

Differential Microvasculature Dysfunction in Living Kidney Donor Transplant Recipients: Nondialyzed versus Dialyzed Chronic Kidney Disease Patients

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Key Words

Vasocontraction • Endothelium-dependent relaxation • Arterial stiffness • Pulse wave velocity • Microcirculation

Abstract

We hypothesized that there was differential vasomotor dysfunction in the microcirculation between nondialyzed and dialyzed chronic kidney disease (CKD) patients. During live donor kidney transplantation procedures, skin arterioles (SkA; internal diameter = $120 \pm 5 \mu\text{m}$) from donors ($n = 27$) and recipients (nondialysis = 15; dialysis = 20) were dissected from the abdominal wall at the incision site. In vivo aortic pulse wave velocity (PWV) was also measured. In the in vitro isometric force measurement, nondialyzed SkA exhibited comparable contraction to donor SkA, whereas dialyzed SkA had 60 and 40–50% increase in contraction in response to depolarization and agonist (that is, phenylephrine, serotonin and endothelin-1) stimulation, respectively. The acetylcholine-induced relaxation in the nondialyzed SkA was decreased by 50% compared with dialyzed SkA. However, pre-incubation with superoxide dismutase greatly enhanced the relaxation response in the nondialyzed, but not in the dialyzed SkA and donor SkA. Pre-incubation with N^G -nitro-L-arginine methyl ester (L-NAME) elevated the resting

tension and left-shifted the concentration response curve of phenylephrine-stimulated contraction in the donor-SkA. L-NAME only increased the resting tension in the nondialyzed vessel. In vitro stiffness positively correlated with PWV ($R^2 = 0.302$, $p = 0.001$), and dialyzed SkA was 60% stiffer than nondialyzed and donor SkA. The acetylcholine relaxation was negatively correlated with PWV in donors and recipients ($R^2 = 0.282$, $p = 0.01$). In conclusion, we have uniquely demonstrated differential microvasculature dysfunction between nondialyzed and dialyzed CKD patients.

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Introduction

There is an increasing recognition that the adverse clinical outcomes associated with chronic kidney disease (CKD) are primarily due to cardiovascular disease. Both large and small vessel disease (such as diabetes mellitus and hypertensive nephrosclerosis) have been described in CKD [1, 2]. Furthermore, the processes of arteriosclerosis and atherosclerosis are both present in CKD, with the former often being more prominent than the latter. The presence of structural and functional alterations in the microcirculation could be considered as an important

link between renal failure and hypertension, ischemic heart disease, and heart failure.

The microvasculature acts on blood pressure propagation and is becoming more evident as the primary determinant of long-term vascular resistance [3, 4]. Functional and mechanical changes in microvasculature have been shown to be correlated with risk for cardiovascular morbidity and mortality [5]. Recent data have also suggested that alterations in small resistance artery morphology may represent the most prevalent and the earliest form of target organ damage at the cardiac level [6] in essential hypertension [7]. The endothelium, by secreting numerous vasoconstrictors or vasodilators, plays an important role in the control of vascular structure and tone [8]. Studies have described reduced endothelium-derived nitric oxide (NO) bioavailability as a contributor to increased arterial stiffness, and elevated pulse wave velocity (PWV) is associated with cardiovascular mortality in dialysis patients [9–11].

Despite the recognition that both macro- and microvascular diseases could be independent contributors to progressive kidney failure [10, 12], the investigation of vascular contractile and endothelial function in the microcirculation in different CKD populations has been limited. Chronic kidney disease has been shown to alter the mechanosensitive and agonist-induced responses of peripheral arterioles, in part by interfering with NO-signaling mechanisms [13, 14]. An increase in contractile response to norepinephrine and endothelin-1 has been demonstrated in isolated human uremic resistance arteries [14]. It has also been suggested that uremia treated with dialysis was not associated with altered sensitivity to agonists [15]. A number of studies which describe changes in contractile and endothelial function in microcirculation from different uremic models and vascular beds have been published [8, 16–18]; however, controversy remains, perhaps due to the different disease models and disease stages studied.

The present study was designed to investigate the impact of uremia/the presence of impaired kidney function on the functional and mechanical properties of isolated resistance arteries from CKD patients. We hypothesized that vasomotor dysfunction and arterial stiffening would be present in the microcirculation from CKD patients, and that these perturbations would be more severe in patients on dialysis compared with those not on dialysis. Using tissue from both donors and recipients who were undergoing living kidney transplantation, we concluded that the endothelium-dependent and endothelium-independent relaxation in resistance vessels was severely sup-

pressed in the nondialyzed patients. In contrast, the microvasculature in the dialyzed group showed increased contraction and stiffness. Uniquely, we correlated the *in vitro* measurements with *in vivo* measurements of vascular stiffness, and describe interesting inverse correlations with the endothelial function. All of these findings serve to increase our appreciation of the microvascular changes which occur in CKD.

Methods

Patient Recruitment and Study Design

Patients who were to undergo live donor kidney transplantation at the St. Paul's Hospital (Vancouver, B.C., Canada) were approached for participation in the study. The study was approved by the ethics board of Providence Health Care and the University of British Columbia. Written informed consent was obtained from donors and recipients prior to the surgery. They agreed to have small samples of skin segment dissected from the incision, and to undergo PWV measurements at the time of transplantation. Medical records were used to obtain demographic data as well as information regarding cardiovascular risk factors and renal function at the time of transplantation. The eGFR was calculated using the MDRD formula (table 1).

