# SERUM ALDOSTERONE LEVEL IN DIABETIC NEPHROPATHY

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**Abstract**

**Background:** Over the last decades, the use of renin–angiotensin system (RAS) blockers has been the mainstay for retarding progression of DKD, along with lifestyle modifications and blood pressure (BP) and glycemic control. Despite the indisputable nephroprotective effects of RAS blockers, accumulated evidence suggests persistence of a high residual risk for CKD and cardiovascular (CVD) progression in these patients, underlining the need for further research to establish novel treatment approaches. Double RAS blockade, initially associated with greater reductions in albuminuria compared to single blockade, was terminally abandoned in the form of a combination of an angiotensin converting enzyme inhibitor (ACEi) with an angiotensin receptor blocker [ARB], or of a renin inhibitor with an ACEi/ARB in diabetics and all other populations, in view of safety concerns raised after publication of two major cardiovascular and renal outcome trials. Steroidal mineralocorticoid receptor antagonists (MRAs) have proven to be effective in the management of primary aldosteronism due to bilateral adrenal hyperplasia or aldosterone-producing adrenal adenomas, in the treatment of resistant hypertension, as well as in the reduction of albuminuria in patients with diabetic and non-diabetic nephropathy (alone or on top of an RAS blocker). Large-scale clinical trials have recently provided evidence of improved renal and cardiovascular outcomes with addition of finerenone to the standard of care in patients with type 2 DM and moderately or severely increased albuminuria

**Keywords:** Diabetic nephropathy, Serum Aldosterone

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**Introduction**

Over the last decades, the use of renin–angiotensin system (RAS) blockers has been the mainstay for retarding progression of DKD, along with lifestyle modifications and blood pressure (BP) and glycemic control. Despite the indisputable nephroprotective effects of RAS blockers, accumulated evidence suggests persistence of a high residual risk for CKD and cardiovascular (CVD) progression in these patients, underlining the need for further research to establish novel treatment approaches ***(1).***

Double RAS blockade, initially associated with greater reductions in albuminuria compared to single blockade, was terminally abandoned in the form of a combination of an angiotensin converting enzyme inhibitor (ACEi) with an angiotensin receptor blocker [ARB], or of a renin inhibitor with an ACEi/ARB in diabetics and all other populations, in view of safety concerns raised after publication of two major cardiovascular and renal outcome trials ***(2).***

In contrast, sodium–glucose cotransporter-2 inhibitors (SGLT2i) have shown to offer kidney protection and are now included in recent KDIGO recommendations as preferred first line treatment in patients with type 2 diabetes mellitus (DM) and estimated glomerular-filtration rate (eGFR) ≥ 30 mL/min/1.73 $m^2$ ***(3).***

Steroidal mineralocorticoid receptor antagonists (MRAs) have proven to be effective in the management of primary aldosteronism due to bilateral adrenal hyperplasia or aldosterone-producing adrenal adenomas, in the treatment of resistant hypertension, as well as in the reduction of albuminuria in patients with diabetic and non-diabetic nephropathy (alone or on top of an RAS blocker) ***(4).***

Moreover, the accumulated clinical trial evidence supporting a significant reduction in mortality and CV events in patients with heart failure (HF) with reduced ejection fraction with MRAs changed the landscape of HF treatment, offering these drug agents a Class 1A recommendation. Subgroup and post hoc analyses of the latter studies showed improved clinical outcomes irrespective of baseline kidney function; however, patients with more advanced CKD stages (i.e., eGFR < 30 mL/min/1.73 $m^2$) had been excluded ***(5).***

The risk of hyperkalemia remains a major determinant of MRA use, especially for the older first- and second-generation steroidal agents spironolactone and eplerenone; an acute, usually reversible, decline in glomerular filtration observed among patients with low eGFR was another problem, particularly when these drugs were used in conjunction with an ACEi or an ARB ***(6).***

In recent years, novel nonsteroidal agents have been developed, i.e., finerenone, esaxerenone and apararenone, with the former having been more extensively studied in diabetic populations. Finerenone belongs to third-generation MRAs and has demonstrated a more potent and selective antagonism for mineralocorticoid receptors (MR) compared to spironolactone and eplerenone, with a balanced distribution between heart and kidney tissue and different agonistic effects with regards to coregulatory molecules, rendering it a more potent anti-inflammatory and anti-fibrotic agent ***(7).***

