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Journal of Hypertension

Endothelial Factors in The Pathogenesis and Treatment of Chronic Kidney Disease Part II: Role in Disease Conditions. A Joint Consensus Statement from the ESH Working Group on Endothelin and Endothelial Factors And The Japanese Society of Hypertension --Manuscript Draft--

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Abstract:	After examining in Part I the general mechanisms of endothelial dysfunction in the kidney vasculature, 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, kidney transplantation, hyperhomocysteinemia and cancer treated with anti-angiogenic therapy. 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.

Prof. Alberto Zanchetti
Editor-in-Chief
Journal of Hypertension

Padova, September 5th, 2017

Dear Prof. Zanchetti,

Thank you for your letter of August 1st concerning the manuscript "Endothelial Factors in The Pathogenesis and Treatment of Chronic Kidney Disease Part II: Role in Disease Conditions. A Joint Consensus Statement from the ESH Working Group on Endothelin and Endothelial Factors And The Japanese Society of Hypertension" (**JH-D-17-00736**).

We valued much the Reviewers' constructive criticisms and implemented all suggested changes. To ease their tracking in the revised version the changes made are highlighted in yellow in the text. We believe that after this revision the manuscript has been improved considerably and can therefore receive a positive evaluation in the present form.

Therefore, on behalf of my coworkers, I would like to submit the revised version of for consideration for publication in Journal of Hypertension.

We are grateful for the additional consideration to our work and remain your sincerely.

Kindest regards,

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JH-D-17-00736, "Endothelial Factors in The Pathogenesis and Treatment of Chronic Kidney Disease **Part II**: Role in Disease Conditions. A Joint Consensus Statement from the ESH Working Group on Endothelin and Endothelial Factors And The Japanese Society of Hypertension"

Reviewer #1:

This is the second part of a large review on endothelial function in relationship to kidney diseases. In general this review is fairly well well-written. The weakness is the unsystematic nature of this multi-author effort. I have the following comments/suggestions for the authors:

1. The lack of focus on endothelial function as related to the GFR and proteinuria in hypertension is a miss. Animal models implicate vascular dysfunction in renal damage in hypertension (reviewed in Am J Physiol Regul Integr Comp Physiol. 2009; 296:R1001-18) an alteration likely contributing to the age-dependent acceleration of renal function loss in this population. Furthermore, studies showing that nitric oxide synthase activity is a major determinant of glomerular haemodynamics in humans (J Hypertension 26:110-116, 2008) and descriptive studies in essential hypertension linking endothelial dysfunction as measured by state of art techniques and the GFR (Circulation 110:821-5; 2004) exist.

RE: We thank the Referee for the constructive criticisms that helped us in the revision of the manuscript. In the revised Introduction we have now clarified that endothelial dysfunction induces renal hemodynamic changes, which favour development of CKD and the age-dependent acceleration of renal function loss in the hypertensive population. Moreover, we reported that proteinuria, the marker of renal microvasculature damage, invariably associates with systemic endothelial dysfunction in hypertension, suggesting a general involvement of endothelium.

The two references (Am J Physiol Regul Integr Comp Physiol. 2009 and Circulation 2004) have been quoted in Part 2, whereas the reference on nitric oxide synthase (JH 2008), being related to the mechanisms, was added in Part 1.

2. Some sections fail to quote and discuss relevant papers. For example, in the part dedicated to the usefulness of ACEi in obesity-related CKD, the authors miss a not so old paper showing that the nephro-protective effect of Ramipril in patients with proteinuric CKD is almost confined to overweight and obese patients (JASN;22:1122-8; 2011).

RE: A comment on the usefulness of ACEi in the obesity nephropathy was added, and the reference has been quoted.

3. The part dealing with endothelial factors in kidney transplantation misses several papers related with factors implicated in the evolution of endothelial function after transplantation, e.g. ADMA (Transplantation. 2005;80:1660-6), biomarkers of mineral disorders (Am J Nephrol. 2013;37:126-34).

RE: The involvement of ADMA and FGF23, and the related references, were added to the text.

4. In the section "Vitamin D, endothelial function, and kidney protection" there is no focus on FGF23, a bone hormone which is probably the most investigated risk factor for cardiovascular disease in CKD. In this respect, experimental studies in vitro and in animal models (Am J Physiol Endocrinol Metab. 2014 Sep 1;307:E426-36.; PLoS One. 2014;9:e93423) and cross-sectional (Kidney Int. 2010;78:679-85) and intervention studies investigating the link between FGF23 and vascular function in CKD exist (Am J Kidney Dis. 2012;59:177-85).