Preparation of Patient Samples

During live donor kidney transplantation, an ellipse of skin approximately 4–5 mm from the abdominal wall to a depth of 10–15 mm was dissected carefully from both donors and recipients. Skin segments were immediately placed into ice-cold RPMI solution in the presence of heparin and transported to the laboratory where it was placed in cold physiological Krebs' solution, pH of 7.4, continuously aerated with a 5% CO₂/95% O₂ mixture. A series of experiments were conducted to test the functional and mechanical characteristics of the vessels obtained.

Note that the number of patient data is included in each figure. Although the total number of subjects is 62, in some experiments there were missing data. This was because, given the nature of the tissue harvested from each patient, we were not able to dissect the same numbers of arterial segments from each individual. We had to take into consideration the healing and cosmetic result as well as the size of surgical scar. From some patients 8–10 segments were obtained, thus allowing us to perform all functional and stiffness measurement listed in the study. However, in those situations where only 2–3 segments were obtained, we selected specific experiments to perform. For example, we would perform KCl, phenylephrine (PE), 5HT contraction, acetylcholine (ACh) relaxation, N-nitro-L-arginine methylester (L-NAME) pretreatment and stiffness measurement. When only limited numbers of vessel segments were obtained, we were unable to perform angiotensin/endothelin contraction, because their effects were not readily reversible.

With respect to the PWV, some data are missing due to the fact that some patients were not available for the PWV measurement (that is, some patients are living in other cities) prior to transplantation surgery.

Table 1. Demographics and clinical features of the patients and living donors

	Donor (n = 27)	Nondialysis (n = 15)	Dialysis (n = 20)	p value
Female, %	70.4	26.7	45.0	0.28
Age, years	51 ± 2.3	53 ± 6.2	55 ± 3.0	0.75
Creatinine, μM	81.4 ± 3.73	423 ± 33.7	NA	NA
eGFR, ml/min/1.73 m ²	74.6 ± 2.59	13.6 ± 1.15	NA	NA
Diabetes, %	0	7	20	0.28
Hypertension, %	0	100	100	NA
Hyperlipidemic, %	0	47	25	0.19
Smokers, %	15	20	25	0.74
Calcium, mM	not collected	2.34 ± 0.04	2.39 ± 0.04	0.34
Phosphate, mM	not collected	1.66 ± 0.11	1.65 ± 0.12	0.95
PTH, pM	not collected	37.1 ± 7.6	39.3 ± 8.9	0.86
Hemoglobin	130 ± 2.6	112 ± 2.9	120 ± 3.0	0.07
Glucose, mM	5.1 ± 0.3	6.3 ± 0.4	8.2 ± 2.1	0.44
Cholesterol, mM	5.2 ± 0.5	4.8 ± 0.4	4.6 ± 0.8	0.79
LDL, mM	3.2 ± 0.4	3.1 ± 0.5	2.8 ± 0.8	0.77
Triglyceride, mM	1.2 ± 0.1	1.7 ± 0.2	1.9 ± 0.2	0.38
Length of dialysis, months	0	0	37.6 ± 9.7	NA
Previous kidney transplant, %	0	13.3	10.0	0.77
Months since GFR <30	0	78.4 ± 25.6	68.2 ± 10.8	0.69
Months since 1st nephrologist consultation	0	141 ± 32.0	104 ± 16.9	0.27
ACE inhibitor, %	0	33.3	15.0	0.21
ARB, %	0	13.3	20.0	0.62
β -Blocker, %	0	40.0	35.0	0.77
Calcium channel blocker, %	0	66.7	20.0	0.004*
Aspirin, %	3.7	26.7	50.0	0.17
Statin, %	0	46.7	25.0	0.19

p values show statistical differences between the living kidney donors and recipients using one-way ANOVA. * $p < 0.05$. NA = Not available.

Isometric Force Measurement

Resistance-sized skin arterioles (SkA; carefully dissected from the subcutaneous fat layer; internal diameter: $120 \pm 5 \mu\text{m}$) were mounted isometrically in a small vessel myograph (A/S Danish Myotechnology, Aarhus N, Denmark) for measuring generated force [19, 20]. Vessel segments were stretched for 20 min at which the resting force was stabilized, and then were challenged twice with 80 mM KCl before experiments. PE (1 nM to 300 μM), endothelin-1 (1–700 nM), angiotensin-II (1–30 nM) and serotonin (1 nM to 1 μM) were added cumulatively to generate concentration-response curves. To evaluate the endothelium-dependent relaxation, vessels were precontracted with 3 μM PE before making cumulative applications of ACh (0.01 nM to 100 μM). The percentage of relaxation compared to the maximal PE-induced contraction was recorded at different concentrations of ACh. The pEC_{50} values (negative logarithm of the concentration giving half the maximum response) were assessed for individual concentration-response curves by nonlinear regression analysis. Sodium nitroprusside (SNP; 0.1 nM to 10 μM) was added to the PE-precontracted vessels to assess the endothelium-independent relaxation. To reveal the endogenous NO production, vessels were pre-incu-

bated with L-NAME (200 μM) for 30 min before PE-induced contraction. To assess whether removal of superoxide affected relaxation, SkA segments were pre-incubated with superoxide dismutase (SOD; 150 units ml^{-1}) for 30 min before precontraction with PE (3 μM), which was followed by the cumulative concentrations of acetylcholine.

In vitro Arterial Stiffness Measurement

Measurements of vessel elasticity were derived from stress-strain curves [19]. Briefly, in a small vessel myograph, a 2-mm vessel segment was stretched by increasing the distance between the 2 stainless wires, and held at each length for 3 min. The procedure was repeated until the vessel was unable to maintain its tension. In the measurement of elasticity, strain increased exponentially as a function of the vessel diameter, eliciting a J-shaped curve. The equation of such an exponential growth is $Y = \text{start} \times \exp(K \times X)$, where Y is stress, X is strain and K is a rate constant, where Y increases exponentially. Steeper slopes indicate increased stiffness and the value of K reflects a numeric indicator of the stiffness of vessels.

