Consensus Document

Endothelial factors in the pathogenesis and treatment of chronic kidney disease Part II: Role in disease conditions: a joint consensus statement from the European Society of Hypertension Working Group on Endothelin and Endothelial Factors and the Japanese Society of Hypertension

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After examining in Part I the general mechanisms of endothelial cell injury in the kidney, the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension and the Japanese Society of Hypertension will herein review current knowledge on the role of endothelial dysfunction in multiple disease conditions that affect the kidney, including diabetes mellitus, preeclampsia, solid organ transplantation, hyperhomocysteinemia and antiangiogenic therapy in cancer. The few available randomized controlled clinical trials specifically designed to evaluate strategies for correcting endothelial dysfunction in patients with hypertension and/or chronic kidney disease are also discussed alongside their cardiovascular and renal outcomes.

Keywords: cancer, diabetes mellitus, endothelin, endothelium, hyperhomocysteinemia, hypertension, kidney, kidney transplantation, preeclampsia

Abbreviations: ACE, angiotensin I converting enzyme; ADMA, asymmetric dimethylarginine; AH, arterial hypertension; Ang II, angiotensin II; ARB, angiotensin AT_1 receptor blocker; BP, blood pressure; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; eNOS, endothelial nitric oxide synthase; ERA, endothelin receptor antagonist; ESRD, end-stage renal disease; ET-1, endothelin-1; ETA, endothelin type A receptor; GFR, glomerular filtration rate; MTHFR, methylene-tetra-hydro-folate reductase; NO, nitric oxide; NOS, nitric oxide synthase; Nox, NADPH oxidase; RAAS, renin–angiotensin–aldosterone system; RCT, randomized controlled clinical trial; ROS, reactive oxygen species; sFlt-1, soluble fms-like tyrosine kinase 1; VEGF, vascular endothelial growth factor

INTRODUCTION

Impaired endothelium-dependent vasodilation, a hallmark of arterial hypertension (AH) and many other cardiovascular disease risk factors and disease conditions, can be an early mechanism leading to cardiovascular damage or, alternatively, a marker of it. Endothelial dysfunction in glomeruli and peritubular vessels affects filtration fraction, resulting in a progressive reduction in the glomerular filtration rate (GFR), extracellular fluid volume expansion, abnormal ion balance and renal hypoxia, all of which ultimately contribute to the age-dependent renal function loss in the hypertensive population and can lead to chronic kidney disease (CKD) [1]. Proteinuria, a marker

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of renal microvasculature damage, is invariably associated with systemic endothelial dysfunction in hypertension, suggesting a general involvement of the endothelium [2].

After examining in Part I the general mechanisms underlying endothelial dysfunction in the kidney, using the same methodology, the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension in conjunction with the Japanese Society of Hypertension will herein review current knowledge on the role of endothelial dysfunction in conditions in which the renal vasculature is deeply affected, such as diabetes mellitus, preeclampsia, kidney transplantation, hyperhomocysteinemia and cancer. The few randomized controlled clinical trials (RCTs) that explored the concept that strategies aimed at correcting endothelial dysfunction in patients with hypertension and/or CKD are also discussed alongside their cardiovascular and renal outcomes.

DIABETIC NEPHROPATHY

Diabetic nephropathy, one of the main preventable causes of reno-parenchymal hypertension and CKD, is characterized by focal and segmental glomerulosclerosis [3], which involves multiple factors including progressive podocyte injury, glomerular fibrosis and loss of glomerular filtration function [4,5], leading to proteinuria, and ultimately to the need for renal replacement therapy [4] (Fig. 1). Proteinuric CKD, and particularly end-stage renal disease (ESRD), not only aggravates hypertension and cardiovascular risk [6], but also poses an economic rapidly growing burden to society [4], making prevention of this disease a critical task for the future.

The pathogenesis of diabetic nephropathy not only involves hyperglycemia and inflammation, but also endothelial and nonendothelial pathways [7,8], including enhanced oxidative stress, renin–angiotensin–aldosterone system (RAAS) and endothelin-1 (ET-1) activation, and inflammatory processes that were discussed in Part I [4].

Obesity, which is frequently associated with insulin resistance and/or diabetes, also leads to focal and segmental glomerulosclerosis, a condition termed 'obesity nephropathy'. Of note, practically all of the aforementioned endothelial mediators of CKD are also implicated in the pathogenesis of diabetic nephropathy [9], as they were found to promote and maintain podocyte injury and glomerular and vascular inflammation [6,10]. Hence, not unsurprisingly, as hypertension is common among these patients, antihypertensive medications targeting endothelial pathways, such as angiotensin I converting enzyme (ACE) inhibitors, angiotensin AT_1 receptor blockers (ARBs) or mineralocorticoid receptor antagonists, have been investigated in diabetic nephropathy. These agents were shown to improve clinical outcome with benefit that exceeded that attributable to changes in blood pressure (BP) [7,11–14]. Of note, the protective effects of ACE inhibitors and ARBs were particularly marked in obesity nephropathy [15] and predictably found to be largely BP-independent [11,13,14], consistent with suggesting their direct renoprotective effects [7].

It has been known for some time that kidney transplantation not only normalizes BP but also reverses hypertensive damage in the heart and retinal arteries of patients with proteinuric renal disease and hypertension [16], indicating the reversibility of end-organ injury. Similarly, regression or partial disease remission was suggested

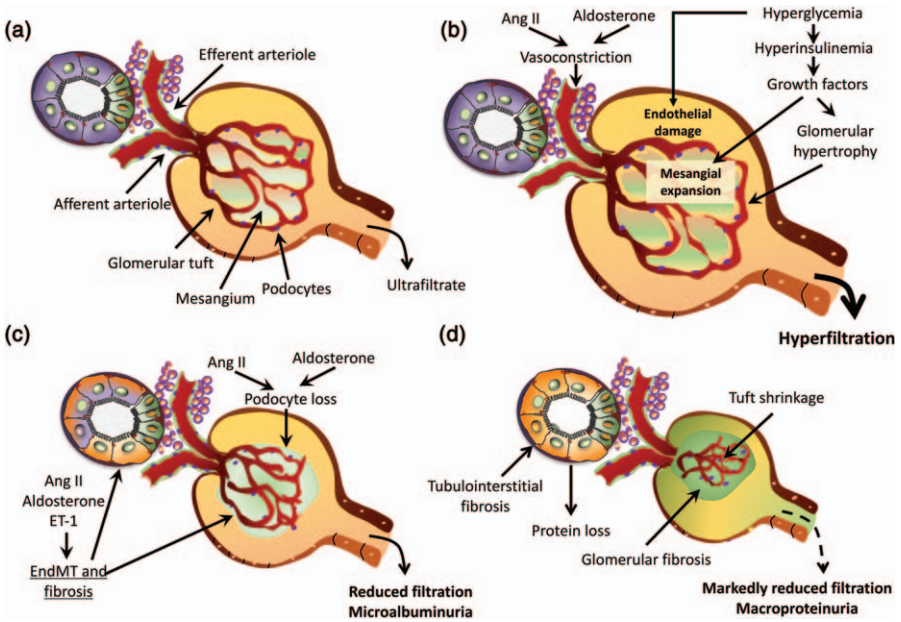

FIGURE 1 Stages of diabetic nephropathy. (a) Structure of glomerulus and tubulus under normal conditions. (b) At an early stage, the high glucose levels cause endothelial injury and, via hyperinsulinemia and release of growth factors, also mesangial expansion and glomerular hypertrophy. Vasoconstriction of the efferent arteriole, mostly induced by angiotensin II and aldosterone, causes hyperfiltration. (c) With time, angiotensin II, aldosterone and endothelin-1 further worsen the endothelial function and induce podocyte loss, causing microalbuminuria and decreased filtration. They also induce endothelial-to-mesenchymal transition and fibrosis. (d) Glomerular and tubulointerstitial fibrosis, by provoking glomerular shrinkage and preventing comunications between endothelial and tubular cells, cause further reduction of glomerular filtration and protein loss. Normal tubular epithelial cells are visualized in violet. Cells undergoing the epithelial to mesenchymal phenotype shift are depicted in orangish-violet, whereas cells entirely transformed into mesenchymal cells and, therefore, able to produce collagen, are visualized in orange.

to occur in patients with diabetic nephropathy treated with ARBs or ACE inhibitors, in whom proteinuria decreased [7]. Reversal of diabetic or nondiabetic focal and segmental glomerulosclerosis and/or proteinuria has also been observed in studies with endothelin receptor antagonists, both experimentally and clinically [17–19]. Currently, a novel approach to interfere with progression of diabetic nephropathy under investigation entails the pharmacological inhibition or downregulation of reactive oxygen species (ROS) generating NADPH oxidases enzymes [20,21] (https://clinicaltrials.gov/ct2/show/NCT02010242).