Large-scale clinical trials have recently provided evidence of improved renal and cardiovascular outcomes with addition of finerenone to the standard of care in patients with type 2 DM and moderately or severely increased albuminuria ***(7).***

***Pleiotropic Effects of Aldosterone in DKD:***

***Mineralocorticoid receptor activation:***

Aldosterone is a steroid hormone that is produced by the adrenal cortex and acts, together with other members of the steroid hormone family (cortisol and corticosterone), as a ligand of MRs. MRs are nuclear receptors, structurally similar to glucocorticoid receptors (GRs), that are expressed in epithelial and non-epithelial tissues, serving as transcription factors of target genes that regulate cellular processes ***(8).***

In epithelial cells of the distal nephron, where both MR and GR are expressed, aldosterone exerts its classical actions with regards to volume depletion and hyperkalemia by regulating sodium, chloride, and potassium handling, through transcription of the epithelial sodium channel (ENaC), Cl−/HCO3− exchangers and ROMK channels ***(9).***

Activation of MR in non-epithelial tissues, including cardiomyocytes, smooth muscle cells, fibroblasts and macrophages in heart tissue, and glomerular podocytes, monocytes and mesangial cells in the kidney tissue, targets expression of genes that are involved in tissue repair and results in excess inflammation and fibrosis. Activation of GRs modulates transcription of responsive genes related to energy homeostasis, response to stress and control of inflammation ***(10).***

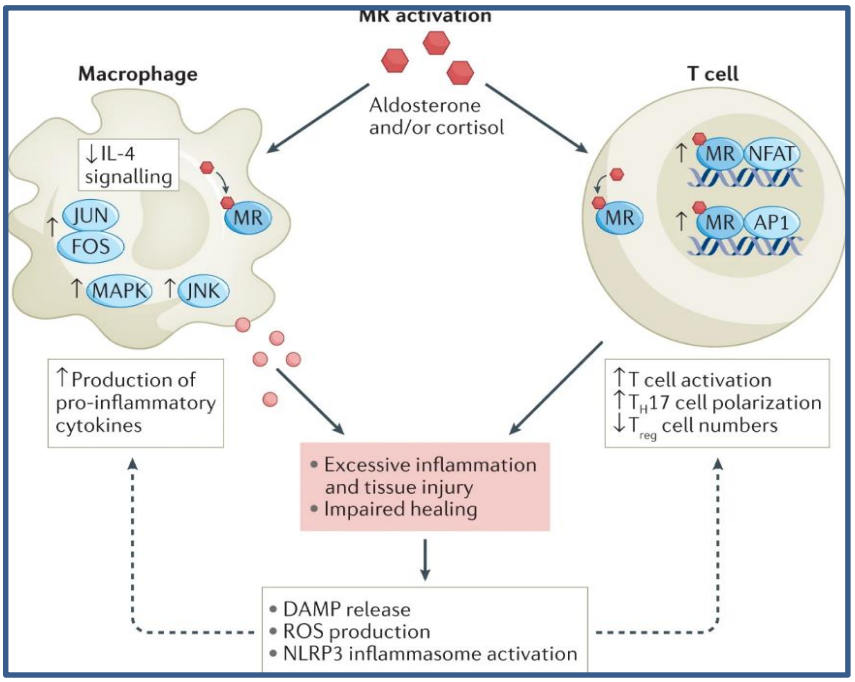


**Figure 1.** Aldosterone-mediated pro-inflammatory effects in macrophages and T cells ***(2).***

While only cortisol binds to the GR, the MR has multiple ligands with high affinity, including both cortisol and aldosterone. Despite the fact that cortisol reaches up to 1000-fold higher concentrations in several tissues, aldosterone is the primary physiological MR ligand in humans. Pre-receptor conversion of cortisol by 11-beta-hydroxysteroid-dehydrogenase-2 (11βHSD2) to inactive cortisone is a key mechanism for maintaining MR selectivity in target tissues and regulating distinct MR functions in the heart and kidneys ***(5).***

Differentiations in the concentrations of 11βHSD2 in each tissue represent the major modulator of this process, with the enzyme being abundantly expressed in distal tubular epithelial cells but not in cardiomyocytes, podocytes, and macrophages, where cortisol is the primary physiological ligand of MRs ***(11).***

During the previous decade, the previously called nongenomic effects of aldosterone were elucidated. The genomic response is the process that includes all the classical steps of cell membrane diffusion of aldosterone: binding to the MR in the cytoplasm, translocation to the nucleus and activation of gene transcription ***(12).***