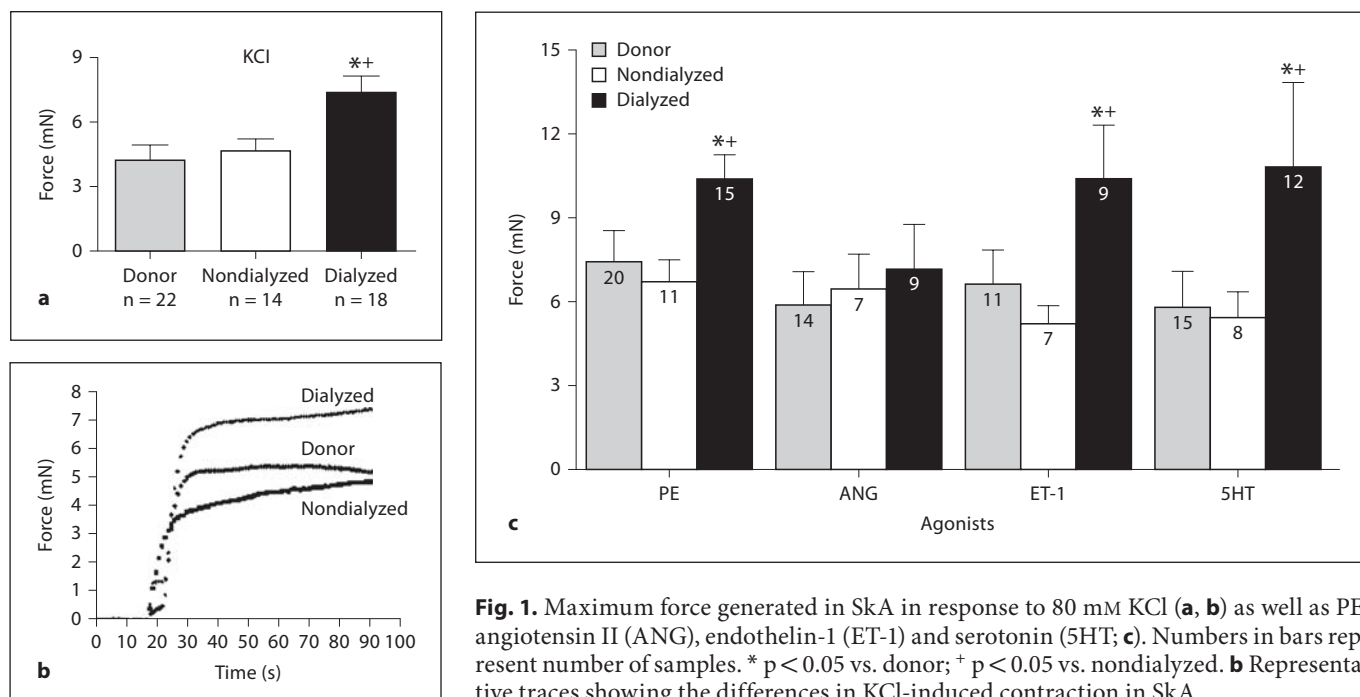


Fig. 1. Maximum force generated in SkA in response to 80 mM KCl (**a, b**) as well as PE, angiotensin II (ANG), endothelin-1 (ET-1) and serotonin (5HT; **c**). Numbers in bars represent number of samples. * $p < 0.05$ vs. donor; + $p < 0.05$ vs. nondialyzed. **b** Representative traces showing the differences in KCl-induced contraction in SkA.

In vivo PWV

All studies were conducted in a quiet, temperature-controlled room. After 15 min of supine rest, peripheral blood pressure was recorded. Using noninvasive methods, radial artery waveforms were obtained with a high-fidelity micromanometer (SPC-301; Millar Instruments, Houston, Tex., USA) applied at the radial pulse as previously described [21]. This generated an augmentation index, a composite measure of systemic arterial stiffness and wave-reflection amplitude or intensity (Sphygmocor; AtCor Medical, Sydney, N.S.W., Australia). Aortic (carotid to femoral) PWV was determined from measurements obtained from carotid and femoral pulses.

Statistics

Data were reported as mean \pm SD for normally distributed data (or median \pm SE for those with nonnormal distribution). Comparisons of unpaired data were performed using unpaired t tests for parametric data, one-way ANOVA for multiple groups and χ^2 tests for nominal data. Correlation plots were analyzed by linear regression; coefficient of determination was calculated from Pearson correlation. Statistical analysis and construction of concentration response curves were performed using GraphPad Prism software (version 4.03). Statistical significance was defined as $p < 0.05$.

Results

Patient Cohort

A total of 62 patients were studied, representing 27 donors and 35 recipients. All agreed to participate in the

study between November 2007 and November 2008. Of the recipients, 15 were nondialysis, 8 were receiving peritoneal dialysis and 12 were on hemodialysis at the time of transplantation and thus tissue harvest and study (table 1). Besides the use of calcium channel blockers, there was no significant difference between nondialyzed and dialyzed groups.

Enhanced Contractile Function in the Microcirculation from Dialyzed Patients

The vasoconstriction of SkA in response to KCl-induced depolarization and receptor-mediated stimulation were not significantly different between donor and nondialyzed groups. However, the depolarization response was markedly elevated in the dialyzed patients by 60% when compared with the nondialysis group (fig. 1a, b).