Statins have also been shown to inhibit inflammatory activation in both endothelial cells and the vasculature [8], but so far the clinical trials conducted in patients with diabetic nephropathy have failed to prove their beneficial effect on the natural history of the disease [6].

Finally, strategies employing preventive measures, such as weight loss/bariatric treatment of obesity [8], lifestyle changes to improve and maintain cardiorespiratory fitness or pharmacological interventions, such as antidiabetic therapy or those targeting the aforementioned endothelial mediators can conceivably reduce BP, delay vascular and renal aging, and thus contribute to an improved overall cardiovascular outcome [8,22–30].

PREECLAMPSIA-ASSOCIATED KIDNEY INJURY

Preeclampsia is the most frequent (prevalence about 3–8%) serious medical complication of pregnancy. It develops through two stages [31,32]: in stage 1, aberrant shallow cytotrophoblast invasion in the maternal spiral arteries supplying the placenta results in poor placentation; in stage 2, this leads to repeated periods of placental hypoxia and reperfusion injury, resulting in oxidative stress and an increased production of placental factors (Fig. 2). Among the latter, soluble fms-like tyrosine kinase 1 (sFlt-1), a splice variant of the membrane-bound vascular endothelial growth factor (VEGF) type 1 receptor originating from placental syncytiotrophoblast, has been widely studied. Others include soluble endoglin, agonistic autoantibodies to the AT_1 receptor, and inflammatory cytokines [33,34]. All these factors, likely in combination with an altered immune system in preeclampsia [35,36], are thought to contribute to generalized endothelial dysfunction, although their precise roles remain unclear. For example, in preeclamptic women, both circulating sFlt-1 and ET-1 levels rise progressively in relation to the severity of preeclampsia [37,38]. These factors affect not only growth and development of the placenta and the fetus, but also the health of endothelial cells and kidney function, including the maintenance of the glomerular filtration barrier [39]. Moreover, elevated sFlt-1 levels, by binding both free VEGF and placental growth factor, disturb the balance between proangiogenic and antiangiogenic factors. Hence, these mechanisms may contribute to hypertension and renal damage in preeclampsia [35].

Accordingly, as discussed below, treatment of cancer patients by blocking angiogenesis via VEGF inhibition (with tyrosine kinase inhibitors, and direct VEGF inhibition or inactivation) resulted in a preeclampsia-like syndrome, featuring hypertension, proteinuria and glomerular endotheliosis [40]. Moreover, VEGF inhibitor-treated cancer patients, like preeclamptic women, display high ET-1 levels, which correlated closely with the degree of VEGF inhibition, as estimated by either the serum sFlt-1 levels or the VEGF inhibitor dose [37]. Multiple regression analysis has pointed to a role for ET-1 as an independent determinant not only of the BP rise and proteinuria, but also of renin suppression in preeclamptic women [37]. Therefore, ET-1 activation seems to be involved in causing both the clinical manifestations of preeclampsia, and the well known paradoxical suppression of renin in this disease. ET-1 additionally acts as an aldosterone secretagogue via endothelin type B (ET_B) receptors [41], whereas autoantibodies to AT_1 receptors, may have similar effects. Obviously, these antibodies should also suppress renin release via AT_1 receptor activation. Consequently, theoretically preeclamptic women would be expected to display an increased aldosterone/renin ratio, due to the opposite effects of

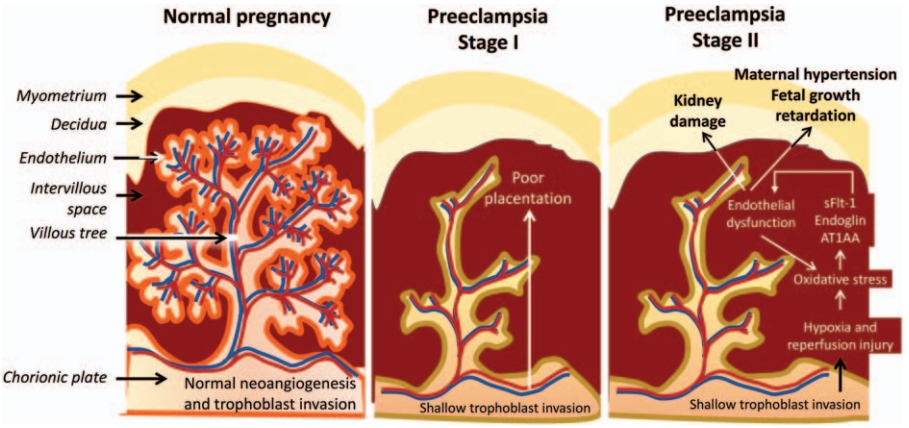

FIGURE 2 Endothelial dysfunction and abnormal placentation in preeclampsia. During normal placentation, cytotrophoblast cells invade the maternal decidual arteries, remodeling them into high-capacitance vessels that supply the placenta and fetus with maternal oxygen and nutrients. In preeclampsia, this process is aberrant. The invasion of the trophoblasts is incomplete, with cytotrophoblast cells only in the superficial layers of the decidua, and the spiral arteries maintain features of high-resistance vessels (stage 1). Shallow trophoblasts invasion leads to placental hypoxia and reperfusion injury, with increased oxidative stress and production of placental factors, mainly soluble fms-like tyrosine kinase 1 (stage II). Soluble fms-like tyrosine kinase 1, combined to soluble endoglin, agonistic autoantibodies to the AT_1 receptor and inflammatory cytokines, causes endothelial dysfunction, finally leading to arterial hypertension and kidney damage in the mother and growth retardation in the fetus.

ET-1 and autoantibodies on renin and aldosterone, but, in contrast with this prediction, no such increase was observed [37], suggesting that multiple complex mechanisms modulate the effects of ET_B receptors and autoantibodies *in vivo*. A possible explanation is that the VEGF-induced increase in adrenal capillary density, which in normal pregnancy upregulates aldosterone production, is disturbed in preeclampsia because sFlt-1 blocks such effects of VEGF [42].

Animal models of preeclampsia support the importance of sFlt-1/ET-1 upregulation in that ET-1 receptor blockade alleviates preeclampsia symptoms, in keeping with the effects seen with these agents in VEGF inhibitor-induced hypertension [37,43,44]. The cause of the rise of ET-1 in preeclampsia remains to be determined. Although acute VEGF inhibition in human umbilical vein endothelial cells did not affect ET-1 release [45], soluble endoglin has been suggested to induce endothelial ET-1 production [46]. If confirmed, this could lead to the development of entirely new treatment targets. Endothelin receptor antagonism, having been linked to teratogenicity, might be feasible only at a late pregnancy stage, when all organs have been formed.

