TESTOSTERONE SUPPLEMENTS ACCELERATE PROGRESSION OF KIDNEY INJURY IN A RAT MODEL OF REDUCED RENAL MASS

RADU ILIESCU[1,2,*], MONICA HĂNCIANU[3], JANE F. RECKELHOFF[2]

[1]Department of Physiology, University of Medicine and Pharmacy "Gr. T. Popa" Iasi,16 Universitatii St., Iasi, Romania,
[2]Department of Physiology, University of Mississippi Medical Center, 2500 North State St., Jackson, MS, USA
[3]Faculty of Pharmacy, University of Medicine and Pharmacy "Gr. T. Popa" Iasi,16 Universitatii St., Iasi, Romania,
**corresponding author: radu.iliescu@umfiasi.ro*

Abstract

Men with end-stage renal disease are frequently given androgen supplements to improve sexual function. Endogenous androgens contribute to hypertension and renal injury in various animal models. We hypothesized that testosterone supplements exacerbate hypertension and renal injury in rats with reduced renal mass (RRM). Rats subjected to surgical ablation of 80% of renal mass were given 8% NaCl diet for 6 weeks. Testosterone was administered in Silastic® pellets throughout the study to groups of rats with intact or ablated kidneys. Arterial pressure was continuously monitored by telemetry. Renal injury was assessed by measurements of urinary protein and neutrophil gelatinase-associated lipocalin (NGAL) excretion. RRM developed hypertension on the high salt diet as compared with intact rats (154±12 vs 111±3mmHg). Testosterone supplementation did not alter the course of hypertension in RRM, nor increased blood pressure in Sham rats (156±12 vs 113±8mmHg, RRM vs Sham). Testosterone-supplemented RRM consistently excreted 20 to 30% more protein than untreated RRM. Urinary levels of NGAL, an index of tubulointerstitial injury, were also higher in RRM as compared to intact rats and were further augmented by testosterone supplements. Our data indicate that testosterone supplements worsen renal injury in a model of chronic hypertensive renal disease without affecting blood pressure.

Rezumat

Terapia de suplimentare cu androgeni este frecvent utilizată la bărbații care suferă de insuficiență renală, cu scopul de a ameliora funcția sexuală. Androgenii endogeni contribuie la hipertensiune și afectarea renală în diferite modele experimentale. Am formulat ipoteza conform căreia suplimentele cu testosteron exacerbează hipertensiunea și boala renală la șobolani cu masa renală redusă (RRM). Șobolanilor supuși ablației chirurgicale a 80% din masa renală li s-a administrat apoi o dietă cu 8% NaCl timp de 6 săptămâni. Testosteronul a fost administrat în pelete de Silastic® pe toată durata studiului grupurilor de șobolani cu ablație renală sau rinichi intacți. Tensiunea arterială a fost monitorizată continuu prin telemetrie. Afectarea renală a fost determinată prin măsurarea excreției urinare de proteine și a lipocalinei asociate gelatinazei neutrofilice (NGAL). Grupul RRM a dezvoltat hipertensiune arterială în timul dietei hipersodate, în comparație cu șobolanii cu rinichi intacți (154±12 față de 111±3mmHg). Suplimentele de testosteron nu au modificat apariția hipertensiunii la RRM tratați și nici nu au crescut tensiunea

arterială la şobolanii intacţi (156±12 faţă de 113±8mmHg, RRM faţă de intact). RRM trataţi cu suplimente de testosteron au avut o excreţie urinară de proteine cu 20 – 30% mai mare decât RRM netrataţi. Nivelul urinar de NGAL, indice al afectării tubulointerstiţiale, a fost de asemenea crescut la RRM faţă de şobolanii intacţi şi a fost crescut suplimentar prin administrarea de testosteron. Datele obţinute indică faptul că suplimentele de testosteron agravează afectarea renală la un model de boală renală cronică hipertensivă, fără a afecta tensiunea arterială.

Keywords: testosterone, blood pressure, renal injury

Introduction

The use of androgen supplements is becoming increasingly prevalent. In addition to anabolic androgen abuse by athletes [1], men who suffer from chronic cardiovascular and renal disease are frequently given testosterone supplements with the purpose of treating osteoporosis, improving libido and overall well-being [2]. However, despite the common use of prescription androgens in men with cardiovascular and renal disease, the long term impact of androgen supplements is not clear.

