Vascular endothelial and myogenic function in renal disease
Ochodnicky, Peter

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 7

Endothelial dysfunction in chronic kidney disease: determinant of susceptibility to end-organ damage and therapeutic response

Peter Ochodnický
Simone Vettoretti
Hendrik Buikema
Robert H. Henning
Richard P.E. van Dokkum
Dick de Zeeuw

*J Nephrol* 2006, 19(3):246-58
Abstract

Endothelial dysfunction (ED) seems to be a crucial mediator of increased cardiovascular risk observed among patients with chronic kidney disease (CKD). Importantly, systemic ED does not only occur in patients with severe renal failure, but also in individuals with earlier stages of CKD. Close association between microalbuminuria and systemic ED renders renal vascular function an important marker for the severity of cardiovascular damage. Furthermore, alterations of the renal endothelium might be actively involved in the progression of renal end-organ damage. Recently, experimental evidence showed that interindividual variability in endothelial function of healthy rats predicts the susceptibility to renal damage and the efficacy of renoprotective treatment. Therefore, a specific manipulation of renal and systemic ED might provide benefit in various stages of CKD. Thus, ED may represent an ideal therapeutic target not only for treatment, but also for primary prevention of renal disease.
Introduction

Chronic kidney disease (CKD) represents a major worldwide health care and economic problem. Apart from the enormous number of patients with chronic renal failure requiring treatment by dialysis or transplantation, there is also a constantly raising number of individuals suffering from earlier stages of CKD. In the United States, a total of 8 million individuals are estimated to have a GFR of less than 60 ml/min/1.73 m$^2$ and another 12 million had evidence of microalbuminuria. CKD is associated with increased prevalence of cardiovascular disease and in fact, patients with CKD have even higher chance to experience a cardiovascular event than to progress to renal failure. Early detection of individuals at risk for renal impairment might therefore prevent both renal failure and cardiovascular-related burden in these patients.

Endothelial injury is currently recognized as a common denominator of vascular damage in various conditions associated with increased cardiovascular risk, including CKD. Several important aspects of endothelial dysfunction (ED) in CKD have recently received a great deal of attention. Firstly, the occurrence of systemic ED in various stages of CKD has been proposed as an explanation for the accelerated rate of cardiovascular events associated with impaired renal function. Moreover, specific intrarenal ED could play an active role in the development and progression of renal damage itself. Finally, recent data suggest that the renal endothelium might be involved in the individual susceptibility to renal damage and might govern the sensitivity to renoprotective treatment. In this chapter, we discuss these aspects of ED in CKD (Figure 1) and propose that the endothelium represents a specific target for early detection and prevention of both renal and cardiovascular damage progression in patients with CKD.

Endothelial dysfunction, general considerations

Based on vascular research carried out over the last 25 years, the endothelium, inner lining of the vasculature, is now recognized as the principal regulator of vascular function (recently reviewed by $^3$). Endothelial dysfunction (ED), defined as alterations in the normal properties of the endothelium that are inappropriate for preservation of organ function, is characterized by loss of protective endothelial characteristics in favour of deleterious mechanisms. ED is now recognized as a crucial event in the initiation and progression of atherosclerosis and a common denominator for conditions associated with elevated cardiovascular risk, including hypertension, diabetes, dyslipidemia, obesity or smoking. In all these states, ED is characterized by altered production and/or decreased bioavailability of nitric oxide (NO), the most extensively studied endothelial mediator, and excessive production of reactive oxygen species.

Several estimates of ED are available in clinics, most of them are however difficult to interpret. Plasma levels of endothelial markers, such as vWF, PAI-1, or soluble adhesive and chemoattractant molecules are frequently used to estimate the state of systemic ED.
Unfortunately, they do not provide any information on endothelial function of clinically relevant vascular beds (e.g. coronary or renal). Localization of ED might be of paramount importance when investigating the role of the endothelium in the progression of specific end-organ damage as the structural and functional properties of the endothelium differ among vascular beds (e.g. renal versus systemic resistance arteries). Specificity of endothelial function measurements might be achieved by assessment of vascular tone in a defined vascular bed (*Table 1*).