KIDNEY TRANSPLANTATION AND TRANSPLANT-ASSOCIATED HYPERTENSION

The global burden of ESRD patients needing renal replacement therapy and transplantation is continuing to rise. This poses a huge financial burden to healthcare systems [47], which will continue to rise with improved survival from cardiovascular disease in these patients. Endothelial dysfunction is held to be central to both CKD development and the cardiovascular continuum as discussed in Part I [48]. Renal transplanted patients are no exception to this, which is clearly understandable given that circulating immune response cells find the endothelium as the first barrier to their attack on the transplanted organ. Although advances in the treatment of acute rejection and short-term graft survival now allow a 90% survival at 1 year [49], long-term success has been more difficult to achieve. Oxidative stress is enhanced in recipients of kidney transplants and starts before transplantation while these patients develop ESRD. It is then aggravated during the ischemia-reperfusion occurring during the grafting, and then further exacerbated as a consequence of long-term immunosuppressant treatment, more with cyclosporine, which causes hypertension and renal damage, than with the newer immunosuppressive drugs like tacrolimus [50]. Moreover, several factors involved in the impaired vasorelaxation of transplanted patients, including ET-1 (see below), asymmetric dimethylarginine (ADMA) and fibroblast growth factor 23 (FGF23) (see section on vitamin D), are held to contribute to renal damage [51,52].

After hepatic, cardiac and renal transplantation, circulating levels of ET-1 increase [53–56], indicating systemic activation of the ET system and decreased ET_B receptor-mediated ET-1 clearance. Accordingly, impaired endothelial cell function associated with ET-1 activation has been observed in human allograft recipients [57]. Cyclosporine is not only a potent stimulus for ET-1 production [58], but also an inhibitor of the L-arginine/nitric oxide (NO) pathway [59]; thereby, it contributes to post-transplant hypertension [60]. Moreover, endothelin type A (ET_A) receptor expression increases in renal allografts [61] and endothelin receptor antagonist (ERA) treatment effectively suppresses fibrotic and proliferative responses in several allografts [62–70]. Accordingly, selective ET_A [71,72], but not mixed ET_A/ET_B blockade [73], largely prevents chronic rejection and renal allograft injury, even in the absence of continued immunosuppression [71] through mechanisms not improved by ARB treatment [74], suggesting that ERA have pronounced and independent immunomodulatory effects in the transplant recipient [75]. However, findings were mostly obtained in experimental models [62–75], whereas RCTs [53–61] were too small to recommend a specific class of drugs to transplant-associated hypertension.

ANGIOGENESIS ANTAGONISTS AND THE RENAL ENDOTHELIN SYSTEM

Angiogenesis is a key process for tumor growth and metastatic spread. This has led to the development and introduction in the clinic of a large number of agents (such as anti-VEGF antibodies and small, orally active receptor tyrosine kinase inhibitors that block the VEGF signaling pathway) aimed at blunting the actions of VEGF. The latter regulates angiogenesis through endothelial cell proliferation and can play an important role in capillary repair in damaged glomeruli [76]. Common adverse effects of these agents are hypertension and kidney injury, which resemble the manifestations of preeclampsia, in which the release of sFlt-1 in the bloodstream, by sequestering VEGF and placenta growth factor, is held to produce an anti-angiogenic state [77]. As in preeclampsia, activation of the ET system occurs in cancer patients treated with the receptor tyrosine kinase inhibitors sunitinib and regorafenib [45,78]. In pregnant rats, the anti-angiogenic factor sFlt-1 causes a rise in BP and expression of the *prepro-ET-1* gene in the renal cortex [79]. Moreover, over expression of sFlt-1 in mice also increases expression of the genes encoding for ET-1 and the ET_A receptor, effects that are amplified in endothelial NO synthase (eNOS)-deficient mice [80]. Thus, inhibition of angiogenesis leads to ET-1 activation, particularly when NO bioactivity is blunted. Accordingly, sunitinib dose-dependently increased plasma ET-1 levels in rats, but unexpectedly did not increase the urinary excretion of ET-1, a marker of renal ET-1 production [81]. Moreover, opposite to what is seen with sFlt-1 administration in pregnant rats, expression of the prepro-ET-1 and endothelin converting enzyme genes were not increased. Notwithstanding this, the non-selective ERA macitentan prevented the rise in BP and proteinuria in sunitinib-exposed rats [82], whereas amlodipine, which similarly lowered BP, did not affect proteinuria [82].

In summary, even though the effects of sFlt-1 and sunitinib on renal expression of the *prepro-ET-1* gene may not be uniform, it can be concluded that antiangiogenic treatment activates the ET-system and that data with ERA treatment support a role of the ET-system in

the development of proteinuria following antiangiogenic treatment.

HYPERHOMOCYSTEINEMIA, ENDOTHELIAL DYSFUNCTION AND RENAL DAMAGE

Hyperhomocysteinemia defined as a total plasma homocysteinemia more than 15 μmol/l, affects 5–7% of the general population and 20–40% of those with coronary atherosclerosis [83,84]. It usually derives from a gene-environment interaction involving a low folate intake and the presence of variants of the methylene-tetrahydro-folatereductase (*MTHFR*) gene (ID 4524), particularly in elderly people and in the presence of reduced GFR [85,86]. Two non-synonymous single nucleotide polymorphisms for the *MTHFR* gene have been described: C677T in exon 4 (Ala222Val) that results in a MTHFR variant with decreased stability to temperature (thermolabile variant), and an A1298C (Glu429Ala) in exon 7 that impairs MTHFR activity, albeit to a lesser extent than C677T [87]. These variants, in the presence of a low-folate supply, lead to hyperhomocysteinemia, which detrimentally affects the endothelium by inducing oxidative stress, with ensuing decreased NO production and NO bioactivity [88], and also accumulation of the endogenous NOS inhibitor ADMA [89].

Declining renal function, alongside aging and left ventricular systolic dysfunction, are recognized factors associated with hyperhomocysteinemia. ESRD patients are expected to develop hyperhomocysteinemia, as the kidney is the major site of homocysteine metabolism, and, moreover, dialysis is associated with loss of water-soluble B vitamins, which are key for maintaining normal plasma homocysteine levels [90]. Hyperhomocysteinemia induces severe oxidative stress, thus leading to oxidation of free or protein-bound thiols and aggravation of endothelial dysfunction [90,91]. Accordingly, the *MTHFR* gene variants have been linked to kidney damage: the C677T variant was found to be associated with CKD in both the cross-sectional Japan Multi-institutional Collaborative Cohort Study [92] and with mortality risk in ESRD patients of the Homocysteinemia in Kidney and End Stage Renal Disease-DNA study [93]. An association was also found between the decline of GFR and A1298C variant in the longitudinal African-Americans Study of Kidney Disease and Hypertension Trial [94].

A recent Cochrane analysis of six studies on the effects of folic acid or vitamins B6 and vitamin B12 in ESRD patients, however, failed to show a decrease in cardiovascular events and/or death, leading the contention that homocysteine-lowering therapies should not be used for cardiovascular risk reduction [95]. This conclusion, however, cannot be taken for granted for the following reason. As there is a linear relationship between plasma homocysteine and risk of cardiovascular events over the entire range of plasma homocysteine values, a benefit from lowering homocysteine may be expected only in those who have overt hyperhomocysteinemia before they develop ESRD [84] and not in the population at large in which the beneficial effect occurring in a subset of the patients can be markedly diluted. In keeping with this prediction, a recent large study in patients with mild-to-moderate CKD found that the combined treatment with enalapril and folic acid supplementation delayed the progression of CKD, as compared with enalapril alone [96]. The concept that lowering plasma hyperhomocysteinemia is beneficial is further supported by evidence that folic acid supplementation protected from cardiovascular diseases patients with low folic acid levels and without preexisting cardiovascular disease [97].

ENDOTHELIAL FACTORS, KIDNEY PROTECTION AND DISEASE PREVENTION

In this era of evidence-based medicine, RCTs are the basis for high level evidence and Class I recommendations. Whether specifically targeting endothelial dysfunction may ultimately improve cardiovascular and renal outcomes in hypertension and CKD patients remains to be demonstrated in adequately designed, long-term RCTs. As a proof-of-principle, a study that used a remote ischemic preconditioning strategy to improve endothelial function reported prevention of acute kidney injury in high-risk patients undergoing cardiac surgery (mean eGFR 56 ml/min per 1.73 m^2) [98], thus supporting the contention that endothelial factors may be targets for kidney protection in humans [99].

