Impaired resistance artery function in patients with end stage renal disease

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TITLE PAGE

Title: Impaired resistance artery function in patients with end-stage renal disease

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ABSTRACT

We investigated an effect of uraemia on structural and functional features of human resistance vasculature. Arteries (≈200 μm) isolated from subcutaneous fat biopsies obtained from 35 end-stage renal disease (ESRD) patients starting peritoneal dialysis and 30 matched controls were studied using isolated small artery bioassays.

Flow-mediated dilatation was attenuated in ESRD patients vs. controls. Nitric oxide (NO) contribution to flow was lacking in ESRD patients, but present in the controls. Asymmetrical dimethyl L-arginine (ADMA) levels were higher in the ESRD group vs. control group. Dilatation to acetylcholine was reduced in ESRD patients vs. controls, but response to NO-donor was similar. Expression of nitrotyrosine and heat shock proteins 70 and 27, but not 90, was increased in arteries from ESRD patients vs. controls. Arterial remodelling was absent in ESRD patients. There was no difference between the groups in myogenic tone, vascular reactivity or sensitivity to several vasoconstrictors. Arterial distensibility, reflecting passive properties of the vascular wall, was reduced in ESRD patients vs. controls. Exclusion of ESRD patients with diabetes and/or cardiovascular disease from analyses had no influence on the main findings.

Thus, uraemia has a strong impact on endothelial function and passive properties of the arterial wall of human peripheral resistance vasculature. The reduced contribution of NO to flow stimulus via enhanced nitrosative stress and higher plasma concentrations of ADMA may confer potential mechanisms behind endothelial dysfunction in the resistance peripheral circulation in ESRD.

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INTRODUCTION

Despite the rapid progress in dialysis treatment, end-stage renal disease (ESRD) patients still die at a markedly accelerated rate, principally from cardiovascular disease (CVD) and infectious complications [1–2]. Multiple mechanisms mediate the elevated risk for cardiovascular events in ESRD, which are complex and involve changes of the whole cardiovascular system. Endothelial dysfunction is a common phenomenon in ESRD, which constitutes an obligatory prodromal phase in cardiovascular complications [3–4]. However, current evidence for endothelial dysfunction in renal disease patients mainly relies on measurements of circulating biomarkers [2] and *in vivo* assessment of endothelial function in larger vessels [5–6]. In addition, studies on vascular structure in ESRD patients have mainly concentrated on larger and medium-size arteries [7–8]. This leaves unaddressed the issue of whether and/or to what extent functional and structural abnormalities may occur concurrently in the resistance circulation. Resistance vessels generally refer to small arteries with diameters ranging from 100 to 300 μm, which are directly involved in the control of blood flow to target organs and peripheral resistance. Abnormalities in this circulation will directly contribute to the development of hypertension, which is common in ESRD patients.

Current evidence that renal diseases affect endothelial function of resistance arteries has been derived mainly from animal studies [9–10]. Only Morris *et al.* [11] reported a reduced agonist-induced endothelium-dependent dilatation in small arteries isolated from patients with chronic renal failure. The technique of venous occlusion plethysmography has also been used [12–13], suggesting an impairment of the nitric oxide (NO) component in the abnormal dilatation to acetylcholine (ACh) in the forearm circulation [14], reflecting, though, the combined responses, not only of the resistance vasculature, but also of conduit arteries and venous circulation [15]. To date, however, in ESRD patients there has been no study to directly assess resistance artery response to a stimulus of flow, which is recognised as the most important physiological regulator of endothelial function and NO release. Furthermore, there has been no study that combines assessment of several parameters that confer resistance artery maintenance in ESRD.

In the present study we hypothesized that ESRD patients will exhibit several abnormalities at the level of resistance circulation via altered dilatory and constrictor responses, as well as via occurrence of structural changes. The impaired endothelial function will be conferred by changes in NO bioavailability. All these abnormalities will contribute to an increased peripheral resistance in patients with ESRD.

We tested this hypothesis by investigating isolated, pressurized subcutaneous resistance arteries from ESRD patients and healthy controls under conditions representative of an *in vivo* physiological performance. Responses to physical forces created by intraluminal flow and pressure as the most relevant physiological stimulators for the resistance artery tone were investigated. Furthermore, passive properties of the vascular wall (distensibility), remodelling process, as well as the contribution of NO to flow responses, were studied. Wire myography technique was implemented to test vascular reactivity/sensitivity to a number of pharmacological vasoconstrictor agonists and the role of NO in these responses was also assessed. Finally, in these vessels we analysed by immunohistochemistry the expression of heat shock proteins (HSPs) 27, 70 and 90, which, it has been suggested, reflect endothelial stress [16–18] and expression of nitrotyrosine [19] to link endothelial abnormalities to a pro-oxidative phenotype. We also measured the endogenous inhibitor of NO-synthase (NOS) asymmetrical dimethyl L-arginine (ADMA) that has been introduced as an additional cardiovascular risk factor in ESRD [20].

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METHODS

Participants

A subcutaneous fat biopsy was obtained from 35 ESRD patients (25 males; median age 58 yrs, range 22–79 yrs) at the time of peritoneal dialysis catheter insertion. In order to establish homogeneity, only patients starting dialysis treatment *de novo* were included. Exclusion criteria were clinical signs of acute infection, active vasculitis or liver disease at the time of evaluation. Control tissue was obtained from 30 age- and gender-matched healthy volunteers (23 males; median age 54 yrs, range 29–74 yrs) without documented renal, cardiovascular, mental or diabetic disease, who underwent hernia repair or laparoscopic cholecystectomy. All subjects gave informed consent, and the ethics committee at Karolinska University Hospital, Stockholm, Sweden, approved the protocol.