*Figure 1. The role of renal and systemic endothelial function in the progression of the end-organ damage in various stages of chronic kidney disease.*

Renal endothelial dysfunction might be involved in the susceptibility of the individual to renal injury, the progression of renal end-organ damage to the stage of renal insufficiency and may possibly co-determine the variability in renoprotective therapeutic response. Microalbuminuria and ESRD are also associated with systemic endothelial dysfunction, which is critically involved in the development of cardiovascular end-organ damage. The exact relations between renal and systemic endothelial function are not completely understood. Arrows depict the mutual relations. The most important mechanisms of endothelial dysfunction are also denoted. NO-nitric oxide, EDHF-endothelium-derived hyperpolarizing factor, ADMA- asymmetric dimethylarginine, HCys- homocysteine, ROS-reactive oxygen species.
Table 1. Methods available for assessment of endothelial function in defined vascular beds in humans.

<table>
<thead>
<tr>
<th>Vascular bed</th>
<th>Method</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic</td>
<td>Flow-mediated dilation (FMD)</td>
<td>Non-invasive measurements of diameter changes in conduit brachial artery by Doppler ultrasound after postischemic induction of flow, mainly NO-dependent</td>
</tr>
<tr>
<td></td>
<td>Forearm blood flow (FBF)</td>
<td>Invasive measurement of flow in forearm microcirculation by strain gauge venous plethysmography, local infusion of endothelium-activating substances (acetylcholine)</td>
</tr>
<tr>
<td>Microcirculation</td>
<td>Non-invasive laser digital Doppler flow measurements in skin microcirculation, endothelium-activating substances (acetylcholine) applied locally by iontophoresis</td>
<td></td>
</tr>
<tr>
<td>Isolated vessels</td>
<td>Subcutaneous arteries from skin biopsies</td>
<td></td>
</tr>
<tr>
<td>Coronary</td>
<td>Coronary angiography</td>
<td>Functional angiogram after intracoronary infusion of acetylcholine</td>
</tr>
<tr>
<td>Renal</td>
<td>Renal blood flow changes after NO-modulating agents</td>
<td>Measurements of renal hemodynamics after L-NAME or L-arginine infusion</td>
</tr>
</tbody>
</table>

Chronic renal failure is associated with systemic endothelial dysfunction

It has long been recognized that the last stage of CKD, end-stage renal disease, is invariably associated with systemic ED\(^7\), a finding that has been interpreted as an important event in the development of renal insufficiency-related cardiovascular complications. This may not be that surprising since renal patients usually have several of the conventional cardiovascular risk factors such as hypertension, dyslipidemia or diabetes, all of which are associated with ED. However, several data suggest that the occurrence of these events can not be solely explained by traditional risk factors and that additional ‘kidney-specific’ mechanisms may contribute to the CKD-related ED.

Many authors report elevated plasma markers of endothelial injury, such as vWF, fibrinogen or thrombomodulin in patients on maintenance dialysis\(^8,9\). Others have confirmed localized impaired endothelium-dependent vasodilation in peripheral vascular beds of uremic patients in vivo\(^7,10,11\), as well as in isolated vascular preparations\(^12\). Interestingly, peripheral vasodilatory ED was not only found in dialyzed subjects, but also in patients with moderate renal failure even when adjusted for hypertension, blood glucose and serum cholesterol\(^13\). In this study, the severity of systemic ED was proportionally
related to the degree of renal dysfunction. A study by Kari et al. demonstrated the presence of ED in uremic children without hypertension and lipid alterations, suggesting a renal failure specific effect. In experimental animal studies, 5/6 nephrectomy resulting in chronic renal failure within several weeks is also associated with ED in both resistance and conduit arteries even without the presence of concomitant hypertension. Therefore, it is now widely accepted that malfunction of the kidneys elicits profound deleterious effects on systemic vascular endothelium.