Although the sensitivity to PE (denoted as pEC_{50} values) was not significantly different among the 3 groups (donor = 6.41 ± 0.11 ; nondialyzed = 6.49 ± 0.28 ; dialyzed = 6.72 ± 0.13), the maximal force (E_{max}) induced by 3 μ M PE in the dialyzed SkA was increased by 47% compared with that of the nondialyzed group. Nevertheless, dialyzed SkA developed 50% more force in response to endothelin-1 and serotonin compared with the other 2 groups. However, the angiotensin II-stimulated contraction was not different among the 3 groups (fig. 1c).

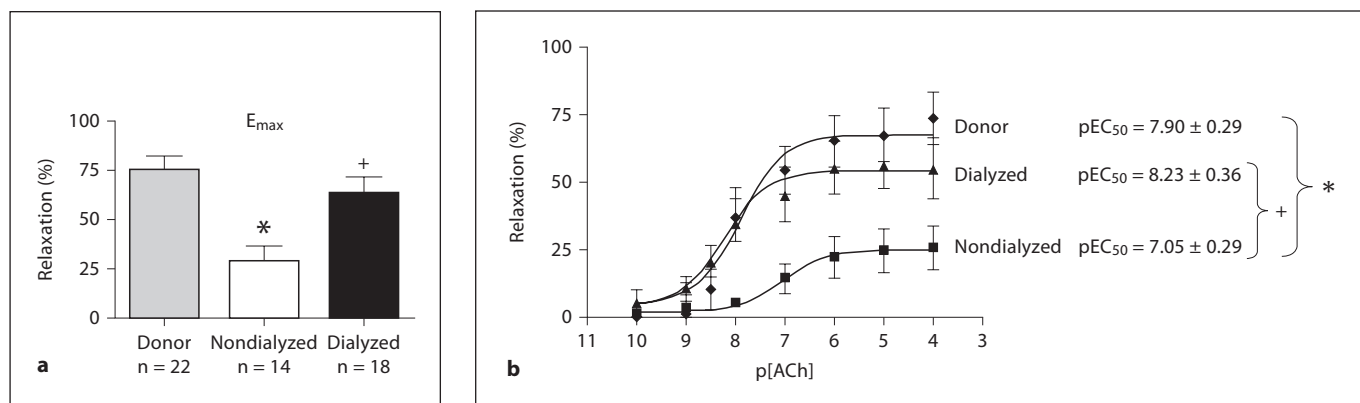


Fig. 2. **a** ACh-induced E_{max} in SkA precontracted with 3 μ M PE. * $p < 0.05$ vs. donor; + $p < 0.05$ vs. nondialyzed. **b** Concentration-response curves of ACh-stimulated relaxation in SkA. The pEC_{50} values of ACh in each group are given. * $p = 0.02$; + $p = 0.045$.

Reduced Endothelium-Dependent and Endothelium-Independent Relaxation in the Microcirculation from Nondialyzed Patients

The SkA from the dialyzed group had similar ACh relaxation response as that from the donors. The relaxation response was significantly ($p < 0.01$) decreased in the nondialyzed group, with a 50% reduction in the E_{max} compared with the other 2 groups (fig. 2a). Nevertheless, the sensitivity to ACh in the nondialyzed group ($pEC_{50} = 7.05 \pm 0.52$) was greatly reduced compared with dialyzed ($pEC_{50} = 8.23 \pm 0.36$) and donor ($pEC_{50} = 7.90 \pm 0.29$) groups, as indicated by the right shift of the ACh-concentration response curves (fig. 2b).

Impact of Exogenous NO

Endothelium-independent relaxation induced by the addition of SNP, an NO donor which bypasses endogenous NO production by endothelial cells, completely dilated PE-precontracted dialyzed and nondialyzed SkA. However, the pEC_{50} value of SNP was significantly reduced ($p < 0.01$) in the nondialyzed group, indicating that the nondialyzed SkA had reduced sensitivity to exogenous NO (fig. 3).

Impact of Superoxide Removal

Excessive amount of reactive oxygen species, termed oxidative stress, contributes to vascular dysfunction and has been suggested to be associated with CKD progression. To test if oxidative stress contributed to the impaired ACh relaxation, SkA was incubated with SOD, the major antioxidant responsible for superoxide removal in the vasculature [22, 23]. SOD greatly improved the ACh re-

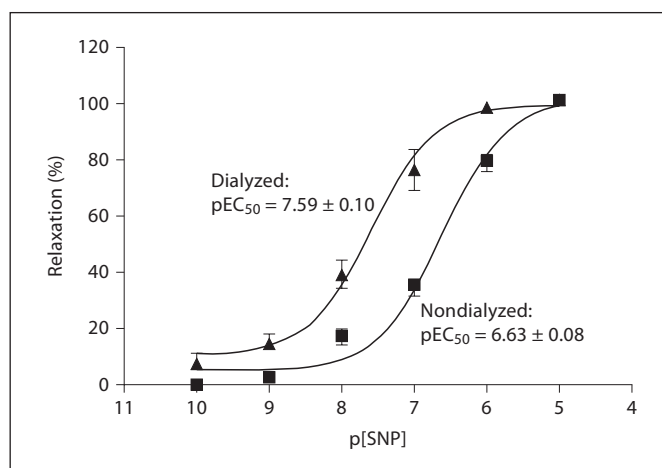


Fig. 3. Concentration-response curves of SNP-stimulated relaxation in nondialyzed and dialyzed SkA. The pEC_{50} values of SNP in each group are given.

laxation in the nondialyzed SkA, with a significant ($p < 0.05$) increase in both ACh E_{max} response and the pEC_{50} values (fig. 4a). Similar improvement was not observed in the dialyzed SkA (fig. 4b) and donor SkA (data not shown).

