]</sup> Reckelhoff reported that increased activity of Ang II stimulates superoxide production through nicotinamide adenine dinucleotide phosphate oxidase, and due to the stimulatory actions of testosterone, this effect is more prominent in the male than female.<sup>[23]</sup> Female hormones that stimulate NO production exert an inhibitory action on Ang II.<sup>[27]</sup> In addition, it is observed that there are increased medullary NOS and NOS in whole kidney homogenates in female than male.<sup>[24,31]</sup> AT<sub>2</sub>R is thought to be associated with the vasodilatory actions of Ang II, which may be mediated by NO.<sup>[11]</sup> A lower ratio of AT<sub>1</sub>R/AT<sub>2</sub>R was reported in female compared with male in blood vessels and kidneys.<sup>[21]</sup> Furthermore, male showed a lower expression of AT<sub>2</sub>R in kidneys compared with female.<sup>[21]</sup> As we observed, the same situation for nitrite is existed with MasR blocker infusion in female versus male but nonsignificantly. In consistence with us, other research reported that the expression and activation of the MasR differ between the sexes, and they have revealed

greater renal ACE<sub>2</sub> and MasR gene expression in female as compared to male normotensive rats,<sup>[32-34]</sup> and therefore, in female, the balance tends to renin angiotensin system (RAS) stimulation toward the depressor arm.<sup>[34]</sup> In the nonrenal vasculature, Ang 1-7 increased production of NO through Mas involved pathways.<sup>[35]</sup> Ang 1-7 induced NO release through stimulating endothelial NOS (eNOS) and these effects were blocked by the A779<sup>[35]</sup> so that is reasonable the NO production decreased compared to Ang II infusion group in female rats. However Ang II acts through different receptors (AT<sub>1</sub>R and AT<sub>2</sub>R), so the effect of Ang II in RBF is dominant and Mas blockade could not change RBF reduction induced by Ang II. Other research also indicated the different response to Ang II related to MasR between male and female.<sup>[36]</sup> In this study, we did not observe any difference in nitrite level of urine in male or female groups. We did not normalized nitrite levels for the urine volume per time unit, and therefore, this limitation may be the cause.

## Conclusion

NO and RBF responses to Ang II administration depend on vasodilator receptors of RAS in a gender-related manner.

## Acknowledgments

We thank Dr. Mehdi Nematbakhsh for his guidance and assistance.

## Financial support and sponsorship

This research was supported by Isfahan University of Medical Sciences (Grant # 393735).

## Conflicts of interest

There are no conflicts of interest.

