doi: 10.20903/zpopov-2022-0020 UDK: 616.69-008.14:616.13-004.6

Ognen IVANOVSKI MD, PhD[1]

# PATHOPHYSIOLOGY OF ERECTILE DYSFUNCTION: FROM BENCH TO BEDSIDE

## Abstract

**Background.** Erectile dysfunction (ED) and cardiovascular diseases share the same risk factors. Atherosclerosis not only affects the coronary arteries but also the penile arteries in men, thus contributing to organic causes of ED. Recently, the hypercholesterolemic atherosclerotic apolipoprotein $E^{-/-}$ deficient ($ApoE^{-/-}$ ) mouse model was introduced as a powerful experimental tool in ED research. The aim of this review is to characterize the development and distribution of atherosclerosis development in $ApoE^{-/-}$ mice and to try to test different treatment strategies in order to prevent or cure ED.

**Experimental model.** Serum cholesterol and triglycerides were significantly increased in $ApoE^{-/-}$ mice as compared to wild type mice. $ApoE^{-/-}$ mice displayed not only fatty streaks, but also widespread fibrous plaques at vascular sites that are typically affected in human atherosclerosis. The atherosclerotic lesions covered 15 ± 1.1 % of the aortic root section, 0.9% ± 0.2 of the aortic luminal surface, 21 ± 4.2 % of the aortic arch region and 26 ± 2.2% of the renal artery. In contrast, no atherosclerotic lesion formation was observed in wild type C57BL6/J mice. Interestingly, no atherosclerosis was observed in penile arteries. However, we have found increased staining of vasculature calcification, nitrotyrosin, macrophage/monocyte content as well as total collagen content at the vascular sites typically affected by atherosclerosis. Uremia significantly increases the degree of atherosclerosis as compared to the controls. Of note, even mild renal dysfunction, for example after uninephrectomy, increases the calcification score and aggravates endothelial function of cavernosal bodies in $apoE^{-/-}$ mice,

[1] University Clinic of Urology, Medical Faculty, University SS Cyril and Methodius, Skopje, North Macedonia; e-mail: ognen.ivanovski@medf.ukim.edu.mk

and this effect might be linked to increased oxidative stress in penile endothelium. Simvastatin significantly improved endothelial function of aortic and cavernosal tissues in uremic ApoE$^{-/-}$ mice via reduction of oxidative stress, and this effect does not seem to be associated with the lipid lowering effect of this drug.

**Conclusions:** The ApoE$^{-/-}$ mouse is a well-characterized model to study disorders associated with hypercholesterolemia and atherosclerosis in cardiovascular research. We anticipate that this mouse model will be useful to test treatment strategies aiming to target both atherosclerosis and erectile dysfunction.

## Erectile dysfunction and cardiovascular disease

Erectile dysfunction (ED) is the persistent inability to achieve or maintain an erection sufficient to permit satisfactory sexual performance, and the resulting stress often impacts interaction with others (1). In the Western industrialized countries, prevalence of ED in the general population is approximately 20 to 30%, with an economical and significant psychological impact (2). In cardiovascular high-risk patients, prevalence of ED rises up to 75%, indicating the strong association of ED with the known cardiovascular risk factors and cardiovascular diseases (CVD) (3).

Atherosclerosis, which can be defined as a chronic and progressive disease characterized by an inflammatory response of the arterial wall, is still a leading cause of death, mainly in the Western world [4,5]. Atherosclerosis is an inflammatory process of the arterial walls and is initiated by endothelial dysfunction accompanied by an imbalance in the production of reactive oxygen species (ROS) and nitric oxide (NO). Erectile dysfunction (ED) and cardiovascular diseases share the same risk factors. Atherosclerosis not only affects the coronary arteries but also the penile arteries in men, thus contributing to organic causes of ED.