Impact of Endogenous NO Removal

To reveal the basal NO production and its impact on resting tension and agonist-induced contraction, vessels were pre-incubated with L-NAME, a specific inhibitor of NO synthase, before stimulation with PE. L-NAME pre-

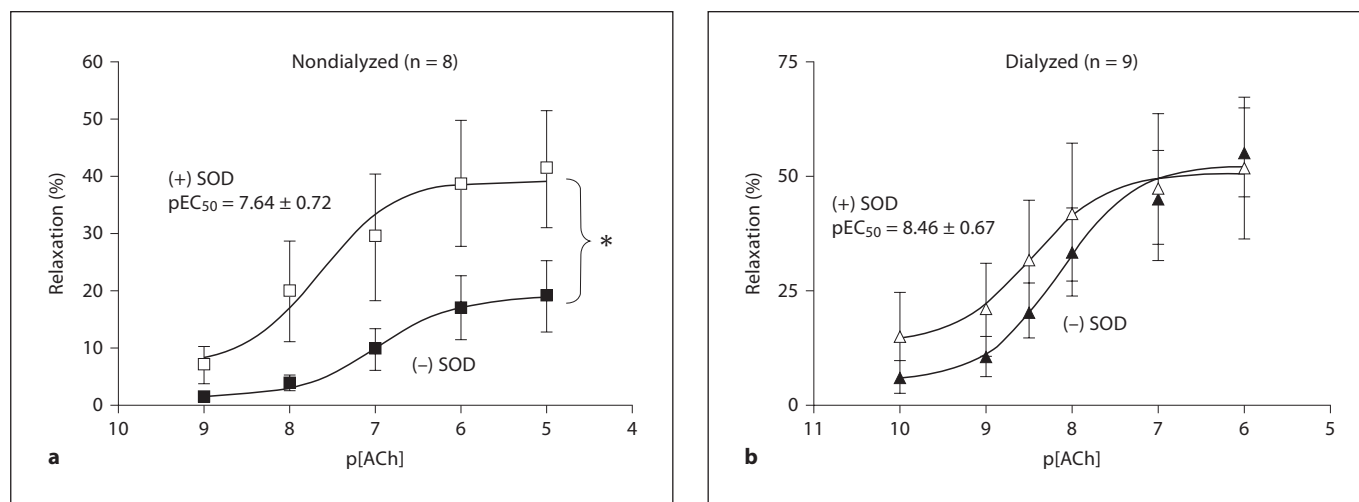


Fig. 4. Concentration-response curves of ACh-stimulated relaxation in the nondialyzed (**a**) and dialyzed SkA (**b**) with (+) or without (–) SOD incubation. * $p < 0.05$.

treatment significantly elevated the resting tension to 29.4 ± 7.9 mN in the donors ($p = 0.019$) and 42.17 ± 8.50 mN in the nondialyzed group ($p = 0.025$), but had no effect in the dialyzed group (from 2.17 ± 1.42 to 4.38 ± 2.81 mN; $p = 0.49$). L-NAME left shifted the concentration-response curve of PE-stimulated contraction in the donor group, and the EC₅₀ value was decreased by 2.4-fold (93.3 nM) compared with that in the absence of L-NAME (427 nM) ($p = 0.05$). However, in two recipient groups, L-NAME did not significantly alter the EC₅₀ values of PE ($p = 0.65$) (fig. 5).

Increased Stiffness in the Microcirculation from Dialyzed Patients

In the in vitro mechanical property measurement, from the stress-strain curves, we found that the K value indicating vessel stiffness of dialyzed SkA was 60% stiffer than that of the nondialyzed and donor groups (fig. 6a, b). Additionally, the in vitro K value of SkA positively correlated with the PWV ($R^2 = 0.301$, $p = 0.001$; fig. 6c).

Vascular Stiffness and Endothelium-Dependent Relaxation

NO-mediated endothelium-dependent relaxation in large artery is suggested to be important in regulating arterial stiffness [9–11]. In SkA, we studied the correlation between ACh response and PWV. In separated groups, the R^2 for nondialysis was 0.17 ($p = 0.16$), the R^2 for dialysis was 0.23 ($p = 0.09$) and the R^2 for donor was 0.44 ($p = 0.004$). However, attempting to elucidate the

validation of PWV in assessing endothelial function in our subjects (donors and recipients), we reported the R^2 from all subjects and the value is 0.28, $p = 0.01$, showing the significant negative correlation between the E_{\max} of ACh relaxation and PWV (fig. 7).

Discussion

By comparing recipients and their kidney donors, and examining human microvasculature, we are the first to clearly report that CKD patients have perturbations in functional and mechanical properties of the microcirculation. Four novel observations arose from this study: (1) contractile function in response to depolarization and agonist (that is, PE, serotonin and endothelin-1) stimulation was significantly elevated in the vessels of dialyzed recipients; (2) endothelium-dependent and endothelium-independent relaxation was impaired in the nondialyzed vessels; (3) SkA from dialyzed patients were stiffer than those from nondialyzed and donor groups; (4) endothelium-dependent relaxation was negatively correlated with vessel stiffness, and there was a significant correlation between in vivo and in vitro stiffness measurements. Thus, we have described a translational experiment which helps define microvascular changes in CKD and correlate those with composite measures of macrovascular stiffness in vivo.