Baseline Laboratory and Clinical Assessments

Clinical history of CVD or diabetes was obtained from medical records. CVD was defined as the presence of ischemic cardiac disease, peripheral vascular disease and/or cerebrovascular disease. Glomerular filtration rate was estimated by the mean of creatinine and urea clearances in ESRD, whereas cystatin-C was used to estimate glomerular filtration rate in controls. Fasting venous blood samples were taken for generation of plasma and serum and stored at –70°C pending further analyses. Serum interleukin-6 was measured on an Immulite® analyser (Siemens Medical Solution Diagnostic, Los Angeles, CA, USA). Circulating ADMA was assessed in serum using commercial ELISA assays (DLD Diagnostika GMBH, Germany). Serum concentrations of albumin (by the bromocresol purple method), creatinine, blood lipids and high-sensitivity C-reactive protein were measured by routine procedures at the Department of Clinical Chemistry at Karolinska University Hospital-Huddinge.

At the time of surgery, a subcutaneous fat biopsy (≈2 × 1.5 × 1.5 cm) was removed from the anterior abdominal wall and immediately placed into cold physiological salt solution (PSS). Arteries with an internal diameter of ≈200 μm were dissected. Depending on the number of arterial segments obtained from the fat biopsy specimen, one or several experiments (flow-mediated dilatation, agonist-induced responses, pressure-induced tonus, distensibility index) were performed.

Pressure myography

The arterial segments were oriented to mimic the direction of flow *in vivo* and mounted between two glass microcannulae in a pressure myograph chamber (Living Systems Instrumentation Inc., USA). Intraluminal pressure (60 mmHg) was maintained by a servo-controlled pump. The dimensions of the cannulated artery (internal diameter and wall thickness) were continuously monitored via a video dimension analyser. The organ bath was superfused with ≈37°C PSS gassed with 5% CO_2 in O_2. Each artery was equilibrated for 60 min. The viability tests were carried out by examining the responses to norepinephrine (NE, 1 μmol/l), and endothelial function was confirmed by relaxation to ACh (1 μmol/l).

Assessment of vascular function: flow-mediated dilatation

Arteries from 27 ESRD patients and 22 controls were used to investigate flow-mediated dilatation. After the equilibration period, the intraluminal pressure was gradually increased from 60 to 80 mmHg for arteries with an internal diameter >200 μm, and the internal diameter was recorded after 20 min. In contrast, for arteries with an internal diameter <200 μm, intraluminal pressure was permanently kept at 60 mmHg. A flow response curve to increasing intraluminal flow by stepwise increase from 0 to 180 μl/min every 3 min was performed on the preconstricted artery to ≈50% of the initial diameter. In a separate experimental setup

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including arteries from ESRD patients (n = 9) and Controls (n = 9), flow response curves were obtained before and after incubation with NO synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine-methyl ester (L-NAME) (300 μmol/l, 30 min).

Agonist-induced responses

Endothelium-dependent response to ACh was tested in 13 ESRD patients and 9 controls, whereas endothelium-independent dilatation to sodium nitroprusside (SNP) was performed in 12 ESRD patients and 10 controls. Equilibrated arteries at 60 mmHg were superfused with NE (1μmol/l) for 20 min in order to induce a stable constriction. ACh (3 nM–1 μmol/l) or SNP (0.1 μmol/l –0.1 mmol/l) were added in NE-contained PSS and concentration-response curves were assessed. Each concentration of the agonist was extraluminally perfused for 3 min, while the changes in the diameter were constantly recorded.

Pressure-induced myogenic tone

The response to changes in pressure was evaluated in arteries from 26 patients with ESRD and 24 controls. After a 20 min artery equilibration at 20 mmHg, intraluminal pressure was gradually increased up to 120 mmHg. Internal artery diameter was recorded after each pressure increment, which was maintained for 5–7 min in order to reach a steady state diameter. Thereafter, PSS was replaced with PSS without Ca^{2+} (Ca^{2+}-free PSS) to determine the passive diameter curve in response to stepwise pressure increase.

Wire myography

The subcutaneous arteries were mounted on a 4-chamber Danish Myotechnology M610 wire myograph, as described elsewhere [21]. Cumulative concentration-response curves were constructed for phenylephrine (selective agonist of α_1-adrenergic receptors, 10 nmol/l – 0.03 mmol/l), NE (non-selective agonist of adrenergic receptors, 10 nmol/l – 0.03 mmol/l), angiotensin II (Ang II, 0.1 – 30 nmol/l) or endothelin-1 (ET-1, 0.1 – 30 nmol/l) before and after incubation with the NOS inhibitor L-NAME (300 μmol/l, 30 min).

Immunohistochemistry

Cryosections (7 μm thick) of subcutaneous arteries were immunostained with antibodies against HSPs 90, 70, 27 and nitrotyrosine. Antibody concentrations were 10 μg/ml for anti-HSP90, 0.66 μg/ml for anti-HSP70, 5 μg/ml for anti-HSP27 and 20 μg/ml for anti-nitrotyrosine. Negative controls with 0.1% Tween in 3% phosphate buffered saline (PBS) and in PBS without primary antibodies were used.

Chemicals and solutions

The composition of PSS (mmol/l) was NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, ethylenediamine-tetraacetic acid 0.026, and glucose 5.5; pH 7.4. Relaxing solution was Ca^{2+}-free PSS supplemented with papaverine (0.1 mmol/l) and ethylene glycol-bis-(β-aminoethyl ether) tetraacetic acid (1 mmol/l). NE was dissolved directly in PSS, whereas for ACh, phenylephrine, Ang II, ET-1 and SNP stock solutions were prepared in distilled water and further dissolved in PSS. All chemicals were obtained from Sigma-Aldrich Sweden AB (Sweden). Monoclonal mouse antibodies used for HSP27 (ab8600), HSP70 (ab6535), HSP90 (ab1429) and nitrotyrosine (ab7048), and secondary biotinylated polyclonal goat anti-mouse IgG antibody (ab6788) were all obtained from Abcam plc (UK, Cambridge).