We suggest that a holistic approach to treat hypertension, dyslipidemia and diabetes mellitus (with/without diabetic nephropathy) should be the optimal strategy to prevent CKD, or at least retard its progression. However, studies involving specific targets suggests the possibility of achieving additional benefits. For example, in four small trials, the addition of spironolactone for 60 days, on top of RAAS inhibition, reduced microalbuminuria, and decreased BP and eGFR, without altering plasma biomarker concentrations of endothelial dysfunction [100]. Aldosterone breakthrough may occur in patients with diabetic nephropathy treated with an ACE inhibitor or an ARB, likely because the secretion of aldosterone is controlled by multiple mechanisms, besides Ang II [101,102]. Notably, aldosterone breakthrough has been associated with worsening of microalbuminuria [103], which might explain why low-dose spironolactone (25 mg/day) on top of standard antihypertensive treatment reduced microalbuminuria despite no significant BP changes [104]. Moreover, spironolactone induced a sustained antiproteinuric effect when added on top of an ACE inhibitor or an ARB in a longer (1-year) study [103]. Similarly, in a larger RCT, eplerenone (50–200 mg/day) was more effective in reducing microalbuminuria than amlodipine in spite of similar lowering BP and pulse pressure in patients with systolic hypertension [105].

In CKD patients, the use of mineralocorticoid receptor antagonists, especially if combined with ACE inhibitors and/or ARBs, has been limited by the fear of hyperkalemia [106,107]. However, serious hyperkalemia developed in less than 1% of the patients with nondiabetic I–III CKD stages (eGFR 30–89 ml/min per 1.73 m^2) receiving 25 mg spironolactone on top of ARB or ACE inhibition, whereas a

significant decrease in urinary albumin excretion greater or equal to 50% was observed in 35% of the patients [108]. Nonetheless, even though in most cases mineralocorticoid receptor antagonists are effective and well tolerated [109], caution should be exercised in prescribing these agents to CKD patients with an eGFR less than 45 ml/min per 1.73 m^2 and serum K^+ levels more than 4.5 mmol/l on appropriately dosed diuretic treatment, as these features predict the development of hyperkalemia.

Several small-sized short-term RCTs were performed to target the molecular pathways involved in endothelial dysfunction in CKD and hypertension [110–114]. In an attempt to improve endothelial function using antioxidant therapy, a double-blind pilot RCT was performed in nine CKD patients with stable chronic heart failure, treated with placebo or *N*-acetylcysteine (500 mg orally twice daily) for 28 days followed by a wash-out period (>7 days) and cross-over to the other treatment. This study showed that *N*-acetylcysteine therapy was associated with improved forearm blood flow after ischemia caused by supra-systolic pressure of 200 mmHg [110].

Cilostazol, a phosphodiesterase inhibitor with antiplatelet/antithrombotic effects, used in chronic peripheral arterial disease, induces vasodilatation and inhibits vascular smooth muscle cells proliferation [111]. In a small single-blinded study, patients with peripheral arterial disease and diabetic nephropathy [baseline eGFR of 73 (placebo)–77 (cilostazol) ml/min per 1.73 m^2] were randomized to oral cilostazol (100 mg b.i.d.) or placebo for 1 year. Microalbuminuria and albumin–creatinine ratio were significantly reduced in the cilostazol group as compared with the placebo group, alongside a decrease in the plasma concentration of endothelial (leukocyte adhesion molecules) markers E-selectin and vascular cell adhesion molecule-1 (VCAM-1), but no changes in BP or eGFR [111].

Phosphodiesterase type 5 (PDE5), expressed in endothelial, glomerular, mesangial, cortical tubular and inner medullary collecting duct cells, degrades cyclic guanosine monophosphate, and experimental data suggest that PDE5 inhibitors can be useful in preventing CKD. Sildenafil, one PDE5 inhibitor, prevented glomerular hypertension and hyperfiltration in rats with subtotal nephrectomy [115,116] and reduced protein excretion in streptozotocin-induced diabetes [117]; it also improved flow-mediated vasodilation in diabetic men [118,119]. Vardenafil, another PDE5 inhibitor, also reduced proteinuria in rat streptozocin-induced type 1 diabetes mellitus, and restored nephrin and podocin expression in podocytes [120]. Whether these agents help in maintaining the kidney function in patients remains to be tested in long-term RCTs.

ADMA is a by-product of the methylation of arginine residues, which acts as a competitive inhibitor of L-arginine to reduce NO production, and also causes decoupling of eNOS leading to ROS production instead of NO [121]. The circulating levels of ADMA are increased in CKD in proportion to the severity of renal impairment and predict cardiovascular outcomes [122]. Oxidative stress also increases ADMA concentration by upregulating the synthetic enzyme protein arginine methyltransferase-1 and downregulating dimethylarginine dimethylaminohydrolase, the enzyme degradating ADMA [123]. Vitamin E supplementation and (transiently) intravenous ascorbic acid level reduce ADMA levels in patients with CKD [124], suggesting that this may represent an important mechanism by which antioxidants exert a beneficial cardiovascular effect.

High uric acid levels promote oxidative stress and might induce endothelial dysfunction. However, few studies that investigated if allopurinol could restore endothelial function in hyperuricemic patients, have given quite heterogeneous results, with some suggesting a benefit and others no effect [112,125,126].

VITAMIN D, ENDOTHELIAL FUNCTION AND KIDNEY PROTECTION

Endothelial cells not only express the vitamin D receptor, but also respond to calcitriol, the active form of vitamin D. A placebo-controlled RCT investigated the effect of a 12-week treatment with calcitriol on endothelial function in patients with stage 3–4 CKD [127].

The vitamin D receptor activator paricalcitol, given at a dose (2 μg/day) that did not affect endothelium-independent vasodilation and BP, improved endothelium-dependent vasodilation, and slightly lowered eGFR [−3.2 ml/min per 1.73 m^2 (−4.9 to −1.4), $P < 0.001$], two beneficial effects that disappeared after drug withdrawal [127]. Significantly, the beneficial effect of paricalcitol was maximal in patients with no or minimal changes in serum phosphate levels and was abolished in patients with hyperphosphatemia. Hence, the endothelium-protective effect of vitamin D receptor activation might be potentiated by phosphate lowering interventions [113].

Another RCT using placebo, 1 or 2 μg of paricalcitol daily for 3 months in 36 nondiabetic CKD patients (mean eGFR 40 ml/min per 1.73 m^2) reported a decline in endothelial function, which occurred in the group receiving the highest dose of paricalcitol, with no changes in BP, eGFR and microalbuminuria [128]. By contrast, in a double-blind, placebo-controlled RCT conducted in patients with type 2 diabetes and stage 3 or 4 CKD, paricalcitol 1 μg daily had no effect on endothelial function, measured by brachial artery flow-mediated dilation, or plasma biomarkers of inflammation and oxidative stress. A smaller RCT performed with oral ergocalciferol, or placebo, over 6 months, in patients with non-diabetic CKD stage 3–4 and concomitant vitamin D deficiency, showed that a high-dose ergocalciferol therapy improved microcirculatory function and reduced oxidative stress, without altering BP, eGFR or albuminuria [129].

In a small RCT, phosphate-lowering treatment with sevelamer improved flow-mediated vasodilatation and FGF23 levels, alongside flow-mediated vasodilatation [130]. FGF23 is a hormone-regulating serum phosphate and vitamin D, whose plasma levels are markedly elevated in patients with CKD [130]. The findings of the RCT, along with the evidence that FGF23 impairs vasorelaxation by decreasing NO bioavailability [131], suggest that FGF-23 contributes to vascular dysfunction in patients with stage 4 CKD and, therefore could be a target for pharmacologic intervention. However, larger and longer intervention studies are necessary to determine whether there is a protective effect of vitamin D and/or other factors affecting calcium/phosphate metabolism on the kidney.