This process results in an increase of ENaC concentration in epithelial cells (i.e., distal tubule, colon) and can be evidenced 30–60 min post aldosterone release/administration. In addition to genomic actions, rapid effects of aldosterone have been described that cannot be explained by the traditional pathway, nor be blocked by inhibition of the transcriptional process molecules such as actinomycin D and aldosterone blockers, now considered to be mediated by MRs ***(7).***

These rapid effects are associated with enhanced activity of the Na+-K+-2Cl− cotransporter and Na+-K+-ATPase in the heart, and of the Na+-H+ antiporter, the ENaC and Na+-K+-ATPase in the kidney and are connected to subcellular trafficking. In addition to the above, over the past years the crucial role of coregulatory molecules in mediating the genomic response to nuclear receptor activation has emerged ***(1).***

Accumulated evidence suggests that upon ligand binding, the transcription of effector proteins is modulated via recruitment of coactivator or corepressor proteins according to distinct MR conformations induced by binding of different agonist ligands. Ligand-selective peptides acting as potent antagonists of the MR-mediated transcription process were identified about a decade ago ***(6).***

***Potential Mechanisms of Aldosterone-Induced DKD:***

In 10–53% of patients that initiate an ACEi/ARB, plasma aldosterone levels tend to rise again 6–12 months later, leaving them exposed to the deleterious proinflammatory and profibrotic effects of aldosterone in the kidneys, heart, and vessels. It has been speculated that this phenomenon of “aldosterone breakthrough” may represent a major cause of accelerated GFR decline in patients with type 1 diabetes and DKD and a poorer antiproteinuric response despite use of a single RAS blockade ***(7).***

Inappropriate activation of MRs by aldosterone in podocytes, monocytes and mesangial cells in the kidney induces monocyte and macrophage infiltration and promotion of glomerulosclerosis and interstitial fibrosis. In the heart, overactivation of MRs promotes cardiac fibrosis, increased collagen synthesis and cardiac remodeling and hypertrophy ***(5).***

An additional negative inotropic effect of aldosterone, counteracting the positive inotropic effect of angiotensin-II, has also been described. Moreover, in the experimental model of streptozotocin-induced diabetes, a classical model of type 1 DM, overexpression of mRNA of MR, NADPH oxidase and collagen I/IV was described, resulting in collagen deposition in glomerular and tubulointerstitial areas in the kidney ***(2).***
Local production of aldosterone in mesangial cells induced by angiotensin-II, high glucose and LDL has also been associated with the pathogenesis of DKD through MR activation. Administration of spironolactone was shown to block MR overexpression and reduce collagen deposition in streptozotocin-induced diabetic rats ***(7).***
Similarly, administration of spironolactone, eplerenone and finerenone in classical experimental models of DKD due to type 2 DM led to amelioration of glomerulosclerosis and macrophage infiltration, prevention of podocyte injury and reduction of proteinuria and NGAL expression ***(6).***

The crucial role of GR in the progression of DKD has recently emerged. In an experimental model of streptozotocin-induced diabetes in podocyte GR knockout mice, the loss of podocyte GR resulted in enhanced Wnt signaling, higher expression of transforming growth factor-β and β-catenin and disturbed fatty acid metabolism, accompanied by histological evidence of worsened fibrosis, increased collagen deposition, as well as mesenchymal transition of the glomerular endothelium and glomerulomegaly ***(7).***
Similarly, the loss of the endothelial GR has been shown to induce upregulation of Wnt/β-catenin signaling and to promote angiogenesis and mesenchymal transition in tubular epithelial cells in diabetic experimental models. The above findings suggest the presence of a podocyte–endothelial cell crosstalk that is regulated by GR; this probably represents an additional mechanism in the development of diabetic nephropathy, on which more light should be shed in the future ***(1).***