Calculations and statistical analysis

Relaxation to flow, ACh and SNP were calculated as a percentage change in internal diameter in response to stimulation, divided by the difference in internal diameter before and after

preconstriction. Wall shear stress (τ, dyn/cm^2) was calculated using the Hagen-Poiseuille formula: $\tau = 4 \times \eta \times Q \times 10^9 / \pi r^3$, where η is viscosity of the perfusate (poise – dyn s/cm^2), Q is flow rate (μl/s) and r is artery radius (μm). The factor of 10^9 in the equation is to correct the use of μl/s for flow and μm for artery radius (1μl = 10^9 μm^3). Viscosity of PSS was assumed as 0.007 poise at 37°C. Cross-sectional area (CSA) was calculated as: $CSA = (\pi/4) \times (D_e^2 - D_i^2)$, where D_e and D_i are external and internal diameters, respectively. Wall lumen ratio was defined as: wall thickness / D_i. Pressure-induced myogenic tone was calculated as: *Myogenic tone (%)* $= 100 \times (D_{i\ Ca^{2+}\text{-free PSS}} - D_{i\ PSS}) / D_{i\ Ca^{2+}\text{-free PSS}}$, where D_i is the internal diameter of the artery. Distensibility index was calculated as: *Distensibility index (%)* $= (D_i - D_0) \times 100$, where D_i is the internal diameter in Ca^{2+}-free PSS at different steps of pressure and D_0 is the internal diameter in Ca^{2+}-free PSS at 5 mmHg.

In tables and figures, results are expressed as the mean ± standard error of the mean, and the mean ± standard deviation or median and range, as appropriate. Baseline characteristics of the patients and arteries used were analysed by conventional parametric and non-parametric methods as appropriate. Differences in artery responses between patients and controls were determined by two-way repeated measures analysis of variance (ANOVA). The semi-quantitative analysis for immunohistochemistry staining intensity was assessed by the average blind score of three different observers, using a scale between 0 and 3, where 0 was absence of staining and 3 corresponded to maximal intensity. In addition, a computer image analysis software (ImageJ), available at http://rsb.info.nih.gov/ij/index.html, was used for quantitative analysis of immunostaining [22–23]. The image area, including vascular wall and lumen on each cross-section, was outlined manually and proportional staining (%) within the marked area was quantified. The examiner was blinded and the threshold level for detection was based on ability to distinguish the specific target staining vs. the non-specific background. For a valid comparison between different images, the threshold levels were maintained similarly. The results were evaluated with Mann-Whitney U test for non-parametric comparisons. All calculations and statistical analyses were performed using STATISTICA (ver. 8.0, StatSoft, Uppsala, Sweden,). All comparisons were considered statistically significant if $P < 0.05$.

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RESULTS

Participants

Baseline clinical and laboratory characteristics of patients in the ESRD group, ESRD sub-group (ESRD patients without CVD and/or diabetes mellitus) and controls enrolled in the study are shown in Table 1. Age, gender, and smoking status were similar between groups. The body mass index was lower in ESRD group and sub-group versus controls. Whereas inflammation markers (interleukin-6 and high-sensitivity C-reactive protein) and ADMA were elevated in the ESRD group and sub-group, total cholesterol was significantly lower in the ESRD group vs. controls, but similar between the ESRD sub-group and controls. No significant difference in blood pressure was observed between the groups.

Vascular Structure

Baseline characteristic of pressurized subcutaneous resistance arteries used in the functional study are summarized in Table 2. The arteries from all groups had similar diameters, wall thickness, wall-lumen ratio and cross-sectional area.

Vascular Function

Flow-mediated dilatation was significantly attenuated in the ESRD patients (Figure 1a), and increments of intraluminal flow led to a much steeper rise in wall shear stress values compared to the control group (Figure 1b). Incubation with a NOS inhibitor, L-NAME, did not affect flow-mediated dilatation in arteries from ESRD patients (Figure 2a). In contrast, L-NAME significantly reduced flow-mediated responses in arteries from controls (Figure 2b) to a level comparable to that obtained in arteries from ESRD before NOS inhibition (Figure 2a).

ACh and SNP induced a robust, concentration-dependent dilatation in both ESRD and control arteries. Whereas a significant decrease in endothelium-dependent dilatation to ACh was observed in the ESRD group versus controls (Figure 3a), the SNP-induced dilatation was similar between the groups (Figure 3b). There was no difference in myogenic tone between the two groups (Figure 4a) or in vascular reactivity or sensitivity to Ang II, ET-1 or agonists of adrenergic receptors (Table 3). Moreover, NOS inhibition had no effect on vascular reactivity or sensitivity to the above-mentioned agonists in isolated arteries from either group studied (Table 3). In contrast, arterial distensibility was significantly reduced in ESRD patients in comparison to controls (Figure 4b).

In order to eliminate the possible interference of co-morbidities, ESRD patients without CVD (based on clinical history from medical records) and/or diabetes mellitus were divided in a sub-group and compared with controls. Briefly, we observed similar results as above (Figure 5). Also, no difference in myogenic tone (e.g. at 120 mmHg 13 ± 3% in ESRD, n = 14 vs. 12 ± 2% in controls, n = 24) or SNP response (e.g. % maximum relaxation at 0.1 mmol/l of SNP: 61 ± 7 in ESRD, n = 7 vs. 69 ± 7 in controls, n = 10) was observed between the groups.

Heat shock proteins and nitrotyrosine expression in the vascular wall

The intensity of immunohistochemistry staining for HSP27, HSP70, HSP90 and the oxidative stress marker nitrotyrosine within the vascular wall of resistance subcutaneous arteries was compared between ESRD patients and controls. Both the semi-quantitative and computer imaging analysis (Table 4) showed higher expression patterns of HSP27, HSP70 and nitrotyrosine in arteries from the ESRD group vs. controls (Figure 6).