Men progress to chronic renal failure at a more rapid rate than do women, even for similar levels of blood pressure [3]. Experimental data indicate that male spontaneously hypertensive rats (SHR) exhibit greater renal injury and reduction in GFR than do age-matched females. Gonadectomy of male SHR reduces blood pressure but also protects against the increase in proteinuria [4]. In a non-genetic model of hypertension, the renal wrap hypertensive model, castration of male rats attenuated glomerular injury and proteinuria, and treatment of castrated rats with dihydrotestosterone (DHT), exacerbated glomerular injury [5]. Thus, there is compelling evidence that endogenous androgens promote renal injury and declines in renal function. Whether exogenous androgens can induce or exacerbate renal injury is not clear.

We hypothesized that testosterone supplementation to rats mimicking the clinical situation of chronic kidney disease (CKD) will accelerate the progression of renal injury. To test this hypothesis, we administered testosterone supplements to rats in which kidney disease was induced by surgical ablation of 80% of the renal mass and concomitant administration of a high salt diet. As systemic hypertension is a major determinant of the progression of renal disease in this model, we monitored blood pressure continuously over the course of 8 weeks, using telemetry, to obtain an accurate evaluation of the effects that testosterone supplements may have on the development of hypertension in this model.

Materials and Methods

Thirty two Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used in the study. Rats were housed in temperature-controlled rooms with a constant light/dark cycle and free access to water and food. All protocols complied with the *Guidelines for the Care and Use of Laboratory Animals* by the National Institutes of Health, and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. At 10 weeks of age, under isoflurane anesthesia, the rats were implanted with radiotelemetry transmitters (TA11PA-C40; Data Sciences International, St. Paul, MN) in the aorta, as previously described [6]. The blood pressure signal was thereafter acquired for 5 sec, every 5 min, throughout the duration of the experiment. During the same surgical procedure, the rats were subjected to right nephrectomy followed by surgical removal of the upper and lower poles of the left kidney [7], thereby creating a reduction of renal mass of ~ 80% (RRM). In another group of rats, the kidneys were mobilized, but not cut (Sham). The rats were allowed to recover for at least 1 week, or until the normal variability of cardiovascular parameters was restored. Following 1 week of baseline recording (Figure 1), 8 RRM and 8 sham-operated rats were implanted subcutaneously with Silastic® pellets containing Testosterone propionate, as previously described (Testo) [8].

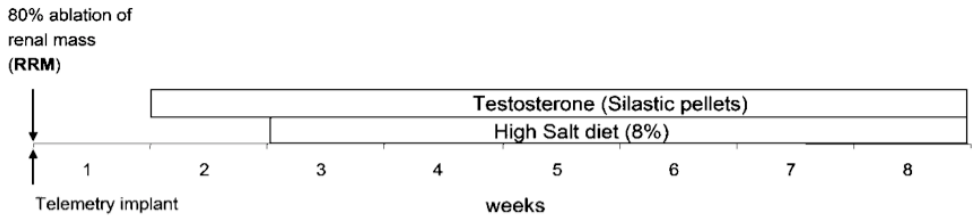

Figure 1
Experimental timeline

Starting on the 3rd week the rats were switched from the normal rat diet to a high-salt diet (8% NaCl, AIN76, Harlan Teklad) for the remaining 6 weeks of the experiment. On weeks 2, 4, 6 and 8, rats were placed in metabolic cages for 24-h urine collection and measurement of total urinary protein excretion (BCA, Pierce, IL), as a marker of glomerular injury. On week 8, the urinary excretion of neutrophil gelatinase associated lipocalin (NGAL, marker of tubular injury) was determined by immunoblotting using a commercially available antibody (R&D Systems) [9]. At the end of the

experiment, the animals were sacrificed and the kidneys were removed and prepared for histological examination of the degree of fibrosis (Mason's Trichrome, as previously described [10]). The concentration of testosterone in plasma was determined by radioimmunoassay [10] and the seminal vesicles were weighed.

Statistical analyses: results are expressed as mean ± SEM. Mean arterial pressure (MAP) values were obtained by averaging all 24-h values during each week. Comparisons for multigroup and multifactorial analysis were performed by two-way ANOVA and by using the Student-Newman-Keul's method for multiple-comparison procedures. The criterion for significant differences between groups of study was $p < 0.05$.

Results and Discussion

As shown in Figure 2, blood pressure increased in rats with reduced renal mass upon salt loading, as compared to sham operated animals.