The majority of the studies investigating the mechanisms of ED in chronic renal failure has focused on the systemic NO deficiency as the principal event leading to ED. Functional evidence of impaired stimulated NO bioactivity in forearm vasculature of hemodialysis patients was provided by Passauer et al. Mechanisms responsible for altered NO activity have not been completely elucidated yet, however some non-traditional risk factors relatively specific for uremia are being extensively discussed. Firstly, chronic renal failure is believed to be a state of excessive oxidative stress, as demonstrated in numerous experimental and clinical studies. Reactive oxygen species result in premature inactivation of NO and its decreased bioavailability. The involvement of increased oxidative stress in systemic ED is further supported by a direct relation between endothelium-dependent vasodilation and plasma markers of oxidative stress and beneficial effect of antioxidant treatment on ED in patients with chronic renal failure.

Hyperhomocysteinemia might be a specific condition responsible for excessive generation of reactive oxygen species in chronic renal failure as it is found in the majority of renal patients and has been shown to adversely affect NO production and endothelial function. Finally, in dialyzed patients elevated plasma levels of endogenous competitive inhibitors of constitutive nitric oxide synthase (eNOS), such as asymmetric dimethylarginine (ADMA), are found. It has been proposed that this substance may in vivo reach the plasma levels relevant for significant inhibition of NO production. Thus, while prevailing evidence shows that chronic renal failure is a state of systemic NO deficiency, the exact role of ‘kidney-specific’ mechanisms has yet to be characterized.

Although not yet systematically addressed, several data suggest that mechanisms other than reduced NO bioavailability might be responsible for ED in patients with chronic renal failure. Additionally to NO, prostaglandins and endothelium-derived hyperpolarizing factor (EDHF) are released from the endothelium upon stimulation with acetylcholine. Most of the authors did not observe any contribution of prostaglandins to ED in experimental models of chronic renal failure, arguing against the important role of these mediators in renal insufficiency-related systemic ED. However, one human study reported that inhibition of cyclooxygenase by diclofenac acutely improves endothelium-dependent dilation in the forearm of the patients with mild to moderate renal insufficiency. The authors speculate that beneficial effect of diclofenac might be attributed to inhibition of prostanoid endothelium-derived contracting factor (EDCF) or decreased production of cyclooxygenase-derived free radicals. In addition to nitric oxide and prostaglandins, yet another mediator, termed EDHF, is responsible for acetylcholine-induced endothelial
Endothelial dysfunction and chronic kidney disease

relaxation. We\textsuperscript{29} and others\textsuperscript{15,16} demonstrated reduced EDHF-dependent dilation in small resistance and conduit arteries of 5/6 nephrectomized rats. Under normal physiological conditions EDHF is responsible for the majority of the acetylcholine-induced relaxation in small arteries, where it prevails over NO-dependent mechanisms\textsuperscript{30}. However, the role of EDHF in ED is not clearly defined since its identity remains illusive. Several mediators, such as cytochrome P450 metabolites of arachidonic acid, potassium ions, hydrogen peroxide or gap junctions have been proposed to account for EDHF-mediated dilation in various vascular beds\textsuperscript{31}. It is however recognized that all these mechanisms involve the opening endothelial Ca\textsuperscript{2+}-sensitive potassium channels with small (SK\textsubscript{Ca}) and intermediate (IK\textsubscript{Ca}) conductance. Kohler et al. showed altered expression of these channels in vascular endothelium of 5/6 nephrectomized rats\textsuperscript{32}. However, the only study addressing EDHF-mediated vasodilation in uremic humans failed to show any changes in forearm non-NO/prostaglandin vasodilation in hemodyalized patients\textsuperscript{11}. Additional studies need to be performed to answer the question whether impaired EDHF-dependent ED, as observed in animal models, may occur also in humans and to which extent it may participate in the development of renal insufficiency-related cardiovascular complications.