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DISCUSSION

To the best of our knowledge, our *ex vivo* study is the first to extensively characterize changes present in the peripheral resistance vasculature in ESRD patients. Here, we show for the first time that endothelium-dependent dilatation to flow-mediated shear stress is impaired in the resistance arteries from patients with ESRD. The obvious absence of NO contribution to flow response in the ESRD group contrasts with data obtained in controls, in which NO played a predominant role. The presence of endothelial dysfunction in the resistance vasculature of the ESRD subjects was further strengthened by a reduced dilatation to ACh, but by preserved response to a NO donor. Moreover, vascular distensibility was also impaired in arteries from the ESRD group compared to controls. The renal failure had, however, no effect on contractile responses to Ang II, ET-1, NE and phenylephrine, or on pressure-induced myogenic tone and remodelling process. Increased expressions of nitrotyrosine and heat shock proteins 70 and 27, but not 90, were observed in artery walls from the ESRD group in comparison with controls. Elevated plasma levels of ADMA were found in ESRD patients vs. controls. Exclusion of ESRD patients with diabetes and/or CVD had no impact on experimental outcomes.

Blunted flow-mediated dilatation and the lack of NO contribution to this response in human peripheral resistance vasculature extend and complement previous reports, but in other vascular beds [6,11–14,24], further emphasizing the general state of endothelial dysfunction and the significant role of NO in this high-risk patient group. Our study also suggests potential mechanisms behind small artery dysfunction in ESRD. Thus, the increased expression of nitrotyrosine in arteries from the ESRD group may imply the enhancement of free radical production towards a pro-oxidant environment and NO degradation. The elevated circulating levels of ADMA in ESRD patients will favour decreased NO production.

Similar responses to a number of pharmacological agonists tested, as well as similar pressure-induced myogenic tone between arteries from ESRD and control groups, argue against the altered vascular smooth muscle function in resistant arteries from patients with ESRD. However, alterations in the passive properties of the arterial wall (reduced distensibility) strengthen a link between ESRD and increased vascular stiffness [25–26]. Since exclusion of patients with diabetes and/or CVD had no impact on experimental outcomes, it is likely that uraemia *per se*, and not existing co-morbidities, is the main cause of endothelial dysfunction and altered passive properties of the resistance vasculature. Thus, our findings may contribute to the elucidation of mechanistic links between uraemia and vascular abnormalities.

Wall shear stress is the main determinant of flow-mediated dilatation, which in small arteries is an important physiological regulator of tissue perfusion [27]. In our study we characterized the effects of increases in intraluminal flow on artery diameter by calculating wall shear stress. In controls, the rise in flow slightly increased wall shear stress. The increments in flow rate resulted in the substantial increases in lumen diameter that prevented further enhancement of wall shear stress, thus providing a functional adaptation to rapid changes in flow rate [10, 27]. ESRD patients, however, demonstrated a compromised adaptation, as the increments in intraluminal flow increased shear stress values continuously, due to impaired dilatory response. This may suggest that the efficacy of shear stress (higher wall shear stress values for the same flow rate) as a dilatory stimulus is impaired in the uraemic environment.

In *ex vivo* set-up, shear stress values, as a function of changes in flow, are usually assessed at constant fluid viscosity. When extrapolating our data to an *in vivo* situation, it should be emphasized that shear stress depends on blood viscosity, which is directly related to haemoglobin concentration. Therefore, abnormalities in flow-mediated responses at the level of resistance arteries may be further amplified *in vivo* by anaemia that is well known to worsen as kidney disease progresses. Taken together, we postulate that in ESRD the reduced

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efficiency of shear stress to induce relaxation, as presented in our study, along with anaemia-related low blood viscosity present in the *in vivo* situation, may further impede small artery wall 'sensitivity' to this physiological stimulus. Indeed, abnormal shear stress values in the brachial artery *in vivo* have been found in ESRD patients, and anaemia correction led to improved flow responses and enhanced arterial wall sensitivity to this mechanical stimulation [6].

The significantly reduced response to flow after NOS inhibition occurred in arteries from controls, further supporting the importance of NO in flow-mediated dilatation of isolated resistance arteries from healthy volunteers [28–29]. In ESRD arteries, however, the contribution of NO to flow responses was lacking. This observation concurs with evidence that so far has only been reported in animal studies [9–10], and is indirectly supported by *in vivo* investigations in ESRD patients [20,30–31].

Considering the complexity of NO deficiency in uraemia [32], we suggest that changes in NO bioavailability merits particular attention. Based on the enhanced pattern of nitrotyrosine staining (marker for peroxynitrite formation) in the resistance artery wall and elevated levels of circulating ADMA (endogenous inhibitor of NO production), our study suggests that decreased bioavailability of NO could serve as a potential mechanism behind impaired flow-mediated dilatation in resistance vasculature of ESRD patients. Our suggestion is further strengthened by *in vivo* studies showing negative relationships between markers of oxidative stress [24], ADMA and flow-mediated dilatation in ESRD subjects [33]. In addition, the similar expression of HSP90 in the vascular wall of ESRD patients and controls argues against NO deficiency via uncoupling of endothelial NOS (eNOS) caused by HSP90 insufficiency [18]. However, limited availability of other co-factors may also contribute to eNOS uncoupling, with a subsequent decrease in NO signalling and an increase in eNOS-derived superoxide generation [34]. Finally, despite the observation of higher expression of HSP70 and HSP27 in the arterial walls of the ESRD group, their contribution to impaired endothelial function is not yet clear and as such requires further investigation. However, some evidence suggests that increased expression of HSP 70 and 27 might reflect a compensatory role against endothelial dysfunction [16–17].