CONCLUSION AND RECOMMENDATIONS

Diabetes mellitus, preeclampsia, solid organ transplantation, hyperhomocysteinemia and antiangiogenic therapy in cancer are all factors that deeply affect endothelial function favoring the development of kidney damage and amplifying injury primarily induced by metabolic abnormalities. As in all diseases, a better understanding of the underlying mechanisms will improve prevention and treatment of kidney disease. However, so far translation of new generated knowledge into clinical practice has been slow with current pharmacologic tools, likely because of the difficulty of disentangling the relative role of each putative pathogenic factor. Hence, further specific research is needed particularly in the field of RCTs focused at testing strategies for preserving endothelial function and GFR. To this end, this working group welcomes and supports the planning of integrated research efforts from all investigators who share an interest for the endothelium and preservation of renal health.

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Conflicts of interest

M.B. serves as a consultant to AbbVie, Inc. The other authors have no financial disclosures and competing interest statement to declare.

REFERENCES

1. Ponnuchamy B, Khalil RA. Cellular mediators of renal vascular dysfunction in hypertension. *Am J Physiol Regul Integr Comp Physiol* 2009; 296:R1001–R1018.
2. Perticone F, Maio R, Tripepi G, Zoccali C. Endothelial dysfunction and mild renal insufficiency in essential hypertension. *Circulation* 2004; 110:821–825.
3. Preston RA, Singer I, Epstein M. Renal Parenchymal Hypertension: current concepts of pathogenesis and management. *Arch Intern Med* 1996; 156:602–611.
4. Kohan DE, Barton M. Endothelin and endothelin antagonists in chronic kidney disease. *Kidney Int* 2014; 86:896–904.
5. Ruggenenti P, Porrini EL, Gaspari F, Motterlini N, Cannata A, Carrara F, *et al.* Glomerular hyperfiltration and renal disease progression in type 2 diabetes. *Diabetes Care* 2012; 35:2061–2068.
6. Barton M. Reversal of proteinuric renal disease and the emerging role of endothelin. *Nat Clin Pract Nephrol* 2008; 4:490–501.
7. Cortinovis M, Ruggenenti P, Remuzzi G. Progression, remission and regression of chronic renal diseases. *Nephron* 2016; 134:20–24.
8. Barton M. Prevention and endothelial therapy of coronary artery disease. *Curr Opin Pharmacol* 2013; 13:226–241.
9. D'Agati VD, Chagnac A, de Vries APJ, Levi M, Porrini E, Herman-Edelstein M, Praga M. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol* 2016; 12:453–471.
10. Rabelink TJ, Koomans HA. Endothelial function and the kidney. An emerging target for cardiovascular therapy. *Drugs* 1997; 53 (Suppl 1): 11–19.
11. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, *et al.* Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; 345:851–860.
12. Patel V, Panja S, Venkataraman A. The HOPE Study and MICRO-HOPE Substudy: effects of ramipril on cardiovascular and microvascualr outcomes in people with diabetes mellitus. *Br J Diab Vasc Dis* 2001; 1:44–51.
13. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, *et al.* Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; 345:861–869.
14. Parving H-H, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; 345:870–878.
15. Schlaich MP, Schmitt D, Ott C, Schmidt BM, Schmieder RE. Basal nitric oxide synthase activity is a major determinant of glomerular haemodynamics in humans. *J Hypertens* 2008; 26:110–116.
16. Curtis JJ, Luke RG, Dustan HP, Kashgarian M, Whelchel JD, Jones P, Diethelm AG. Remission of essential hypertension after renal transplantation. *N Engl J Med* 1983; 309:1009–1015.
17. Ortmann J, Amann K, Brandes RP, Kretzler M, Munter K, Parekh N, *et al.* Role of podocytes for reversal of glomerulosclerosis and proteinuria in the aging kidney after endothelin inhibition. *Hypertension* 2004; 44:974–981.
18. Culshaw GJ, MacIntyre IM, Dhaun N, Webb DJ. Endothelin in non-diabetic chronic kidney disease: preclinical and clinical studies. *Semin Nephrol* 2015; 35:176–187.
19. de Zeeuw D, Coll B, Andress D, Brennan JJ, Tang H, Houser M, *et al.* The endothelin antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetic nephropathy. *J Am Soc Nephrol* 2014; 25:1083–1093.
20. Meyer MR, Fredette NC, Daniel C, Sharma G, Amann K, Arterburn JB, *et al.* Obligatory role for GPER in cardiovascular aging and disease. *Sci Signal* 2016; 9:ra105.
21. Meyer MR, Barton M. GPER blockers as Nox downregulators: a new drug class to target chronic noncommunicable diseases. *J Steroid Biochem Mol Biol* 2017; doi: 10.1016/j.jsbmb.2017.03.019. [Epub ahead of print].
22. Joris PJ, Plat J, Kusters YH, Houben AJ, Stehouwer CD, Schalkwijk CG, *et al.* Diet-induced weight loss improves not only cardiometabolic risk markers but also markers of vascular function: a randomized controlled trial in abdominally obese men. *Am J Clin Nutr* 2017; 105: 23–31.
23. Lee GK, Cha YM. Cardiovascular benefits of bariatric surgery. *Trends Cardiovasc Med* 2016; 26:280–289.
24. Cefalu WT, Bray GA, Home PD, Garvey WT, Klein S, Pi-Sunyer FX, *et al.* Advances in the science, treatment, and prevention of the disease of obesity: reflections from a diabetes care editors' expert forum. *Diabetes Care* 2015; 38:1567–1582.
25. MacLaughlin HL, Hall WL, Condry J, Sanders TA, Macdougall IC. Participation in a structured weight loss program and all-cause mortality and cardiovascular morbidity in obese patients with chronic kidney disease. *J Ren Nutr* 2015; 25:472–479.
26. Kwok CS, Pradhan A, Khan MA, Anderson SG, Keavney BD, Myint PK, *et al.* Bariatric surgery and its impact on cardiovascular disease and mortality: a systematic review and meta-analysis. *Int J Cardiol* 2014; 173:20–28.
27. Brunner EJ, Shipley MJ, Ahmadi-Abhari S, Tabak AG, Mceniery CM, Wilkinson IB, *et al.* Adiposity, obesity, and arterial aging: longitudinal study of aortic stiffness in the Whitehall II Cohort. *Hypertension* 2015; 66:294–300.
28. Hruby A, Manson JE, Qi L, Malik VS, Rimm EB, Sun Q, *et al.* Determinants and consequences of obesity. *Am J Public Health* 2016; 106:1656–1662.
29. Litwin M, Feber J, Niemirska A, Michalkiewicz J. Primary hypertension is a disease of premature vascular aging associated with neuro-immuno-metabolic abnormalities. *Pediatr Nephrol* 2016; 31:185–194.
30. Yang HC, Fogo AB. Fibrosis and renal aging. *Kidney Int Suppl* 2014; 4:75–78.

31. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005; 308:1592–1594.
32. Seki H. Balance of antiangiogenic and angiogenic factors in the context of the etiology of preeclampsia. *Acta Obstet Gynecol Scand* 2014; 93:959–964.
33. Naljayan MV, Karumanchi SA. New developments in the pathogenesis of preeclampsia. *Adv Chronic Kidney Dis* 2013; 20:265–270.
34. Verdonk K, Visser W, Van Den Meiracker AH, Danser AH. The renin-angiotensin-aldosterone system in preeclampsia: the delicate balance between good and bad. *Clin Sci (Lond)* 2014; 126:537–544.
35. Spradley FT, Palei AC, Granger JP. Immune mechanisms linking obesity and preeclampsia. *Biomolecules* 2015; 5:3142–3176.
36. Tomimatsu T, Mimura K, Endo M, Kumasawa K, Kimura T. Pathophysiology of preeclampsia: an angiogenic imbalance and long-lasting systemic vascular dysfunction. *Hypertens Res* 2017; 40: 305–310.
37. Verdonk K, Saleh L, Lankhorst S, Smilde JE, van Ingen MM, Garrelds IM, *et al*. Association studies suggest a key role for endothelin-1 in the pathogenesis of preeclampsia and the accompanying renin-angiotensin-aldosterone system suppression. *Hypertension* 2015; 65:1316–1323.
38. Aggarwal PK, Chandel N, Jain V, Jha V. The relationship between circulating endothelin-1, soluble fms-like tyrosine kinase-1 and soluble endoglin in preeclampsia. *J Hum Hypertens* 2012; 26:236–241.
39. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Preeclampsia. *Lancet* 2010; 376:631–644.
40. Kappers MH, Smedts FM, Horn T, van Esch JH, Sleijfer S, Leijten F, *et al*. The vascular endothelial growth factor receptor inhibitor sunitinib causes a preeclampsia-like syndrome with activation of the endothelin system. *Hypertension* 2011; 58:295–302.
41. Belloni AS, Rossi GP, Andreis PG, Neri G, Albertin G, Pessina AC, Nussdorfer GG. Endothelin adrenocortical secretagogue effect is mediated by the B receptor in rats. *Hypertens* 1996; 27:1153–1159.
42. Gennari-Moser C, Khankin EV, Escher G, Burkhard F, Frey BM, Karumanchi SA, *et al*. Vascular endothelial growth factor-a and aldosterone: relevance to normal pregnancy and preeclampsia. *Hypertension* 2013; 61:1111–1117.
43. Alexander BT, Rinewalt AN, Cockrell KL, Massey MB, Bennett WA, Granger JP. Endothelin type a receptor blockade attenuates the hypertension in response to chronic reductions in uterine perfusion pressure. *Hypertension* 2001; 37:485–489.
44. Saleh L, Verdonk K, Visser W, van den Meiracker AH, Danser AHJ. The emerging role of endothelin-1 in the pathogenesis of preeclampsia. *Ther Adv Cardiovasc Dis* 2016; 10:282–293.
45. Kappers MH, van Esch JH, Sluiter W, Sleijfer S, Danser AH, van den Meiracker AH. Hypertension induced by the tyrosine kinase inhibitor sunitinib is associated with increased circulating endothelin-1 levels. *Hypertension* 2010; 56:675–681.
46. Gregory AL, Xu G, Sotov V, Letarte M. Review: the enigmatic role of endoglin in the placenta. *Placenta* 2014; 35 (Suppl):S93–S99.
47. Meguid El Nahas A, Bello AK. Chronic kidney disease: the global challenge. *Lancet* 2005; 365:331–340.
48. Johnson RJ, Nangaku M. Endothelial dysfunction: the secret agent driving kidney disease. *J Am Soc Nephrol* 2016; 27:3–5.
49. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* 2011; 11:450–462.
50. Sahin G, Akay OM, Uslu S, Bal C, Yalcin AU, Gulbas Z. Association between endothelial and platelet function markers and adiponectin in renal transplanted recipients on cyclosporine and tacrolimus immunosuppression based therapy. *Nephrology* 2015; 20:392–398.
51. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Kilic S, Turker T, *et al*. Longitudinal analysis of vascular function and biomarkers of metabolic bone disorders before and after renal transplantation. *Am J Nephrol* 2013; 37:126–134.
52. Yilmaz MI, Saglam M, Caglar K, Cakir E, Ozgurtas T, Sonmez A, *et al*. Endothelial functions improve with decrease in asymmetric dimethylarginine (ADMA) levels after renal transplantation. *Transplantation* 2005; 80:1660–1666.
53. Stockenhuber F, Gottsauner-Wolf M, Marosi L, Liebisch B, Kurz RW, Balcke P. Plasma levels of endothelin in chronic renal failure and after renal transplantation: impact on hypertension and cyclosporin A-associated nephrotoxicity. *Clin Sci (Lond)* 1992; 82:255–258.
54. Textor SC, Wilson DJ, Lerman A, Romero JC, Burnett JC Jr, Wiesner R, *et al*. Renal hemodynamics, urinary eicosanoids, and endothelin after liver transplantation. *Transplantation* 1992; 54:74–80.
55. Lerman A, Kubo SH, Tschumperlin LK, Burnett JC Jr. Plasma endothelin concentrations in humans with end-stage heart failure and after heart transplantation. *J Am Coll Cardiol* 1992; 20:849–853.
56. Kumano K, Masui N, Soh S, Mashimo S, Endo T. Plasma endothelin level following kidney transplantation. *Transplant Proc* 1994; 26: 2114–2116.
57. Weis M, Wildhirt SM, Schulze C, Rieder G, Wilbert-Lampen U, Wolf WP, *et al*. Endothelin in coronary endothelial dysfunction early after human heart transplantation. *J Heart Lung Transplant* 1999; 18:1071–1079.
58. Haug C, Duell T, Voisard R, Lenich A, Kolb HJ, Mickley V, *et al*. Cyclosporine A stimulates endothelin release. *J Cardiovasc Pharmacol* 1995; 26 (Suppl 3):S239–S241.
59. Gaston RS, Schlessinger SD, Sanders PW, Barker CV, Curtis JJ, Warnock DG. Cyclosporine inhibits the renal response to L-arginine in human kidney transplant recipients. *J Am Soc Nephrol* 1995; 5:1426–1433.
60. Textor SC, Canzanello VJ, Taler SJ, Wilson DJ, Schwartz LL, Augustine JE, *et al*. Cyclosporine-induced hypertension after transplantation. *Mayo Clin Proc* 1994; 69:1182–1193.
61. Nakatani T, Tanabe S, Han YS, Kayo S, Yoshimi N, Hai E, *et al*. Enhanced expression of endothelin-A receptor in human transplant renal arteriosclerosis. *Int J Mol Med* 2003; 11:153–156.
62. Okada K, Nishida Y, Murakami H, Sugimoto I, Kosaka H, Morita H, *et al*. Role of endogenous endothelin in the development of graft arteriosclerosis in rat cardiac allografts: antiproliferative effects of bosentan, a nonselective endothelin receptor antagonist. *Circulation* 1998; 97:2346–2351.
63. Fukunaga K, Takada Y, Taniguchi H, Yuzawa K, Otsuka M, Todoroki T, *et al*. Protecting the viability of hepatic allografts procured from nonheart-beating donors by blockade of endothelin and platelet activating factor in porcine liver transplantation. *Int Surg* 1998; 83:226–231.
64. Fukunaga K, Takada Y, Mei G, Taniguchi H, Seino K, Yuzawa K, *et al*. An endothelin receptor antagonist ameliorates injuries of sinusoid lining cells in porcine liver transplantation. *Am J Surg* 1999; 178: 64–68.
65. Fukunaga K, Takada Y, Taniguchi H, Mei G, Seino KI, Yuzawa K, *et al*. Endothelin antagonist treatment for successful liver transplantation from nonheart-beating donors. *Transplantation* 1999; 67:328–332.
66. Tang JL, Aitouche A, Subbotin V, Salam A, Sun H, Gandhi C, *et al*. Endothelin-1 receptor blockade and its effect on chronic rejection. *Transplant Proc* 1999; 31:1249.
67. Fukunaga K, Takada Y, Taniguchi H, Otsuka M, Fukao K. Endothelin antagonist improves viability of liver grafts from nonheart-beating donors. *Transplant Proc* 1999; 31:460–461.
68. Shennib H, Lee AG, Kuang JQ, Yanagisawa M, Ohlstein EH, Giaid A. Efficacy of administering an endothelin-receptor antagonist (SB209670) in ameliorating ischemia-reperfusion injury in lung allografts. *Am J Respir Crit Care Med* 1998; 157:1975–1981.
69. Uhlmann D, Ludwig S, Escher E, Armann B, Gäbel G, Teupser D, *et al*. Protective effect of a selective endothelin a receptor antagonist (BSF 208075) on graft pancreatitis in pig pancreas transplantation. *Transplant Proc* 2001; 33:3732–3734.
70. Uhlmann D, Ludwig S, Escher E, Armann B, Gabel G, Teupser D, *et al*. Attenuation of endothelin expression and histologic changes by administration of a selective endothelin-A receptor antagonist in pig pancreas transplantation. *Transplant Proc* 2002; 34:2362–2363.
71. Orth SR, Odoni G, Amann K, Strzelczyk P, Raschack M, Ritz E. The ET(A) receptor blocker LU 135252 prevents chronic transplant nephropathy in the 'Fisher to Lewis' model. *J Am Soc Nephrol* 1999; 10:387–391.
72. Braun C, Conzelmann T, Vetter S, Schaub M, Back WE, Yard B, *et al*. Prevention of chronic renal allograft rejection in rats with an oral endothelin A receptor antagonist. *Transplantation* 1999; 68: 739–746.
73. Braun C, Conzelmann T, Vetter S, Schaub M, Back WE, Kirchengast M, *et al*. Treatment with a combined endothelin A/B-receptor antagonist does not prevent chronic renal allograft rejection in rats. *J Cardiovasc Pharmacol* 2000; 36:428–437.