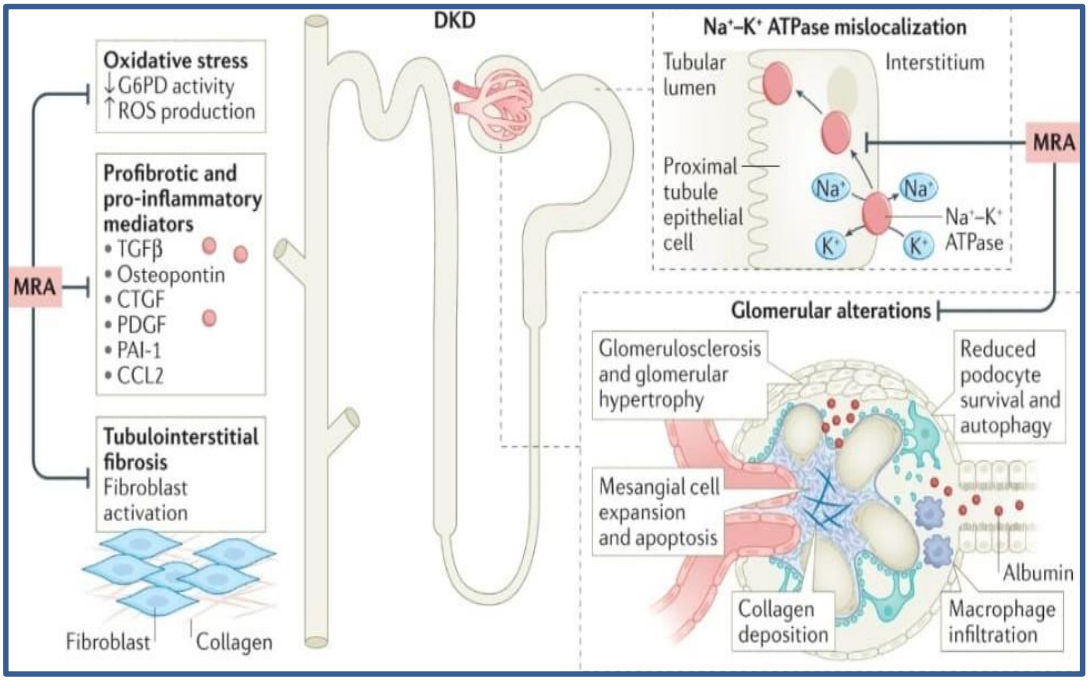


**Figure 2.** Aldosterone and DKD ***(2).***

***Mineralocorticoid Receptor Antagonism in DKD:*** After considerable preclinical data yielding information on the pivotal role of MR overactivation in the progression of kidney and cardiac injury were available, several clinical studies embarked to investigate the potential benefits from MR blockade in patients with diabetic and non-diabetic kidney disease ***(7).***
During the previous two decades, the non-selective MRA spironolactone and the selective eplerenone were extensively studied mainly for their anti-albuminuric effects. These older classical steroidal MRAs function as active competitors to

aldosterone for binding to the MR ligand-binding pocket, destabilizing the active conformation of the receptor ***(4).***

They prevent recruitment of transcriptional coactivators in the presence of aldosterone, but in the absence of aldosterone they exhibit partially agonistic coactivator recruitment properties. They both lack tissue and ligand specificity, while spironolactone additionally lacks receptor specificity ***(13).***

***Mineralocorticoid Receptor Antagonists:***

***Spironolactone:***

Spironolactone is a first-developed MRA, and its structure is based on progesterone, which has an antagonistic effect on MR. Spironolactone binds to MR at the same site of aldosterone. As spironolactone is not a selective MRA and has an agonistic effect on progesterone receptor as well as an antagonistic effect on androgen receptor, its use causes gynecomastia. The effects of spironolactone on DKD have been widely investigated ***(6).***

Spironolactone has been shown to attenuate aldosterone-induced apoptosis in cultured mesangial cells by activating the Wnt signaling pathway. Spironolactone has been shown to attenuate high-glucose-induced podocyte apoptosis. Consistent with this observation, spironolactone has been shown to prevent high-glucose-induced podocyte injury by reducing SGK1 and NADPH oxidase activity ***(3).***

***Eplerenone***:

Eplerenone is a steroidal MRA that has a higher selectivity for MRAs than spironolactone. The beneficial effects of eplerenone have been extensively investigated in patients with hypertension and heart failure. In addition, eplerenone has been shown to reduce morbidity and mortality in patients with left ventricular dysfunction after myocardial infarction via its favorable effects on fibrosis and cardiovascular remodeling ***(2).***

The administration of eplerenone has been shown to attenuate renal fibrosis by inhibiting the TGF-β and collagen IV expression in hypertensive diabetic rats. Many studies demonstrated that eplerenone reduced proteinuria by preventing podocyte injury and glomerulosclerosis in Dahl salt-hypertensive rats ***(1).***

The inhibition of oxidative stress by eplerenone appears to be involved in this observation, as reported by several authors that eplerenone attenuates proteinuria in uninephrectomized rats with aldosterone infusion by downregulating SGK1 and subsequently increasing the NADPH oxidase activity in podocytes ***(7).***