Despite existing debate as to whether myogenic tone abnormalities may serve as a cause or a consequence of elevated blood pressure [35–36], we proposed that enhanced pressure-dependent myogenic constriction would contribute to the elevated peripheral resistance in ESRD. However, even if a slight tendency existed for enhanced myogenic tone in ESRD patients, our study did not support this. The observed preservation of myogenic tone concurs with the previous animal report on uraemic hypertension [37]. It might be anticipated that treatment for blood pressure control could also normalize the level of myogenic tone. This suggestion is supported by a number of studies on hypertensive animals after anti-hypertensive treatment [38–39]. However, we cannot exclude the possibility that increased peripheral resistance *in vivo* take place by other means in patients with ESRD. For example, enhanced levels of local and circulating endothelium-derived vasoconstrictors, such as ET-1 [40], Ang II [41] or the activated sympathetic nervous system [42] could amplify the myogenic tone via constriction. Conversely, our data on wire-mounted arteries indicated similar constriction in response to ET-1, Ang II and agonists of adrenergic receptors, indicating that increased local concentrations of vasoconstrictors, rather than changes in sensitivity of the arterial wall to them, could be involved in ESRD. Our data is in line with a previous report on subcutaneous arteries that showed similar response to vasoconstrictors between controls and chronic intermittent dialysis patients [43], but is in contrast with the study by Morris *et al.* [11], in which a more sustained constriction at highest concentrations of ET-1 and NE were observed in ESRD. The reasons for difference between studies are largely unknown and further studies are warranted.

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As the internal diameter, wall thickness, wall-lumen ratio and cross-sectional area did not differ between ESRD patients and controls, our study fails to show vascular remodelling in the resistance subcutaneous arteries from patients with ESRD. Although our observation agrees with one very early report on isolated subcutaneous arteries in advanced human uraemia [43], it opposes the observed vascular remodelling in the microcirculation of the heart [44]. Clearly, further studies are needed to evaluate whether regional differences are operative in this process. The lack of remodelling in ESRD arteries could be attributed to antihypertensive treatment, as several studies have provided direct evidence of a corrective effect of different antihypertensive drugs on structural parameters of resistance arteries [45]. Because the correction of wall structure by antihypertensive treatments has been shown to be accompanied by normalization of myogenic tone [39, 46], the simultaneous absence of remodelling and preserved myogenic tone in our ESRD patients seems to be logical.

Since the elastic properties of the vessels in this study were tested *ex vivo* (without flow and active tone in maximally dilated condition), the decreased distensibility is directly related to the vessel wall composition, which is determined by extracellular matrices, such as collagen and elastin [47]. Although the mechanisms by which this occurs are less well understood, the renal diseases may exert detrimental effects on collagen and elastin functionality, particularly when an increased exposure to glycation and oxidation products is demonstrated [48–49]. This may lead to the loss of fibre flexibility, altered conformation and elevated susceptibility to enzymatic digestion [50]. The decreased distensibility of the resistance vasculature and the proposed modifications of the passive elements of the vascular wall may also contribute to vascular complications upstream and serve as a possible common pathway to explain the cardiovascular risk linked to ESRD [7].

The present study should be considered with the following caveats: although we have studied a relatively homogenous group of incident dialysis patients, the findings regarding remodelling and myogenic tone should be considered with caution, due different pharmacological treatments. Since direct vascular effects of anti-hypertensives could not be ruled out, it should be noted that these drugs may reverse the remodelling process and reduce myogenic tone [51–53]. Moreover, as the presence of CVD was based solely on clinical grounds, the true prevalence of CVD in these patients may have been underestimated. A larger patient cohort would possibly have permitted further associations between biochemical markers and functional and structural findings in isolated resistance arteries that would allow us to gather new insights into possible mechanisms. The future aim would be to extend the investigations in the same cohort, but in other resistance vascular beds, particularly to those of critical importance like coronary and cerebral circulations, or to larger elastic and muscular arteries.

In summary, our e*x vivo* study provides new insights into how renal failure affects human resistance artery function. We show for the first time that uraemia primarily targets endothelial maintenance via impaired response to flow-mediated shear stress and absence of NO contribution to this stimulation, as well as via blunted dilatation to endothelium-dependent agonist. Overall, these findings further highlight the general state of endothelial dysfunction in ESRD. The changes in NO bioavailability via enhanced degradation and/or reduced production may serve as a potential mechanism behind endothelial dysfunction in the peripheral resistance circulation in patients with ESRD. Renal failure also affects passive properties of the vascular wall (reduced distensibility), which strengthens an association between ESRD and increased arterial stiffness. Such changes in the peripheral resistance circulation will predispose this patient group to increased cardiovascular risk. Therefore, association between hypertension, cardiovascular diseases, cerebral ischemic attacks and renal failure might be mediated, at least partly, by functional alterations at the level of microcirculation.

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AUTHOR CONTRIBUTIONS

Study concept and design: K Kublickiene, P Stenvinkel, JJ Carrero, N Luksha, L Luksha
Experimental part: N Luksha, L Luksha, K Kublickiene, JJ Carrero, F Hammarqvist
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FIGURE LEGENDS

Figure 1. Flow-mediated dilatation (a) and wall shear stress (τ, b) in norepinephrine pre-constricted resistance subcutaneous arteries from end-stage renal disease (ESRD) patients and controls. The number of participants in the groups is indicated in parenthesis. * $P < 0.05$.

Figure 2. Flow-mediated dilatation in physiological salt solution (PSS) and after incubation with N^{ω}-nitro-L-arginine-methyl ester (L-NAME) in norepinephrine pre-constricted resistance subcutaneous arteries from end-stage renal disease (ESRD) patients (a) and from controls (b). The number of participants in the groups is indicated in parenthesis. * $P < 0.05$.

Figure 3. Concentration response curves to acetylcholine (a, ACh) and sodium nitroprusside (b, SNP) in resistance subcutaneous arteries from end-stage renal disease (ESRD) patients and controls. The number of participants in the groups is indicated in parenthesis. * $P < 0.05$.

Figure 4. Pressure-induced myogenic tone (a) and distensibility (b) in response to changes of intraluminal pressure in resistance subcutaneous arteries from end-stage renal disease (ESRD) patients and controls. The number of participants in the groups is indicated in parenthesis. * $P < 0.05$.