RE: Following your suggestion, we added information on the role of FGF23 and mentioned the RCT in stage 4 CKD patients. The references were quoted.

Reviewer #2:

In this second part of the review on the role of endothelial factors in the pathogenesis of renal diseases, the authors discuss the potential role of the endothelium and its dysfunction in several circumstances of renal diseases such as diabetes nephropathy, obesity, pregnancy, cancer therapy, vitamin deficiency and transplantation. The authors convincingly demonstrate that the contribution of endothelial dysfunction is almost always present in these renal diseases but whether it represents a primary or a secondary event is not always clear and the effect of interventions is so far rather scarce in most situations.

RE: We thank the Referee for his/her comments and criticisms, which helped us in the revision of the manuscript. The lack of clear evidences mostly derives from the lack of large RCTs designed to test the effects of drugs on specific disease-associated CKD. Hence, whether endothelial dysfunction is a primary or a secondary event remains largely known in most conditions. We hope that our review could stimulate RCTs in this field.

Comments:

1. Compared to the first part of the review this second part is less well elaborated and sometimes a bit superficial just aligning more or less convincing studies. The authors do not always make it clear that interventional results are only obtained in animal models (for ex in transplantation) and that so far, there is no clear evidence from clinical trials that any interventions focused on endothelial function (if possible) are really effective in most of the clinical situations described in the review. Thus authors should be more clear on the level acquired so far in each pathology: this could perhaps be included in a table. At this stage no recommendations can be made for clinicians.

RE: We agree that many findings were obtained from experimental studies and RCTs were too small to make recommendations. This was true particularly for transplantation-associated hypertension and hence we added a note at the end of the section. Moreover, we distinguished the studies on humans from those performed in experimental models.

2. It should also be mentioned that it is often difficult to disentangle with actual drugs the respective role of pathogenic factors such as high BP, AGES, ROS and so on, as interfering on one parameter affects the others.

RE: We added a comment on the difficulties of identifying the role of each pathogenic factor on endothelial dysfunction in the Conclusions.

3. On page 6 when the authors say that the effects of RAS blockade is largely independent of BP, this is partially true. Indeed, what is the evidence that these agents protect the kidney when no change in BP occurs? This is why authors talk about effects beyond BP.

RE: Thank you for this suggestion. We added a comment on this issue and quoted Micro-HOPE, Renaal, IRMA-2 and IDNT studies.

Minor: relevant figures could be added to illustrate the potential role of endothelial dysfunction in some of the described diseases.

RE: An additional figure illustrating the stages of diabetic nephropathy was added (Figure 1).

Abbreviations

Ang II: angiotensin II

ACE: angiotensin I converting enzyme

ADMA: asymmetric dimethylarginine

ARB: angiotensin AT₁ receptor blocker

BP: blood pressure

CKD: chronic kidney disease

CV: cardiovascular

eGFR: estimated glomerular filtration rate

ERA: endothelin receptor antagonist

eNOS: endothelial nitric oxide synthase

ESRD: end-stage renal disease

ET-1: endothelin-1

GFR: glomerular filtration rate

MTHFR: methylenetetrahydrofolatereductase

NOS: nitric oxide

NOS: nitric oxide synthase

Nox: NADPH oxidases

RAAS: renin-angiotensin-aldosterone system

RCT: randomized controlled clinical trials

ROS: reactive oxygen species

sFlt-1: soluble Fms-like tyrosine kinase 1

VEGF: vascular endothelial growth factor

Condensed abstract

After examining in Part I the general mechanisms of endothelial dysfunction in the kidney vasculature, the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension and the Japanese Society of Hypertension herein review current knowledge on the role of endothelial dysfunction in multiple disease conditions that affect the kidney. The few 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.

**Endothelial Factors in The Pathogenesis and Treatment
of Chronic Kidney Disease Part II: Role in Disease Conditions.
*A Joint Consensus Statement from the ESH Working Group on Endothelin and
Endothelial Factors And The Japanese Society of Hypertension***

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Abstract

After examining in Part I the general mechanisms of endothelial dysfunction in the kidney vasculature, 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, kidney transplantation, hyperhomocysteinemia and cancer treated with anti-angiogenic therapy. 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.