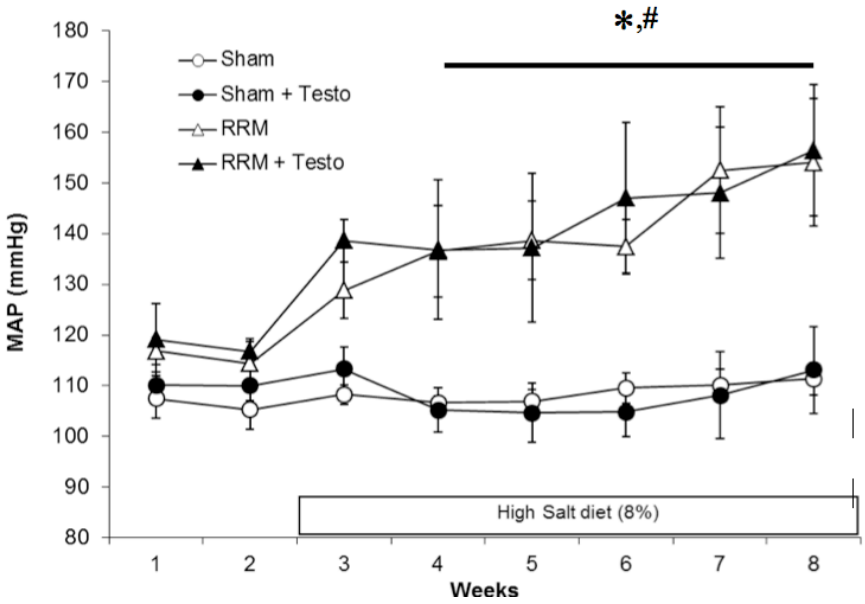

Figure 2

Mean arterial pressure measured by telemetry;
* p<0.05, RRM vs. Sham, # p<0.05 RRM+Testo vs. Sham+Testo

This increase was evident as early as the first week on the high salt diet and hypertension continued to progress thereafter, reaching 154 ± 12 mmHg at the end of the experiment. Testosterone supplementation in RRM rats did not alter the course of hypertension (p >0.05 RRM vs RRM+Testo,

for all time points). Furthermore, neither salt loading nor administration of testosterone supplements to sham-operated control rats had an effect on their blood pressure, which remained at levels similar to those found during the control period throughout the experiment.

Urinary protein excretion was used as a dynamic marker of the progression of of glomerular injury. As shown in Figure 3, significant proteinuria developed in RRM rats during the high salt diet.

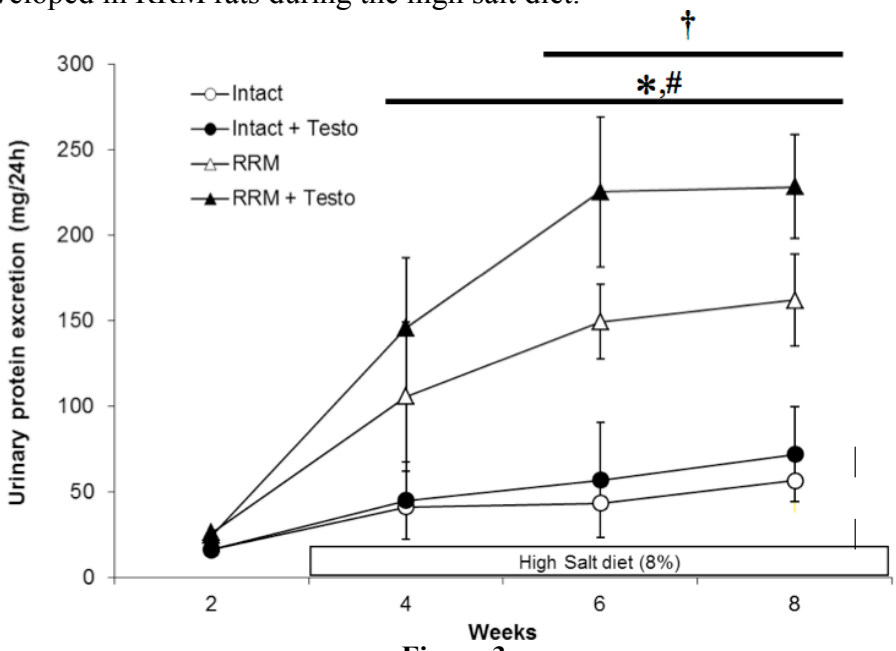

Figure 3

Urinary protein excretion; * $p<0.05$, RRM vs. Sham, # $p<0.05$ RRM+Testo vs. Sham+Testo, †, $p<0.05$ RRM+Testo vs. RRM