Figure 5. Flow-mediated dilatation (a), wall shear stress (b, τ), concentration response curves to acetylcholine (c, ACh) and distensibility (d) in resistance subcutaneous arteries from end-stage renal disease (ESRD) patients without a clinical history of diabetes mellitus and/or cardiovascular disease vs. controls. The number of participants in the groups is indicated in parenthesis. * $P < 0.05$.

Figure 6. Immunohistochemistry of heat shock protein 27 (A, B, C, E), 70 (D, F, G, H) and nitrotyrosine (I, J, K, M) and negative control (L) in resistance subcutaneous arteries from end-stage renal disease (ESRD) patients and controls. The areas denoted by the boxes are magnified and presented in separate pictures (C, D, E, F, K, M). All images are ×40; scale bar: 50μm.

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Figure 1.

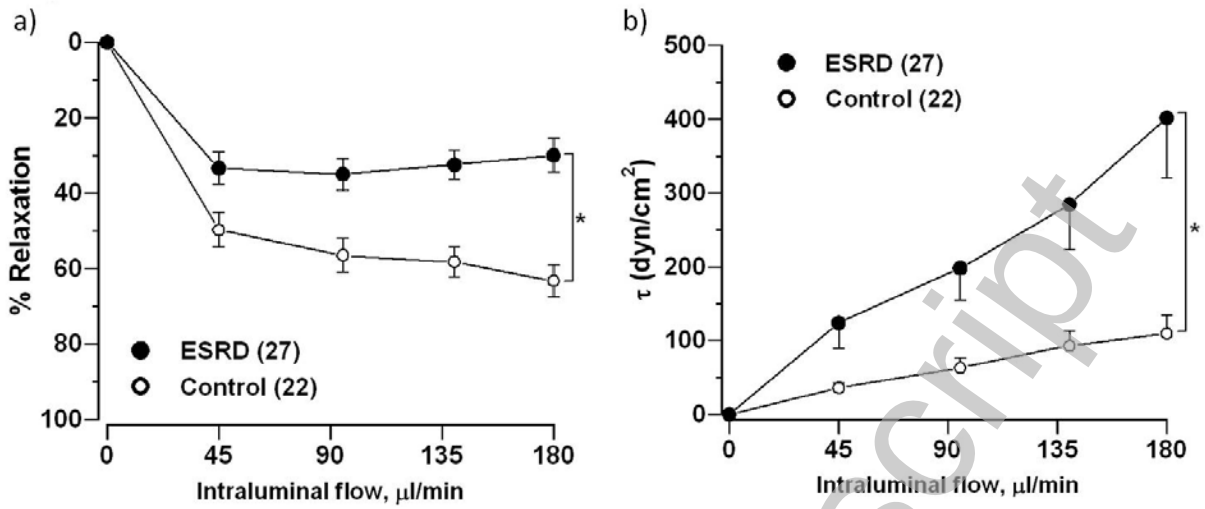

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Figure 2.

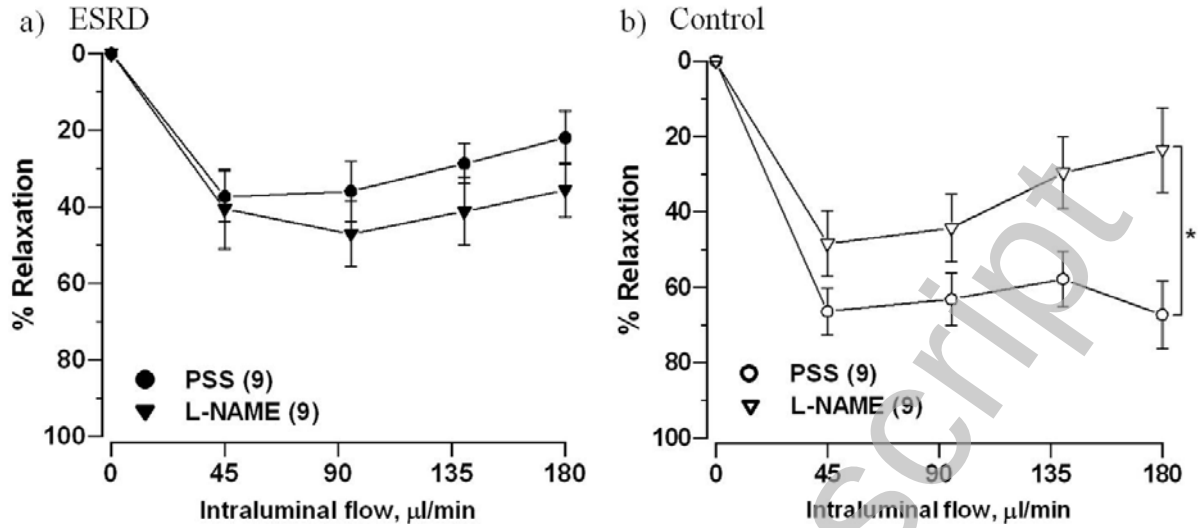

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Figure 3.