Key Words: diabetes mellitus; endothelin; endothelium; hypertension; kidney; diabetes mellitus; preeclampsia; kidney transplantation; hyperhomocysteinemia; cancer.

Abbreviations

Ang II: angiotensin II

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eNOS: endothelial nitric oxide synthase

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ET-1: endothelin-1

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MTHFR: methylenetetrahydrofolatereductase

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Nox: NADPH oxidases

RAAS: renin-angiotensin-aldosterone system

RCT: randomized controlled clinical trials

ROS: reactive oxygen species

sFlt-1: soluble Fms-like tyrosine kinase 1

VEGF: vascular endothelial growth factor

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1. Introduction

Impaired endothelium-dependent vasodilation, a hallmark of arterial hypertension and many other cardiovascular (CV) disease risk factors and conditions, can be an early mechanism leading to CV 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 to chronic kidney disease (CKD) [1]. Proteinuria, a marker of renal microvasculature damage, is invariably associated with systemic endothelial dysfunction in hypertension, suggesting a general involvement of 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 where the renal vasculature is deeply affected, as diabetes mellitus, preeclampsia, kidney transplantation, hyperhomocysteinemia and cancer. The few randomized controlled clinical trials that explored the concept that strategies aimed at correcting endothelial dysfunction in patients with hypertension and/or chronic kidney disease are also discussed alongside their cardiovascular and renal outcomes.

2. 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 CV 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 non-endothelial pathways [7,8], including enhanced oxidative stress, renin-angiotensin-aldosterone system (RAAS) and endothelin-1 (ET-1) activation, and inflammatory processes, as 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 antihypertensive medications targeting endothelial pathways, such as angiotensin I converting enzyme (ACE) inhibitors, angiotensin AT₁ receptor blocker (ARBs) or mineralocorticoid receptor antagonists, have been investigated in diabetic nephropathy, as hypertension is common among these patients. **These agents were shown to improve clinical outcome with benefit that exceeded that attributable to changes in blood pressure** [7,11–14]. Of note, the protective effect of ACE inhibitors and ARBs was **particularly marked in obesity nephropathy [15]** and predictably found to be largely blood pressure (BP)-independent [11,13,14], consistent with suggesting their direct reno-protective 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 to occur in patients with diabetic nephropathy treated with ARBs or ACE inhibitors, in whom proteinuria decreased [7]. Reversal of diabetic or non-diabetic focal and segmental glomerulosclerosis and/or proteinuria has also been observed in studies with ET-1 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 (Nox) 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 preventative measures, such as weight loss /bariatric treatment of obesity [8], lifestyle changes to improve and maintain cardiorespiratory fitness or pharmacological interventions, such as anti-diabetic therapy or those targeting the aforementioned endothelial mediators can conceivably reduce BP, delay vascular and renal aging, and thus contribute to an improved overall CV outcome [8,22–30].

3. 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 VEGF type 1 receptor originating from placental syncytiotrophoblasts, has been widely studied. Others include soluble endoglin, agonistic auto-antibodies to the AT₁ 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 pro- and anti-angiogenic factors. Hence, these mechanisms may contribute to arterial hypertension and renal damage in preeclampsia [35].

Accordingly, as discussed later, 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 pre-eclamptic 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 ET_B receptors [41], while auto-antibodies to the Angiotensin II (Ang II) type 1 receptor might do the same. Obviously, these antibodies should also suppress renin release via AT₁ receptor activation. Consequently, theoretically preeclamptic women would be expected to display an increased aldosterone/renin ratio, due to the opposite effects of ET-1 and auto-antibodies 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 inhibition-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. ET receptor antagonism, having been linked to teratogenicity, might be feasible only at a late pregnancy stage, when all organs have been formed.

4. Kidney transplantation and transplant-associated hypertension

The global burden of ESRD patients needing renal replacement therapy and transplantation is continuing

to rise. This is a huge financial burden to health care systems [47], which will continue to rise with improved survival from CV disease in these patients. Endothelial dysfunction is held to be central to both CKD development and the CV 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 during ESRD. It is then aggravated during the ischemia-reperfusion occurring with 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), ADMA, and 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, 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], while RCTs [53–61] were too small to recommend a specific class of drugs to transplant-associated hypertension.