## Explanation of the model

Recently, the hypercholesterolemic atherosclerotic apolipoprotein ApoE deficient (ApoE$^{-/-}$) mouse model was introduced as a powerful expe-

rimental tool in ED research. We and others have addressed important questions more directly in a mouse model of accelerated atherosclerosis (6), vascular calcification (7) and ED (8). Since wild type rats or mice do not easily develop atheromatous lesions, we used the most common mouse models to study atherogenic mechanisms, namely the apolipoprotein E gene knock-out ($ApoE^{-/-}$) model. $ApoE^{-/-}$ mice have delayed clearance of lipoproteins, and when placed on low-cholesterol, low-fat diets, their total serum cholesterol levels reach 11 to 13 mM as a result of the accumulation of chylomicrons and cholesterol-rich VLDL remnants, as compared with 2 to 3 mM in wild-type mice. Importantly, these genetically engineered mice develop not only fatty streaks but also widespread fibrous plaques at vascular sites that are typically affected in human atherosclerosis (9). Atherosclerotic lesion formation was evaluated with oil-red O staining on the following vascular sites: aortic root, aortic arch with its main branches: brachiocephalic artery, right common carotid artery and left subclavian artery, longitudinal aorta and renal arteries. Serum total cholesterol, triglycerides and urea levels were determined. The results were compared with a group of wild type C57BL6/J male mice. Interestingly, we have discovered that atherosclerotic lesions covered 15 ± 1.1 % of the aortic root section, 0.9% ± 0.2 of the aortic luminal surface, 21 ± 4.2 % of the aortic arch region and 26 ± 2.2% of the renal artery. In contrast, no atherosclerotic lesion formation was observed in wild type C57BL6/J mice. In addition, no atherosclerosis was observed in penile arteries in both types of mice. However, microscopic structural changes of the arterial wall were observed (see below).

## Erectile dysfunction in uremia

Chronic kidney disease (CKD) in males causes sexual dysfunction with a prevalence ranging between 71% and 98% [10]. ED's physiopathology in uremia is complex and multifactorial, involving a combination of classical risk factors (obesity, glucose intolerance, hypercholesterolemia, smoking, and hypertension) and specific uremia-related risk factors (increased oxidative stress, endothelial dysfunction, inflammation). Endothelial dysfunction is associated with loss of nitric oxide (NO) bioavailability due to either reduced formation or accelerated degradation of NO. In turn, degradation of NO by increased reactive oxidant species including supero-

xide anion and oxidized lipoproteins might be a major mechanism underlying ED in these patients [11]. In addition to the uremic milieu, peripheral neuropathy, autonomic insufficiency, peripheral vascular disease, and pharmacologic therapy all play an important role in the genesis of this problem.

Endothelial dysfunction and accelerated atherosclerosis are some of the primary causes of morbidity and mortality in patients with CKD [12]. ED and CVD share the same risk factors. Accelerated atherosclerosis not only affects the coronary arteries but also may affect penile arteries in men, thus contributing to arteriogenic causes of ED in CKD patients. Thus atherosclerosis as a general health problem may be a link between these two entities [13]. We have recently published the effect of CKD on vascular calcification and endothelial function of cavernosal bodies in apoE$^{-/-}$ mice as a new model of ED research (14). We have created 2 uremic groups with different degree of uremia by using serum urea levels to assess renal function. At 16 weeks after uremia-inducing surgery, serum urea concentrations in uremic mice were 200% increased, as compared with those in the sham-op group. Likewise, uninephrectomized mice showed significant increase in serum urea level compared to the controls. Serum total cholesterol and triglyceride levels also were significantly higher in uremic and uninephrectomized mice. Both groups had higher serum calcium concentrations), whereas the serum phosphate level did not differ between groups (P = NS, Table 1). Mean arterial blood pressure did not differ between the groups, either. Atherosclerotic lesions in thoracic aorta were significantly larger both uremic groups compared to the non-uremic controls. There was no atheromatous lesions in cavernosal bodies or penile arteries observed in any group. However, uremic animals showed a significant increase in calcification score in both aorta and cavernosal bodies when compared with controls (Figure 1A, 1B). In addition, calcification score in the cavernosal bodies was significantly higher in uninephrectomized mice, as compared with the controls (Figure 1B). No such difference was observed in the aorta between uninephrectomized mice and the controls (Figure 1A). To investigate whether uremia had an impact on the cavernosal body's morphology, detailed histological studies analyzing nitrotyrosine expression, collagen content, and macrophage infiltration, were performed. Collagen content was higher in cavernosal bodies of uremic mice than in those of the controls. (Table 2, Figure 2A). Furthermore, uremic mice showed increased nitrotyrosine expression in the cavernosal bodies

compared with sham-operated controls (Table 2, Figure 2B). The percentage of the lesion cross-section area occupied by macrophages, as revealed by MOMA-2 staining, was comparable between the 3 groups (P=NS, Table 2).