***Finerenone***:

Steroid hormone receptors, including MR, have been shown to interact with cofactors that affect gene transcription, and steroidal MRAs can interact with cofactors, leading to their functioning as partial MR agonists. Therefore, the actions of nonsteroidal MRAs differ from those of steroidal MRAs, such as spironolactone and eplerenone, as nonsteroidal MRA blocks the MR as a bulky and passive antagonist ***(7).***

Finerenone has been developed as a potent and selective nonsteroidal MRA. Finerenone inhibits cofactor recruitment to the MR in the absence of aldosterone and functions as an inverse agonist. In addition, the gene regulation profile by finerenone differs from that for steroidal MRAs. Finerenone has more potent antifibrotic activity than eplerenone. For instance, differential MR cofactor modulation is proposed to be associated with finerenone-specific amelioration of tenascin X (TNX), an MR target gene that is a crucial regulator of fibrosis ***(4).***

In addition, finerenone is expected to carry a lower risk of hyperkalemia than steroidal MRAs. One possible mechanism involves its tissue distribution. A study using [14C]-labelled finerenone demonstrated a balanced kidney-heart distribution, although spironolactone and eplerenone showed a dominant distribution in the kidney compared with the heart ***(5).***

These differences may affect the sodium and potassium balance. The pharmacokinetics of finerenone are also different from those of spironolactone and eplerenone, as finerenone has no active metabolites and a short half-life (2 h). In contrast, spironolactone is a prodrug that has multiple active metabolites with long half-lives (14–16 h), and eplerenone has no active metabolites with a half-life of 4–6 h ***(2***

***Canrenone***:

Canrenone, an active metabolite of spironolactone, was expected to have fewer side effects than spironolactone by preventing the formation of intermediate products with antiandrogenic and progestational actions. It was approved for clinical use in Europe; however, its use was limited by significant hyperkalemia associated with lack of receptor-specific selectivity, similar to a first-generation MRA ***(6).***

***Esaxerenone***:

Esaxerenone was developed as a potent and selective non-steroidal MRA. Esaxerenone is

effective and well-tolerated with a BP-lowering activity equivalent to or better than that of eplerenone. Esaxerenone has been shown to bind to MR through the MR ligand binding domain and large side chains, thereby demonstrating a high affinity and selectivity for MR. In addition, it has long a half-life of 18.6–25.1 h ***(14).***

***Apararenone***:
Apararenone is another compound belonging in the nonsteroidal family, exhibiting highly selective MRA activity ***(1).***

***Future Directions and Perspectives:***
Overall, many studies provide strong evidence of the beneficial effects of MRAs towards reduction of albuminuria and protection against CKD and CVD progression in patients with DKD. The landscape of treatment of this disease is expected to change in the near future; the use of finerenone will probably be incorporated in forthcoming guidelines and consensus statements of major organizations as first-line treatment for DKD along with SGLT2is to maximize cardio- and nephroprotection ***(15).***

Further controlled trials with cardiovascular and renal outcomes are necessary to shed more light on the presence or absence of similar beneficial effects of the other two nonsteroidal agents, esaxerenone and apararenone. Even with widespread use of SGLT2 inhibitors and GLP-1 receptor agonists, a substantial residual risk of DKD progression remains. Nonsteroidal MRAs potentially complement this risk ***(13).***

However, some clinical questions need to be addressed. First, it needs to be clarified at which stage of DKD MRA should be started. Second, it remains unclear whether monotherapy of nonsteroidal MRAs is effective on DKD ***(5).***

Third, it will be necessary to elucidate what kind of anti-diabetic drugs and nonsteroidal MRAs are effective in combination. To clarify these points will determine the position of nonsteroidal MRAs in DKD treatment. Currently, the development of novel therapeutic agents for DKD that target inflammation and fibrosis is in progress ***(7).***

Among them, JAK/STAT inhibitors have been shown to exert renoprotective effects in patients with DKD. It is important that finerenone have both renal and cardiovascular protective effects clinically that have not yet been clarified with these developing drugs. Combined effect of these drugs and nonsteroidal MRA can also be expected ***(14).***
Unlike SGLT2 inhibitors and incretin-based drugs, the dose of nonsteroidal MRAs can be adjusted for renoprotection without consider in an advantage-lowering effects in patients with DKD, which may be advantage of nonsteroidal MRAs. Furthermore, expectations for nonsteroidal MRAs go beyond cardiorenal protection ***(15).***

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