5. 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 vascular endothelial growth factor. 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, where the release of sFlt-1 in the bloodstream, by sequestering VEGF and placenta growth factor, is held to produce an antiangiogenic state [77]. As in preeclampsia, activation of the ET system occurs in cancer patients treated with the receptor tyrosine kinase inhibitors sunitinib and regoratorafenib [45,78]. In pregnant rats the antiangiogenic factor sFlt-1 causes a rise in BP and expression of the preproET-1 gene in the renal cortex [79]. Moreover, overexpression of sFlt-1 in mice also increases renal expression of preproET-1 and ET_A receptor, effects that are amplified in endothelial nitric oxide 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 seen with sFlt-1 administration in pregnant rats, expression of the preproET-1 and endothelin converting enzyme genes were not increased. Notwithstanding this, the mixed ERA macitentan prevented the rise in BP and proteinuria in sunitinib-exposed rats [82], while 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 pre-pro-ET-1 gene may not be uniform, it can be concluded that anti-angiogenic 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.

6. Hyperhomocysteinemia, endothelial dysfunction and renal damage

Hyperhomocysteinemia defined as a total plasma homocysteinemia ≥ 15 $\mu\text{mol/L}$, 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-tetra-hydro-folatereductase (MTHFR) gene (ID 4524), particularly in elderly people and in the presence of reduced eGFR [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 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 NO synthase inhibitor asymmetric dimethylarginine (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, because 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 (J-MICC) Study [92] and with mortality risk in ESRD patients of the Homocysteinemia in Kidney and End Stage Renal Disease (HOST)-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 (AASK) Trial [94].

A recent Cochrane analysis of 6 studies on the effects of folic acid or vitamins B6 and vitamin B12 in ESRD patients, however, failed to show a decrease in CV events and/or death, leading the contention that homocysteine-lowering therapies should not be used for CV risk reduction [95]. This conclusion, however, cannot be taken for granted for the following reason. Since there is a linear relationship between plasma homocysteine and CV risk 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 where the beneficial effect seen in a subset of the subjects 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 to enalapril alone [96]. The concept that lowering plasma hyperhomocysteinemia is beneficial is further supported by evidence that folic acid supplementation protected from CVD patients with low folic acid levels and without preexisting CV disease [97].

7. Endothelial factors, kidney protection, and disease prevention

In this era of evidence-based medicine randomized controlled clinical trials (RCTs) are the basis for high level evidence and Class I recommendations. Whether specifically targeting endothelial dysfunction may ultimately improve CV 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/1.73 m²)[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, hyperlipidemia, 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, improved 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 enhanced microalbuminuria [103], which might explain why low dose spironolactone (25 mg daily) 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 to 200 mg daily) 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 hyperkalaemia developed in less than 1% of the patients with non-diabetic I-III CKD stages (eGFR 30-89 mL/min/1.73m²) 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 safe [109], caution should be exercised in prescribing these agents to CKD patients with an eGFR < 45 mL/min/1.73m² and serum K⁺ levels > 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 the endothelial function with an 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 suprasystolic 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/1.73 m²) were randomized to oral cilostazol (100 mg b.i.d.) or placebo for one year. Microalbuminuria and albumin-creatinine ratio were significantly reduced in the cilostazol group as compared to 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 the endothelial, glomerular, mesangial, cortical tubular, and inner medullary collecting duct cells, degrades cGMP, 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 streptozotocin-induced type 1 diabetes mellitus, and restored nephrin and podocin expression in podocytes [120]. Whether these agents help in maintaining the kidney function 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 (PRMT-1) and downregulating dimethylarginine dimethylaminohydrolase (DDAH), the enzyme degrading 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].

8. Vitamin D, endothelial function, and kidney protection

As 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/d) that did not affect endothelium-independent vasodilation and BP, improved endothelium-dependent vasodilation, and slightly lowered eGFR (-3.2 mL/min per 1.73 m² (-4.9 to -1.4), $P < 0.001$), two changes that disappeared after drug withdrawal [127]. Interestingly, 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 non-diabetic CKD patients (mean eGFR 40 ml/min/1.73 m²) 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 fibroblast growth factor 23 (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 establish a protective effect of vitamin D and/or other factors affecting calcium/phosphate metabolism on the kidney.