Although it is now established that chronic renal failure represents a state of systemic NO and possibly EDHF-mediated ED, the role of ED in the excessive occurrence of cardiovascular risk of renal patients will have to be further clarified. Pannier et al. reported a direct relation between forearm post-ischemic endothelial vasodilation and markers of end-organ cardiovascular damage including intima-media thickness of common carotid artery and left ventricular hypertrophy\textsuperscript{33} in subjects on hemodialysis. Several plasma markers of ED or molecules potentially involved in ED, including ADMA, hyperhomocysteine and PAI-1\textsuperscript{34-37}, all have been shown to be related with future cardiovascular morbidity and mortality in longitudinal studies in hemodialysis patients. So far, the only evidence available for such prognostic value of specific vasomotor endothelial dysfunction has been reported by London et al.\textsuperscript{38} They have shown that flow-mediated vasodilation was related with all-cause mortality in patients with end-stage renal disease with 60 months median follow-up. Thus, the current data support the view of systemic ED being a cardiovascular risk factor in patients with chronic renal failure.

Systemic endothelial dysfunction and microalbuminuria

Although most of the clinical data regarding systemic ED in CKD has been collected in uremic patients on dialysis, ED is often present in milder stages of renal insufficiency\textsuperscript{13,39}. Moreover, the severity of ED might be related to the degree of renal insufficiency in renal patients\textsuperscript{22}, further suggesting a crucial link between kidney and systemic vascular damage. Interestingly, systemic ED can be already detected in subjects with minor increases in urinary protein excretion, which may be a marker of initial renal injury. A minor increase in protein (albumin) excretion (microalbuminuria) might be interpreted as the reflection of impaired glomerular endothelial function. Therefore it has been hypothesized (Steno hypothesis) that microalbuminuria reflects the specific renal feature of generalized vascular
Chapter 7

Dysfunction. The view of the kidney as an organ reflecting general vascular damage has stemmed from numerous studies showing the predictive value of microalbuminuria for cardiovascular mortality in diabetic and high risk non-diabetic individuals. Recently, the PREVEND study reported predictive value of microalbuminuria for all-cause mortality in the general population with low cardiovascular risk, suggesting that minor renal injury might be an early indicator of systemic vascular damage.

ED estimated from elevated plasma levels of endothelial markers or measured as endothelium-dependent dilation in systemic vascular beds is found in diabetic or hypertensive patients with microalbuminuria compared to normoalbuminemic counterparts. Furthermore, a direct inverse relationship has been found between systemic acetylcholine-induced dilation and microalbuminuria in diabetic patients. Finally, microalbuminuria is associated with increased permeability to albumin in peripheral vessels in both diabetics and clinically healthy subjects. Altered endothelium-dependent vasodilation could be also found in clinically healthy subjects with microalbuminuria as well as in subjects with asymptomatic proteinuria, e.g. in populations with minimal presence of other cardiovascular risk factors. At present, it remains unclear whether minor renal damage itself adds a new risk to the systemic vasculature or whether microalbuminuria represents just a reflection of a more severe, generalized cardiovascular phenotype. In support of the latter hypothesis, some authors suggest that generalized ED precedes the development of microalbuminuria, as the plasma levels of endothelial markers such as vWF are strongly related to de novo development of microalbuminuria in the follow-up of diabetic or healthy cohorts.