The amount of urinary protein excretion significantly increased over the course of 4 weeks of high salt diet (105 mg/day on week 4 vs 162 mg/ day on week 8). Moreover, administration of testosterone supplements to RRM rats led to a marked exacerbation of glomerular injury, as evidenced by the significantly higher proteinuria on weeks 6 and 8 of the experimental period as compared to RRM without testosterone ($p<0.05$). Salt loading in rats with intact kidneys (Sham) did not have an effect on urinary protein excretion, irrespective of whether testosterone supplements were administered.

Furthermore, in order to evaluate tubulointerstitial injury, urinary excretion of NGAL, an early biomarker of tubular damage [11] was evaluated by immunobloting. As shown in Figure 4, rats with RRM and salt

loading excreted significantly higher amounts of NGAL when administered testosterone supplements.

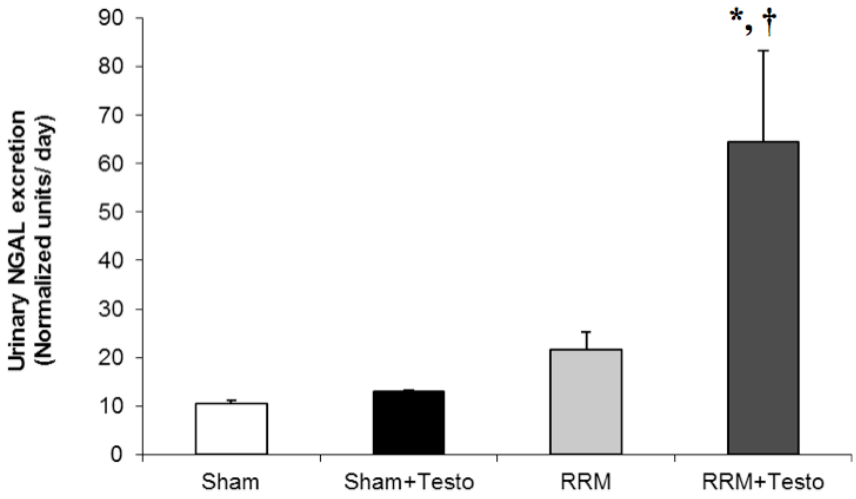

Figure 4
Urinary NGAL excretion;
* p<0.05, RRM+Testo vs. Sham, †, p<0.05 RRM+Testo vs. RRM

Histological analysis revealed that rats with reduced renal mass developed significant segmental glomerulosclerosis and tubulointerstitial fibrosis. Administration of testosterone supplements to rats with RRM and salt loading aggravated renal structural lesions, as evidenced by extensive areas of complete loss of structural renal integrity, glomerulosclerosis affecting the entire glomerular area and tubular dilation with loss of the normal epithelium (Figure 5).

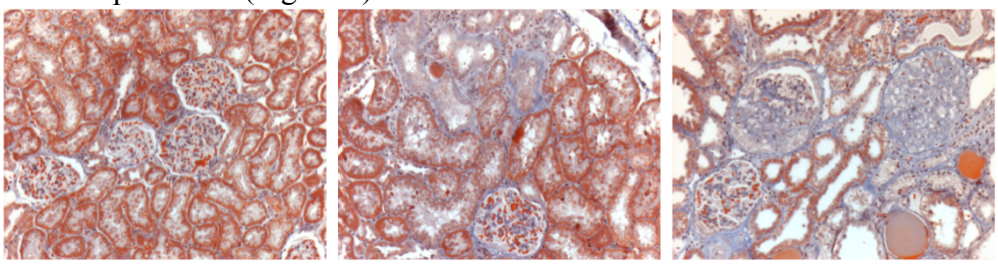

Figure 5
Representative histological sections stained with Mason's Trichrome. Segmental glomerulosclerosis and tubulointerstitial fibrosis is present in RRM rats. Areas of loss of renal structural integrity in RRM+Testo rats. Intact+Testo were not different to Sham and slides are omitted for clarity