## Partial nephrectomy and atherosclerosis

Radical nephrectomy (RN) as compared to partial nephrectomy (PN) increases the risk of CKD in patients with kidney tumors. CKD is a significant risk factor for cardiovascular events and death. Given equivalent oncological efficacy of both surgical approaches in patients with small (15) and large renal tumors (16), several recent studies report that patients treated with PN have better cardiovascular health and global survival rates than those undergoing RN (17, 18) This survival advantage was also observed after surgery for histologically benign renal tumors, comparing PN with RN (19) Thus, the widespread use of RN may result in an unnecessary removal of functional nephron mass in many patients with kidney tumors and increase the risk of cardiovascular and all-cause mortality, particularly in individuals with small kidney tumors who are unlikely to die of renal cancer.

There is an increasing body of literature on the deleterious effects of decreased renal function on overall survival in the general population (20). After stratification for age, gender, race, and the presence or absence of diabetes, cardiovascular mortality in patients with CKD is 10 to 20 times higher than in the general population (21) due to a variety of CKD-linked complications including accelerated atherosclerosis and arterial stiffening.

The association between poor renal functional outcomes and cardiovascular outcomes is relevant for kidney cancer management as well. Several studies have demonstrated poor kidney function outcomes in patients treated with RN rather than PN (22, 23). Recently, Huang et al reported that a 3-year probability of freedom from new onset CKD was only 35% in patients who underwent RN compared with 80% in those who underwent PN. The average excess loss of renal function observed with RN was associated with a 25% increase in the risk of cardiac death and a 17% increase in the risk of death from any cause (19)

The clinical significance of iatrogenic CKD and in particular the impact of kidney surgery on long-term cardiovascular outcomes remain

research topics of great importance. Even mild CKD is associated with numerous metabolic and endocrine disturbances, including arterial hypertension, abnormalities of calcium and phosphate metabolism, and a state of chronic inflammation and oxidative stress. They contribute to the development and progression of arteriosclerosis, atherosclerosis, vascular and valvular calcification, and cardiac disease.

It is important to further investigate the pathophysiological mechanisms involved in the increased cardiovascular risk of patients with mild CKD. We have shown, for the first time, that PNX, in contrast to UNX, does not stimulate CKD-enhanced atherosclerosis progression in the experimental model of the apoE$^{-/-}$ mouse. Our study lends further support to the still controversial view that even mild renal dysfunction, such as that occurring after unilateral nephrectomy, may cause an increase in atherosclerotic plaque size in presence of pre-existing atheromatous disease. Our finding is in agreement with two other recent reports on the deleterious effects of UNX on the apoE$^{-/-}$ mouse model (14-26) and extends these results by showing that unlike UNX, PNX prevents accelerated atherosclerosis and plaque composition changes in this mouse model.

Partial nephrectomy preserves functional nephron mass and offers cancer specific survival, equivalent to that of radical nephrectomy. The results obtained in our experimental study, if they can be extrapolated to the human condition, represent further support for an expanded use of nephron sparing techniques among patients with kidney cancer. In addition, we have shown that even uninephrectomy has a deterious effect on erectile function by making structural changes to the cavernosal bodies.