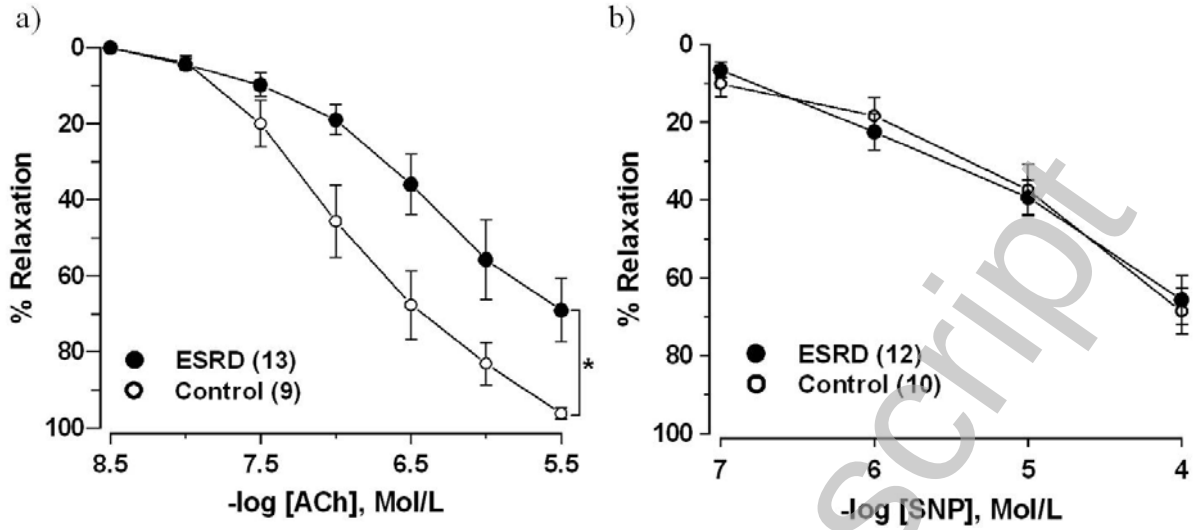

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Figure 4.

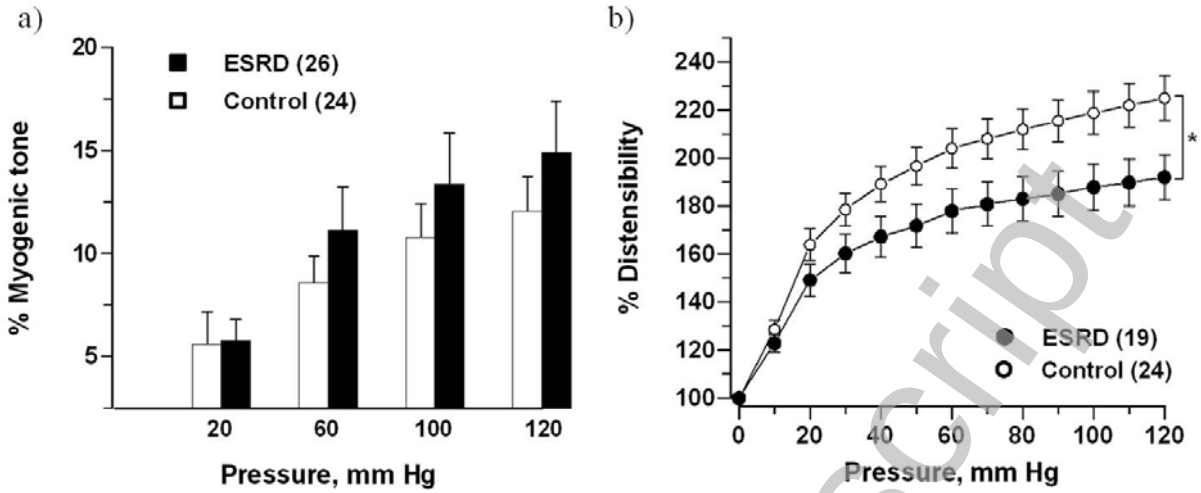

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Figure 5.

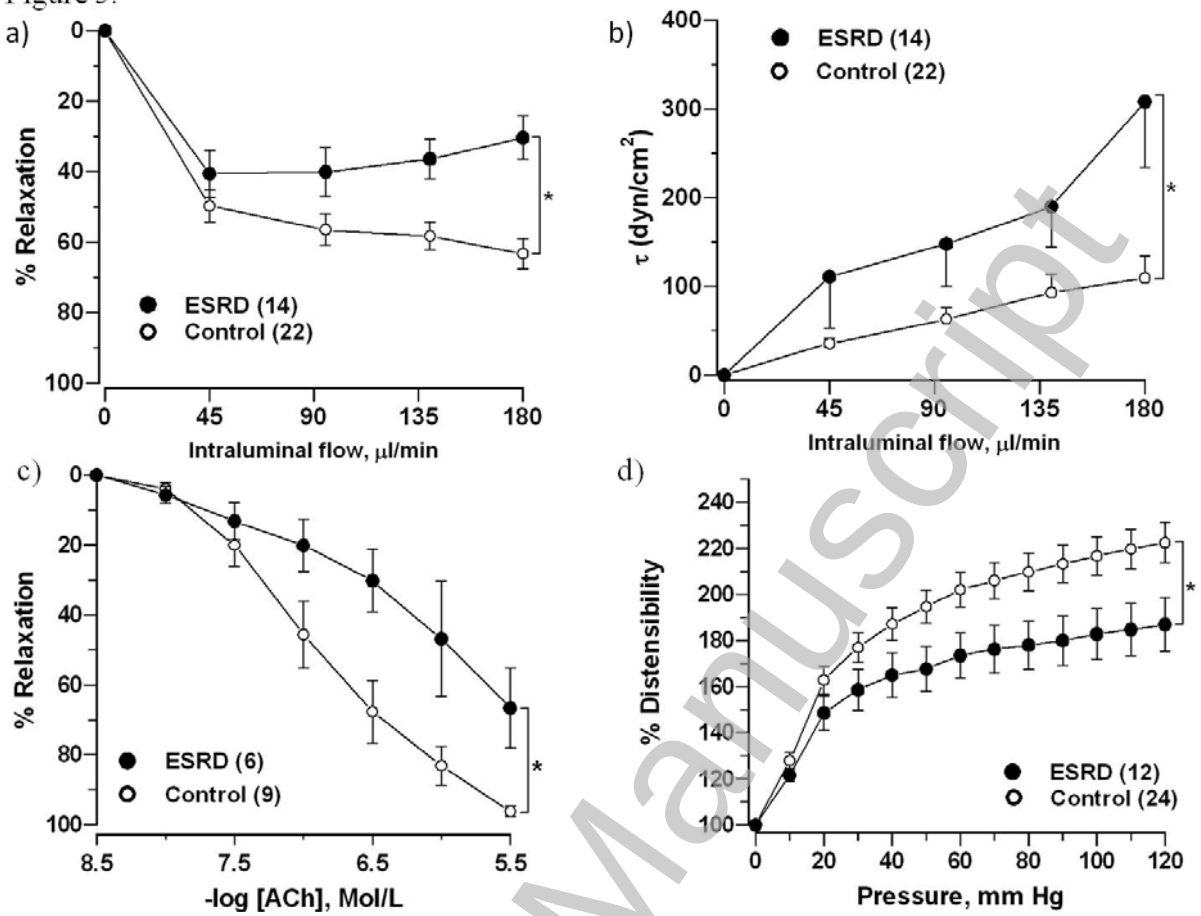

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Figure 6.