The contractile function in response to membrane depolarization and agonist (that is, PE, serotonin and endo-

thelin-1) stimulation was markedly increased in the dialyzed SkA compared with that in the nondialyzed SkA, which demonstrated comparable responses with the donor (fig. 1). Since small vessels play an important role in regulating blood pressure and are sensitive to blood volume expansion, the increased sensitivity to membrane depolarization and agonist stimulation may be related to aberrant control of blood pressure and body fluid in the dialyzed patients. The increased plasma levels of vasoconstrictors, that is, endothelin-1, serotonin and norepinephrine, have been reported in end-stage CKD patients, which could result from decreased metabolic clearance rate, and stimulated synthesis caused by exaggerated sympathetic nervous activity, hypoxia, hemodynamic shear stress, increased blood pressure and other promoter cytokines present in uremia [24–32]. There was remarkable contraction in response to endothelin-1 (fig. 1c) [14, 33]; however, a reduced expression of endothelin-1 receptor has been suggested in the vasculature, probably resulting from the prolonged exposure to the excessive secretion of endothelin-1 [34]. The increased plasma, urinary and vascular levels of endothelin-1 were correlated with blood pressure and the degree of renal damage (proteinuria, creatinine and glomerular sclerosis) [32, 35]. The exaggerated endothelin-1 response has been suggested to be associated with elevated PWV, left ventricular hypertrophy, prevalence of atherosclerotic plaques and arterial intima-media thickening in CKD patients [26, 36]. In dialysis patients, the high serotonin response may implicate platelet and coagulation abnormalities, hypo- and hypertension [27], and the increased reactivity to serotonin could be due to its interaction with sympathetic nerves [33]. High circulating level of norepinephrine would predict adverse cardiovascular outcomes among hemodialysis patients and is strongly associated with left ventricular concentric hypertrophy and left ventricular systolic dysfunction [29, 30]. The comparable angiotensin II-induced contraction between donor and recipient groups agreed with the previous studies [37, 38], but it appeared to be conflicting since angiotensin II is believed to be one of the important vasoconstrictors causing hypertension in CKD. Mechanical forces including pressure and shear stress could regulate the production of angiotensin II [39]. As the hemodynamic shear stress may vary between healthy and CKD resistance arteries, we believed that it could affect the local angiotensin II level, receptor expression and its downstream vascular responses [40]. Nevertheless, since our recipient cohorts were well enough to undergo kidney transplantations, their cardiovascular risk burden was relatively lower

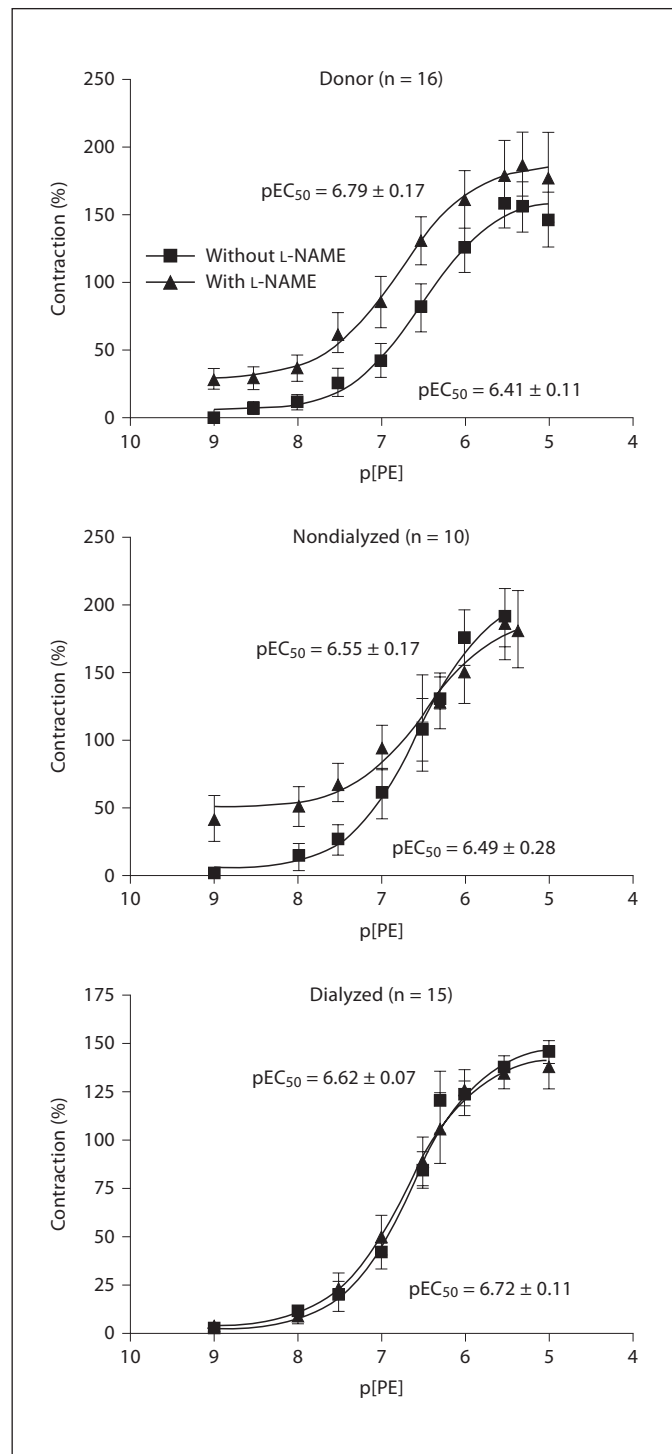


Fig. 5. Effect of L-NAME pretreatment on the concentration-response curves of PE-induced contraction in the SkA from donors, nondialyzed and dialyzed recipients.