Only a small number of studies addressed the mechanisms responsible for selective contribution of increased urinary protein (albumin) excretion to systemic ED. Elliot et al. has found blunted vasoconstriction in response to the NOS inhibitor L-NAME in forearm vasculature of insulin-dependent diabetic patients with microalbuminuria as compared to normoalbuminuria, suggesting loss of NO-dependent vasodilation. In another study, Stehouwer et al. demonstrated that the presence of microalbuminuria was associated with impaired flow (NO)-mediated systemic vasodilation in elderly individuals with and without diabetes. Mechanisms responsible for potential NO deficiency in microalbuminuric patients might involve excessive oxidative stress. Giner et al. found the elevated levels of markers of peripheral oxidative stress in hypertensive patients with microalbuminuria compared to normoalbuminurics. Similar results were reported from diabetic patients. Recently, higher plasma levels of homocysteine were related with the occurrence of microalbuminuria in diabetic populations, rendering homocysteinemia a potential mechanism responsible for increased oxidative stress and ED in microalbuminuria. Interestingly, animal data suggest that systems other than NO might be affected by minor renal impairment. EDHF–mediated dilation is reduced in coronary arteries of Munich Wistar Fromter (MWF) rat model of spontaneous albuminuria. In yet another model of spontaneous renal disease, the Fawn-Hooded rat, slightly elevated urinary protein excretion at a young age has already been associated with a selective EDHF defect in systemic
resistance arteries. However, since in such animal models it is difficult to dissociate between the effect of renal impairment itself and concomitant hypertension, the altered EDHF-mediated relaxation in systemic vasculature related to minor renal injury will need to be further confirmed.

Several studies investigated whether markers of systemic ED are related to cardiovascular risk in microalbuminuric patients. Plasma markers related to ED, such as levels of VCAM-1 and homocysteine, predicted mortality in diabetic patients with microalbuminuria. However, no such prognostic value has been reported for endothelium-dependent vasodilation. Nevertheless, the relation between putative renal and systemic ED seems to be established and may help to explain the increased occurrence of cardiovascular complications in patients with minor renal injury.

**Renal endothelial dysfunction and the progression of renal damage**

It is generally agreed that ED might be crucially involved in the development of systemic end-organ damage associated with renal dysfunction. However, ED might have profound consequences for the progression of damage in the kidney itself. Proteinuria or microalbuminuria may be the reflection of elevated glomerular permeability, in which endothelium might be involved. It suggests that at least some aspects of endothelial dysfunction might be present in very early stages of renal disease progression. Microalbuminuria predicts the rate of progression of renal function loss in diabetic patients and general population, raising the possibility that renal ED is involved in the progression of renal disease. Interestingly, the conditions associated with increased risk of renal impairment, including hypertension, diabetes or aging are all characterized by impaired renal endothelial function both in humans and experimental animals.

Endothelial injury might play a prominent role in hemodynamic adaptations of the kidney to renal injury, which are responsible for the progressive nature of kidney damage. According to the most prevailing concept, remnant nephrons, e.g. those surviving the initial damaging event, adapt to the increased hemodynamic load by allowing higher intraglomerular pressure and hyperfiltration to compensate for reduced filtration. Glomerular hyperfiltration seems to represent the key event in the development of glomerulosclerosis, thus creating a vicious circle of additional nephron loss. Endothelium-derived NO has been long defined as a key regulator of glomerular pressure and renal perfusion. Administration of the NO synthase inhibitor L-NAME leads to severe glomerular hypertension, glomerulosclerosis, interstitial fibrosis and eventually renal failure in rats. On the other hand, administration of NO precursor L-arginine attenuates proteinuria and glomerular hypertension in experimental chronic renal failure induced by 5/6 nephrectomy. Interestingly, direct renal hemodynamic NO dysfunction has been scarcely addressed in chronic renal failure. Recently, Okuda et al. reported impaired endothelium-mediated vasodilation of afferent and efferent arterioles in subtotally nephrectomized dogs, which was attributed to the increased levels of endogenous NO synthase inhibitor ADMA due to lower activity of its degrading enzyme dimethylarginine.
dimethylaminohydrolase (DDAH). These data suggest that increased ADMA levels might represent the important mechanism of renal ED. However, reduction in other than hemodynamic properties of NO, including inhibition of mesangial growth and extracellular matrix production, limitation of leucocyte influx and adhesion, may importantly contribute to the progression of renal disease. Kang et al. highlighted the role of proliferative properties of glomerular microvascular endothelium in the progression of renal disease. They showed that L-NAME treatment lead to the loss of microvascular endothelium and loss of the endothelial proliferative response in 5/6 nephrectomized rats. Overall, although not precisely characterized, renal ED might crucially contribute to the development and progression renal end-organ damage.