74. Adams J, Odoni G, Ogata H, Viedt C, Amann K, Ritz E, Orth SR. Combination treatment with an ET(A)-receptor blocker and an ACE inhibitor is not superior to the respective monotherapies in attenuating chronic transplant nephropathy in a 'Fisher-to-Lewis' rat model. *Nephrol Dial Transplant* 2002; 17:780–787.
75. Lattmann T, Hein M, Horber S, Ortmann J, Teixeira MM, Souza DG, *et al.* Activation of pro-inflammatory and anti-inflammatory cytokines in host organs during chronic allograft rejection: role of endothelin receptor signaling. *Am J Transplant* 2005; 5:1042–1049.
76. Masuda Y, Shimizu A, Mori T, Ishiwata T, Kitamura H, Ohashi R, *et al.* Vascular endothelial growth factor enhances glomerular capillary repair and accelerates resolution of experimentally induced glomerulonephritis. *Am J Pathol* 2001; 159:599–608.
77. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, *et al.* Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003; 111:649–658.
78. de Jesus-Gonzalez N, Robinson E, Penchev R, von Mehren M, Heinrich MC, Tap W, *et al.* Regorafenib induces rapid and reversible changes in plasma nitric oxide and endothelin-1. *Am J Hypertens* 2012; 25:1118–1123.
79. Murphy SR, LaMarca BB, Cockrell K, Granger JP. Role of endothelin in mediating soluble fms-like tyrosine kinase 1-induced hypertension in pregnant rats. *Hypertension* 2010; 55:394–398.
80. Li F, Hagaman JR, Kim HS, Maeda N, Jennette JC, Faber JE, *et al.* eNOS deficiency acts through endothelin to aggravate sFlt-1-induced preeclampsia-like phenotype. *J Am Soc Nephrol* 2012; 23: 652–660.
81. Lankhorst S, Baelde HJ, Kappers MH, Smedts FM, Hansen A, Groningen MCC, *et al.* Greater sensitivity of blood pressure than renal toxicity to tyrosine kinase receptor inhibition with sunitinib. *Hypertension* 2015; 66:543–549.
82. Lankhorst S, Kappers MH, van Esch JH, Smedts FM, Sleijfer S, Mathijssen RH, *et al.* Treatment of hypertension and renal injury induced by the angiogenesis inhibitor sunitinib: preclinical study. *Hypertension* 2014; 64:1282–1289.
83. Schini-Kerth VB. Homocysteine, a proinflammatory and proatherosclerotic factor: role of intracellular reactive oxygen species. *Circ Res* 2003; 93:271–273.
84. Rossi GP, Maiolino G, Seccia TM, Burlina A, Zavattiero S, Cesari M, *et al.* Hyperhomocysteinemia predicts total and cardiovascular mortality in high-risk women. *J Hypertens* 2006; 24:851–859.
85. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, *et al.* A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; 10:111–113.
86. Cesari M, Zanchetta M, Burlina A, Pedon L, Maiolino G, Sticchi D, *et al.* Hyperhomocysteinemia is inversely related with left ventricular ejection fraction and predicts cardiovascular mortality in high-risk coronary artery disease hypertensives. *Arterioscler Thromb Vasc Biol* 2005; 25:115–121.
87. Martin YN, Salavaggione OE, Eckloff BW, Wieben ED, Schaid DJ, Weinshilboum RM. Human methylenetetrahydrofolate reductase pharmacogenomics: gene resequencing and functional genomics. *Pharmacogenet Genomics* 2006; 16:265–277.
88. Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. *Circulation* 1997; 95:1119–1121.
89. Stühlinger MC, Oka RK, Graf EE, Schmölzer I, Upson BM, Kapoor O, *et al.* Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. *Circulation* 2003; 108:933–938.
90. Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 1997; 52:10–20.
91. Ostrakhovitch EA, Tabibzadeh S. Homocysteine in chronic kidney disease. *Adv Clin Chem* 2015; 72:77–106.
92. Hishida A, Okada R, Guang Y, Naito M, Wakai K, Hosono S, *et al.* MTHFR, MTR and MTRR polymorphisms and risk of chronic kidney disease in Japanese: cross-sectional data from the J-MICC Study. *Int Urol Nephrol* 2013; 45:1613–1620.
93. Jamison RL, Shih MC, Humphries DE, Guarino PD, Kaufman JS, Goldfarb DS, *et al.* Effect of the MTHFR C677T and A1298C polymorphisms on survival in patients with advanced CKD and ESRD: a prospective study. *Am J Kidney Dis* 2009; 53:779–789.
94. Fung MM, Salem RM, Lipkowitz MS, Bhatnagar V, Pandey B, Schork NJ, O'Connor DT. Methylenetetrahydrofolate reductase (MTHFR) polymorphism A1298C (Glu429Ala) predicts decline in renal function over time in the African-American Study of Kidney Disease and Hypertension (AASK) Trial and Veterans Affairs Hypertension Cohort (VAHC). *Nephrol Dial Transplant* 2012; 27:197–205.
95. Nigwekar SU, Kang A, Zoungas S, Cass A, Gallagher MP, Kulshrestha S, *et al.* Interventions for lowering plasma homocysteine levels in dialysis patients. *Cochrane Database Syst Rev* 2016;CD004683.
96. Xu X, Qin X, Li Y, Sun D, Wang J, Liang M, *et al.* Efficacy of folic acid therapy on the progression of chronic kidney disease: the renal substudy of the China Stroke Primary Prevention Trial. *JAMA Intern Med* 2016; 176:1443–1450.
97. Li Y, Huang T, Zheng Y, Muka T, Troup J, Hu FB. Folic acid supplementation and the risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. *J Am Heart Assoc* 2016; 5:e003768.
98. Zarbock A, Schmidt C, Van Aken H, Wempe C, Martens S, Zahn PK, *et al.* Effect of remote ischemic preconditioning on kidney injury among high-risk patients undergoing cardiac surgery: a randomized clinical trial. *JAMA* 2015; 313:2133–2141.
99. Pan JS, Sheikh-Hamad D. Remote ischemic preconditioning for kidney protection. *JAMA* 2015; 313:2124–2125.
100. Nielsen SE, Schjoedt KJ, Rossing K, Persson F, Schalkwijk CG, Stehouwer CD, *et al.* Levels of NT-proBNP, markers of low-grade inflammation, and endothelial dysfunction during spironolactone treatment in patients with diabetic kidney disease. *J Renin Angiotensin Aldosterone Syst* 2013; 14:161–166.
101. Schjoedt KJ, Andersen S, Rossing P, Tarnow L, Parving HH. Aldosterone escape during blockade of the renin-angiotensin-aldosterone system in diabetic nephropathy is associated with enhanced decline in glomerular filtration rate. *Diabetologia* 2004; 47:1936–1939.
102. Rocha R, Stier CT Jr. Pathophysiological effects of aldosterone in cardiovascular tissues. *Trends Endocrinol Metab* 2001; 12:308–314.
103. van den Meiracker AH, Baggen RG, Pauli S, Lindemans A, Vulto AG, Poldermans D, Boomsma F. Spironolactone in type 2 diabetic nephropathy: effects on proteinuria, blood pressure and renal function. *J Hypertens* 2006; 24:2285–2292.
104. Nielsen SE, Persson F, Frandsen E, Sugaya T, Hess G, Zdunek D, *et al.* Spironolactone diminishes urinary albumin excretion in patients with type 1 diabetes and microalbuminuria: a randomized placebo-controlled crossover study. *Diabet Med* 2012; 29:e184–e190.
105. White WB, Duprez D, St Hillaire R, Krause S, Roniker B, Kuse-Hamilton J, Weber MA. Effects of the selective aldosterone blocker eplerenone versus the calcium antagonist amlodipine in systolic hypertension. *Hypertension* 2003; 41:1021–1026.
106. Schepkens H, Vanholder R, Billiouw JM, Lameire N. Life-threatening hyperkalemia during combined therapy with angiotensin-converting enzyme inhibitors and spironolactone: an analysis of 25 cases. *Am J Med* 2001; 110:438–441.
107. Cruz CS, Cruz AA, Marcílio de Souza CA. Hyperkalaemia in congestive heart failure patients using ACE inhibitors and spironolactone. *Nephrol Dial Transplant* 2003; 18:1814–1819.
108. Edwards NC, Steeds RP, Chue CD, Stewart PM, Ferro CJ, Townend JN. The safety and tolerability of spironolactone in patients with mild to moderate chronic kidney disease. *Br J Clin Pharmacol* 2012; 73:447–454.
109. Khosla N, Kalaitzidis R, Bakris GL. Predictors of hyperkalemia risk following hypertension control with aldosterone blockade. *Am J Nephrol* 2009; 30:418–424.
110. Camuglia AC, Maeder MT, Starr J, Farrington C, Kaye DM. Impact of *N*-acetylcysteine on endothelial function, B-type natriuretic peptide and renal function in patients with the cardiorenal syndrome: a pilot cross over randomised controlled trial. *Heart Lung Circ* 2013; 22:256–259.
111. Tang WH, Lin FH, Lee CH, Kuo FC, Hsieh CH, Hsiao FC, Hung YJ. Cilostazol effectively attenuates deterioration of albuminuria in patients with type 2 diabetes: a randomized, placebo-controlled trial. *Endocrine* 2014; 45:293–301.
112. Jalal DI, Decker E, Perrenoud L, Nowak KL, Bispham N, Mehta T, *et al.* Vascular function and uric acid-lowering in stage 3 CKD. *J Am Soc Nephrol* 2017; 28:943–952.
113. Zoccali C, Torino C, Curatola G, Panuccio V, Tripepi R, Pizzini P, *et al.* Serum phosphate modifies the vascular response to vitamin D receptor activation in chronic kidney disease (CKD) patients. *Nutr Metab Cardiovasc Dis* 2016; 26:581–589.