## Treatment measures

Statin is an inhibitor of the enzyme 3-hydroxy-methylglutaryl-CoA reductase. It suppresses the conversion of 3-hydroxy-methylglutaryl-CoA to mevalonate, which is the rate-limiting step in *de novo* synthesis of cholesterol. 10 Recent evidence suggests that even in the absence of cholesterol lowering, statins may be beneficial in the treatment of ED and endothelial dysfunction, due to direct anti-inflammatory and anti-oxidative actions at the arterial site. These local actions may occur via an upregulation of endothelial nitric oxide (NO) synthase with increased bioavailability of NO, a

decrease in cellular proliferation, an increase in apoptosis and/or an interference with local oxidative injury [6]. Many of these pleiotropic statin effects are mediated by their ability to block the production of isoprenoid intermediates such as farnesyl or geranylgeranyl pyrophosphates. These isoprenoid derivatives play an important role in the activation of small GTP-binding proteins, including Rho, Ras, and Rac, through an isoprenylation process [7]. Moreover, a mevalonate pathway–independent effect of statins has been reported previously, although the mechanism has not yet been elucidated [8].

Several studies found that statins could rapidly improve endothelial function, even before changing the lipid profile.12, 13. However, the precise involvement of such pleiotropic effects in the efficacy of statins in patients remains unclear because of the difficulty of separating lipid-related versus non-lipid related responses in clinical trials. In addition, some studies suggested that statin therapy was associated even with reduced levels of testosterone and even symptoms of hypogonadism. 15, 16

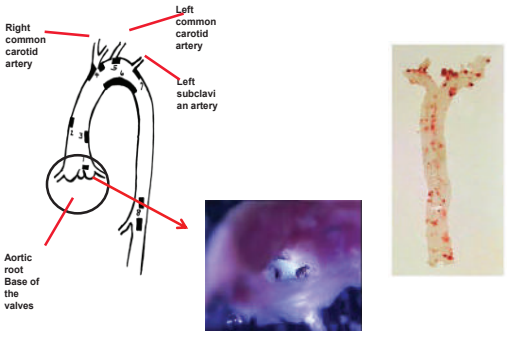
One of the differences with the clinical setting, however, is that, in apolipoprotein E deficient models, statins do not decrease serum cholesterol levels. Therefore, any potential statin benefit in these animals can be interpreted in the absence of a change of this confounding variable [9].

Simvastatin treatment led to a significant decrease in both nitrotyrosine expression and collagen content in the corpus cavernosum and in the atheromatous plaques of CKD mice (Fisher's exact test, p 0.02, table 2). As revealed by monocyte-macrophage-2 staining, the percent of lesion cross-sectional area occupied by macrophages was comparable in the 4 groups.

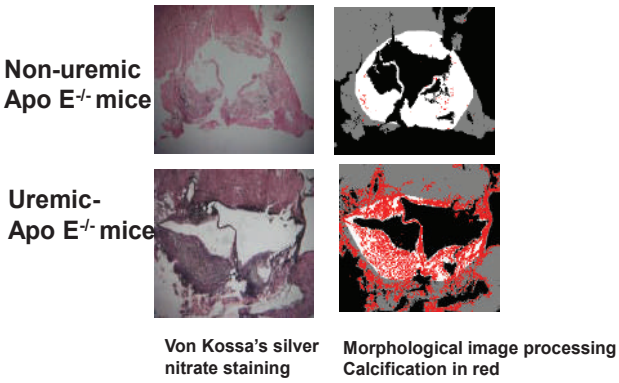
We evaluated the effects of the HMG-CoA reductase inhibitor on uremia enhanced atherosclerosis and vascular calcification in apoE$^{-/-}$ mice with superimposed CKD. We found that CKD enhanced aortic plaque development in apoE$^{-/-}$ mice compared with the controls, in agreement with previous reports by us and others.10, 12 Simvastatin treatment did not reduce the atherosclerotic lesions in the aortic root or thoracic aorta in this experimental model, in line with its failure to decrease serum total cholesterol. Nevertheless, simvastatin therapy led to a significant decrease in intima calcium content, that is, the calcium content of atheromatous plaques. It did not, however, change medial calcium deposition. Vascular calcification is a prominent feature of CKD13, and it is predictive of increased