**Renal endothelial function and variation in the susceptibility to renal damage**

Several authors have tried to linked endothelial damage in the kidney to progression of renal damage, recognizing renal ED as an early event in the renal pathophysiology. This has led to the hypothesis that renal endothelium is not only involved in the progression, but also in the susceptibility to renal damage. Interindividual differences in susceptibility to the development of renal disease are long recognized from clinical studies. The systemic factors including hypertension or diabetes represent the ultimate risk for renal damage, however the majority of patients with these disorders would never develop nephropathy. Additional evidence of the predisposition to renal disease is represented by clustering of renal disease with a specific racial and ethnic background. Comparably, specific animal strains spontaneously developing renal disease have been described suggesting a role of genetic components in susceptibility to CKD. Although the genetic background of CKD is a subject of intensive research, the involved genes have not been identified yet.

The role of the endothelium and/or NO in the predisposition to renal disease has been recently proposed by some investigators. An inbred strain of the Wistar-Furth rat is largely resistant to the development of renal damage after 5/6 nephrectomy or after puromycin aminonucleoside injection as compared to the widely used Sprague-Dawley strain. Low level of NOS inhibition with no impact on disease progression in the Sprague-Dawley rat, converted Wistar Furth rat into a model of rapidly progressive renal disease. Similar data were presented by using C57B16 mouse, a strain highly resistant to the development of nephrectomy-induced renal damage. In our laboratory, we addressed the role of endothelial function in the susceptibility to end-organ damage using different approaches. Not only the various inbred strains differ in their sensitivity to organ damage, but also the animals of the same outbred strain such as the Wistar rat. When renal damage is induced by a well-defined uniform damaging procedure, such as 5/6 nephrectomy or standard injection of adriamycin, individual animals develop renal disease of highly variable severity. Some healthy rats seem to be more susceptible to end-organ damage than others. To investigate the factors responsible for this variability, we measured endothelium-mediated vasodilation in small renal arteries of these healthy animals. Following these measurements renal damage was induced. Interestingly, nitric oxide-mediated vasodilatory ability of renal
arteries measured in healthy kidney inversely predicted the subsequent development of proteinuria and focal glomerulosclerosis after 5/6 nephrectomy and adriamycin nephrosis. The rats with more pronounced endothelial nitric oxide-mediated relaxation seem to be protected against end-organ damage. This is in agreement with the protective role of nitric oxide against the development of renal damage. However, many questions remain unanswered. We measured vasomotor endothelial function, but it is unclear whether the renal endothelium provides direct hemodynamic protection against renal damage, or whether it is just a reflection of other protective properties of nitric oxide. Support for the latter explanation comes from the fact that the predictive value of NO-mediated dilation is found in models with various etiology of renal disease, e.g. 5/6 nephrectomy with hemodynamic-mediated injury and adriamycin nephrosis with nephrotoxic agent-induced damage. Interestingly, recent data suggest that the predictive value of endothelial vasomotor function is found in yet another rat model, in which renal damage is induced by combined unilateral nephrectomy and myocardial infarction. The second important question is whether this predictive ability of renal endothelium may have a genetic basis. Polymorphisms of endothelial nitric oxide synthase have been associated with end-stage renal disease in several studies. Clearly, additional experiments need to be performed, however the current evidence point out that interindividual differences in endothelial function might account for the variability in the susceptibility to renal injury.