9. Conclusions and recommendations

Diabetes mellitus, preeclampsia, kidney transplantation, hyperhomocysteinemia and anti-angiogenic

therapy are all factors that deeply affect endothelial function favouring 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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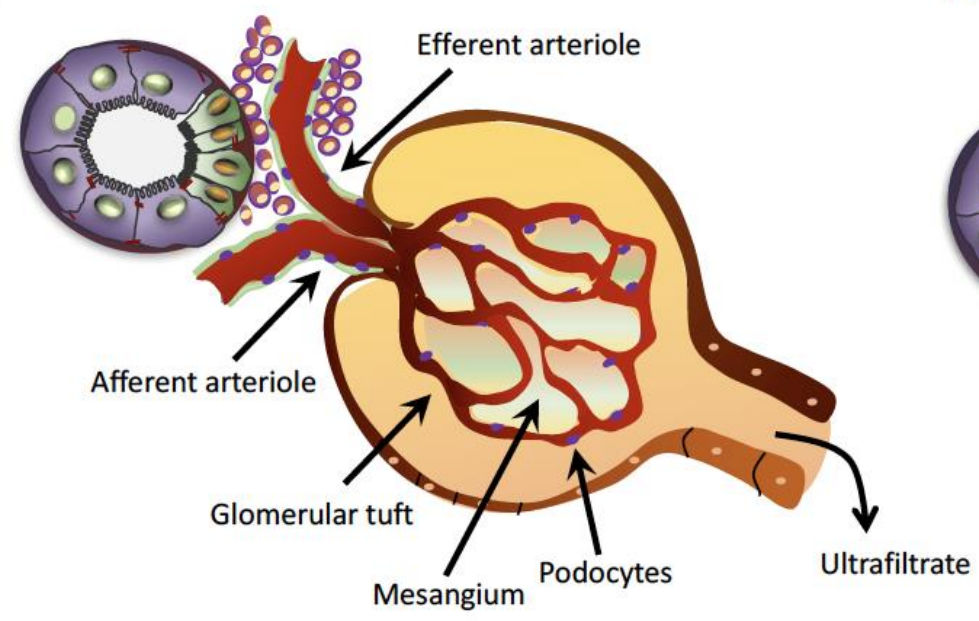
Legends to the Figure

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 (EndMT) and fibrosis. D. Glomerular and tubulointerstitial fibrosis, by provoking glomerular shrinkage and preventing communications between endothelial and tubular cells, cause further reduction of filtration and protein loss.

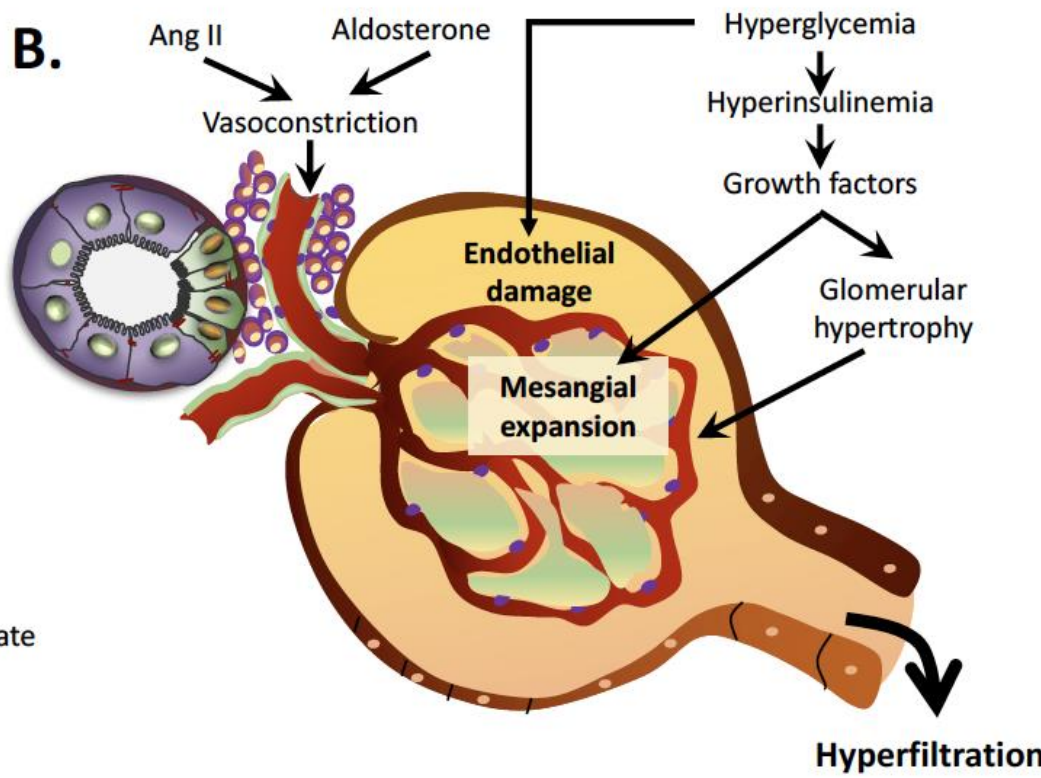
Figure 2. Endothelial dysfunction and abnormal placentation in preeclampsia. During normal placentation, cytotrophoblast cells invade the maternal decidual arteries, remodeling them into high capacitance vessels that supply the placenta and fetus with maternal oxygen and nutrients. In preeclampsia, this 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 sFlt-1 (stage II). sFlt-1, combined to soluble endoglin, agonistic auto-antibodies to the AT₁ receptor and inflammatory cytokines causes endothelial dysfunction, finally leading to arterial hypertension and kidney damage in the mother and growth retardation in the fetus.