## References

- Phillips L, Toledo AH, Lopez-Neblina F, Anaya-Prado R, Toledo-Pereyra LH. Nitric oxide mechanism of protection in ischemia and reperfusion injury. *J Invest Surg* 2009;22:46-55.
- Ebrahimzadeh M, Nabavi S, Nabavi S, Pourmorad F. Nitric oxide radical scavenging potential of some Elburz medicinal plants. *Afr J Biotechnol* 2013;9:5212-7.
- Finlay S, Jones MC. Acute kidney injury. *Medicine* 2013;41:182-5.
- Viñas JL, Sola A, Genescà M, Alfaro V, Pi F, Hotter G. NO and NOS isoforms in the development of apoptosis in renal ischemia/reperfusion. *Free Radic Biol Med* 2006;40:992-1003.
- Jung KH, Chu K, Ko SY, Lee ST, Sinn DI, Park DK, *et al.* Early intravenous infusion of sodium nitrite protects brain against *in vivo* ischemia-reperfusion injury. *Stroke* 2006;37:2744-50.
- Tripatara P, Patel NS, Webb A, Rathod K, Lecomte FM, Mazzon E, *et al.* Nitrite-derived nitric oxide protects the rat kidney against ischemia/reperfusion injury *in vivo*: Role for xanthine oxidoreductase. *J Am Soc Nephrol* 2007;18:570-80.
- de Gasparo M. Angiotensin II and nitric oxide interaction. The Role of Nitric Oxide in Heart FailureSpringer (USA); 2004. p. 137-48.
- Zhou MS, Adam A, Raij L. Review: Interaction among angiotensin II, nitric oxide and oxidative stress. *J Renin Angiotensin Aldosterone Syst* 2001;2 1 Suppl:S59-63.
- Schiavone MT, Santos RA, Brosnihan KB, Khosla MC, Ferrario CM. Release of vasopressin from the rat hypothalamo-neurohypophysial system by angiotensin-(1-7) heptapeptide. *Proc Natl Acad Sci U S A* 1988;85:4095-8.
- Heitsch H, Brovkovich S, Malinski T, Wiemer G. Angiotensin-(1-7)-stimulated nitric oxide and superoxide release from endothelial cells. *Hypertension* 2001;37:72-6.
- Siragy HM, Carey RM. The subtype 2 (AT<sub>2</sub>) angiotensin receptor mediates renal production of nitric oxide in conscious rats. *J Clin Invest* 1997;100:264-9.
- Palm F, Connors SG, Mendonca M, Welch WJ, Wilcox CS. Angiotensin II type 2 receptors and nitric oxide sustain oxygenation in the clipped kidney of early Goldblatt hypertensive rats. *Hypertension* 2008;51:345-51.
- Santos RA, Ferreira AJ, Pinheiro SV, Sampaio WO, Touyz R, Campagnole-Santos MJ. Angiotensin-(1-7) and its receptor as a potential targets for new cardiovascular drugs. *Expert Opin Investig Drugs* 2005;14:1019-31.
- Oudit GY, Herzenberg AM, Kassiri Z, Wong D, Reich H, Khokha R, *et al.* Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin II-dependent glomerulosclerosis. *Am J Pathol* 2006;168:1808-20.
- Raij L. Workshop: Hypertension and cardiovascular risk factors: Role of the angiotensin II-nitric oxide interaction. *Hypertension* 2001;37(2 Pt 2):767-73.
- Müller V, Losonczy G, Heemann U, Vannay A, Fekete A, Reusz G, *et al.* Sexual dimorphism in renal ischemia-reperfusion injury in rats: Possible role of endothelin. *Kidney Int* 2002;62:1364-71.
- Fekete A, Vannay A, Vér A, Rusai K, Müller V, Reusz G, *et al.* Sex differences in heat shock protein 72 expression and localization in rats following renal ischemia-reperfusion injury. *Am J Physiol Renal Physiol* 2006;291:F806-11.
- Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease: A meta-analysis. *J Am Soc Nephrol* 2000;11:319-29.
- Silbiger SR, Neugarten J. The impact of gender on the progression of chronic renal disease. *Am J Kidney Dis* 1995;25:515-33.
- Valdivielso JM, Crespo C, Alonso JR, Martínez-Salgado C, Eleno N, Arévalo M, *et al.* Renal ischemia in the rat stimulates glomerular nitric oxide synthesis. *Am J Physiol Regul Integr Comp Physiol* 2001;280:R771-9.
- Silva-Antonialli MM, Tostes RC, Fernandes L, Fior-Chadi DR, Akamine EH, Carvalho MH, *et al.* A lower ratio of AT<sub>1</sub>/AT<sub>2</sub> receptors of angiotensin II is found in female than in male spontaneously hypertensive rats. *Cardiovasc Res* 2004;62:587-93.
- Forte P, Kneale BJ, Milne E, Chowienzyk PJ, Johnston A, Benjamin N, *et al.* Evidence for a difference in nitric oxide biosynthesis between healthy women and men. *Hypertension* 1998;32:730-4.
- Reckelhoff JF. Gender differences in the regulation of blood pressure. *Hypertension* 2001;37:1199-208.
- Neugarten J, Ding Q, Friedman A, Lei J, Silbiger S. Sex hormones and renal nitric oxide synthases. *J Am Soc Nephrol* 1997;8:1240-6.
- Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R233-49.
- Baylis C. Changes in renal hemodynamics and structure in the aging kidney; sexual dimorphism and the nitric oxide system. *Exp Gerontol* 2005;40:271-8.

27. Higuchi S, Ohtsu H, Suzuki H, Shirai H, Frank GD, Eguchi S. Angiotensin II signal transduction through the AT1 receptor: Novel insights into mechanisms and pathophysiology. *Clin Sci (Lond)* 2007;112:417-28.
28. Farquharson CA, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation* 2000;101:594-7.
29. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, *et al.* Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. *Circulation* 1996;94:258-65.
30. Prasad A, Tupas-Habib T, Schenke WH, Mincemoyer R, Panza JA, Waclawin MA, *et al.* Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. *Circulation* 2000;101:2349-54.
31. Hennington BS, Zhang H, Miller MT, Granger JP, Reckelhoff JF. Angiotensin II stimulates synthesis of endothelial nitric oxide synthase. *Hypertension* 1998;31(1 Pt 2):283-8.
32. Sampson AK, Moritz KM, Jones ES, Flower RL, Widdop RE, Denton KM. Enhanced angiotensin II type 2 receptor mechanisms mediate decreases in arterial pressure attributable to chronic low-dose angiotensin II in female rats. *Hypertension* 2008;52:666-71.
33. Sampson AK, Moritz KM, Denton KM. Postnatal ontogeny of angiotensin receptors and ACE2 in male and female rats. *Gend Med* 2012;9:21-32.
34. Hilliard LM, Sampson AK, Brown RD, Denton KM. The "his and hers" of the renin-angiotensin system. *Curr Hypertens Rep* 2013;15:71-9.
35. Dilauro M, Burns KD. Angiotensin-(1-7) and its effects in the kidney. *ScientificWorldJournal* 2009;9:522-35.
36. Safari T, Nematbakhsh M, Hilliard LM, Evans RG, Denton KM. Sex differences in the renal vascular response to angiotensin II involves the Mas receptor. *Acta Physiol (Oxf)* 2012;206:150-6.