The data presented above indicate that administration of testosterone supplements in a rat model of chronic kidney disease accelerates the progression of renal injury. Clinical and experimental studies have shown that after the initial loss of a critical mass of nephrons, the remnant nephrons compensate for the overall loss of function by increasing filtration [12]. This maladaptive process leads to progressive sclerosis of the remnant glomeruli, with glomerular hypertension being the key mechanism responsible [13]. Indeed, experimental animal models of renal mass reduction only display significant glomerular damage if systemic hypertension develops [7]. In our model of hypertensive renal injury, administration of testosterone supplements did not alter systemic blood pressure levels. This is in contrast to our previous studies in SHR [8, 14], suggesting that testosterone increases blood pressure by activation of the renin-angiotensin system [4]. Indeed, in experimental models of reduced kidney mass combined with high salt intake, the renin-angiotensin system is suppressed [15]. However, despite similar blood pressure levels, RRM rats administered testosterone in our study displayed more severe glomerular and tubulointerstitial injury than their controls. While the precise mechanism by which testosterone may increase the susceptibility to hypertensive glomerular injury is unknown, it may involve direct vasodilation of the preglomerular vessels [16], with consequent increased transmission of systemic blood pressure to the glomerular capillaries. Alternatively, testosterone may stimulate renal oxidative stress [14] which contributes to glomerular damage. However, this effect manifests only in the context of systemic hypertension since testosterone supplementation in rats with intact kidneys did not affect renal structure and function.

Finally, plasma testosterone concentrations achieved by the slow-release formulation were assessed by radioimmunoassay (Figure 6) and the effect of testosterone on specific target organs was evaluated by the weight of the seminal vesicles indexed for body weight (Figure 7). Testosterone propionate administration by Silastic® pellets led to an approximately 6 fold increase in plasma testosterone concentration both in Sham and RRM rats. The higher testosterone levels were biologically active, as evidenced by a significant increase of seminal vesicle size.

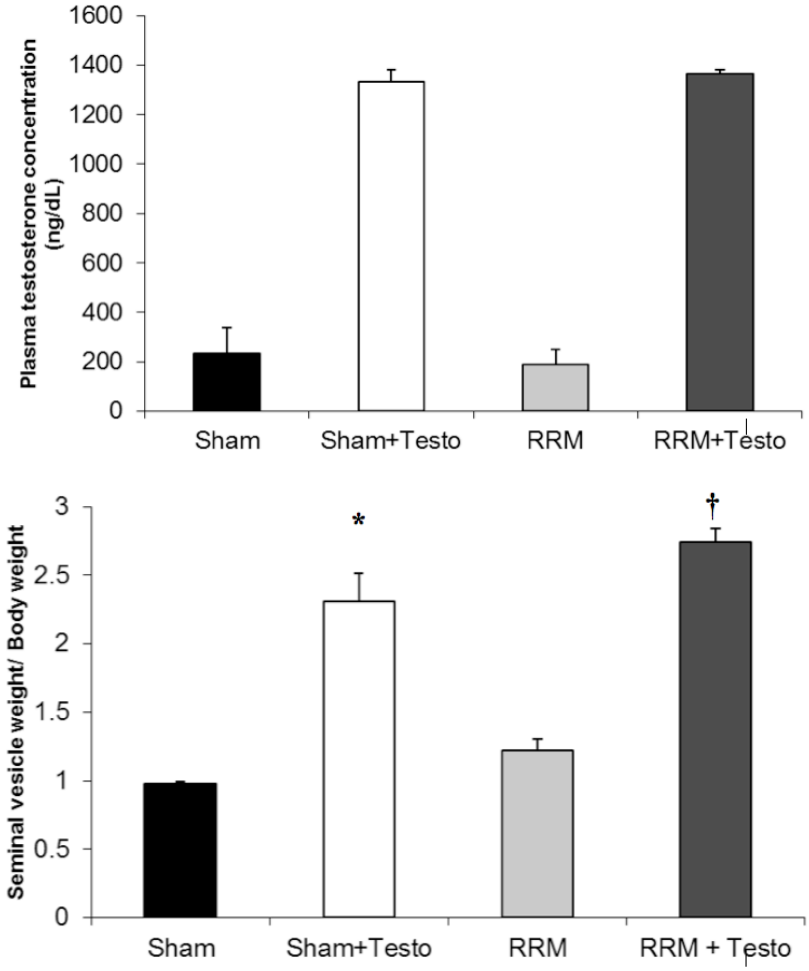

Figure 7
Seminal vesicle weight indexed by body weight;
* $p<0.05$, Sham+Testo vs. Sham, †, $p<0.05$ RRM+Testo vs. RRM