Figure 1

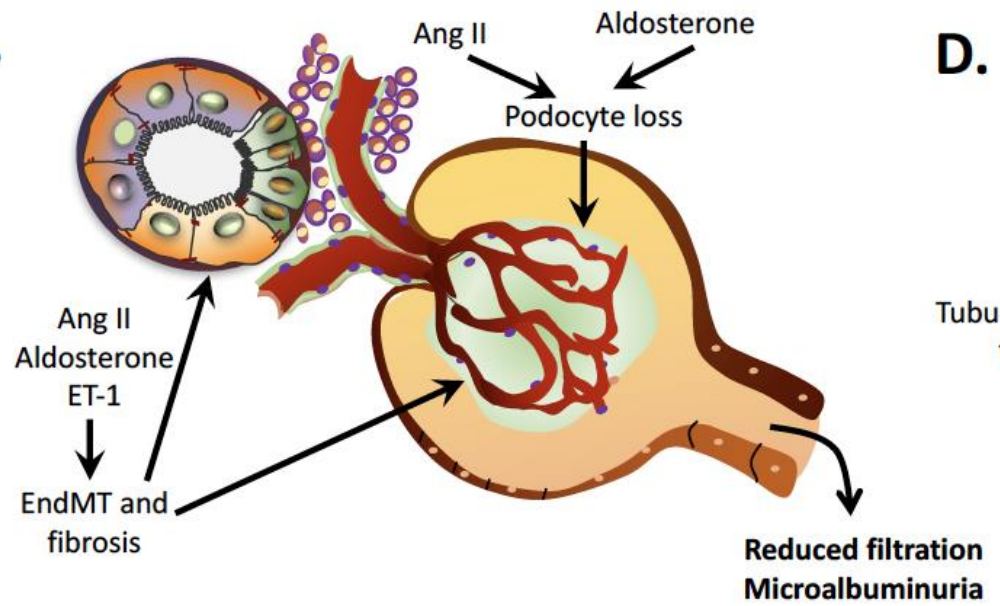
A.



B.



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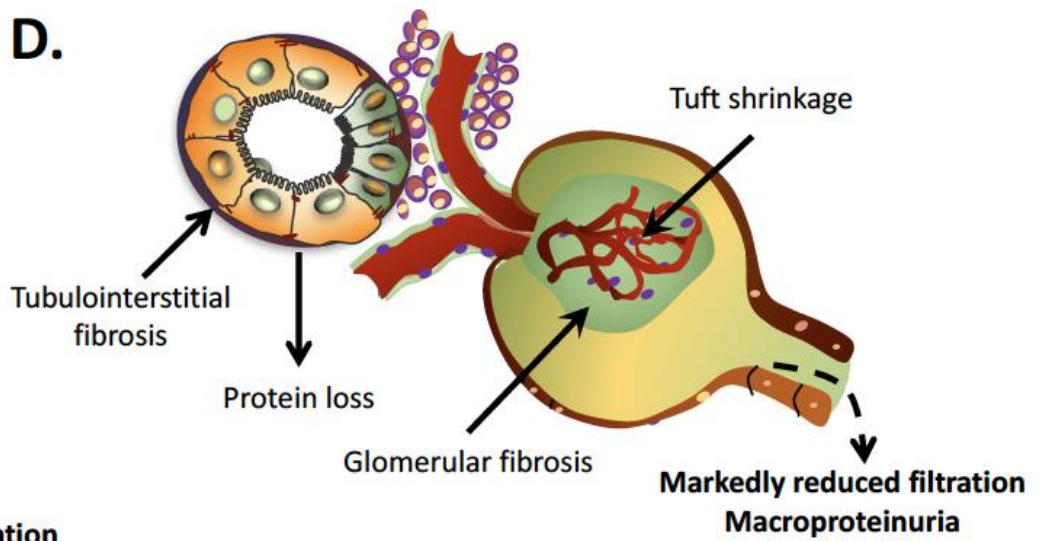