114. Park J, Liao P, Sher S, Lyles RH, Deveaux DD, Quyyumi AA. Tetrahydrobiopterin lowers muscle sympathetic nerve activity and improves augmentation index in patients with chronic kidney disease. *Am J Physiol Regul Integr Comp Physiol* 2015; 308:R208–R218.
115. Tapia E, Sanchez-Lozada LG, Soto V, Manrique AM, Ortiz-Vega KM, Santamaria J, *et al.* Sildenafil treatment prevents glomerular hypertension and hyperfiltration in rats with renal ablation. *Kidney Blood Press Res* 2012; 35:273–280.
116. Rodragguez-Iturbe B, Ferrebuz A, Vanegas V, Quiroz Y, Espinoza F, Pons H, *et al.* Early treatment with cGMP phosphodiesterase inhibitor ameliorates progression of renal damage. *Kidney Int* 2005; 68: 2131–2142.
117. El-Mahdy NA, El-Sayad ME-S, El-Kadem AH. Combination of telmisartan with sildenafil ameliorate progression of diabetic nephropathy in streptozotocin-induced diabetic model. *Biomed Pharmacother* 2016; 81:136–144.
118. Desouza C, Parulkar A, Lumpkin D, Akers D, Fonseca VA. Acute and prolonged effects of sildenafil on brachial artery flow-mediated dilatation in type 2 diabetes. *Diabetes Care* 2002; 25:1336–1339.
119. Burnett AL, Strong TD, Trock BJ, Jin L, Bivalacqua TJ, Musicki B. Serum biomarker measurements of endothelial function and oxidative stress after daily dosing of sildenafil in type 2 diabetic men with erectile dysfunction. *J Urol* 2009; 181:245–251.
120. Fang L, Radovits T, Szabo G, Mozes MM, Rosivall L, Kokeny G. Selective phosphodiesterase-5 (PDE-5) inhibitor vardenafil ameliorates renal damage in type 1 diabetic rats by restoring cyclic 3′,5′ guanosine monophosphate (cGMP) level in podocytes. *Nephrol Dial Transplant* 2013; 28:1751–1761.
121. Sydow K, Münzel T. ADMA and oxidative stress. *Atheroscler Suppl* 2003; 4:41–51.
122. Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino LS, *et al.* Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; 358:2113–2117.
123. Matsuguma K, Ueda S, Yamagishi S, Matsumoto Y, Kaneyuki U, Shibata R, *et al.* Molecular mechanism for elevation of asymmetric dimethylarginine and its role for hypertension in chronic kidney disease. *J Am Soc Nephrol* 2006; 17:2176–2183.
124. Saran R, Novak JE, Desai A, Abdulhayoglu E, Warren JS, Bustami R, *et al.* Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease (CKD): a pilot study. *Nephrol Dial Transplant* 2003; 18:2415–2420.
125. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation* 2006; 114:2508–2516.
126. Borgi L, McMullan C, Wohlhueter A, Curhan GC, Fisher ND, Forman JP. Effect of uric acid-lowering agents on endothelial function: a randomized, double-blind, placebo-controlled trial. *Hypertension* 2017; 69:243–248.
127. Zoccali C, Curatola G, Panuccio V, Tripepi R, Pizzini P, Versace M, *et al.* Paricalcitol and endothelial function in chronic kidney disease trial. *Hypertension* 2014; 64:1005–1011.
128. Lundwall K, Jorneskog G, Jacobson SH, Spaak J. Paricalcitol, microvascular and endothelial function in non-diabetic chronic kidney disease: a randomized trial. *Am J Nephrol* 2015; 42:265–273.
129. Dreyer G, Tucker AT, Harwood SM, Pearse RM, Raftery MJ, Yaqoob MM. Ergocalciferol and microcirculatory function in chronic kidney disease and concomitant vitamin d deficiency: an exploratory, double blind, randomised controlled trial. *PLoS One* 2014; 9:e99461.
130. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Kilic S, Eyileten T, *et al.* Comparison of calcium acetate and sevelamer on vascular function and fibroblast growth factor 23 in CKD patients: a randomized clinical trial. *Am J Kidney Dis* 2012; 59:177–185.
131. Silswal N, Touchberry CD, Daniel DR, McCarthy DL, Zhang S, Andresen J, *et al.* FGF23 directly impairs endothelium-dependent vasorelaxation by increasing superoxide levels and reducing nitric oxide bioavailability. *Am J Physiol Endocrinol Metab* 2014; 307:E426–E436.