Conclusions

Testosterone supplementation in a rat model of reduced renal mass and high salt loading exacerbates progression of renal injury. Testosterone supplementation does not alter the development of hypertension. In the context of the increased use of testosterone supplements in patients with chronic kidney disease, the above mentioned results caution against the potential detrimental effects of testosterone administration, far exceeding the benefits of increased well-being and improvement of muscle and bone strength. Furthermore, the results of this study underline the need for large

clinical trials aimed at evaluating the risk/benefit ratio of testosterone supplements in patients with chronic diseases in general. A potential limitation of the present study is represented by the supraphysiological concentrations of testosterone in plasma. However, since guidelines for administration of testosterone supplementation therapy are currently lacking, the levels of bioactive androgens in patients receiving such supplements are often supraphysiological [17]. Finally, the mechanisms by which androgens increase the susceptibility of the kidneys to pressure-induced damage require further investigation.

References

1. Pitigoi G, Paunescu, C., Mitrea, N., Baconi, D., Paunescu, M., Burcea, C., Arsene, A.L. New approaches regarding the dynamics of the doping pharmacologic agents in sports *Farmacia*. 2012;60(1):111-119
2. Johansen KL. Treatment of hypogonadism in men with chronic kidney disease. *Advances in chronic kidney disease*. 2004;11:348-356
3. Silbiger SR, Neugarten J. The impact of gender on the progression of chronic renal disease. *American journal of kidney diseases : the official journal of the National Kidney Foundation*. 1995;25:515-533
4. Reckelhoff JF, Granger JP. Role of androgens in mediating hypertension and renal injury. *Clinical and experimental pharmacology & physiology*. 1999;26:127-131
5. Ji H, Menini S, Mok K, Zheng W, Pesce C, Kim J, Mulroney S, Sandberg K. Gonadal steroid regulation of renal injury in renal wrap hypertension. *American journal of physiology. Renal physiology*. 2005;288:F513-520
6. Sartori-Valinotti JC, Iliescu R, Yanes LL, Dorsett-Martin W, Reckelhoff JF. Sex differences in the pressor response to angiotensin II when the endogenous renin-angiotensin system is blocked. *Hypertension*. 2008;51:1170-1176
7. Griffin KA, Picken M, Bidani AK. Method of renal mass reduction is a critical modulator of subsequent hypertension and glomerular injury. *Journal of the American Society of Nephrology : JASN*. 1994;4:2023-2031
8. Reckelhoff JF, Zhang H, Granger JP. Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension*. 1998;31:435-439
9. Chandrashekar K, Lopez-Ruiz A, Juncos R, Nath K, Stec DE, Vera T, Liu R, Juncos LA. The modulatory role of heme oxygenase on subpressor angiotensin II-induced hypertension and renal injury. *International journal of hypertension*. 2012;2012:392890
10. Davis DD, Ruiz AL, Yanes LL, Iliescu R, Yuan K, Moulana M, Racusen LC, Reckelhoff JF. Testosterone supplementation in male obese zucker rats reduces body weight and improves insulin sensitivity but increases blood pressure. *Hypertension*. 2012;59:726-731
11. Bârcă M, Baconi DL, Ciobanu AM, Militaru M, Burcea GTA, Bălălău C, Comparative evaluation of methotrexate toxicity as solution for injection and liposomes following a short-term treatment in a murine model of arthritis: Note II. Histopathological changes, *Farmacia*, 2013, 61(5), 939-947
12. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Journal of the American Society of Nephrology : JASN*. 2001;12:1315-1325
13. Griffin KA, Picken MM, Bidani AK. Blood pressure lability and glomerulosclerosis after normotensive 5/6 renal mass reduction in the rat. *Kidney international*. 2004;65:209-218

14. Iliescu R, Cucchiarelli VE, Yanes LL, Iles JW, Reckelhoff JF. Impact of androgen-induced oxidative stress on hypertension in male shr. *American journal of physiology. Regulatory, integrative and comparative physiology*. 2007;292:R731-735
15. Hall JE, Mizelle HL, Brands MW, Hildebrandt DA. Pressure natriuresis and angiotensin II in reduced kidney mass, salt-induced hypertension. *The American journal of physiology*. 1992;262:R61-71
16. Iliescu R, Reckelhoff JF. Testosterone and vascular reactivity. *Clinical science*. 2006;111:251-252
17. Cherrier MM. Androgens and cognitive function. *Journal of endocrinological investigation*. 2005;28:65-75

Manuscript received: November 15th 2013