Fig. 1

Figure 2

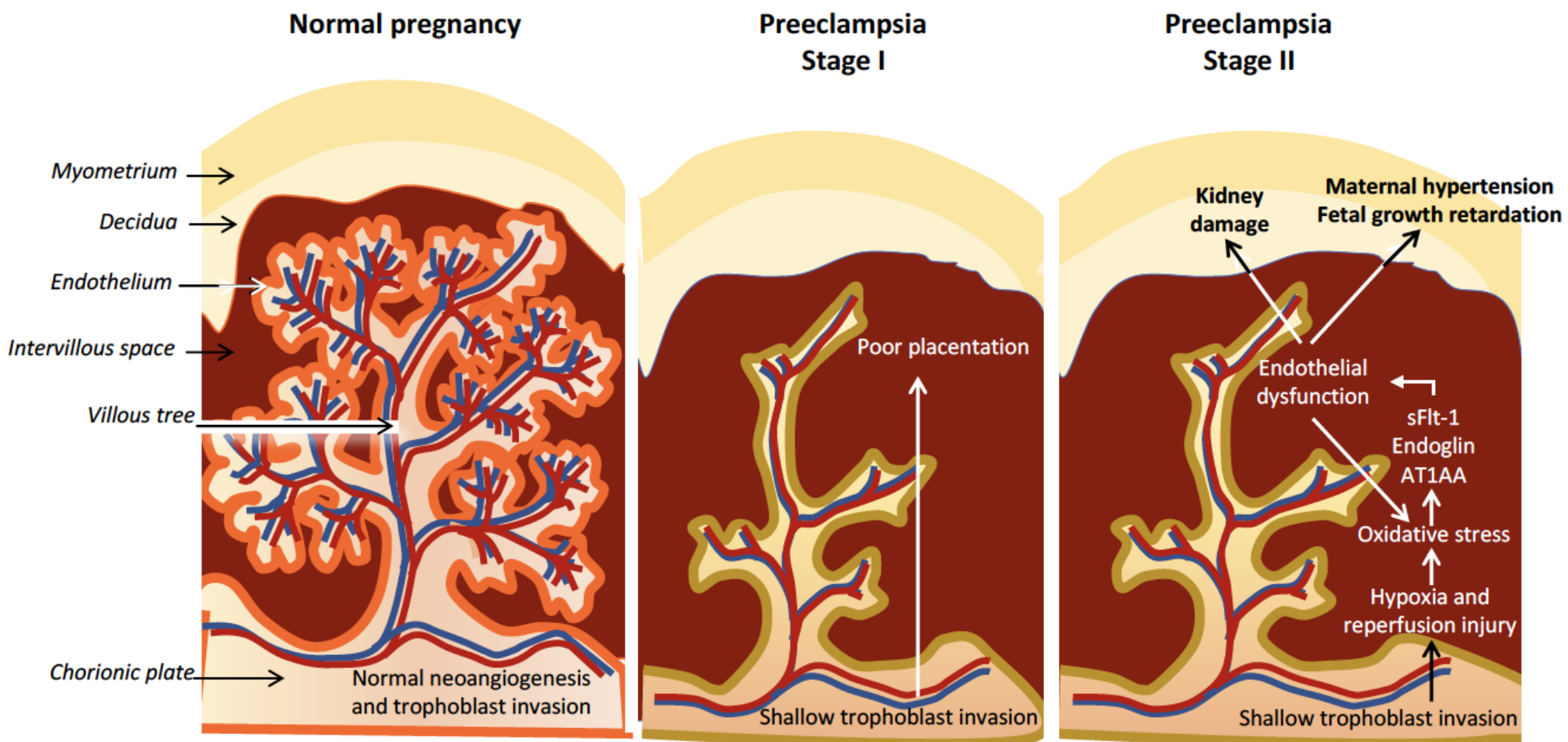


Fig. 2