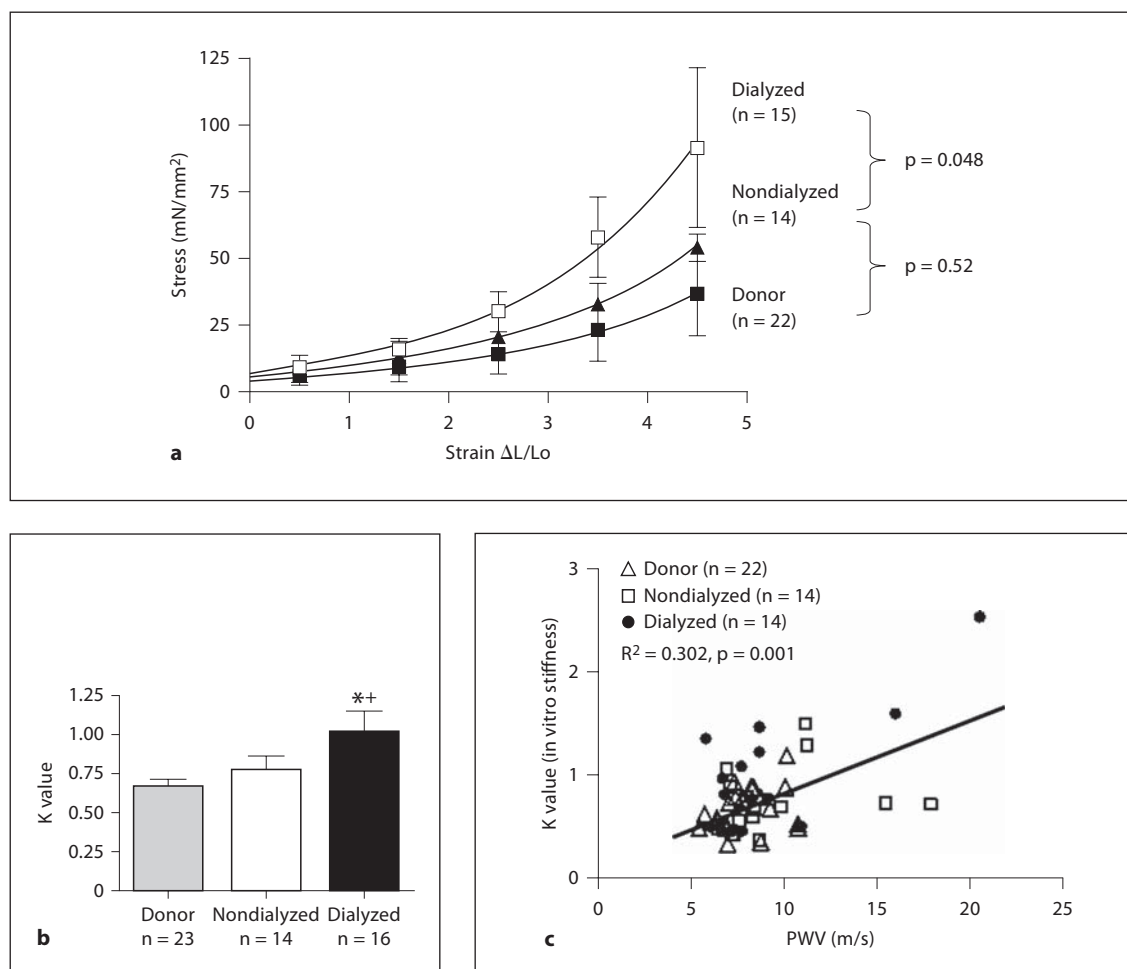


Fig. 6. In vitro and in vivo measurements of arterial stiffness. **a** Stress-strain curves of SkA. Equation of the exponential growth is $Y = \text{start} \times \exp(K \times X)$. Y increases exponentially with rate constant K. Stiffer vessel has larger slope and therefore larger K value. **b** Values of K from SkA. * $p < 0.05$ vs. donor; + $p < 0.05$ vs. nondialyzed. **c** Correlation between K value and PWV in all subjects.

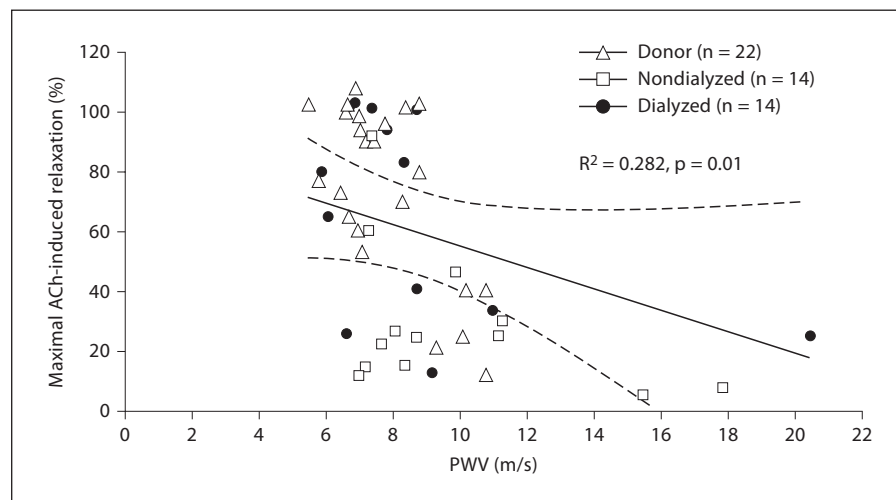
compared with the long-term dialysis patients. We speculate that their renin-angiotensin system could be well controlled by the use of various antihypertensive medications (table 1), resulting in no difference in the angiotensin II response among the 3 groups.

We assessed the bioavailability of NO in the microvasculature by ACh stimulation and L-NAME pre-incubation. The marked reduction in ACh-stimulated endothelium-dependent relaxation in the nondialyzed group (fig. 2) agreed with previous work which demonstrated a decreased relaxation to ACh in the cephalic veins from preemptive patients [41]. However, the elevation of resting tension in the nondialyzed SkA by L-NAME indicated the presence of basal NO in the microcirculation,

while the unresponsiveness to L-NAME in the dialyzed group suggested the absence of basal NO production (fig. 5). This differential NO bioavailability implicates the level of endothelial dysfunction and the deterioration of vascular health during CKD progression: from nondialysis to dialysis status.