cardiovascular morbidity and mortality.13 Coronary artery plaques in patients with end stage renal failure are characterized by increased medial thickness and marked intimal and medial calcification.3 Treatment with statins has been shown to be associated with a decrease in vascular calcification in two retrospective clinical studies 4,14 and in animal models such as the apoE$^{-/-}$ Leiden mouse15 but not in another prospective trial.5 This effect is generally associated with a decrease in serum lipid levels, which was not the case in the current study. The beneficial effect of simvastatin on CKD mice had severe atherosclerotic lesions in the thoracic aorta that were significantly increased compared to those in the non-CKD controls (fig. 2). Long-term Simvastatin treatment did not decrease uremia associated atherosclerosis in the aortic root or in the thoracic aorta. The same was true in the control non-CKD mice (fig. 2). Analyses of corpus spongiosum did not show any Red O Oli positive lesions in all groups. As expected, the apoE$^{-/-}$ mice showed higher plasma cholesterol than the WT animals [17-20], exhibiting approximately a 14-fold increase in total cholesterol, as recently reviewed.

Figure 1



## Figure 2




## Figure 3A

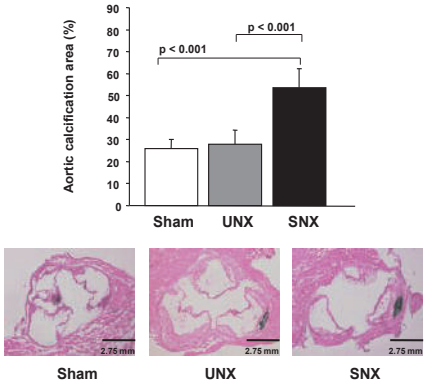


Figure 3A

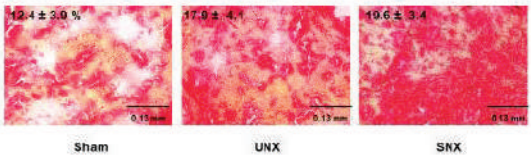
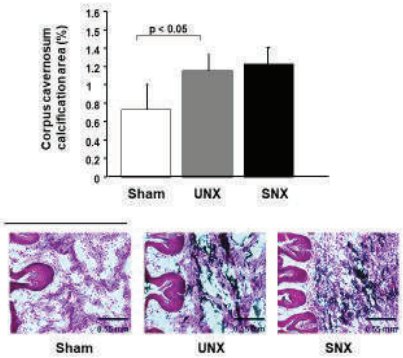



Figure 3B




REFERENCES

1. Lue T.F. Erectile dysfunction. N Engl J Med. 2000; 342: 1802-1813
2. Braun M, Wassmer G, Klotz T, Reifenrath B, Mathers M, Engelmann U. Epidemiology of erectile dysfunction: results of the 'Cologne Male Survey'. Int J Impot Res. 2000; 12: 305-11.
3. Montorsi P, Ravagnani PM, Galli S, Rotatori F, Briganti A, Salonia A, Rigatti P, Montorsi F (2005) The artery size hypothesis: a macrovascular link between erectile dysfunction and coronary artery disease. Am J Cardiol 96 (12B):19M-23M. doi:10.1016/j.amjcard.2005.07.006
4. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med. 1999; 340: 115–126.
   doi: 10.1056/NEJM199901143400207. [PubMed] [CrossRef] [Google Scholar]

5. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011; 473: 317–325.
doi: 10.1038/nature10146. [PubMed] [CrossRef] [Google Scholar]
6. Buzello M, Tornig J, Faulhaber J, Ehmke H, Ritz E, Amann K (2003) The apolipoprotein e knockout mouse: a model documenting accelerated atherogenesis in uremia. Journal of the American Society of Nephrology : JASN 14 (2): 311-316
7. Massy ZA, Ivanovski O, Nguyen-Khoa T, Angulo J, Szumilak D, Mothu N, Phan O, Daudon M, Lacour B, Drueke TB, Muntzel MS (2005) Uremia accelerates both atherosclerosis and arterial calcification in apolipoprotein E knockout mice. Journal of the American Society of Nephrology: JASN 16 (1):109-116. doi:10.1681/ASN.2004060495
8. Xie D, Odronic SI, Wu F, Pippen AM, Donatucci CF, Annex BH (2007) A mouse model of hypercholesterolemia-induced erectile dysfunction. The journal of sexual medicine 4 (4 Pt 1):898-907.
doi:10.1111/j.1743-6109.2007.00518.x
9. 8. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 71 (2): 343-353
10. Navaneethan SD, Vecchio M, Johnson DW, Saglimbene V, Graziano G, Pellegrini F, Lucisano G, Craig JC, Ruospo M, Gentile G, Manfreda VM, Querques M, Stroumza P, Torok M, Celia E, Gelfman R, Ferrari JN, Bednarek-Skublewska A, Dulawa J, Bonifati C, Hegbrant J, Wollheim C, Jannini EA, Strippoli GF Prevalence and correlates of self-reported sexual dysfunction in CKD: a meta-analysis of observational studies. Am J Kidney Dis 56 (4): 670-685
11. Beckman JS, Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271 (5 Pt 1):C1424-1437
12. Foley RN, Parfrey PS, Sarnak MJ (1998) Epidemiology of cardiovascular disease in chronic renal disease. Journal of the American Society of Nephrology : JASN 9 (12 Suppl): S16-23
13. Montorsi P, Ravagnani PM, Galli S, Rotatori F, Briganti A, Salonia A, Rigatti P, Montorsi F (2005) The artery size hypothesis: a macrovascular link between erectile dysfunction and coronary artery disease. Am J Cardiol 96 (12B):19M-23M. doi:10.1016/j.amjcard.2005.07.006
14. Ivanovski O, I, Davceva O, Petrushevska G. Calcification of the cavernosal bodies may be responsible for development of erectile dysfunction in uremic apolipoprotein E deficient (apoE-/-) mice. Rev Int Androl Jan-Mar 2021; 19(1): 25-3

15. Patard JJ, Pantuck AJ, Crepel M, et al. Morbidity and clinical outcome of nephron-sparing surgery in relation to tumour size and indication. Eur Urol. 2007; 52: 148-154.
16. Becker F, Roos FC, Janssen M, et al. Short-term functional and oncologic outcomes of nephron-sparing surgery for renal tumours >/= 7 cm. Eur Urol. 2011; 59: 931-937.
17. Thompson RH, Siddiqui S, Lohse CM, et al. Partial versus radical nephrectomy for 4 to 7 cm renal cortical tumors. J Urol. 2009; 182: 2601-2606.
18. Weight CJ, Larson BT, Fergany AF, et al. Nephrectomy induced chronic renal insufficiency is associated with increased risk of cardiovascular death and death from any cause in patients with localized cT1b renal masses. J Urol. 2010; 183: 1317-1323.
19. Weight CJ, Lieser G, Larson BT, et al. Partial nephrectomy is associated with improved overall survival compared to radical nephrectomy in patients with unanticipated benign renal tumours. Eur Urol. 2010; 58: 293-298.
20. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004; 351: 1296-1305.
21. Foley RN, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol. 1998; 9: S16-23.
22. McKiernan J, Simmons R, Katz J, Russo P: Natural history of chronic renal insufficiency after partial and radical nephrectomy. Urology. 2002; 59: 816-820.
23. Lau WK, Blute ML, Weaver AL, Torres VE, Zincke H. Matched comparison of radical nephrectomy vs nephron-sparing surgery in patients with unilateral renal cell carcinoma and a normal contralateral kidney. Mayo Clin Proc. 2000; 75: 1236-1242.
24. Ivanovski O, Nikolov IG, Davceva O, Gjorgjievska K, Janevska V, Petrushevska G. Compared With Radical Nephrectomy, Nephron-sparing Partial Nephrectomy Protects Apolipoprotein E-deficient Mice From Atherosclerosis Progression. Urology. 2015 May; 85(5)
25. Buzello M, Tornig J, Faulhaber J, et al. The apolipoprotein E knockout mouse: a model documenting accelerated atherogenesis in uremia. J Am Soc Nephrol. 2003; 14: 311-316.
26. Piecha G, Koleganova N, Gross ML, Geldyyev A, Ritz E. Oxidative stress after uninephrectomy alters heart morphology in the apolipoprotein E-/- mouse. J Hypertens. 2008; 26: 2220-2229.