**Endothelial function and variability in renoprotective therapeutic response**

Recognizing the important role of ED in the development of both systemic and renal end-organ damage, the endothelium might represent a valid therapeutic target. A wide spectrum of treatments has been shown to improve ED in several conditions. However, it is very difficult to assess to which extent this improvement contributes to the prevention or regression of end-organ damage, since these beneficial agents often possess antihypertensive, antilipidemic or hypoglycemic properties thus additionally targeting traditional cardiovascular risk factors.

The beneficial effect of several interventions has been tested in patients with chronic renal failure. Interestingly, restoration of renal function, e.g. by transplantation, is associated with reversal of systemic ED. Dialysis might also acutely improve systemic ED, which could be attributed to removal of elevated ADMA or homocysteine levels. In contrast, other studies highlight dialysis-induced oxidative stress, which may eventually lead to impaired endothelial function. Decreasing oxidative stress by antioxidant treatment might improve endothelial function in some vascular beds. Attempts for NO bioavailability restoration either by L-arginine supplementations or by decreasing homocysteine levels with folic acid administration have led to disappointing results. Recently, the beneficial effect of cyclooxygenase inhibition has been reported. Additionally, in experimental settings, selective restoration of EDHF-mediated dilation was achieved by chronic angiotensin AT1 receptor blocker losartan. As previously stated, it is not known whether observed beneficial endothelial effects would translate to the reduction of cardiovascular risk. In this
respect, it is important to mention the results of the recent studies in hemodialysis patients, in which treatment with acetylcysteine\textsuperscript{101} and vitamin E\textsuperscript{102} were associated with a lower incidence of cardiovascular events, suggesting that targeting oxidative stress associated with endothelial dysfunction might prove a useful therapeutic strategy in chronic renal failure.

ED in the peripheral vasculature is also an emerging treatment target in patients with minute renal injury manifested as microalbuminuria. Several studies have been performed in diabetic patients, mainly investigating the effect of drugs interfering with the renin-angiotensin system, such as ACE inhibitors (ACEi) and angiotensin AT\textsubscript{1} receptor blockers (ARB). Although ACEi might improve some aspects of endothelial function in diabetic microalbuminurics, this is not always associated with reduction in microalbuminuria\textsuperscript{103-105}. Furthermore, reduction in microalbuminuria by ARB was not accompanied by improvement of endothelium-dependent vasodilation\textsuperscript{106}. Therefore, it seems that improvement of microalbuminuria is not always related to the improved systemic ED, at least in diabetic patients. The exact relation between renal and systemic ED will have to be defined, since lowering of microalbuminuria efficiently prevents cardiovascular events in both high\textsuperscript{107} and low risk populations\textsuperscript{108}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Renal endothelial function of healthy rat predicts the antiproteinuric effect of ACE inhibition.}
\end{figure}

Endothelium-dependent relaxation measured in small renal arteries of healthy animals (in arbitrary units of Area Under dose-response Curve to Acetylcholine- AUC) predicts the reduction in proteinuria after 10 weeks therapy with lisinopril (2.5mg/kg) preceded by 6 weeks of chronic renal disease induced by 5/6 nephrectomy.
Importantly, specific lowering of microalbuminuria translates in the reduction of renal events in several populations. Parving et al. showed that lowering of albuminuria with ARB irbesartan is dose-dependently associated with reduced progression to diabetic nephropathy in hypertensive type II diabetics independent of blood pressure control\textsuperscript{109}. This may also suggest that reversal of ED might play a role in the inhibition of renal disease progression. Many of the agents known to improve ED, including ACEi, ARB, vasopeptidase inhibitors and probably statins, have been shown to inhibit the progression of renal disease. Furthermore, all these agents have profound effects on renal hemodynamics thus potentially interfering with endothelium-dependent control of renal function.