Endothelial dysfunction has been consistently demonstrated in conduit vessels of patients with renal failure, with a smaller defect seen in the resistance vasculature [14]. However, a recent study demonstrated the prognostic role of endothelial dysfunction in resistance arteries from the population with high cardiovascular risk [5]. A noninvasive laser Doppler flowmetry functional testing of endothelium-dependent microvascular reactivity also

Fig. 7. Correlation between PWV and the maximum ACh-induced relaxation in the 3 groups. The correlation was significant in all studied subjects.



suggested that the microvascular reactivity offers a sensitive characterization of endothelial dysfunction [42]. We believe that further studies are required to assess endothelial function in individuals with GFR between 50 and 80 ml/min to determine the level at which endothelial dysfunction is induced.

The observation of normal endothelium relaxation in the dialyzed SkA is interesting and in conflict with current beliefs that CKD-5 patients have blunted/poor endothelial function. This apparent discordance can be explained in 3 ways. (1) Endothelial function is also modulated by shear stress, a factor dependent on flow that cannot be simulated in the current study. (2) In the nondialyzed group, calcium channel blockers are the preferred medication. Several studies have demonstrated that the effect of different antihypertensive drugs on vascular structure is not the same, with those interfering with the renin-angiotensin system being more effective [43, 44]. (3) It has been shown that physiological flow is reduced in the skin microcirculation of uremics, and this abnormality is rapidly corrected by hemodialysis [45]. Besides, dialysis could remove dimethyl L-arginine and N(G)-monomethyl-L-arginine, an inhibitor of NO synthesis, and thereby improve endothelial relaxation [46].

The impaired endothelium-dependent relaxation in the nondialyzed SkA, interestingly, could be improved by incubation with SOD (fig. 4a). Excessive amount of reactive oxygen species, termed oxidative stress, would contribute to vascular dysfunction in diabetes, heart failure, hypercholesterolemia, hypertension and chronic renal disease. Endothelial function has been shown to be improved by antioxidants, such as SOD, melatonin as well

as vitamins C and E [22, 23]. Accumulation of oxidative stress is caused by an imbalance between the production and neutralization of reactive oxygen species. SOD is the major endogenous antioxidant responsible for superoxide removal. Oxidative stress may cause endothelial dysfunction through several direct and indirect pathways, the most well-known of which is the scavenging of NO by superoxide. Superoxide radicals bind to NO at a rate 3 times faster than they bind to SOD; therefore, excess superoxide production would increase the rate of NO degradation [23]. This also explained the reduced sensitivity to SNP-stimulated relaxation in the nondialyzed SkA as the exogenous NO would readily be neutralized by the augmented amount of reactive oxygen species.

Although endothelial dysfunction in nondialyzed patients may be reversed by dialysis, the increased stiffness (fig. 6a, b) involving the long-term modification of vascular remodeling may not be readily reversed. There is a pronounced intimal thickening and enhanced calcification of the internal elastic lamella in uremic patients, and these intrinsic degenerative changes of the arterial wall reduce vessel distensibility [47]. The elevated peripheral vascular stiffness and impaired endothelial function may increase cardiac afterload and further impair cardiac function in CKD patients. In addition, arterial stiffness is characterized by very steep volume-pressure relationship and is associated with hemodynamic instability in dialyzed patients. This explains that small blood volume increase could produce abnormally high pressure while small decrease in blood volume could cause hypotension.

We also demonstrated the statistically significant correlation between the in vitro assessment of small vessel

stiffness and the in vivo large vessel PWV (fig. 6c). The weak correlation could be due to the fact that the PWV was used to measure the stiffness of large muscular artery, while the in vitro stiffness as indicated by the K value was performed on the skin resistance-sized artery. Irrespective, our additional finding that PWV was negatively related to endothelial function in vitro (fig. 7) supports the current practice that PWV measurements are useful tools by which to assess vascular health [21].

We acknowledge the limitations of this study. (1) All recipients are hypertensive. That clustering of cardiovascular risk factors may have synergistic deleterious effects on the vasculature deserves further investigation [5]. (2) Although the types of dialysis, that is, peritoneal and hemodialysis, could contribute to different clinical outcomes [48], in this study we combined peritoneal and hemodialysis for emphasizing the alterations in microvasculature during CKD progression. (3) Both the nature of the population studied (transplant eligible) and the cross-section design may bring some bias. Since it has been shown that reduced endothelial function is a predictor of cardiovascular events in dialyzed patients, it is possible that dialyzed patients with severe microvascular endothelial dysfunction had already died and the dialyzed patients of this study were survivors. Such a selection bias can explain the seemingly favorable results on the endothelial function from the dialyzed recipients presented herein.

In conclusion, using human tissue, we demonstrate the differential aberrations in vascular function between 2 groups of CKD patients, those exposed and not exposed to dialysis, but both well enough to receive kidney transplantations. The findings raise questions as to the possible impact of dialysis treatment on the microvascular function. The optimal timing of preemptive transplantation remains problematic: these results add interesting insights into vascular health in these patients that may impact on future perspectives. In clinical practice, monitoring the integrity of microcirculation may be helpful, though technically difficult, to assist in diagnosis, risk stratification and monitoring disease progression. The ability to investigate the microvascular structure and function is important in improving our understanding of pathophysiological processes in CKD and the related cardiovascular disease.

Acknowledgments

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