Among the renoprotective treatments, blockade of renin-angiotensin system seem to provide superior protection as compared to other drugs. In patients, ACEi effectively reduce proteinuria, glomerulosclerosis and decline of renal function. The antiproteinuric effect of ACEi is a valid index of therapeutic efficacy since it predicts long-term effect on renal disease\textsuperscript{110}. Comparably to the rate of renal disease progression, the response to ACE inhibition is highly variable among individuals, suggesting that some patients are resistant while others are more sensitive to renoprotection by ACEi\textsuperscript{111}. Several factors have been implicated in this variability of therapeutic response, including variability in severity of systemic complications (e.g. hypercholesterolemia, hypertension, obesity, insulin resistance), volume status and/or genetic background. Given the potential role of endothelium in the therapeutic effects of ACEi, we studied whether variability in renal endothelial function represent a factor involved in variability of ACEi antiproteinuric response. In the experimental setting described above, we measured vasomotor endothelial function in healthy Wistar rats. The animals were subsequently subjected to 5/6 nephrectomy and developed severe renal damage within 6 weeks. From this time point on, the rats were treated by ACEi lisinopril during additional 10 weeks. Antiproteinuric response to ACEi was highly variable in these rats and was not related to the level of blood pressure or volume status. However, ACEi antiproteinuric response was strongly predicted by baseline endothelial function (Figure 2). The rats with more pronounced endothelial vasodilation were found to benefit less from ACEi\textsuperscript{112}. Therefore, in contrast to the predictive value of endothelial function for the progression of renal damage, better renal endothelial function was associated with less beneficial outcome. This suggests that individual variability in antiproteinuric response might be independent of the severity of renal damage and that specific endothelial effects of ACEi might be involved in their renoprotective effects. Indeed, ACEi lowers the level of oxidative stress thereby preventing reduction in NO bioavailability, and retards the degradation of bradykinin, an NO-releasing autacoid. In fact, angiotensin II-induced release of NO is an important physiologic mechanism to counteract the excessive renal vasoconstriction\textsuperscript{113}. The present results only allow speculation on the role of NO in predictive value of endothelial function for antiproteinuric effects of ACEi. It might be hypothesized that individuals with well-preserved endothelium (eventually NO) seem to be less dependent on activity of angiotensin II after the induction of renal damage and thus resistant to the beneficial effects.
of ACEi. Alternatively, the favorable effects of ACEi might be directly related to their NO-releasing ability. Individuals with saturated mechanisms of NO production might be less sensitive for additional ACEi-induced NO release. Clearly, more data on this subject are required, however at present it seems possible that variability in endothelial function among healthy individuals might provide prognostic information on the ACEi therapeutic response.

**Conclusions**

Endothelial dysfunction seems to be a crucial mediator of increased cardiovascular risk observed among patients with CKD. Importantly, systemic ED does not only occur in patients with severe renal failure, but also in the individuals with earlier stages of renal impairment. Close association between microalbuminuria and systemic ED renders renal vascular function an important marker for the severity of cardiovascular damage. Furthermore, changes in renal endothelium might be actively involved in the progression of renal end-organ damage. Recently, variability in endothelial function of healthy individuals emerged to predict the susceptibility to renal damage and the efficacy of renoprotective treatment (Figure 1). Therefore, a specific manipulation of renal and systemic ED might provide benefit in various stages of CKD. ED thus represents an ideal therapeutic target not only for treatment, but also for primary prevention of renal disease.
References


55. Stehouwer CD, Henry RM, Dekker JM, Nijpels G, Heine RJ, Bouter LM. Microalbuminuria is associated with impaired brachial artery, flow-mediated vasodilation in elderly individuals without and with diabetes: further evidence for a link between microalbuminuria and endothelial dysfunction--the Hoorn Study. Kidney Int Suppl 2004; S42-S44


Chapter 7

...oxic modulation of renal and cardiac hemodynamics in type 2 diabetes. Eur J Endocrinol 2002; 146:687-694