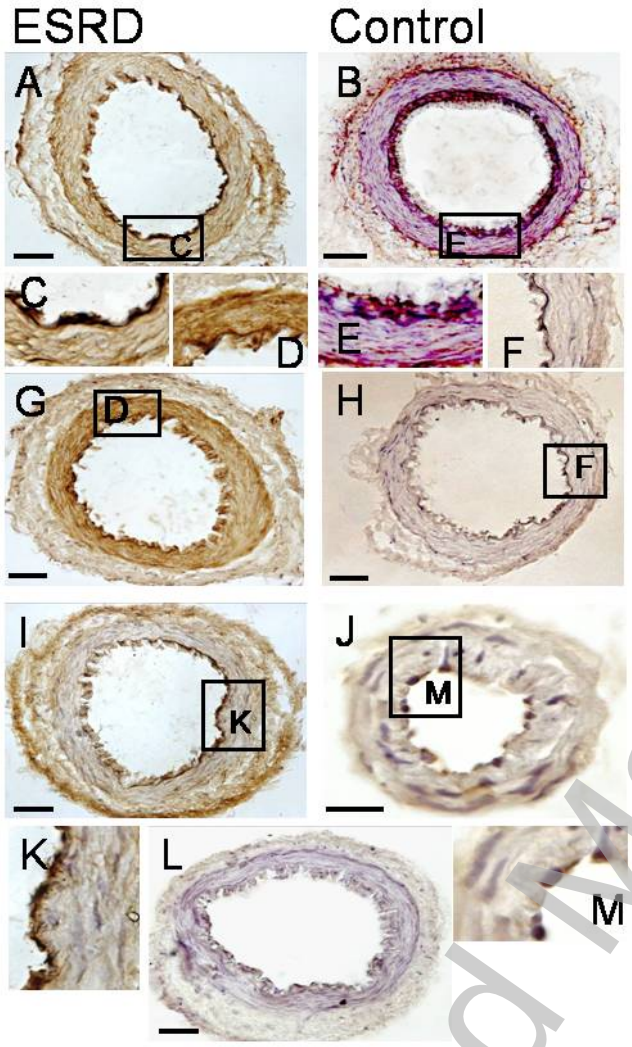

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Table 1. Baseline characteristics of end-stage renal disease (ESRD) patients with cardiovascular disease (CVD) and/or diabetes mellitus (DM) (ESRD group), ESRD patients without co-morbidities (ESRD sub-group) and matched controls.

Parameters	ESRD group (n = 35)	ESRD sub-group (n = 14)	Controls (n = 30)	*P*-value
Age (years)	58 ± 13	54 ± 17	56 ± 12	NS
Males, % (n)	71 (25)	57 (8)	77 (23)	
BMI (kg/m^2)	25 ± 3	24 ± 3	27 ± 4	<0.01
SBP (mmHg)	145 ± 20	139 ± 14	140 ± 16	NS
DBP (mmHg)	86 ± 9	85 ± 11	86 ± 10	NS
Biochemical parameters				
Total cholesterol (mmol/l)	4.6 ± 1.2	5.0 ± 1.1	5.4 ± 1.0	<0.05/NS*
Triglycerides (mmol/l)	1.6 (0.7–5.6)	1.5 (0.8–3.7)	1.3 (0.7–19)	NS
S-albumin (g/l)	35 ± 4	35 ± 3	39 ± 3	<0.05
S-Creatinine (µmol/l)	649 (249–1 069)	688 (440–1 069)	80 (55–105)	<0.001
Haemoglobin (g/l)	111 ± 13	108 ± 17	142 ± 12	<0.001
IL-6 (pg/ml)	6.9 (2.6–31.7)	6.9 (2.6–11.9)	2.7 (1.4–18.1)	<0.001
hsCRP (mg/l)	5 (1.0–24.0)	4 (1.0–24.0)	1.4 (0.5–42.0)	<0.001
GFR (ml/min)	12 ± 3	11 ± 3	89 ± 5	<0.001
ADMA (µmol/l)	0.56 ± 0.1	0.56 ± 0.1	0.49 ± 0.1	<0.05
Calcium (mmol/l)	2.3 ± 0.2	2.3 ± 0.1	2.3 ± 0.1	NS
Phosphate (mmol/l)	1.8 ± 0.5	1.9 ± 0.7	1.0 ± 0.2	<0.001
U-albumin (mg/24h)	804 (6–5 985)	401 (6–5 985)	Not determined	
Co-morbidities				
DM, % (n)	29 (10)	0	0	
CVD, % (n)	40 (14)	0	0	
Treatments				
Antihypertensives % (n):	91 (32)	91 (32)	0	
β-blockers, % (n)	60 (21)	60 (8)	0	
Ca-blockers, % (n)	51 (18)	29 (4)	0	
ACE-inhibitors, % (n)	80 (28)	86 (12)	0	
Statins, % (n)	40 (14)	14 (2)	0	
Erythropoietin, % (n)	83 (29)	93 (13)	0	

Mean ± standard deviation indicated for normally distributed variables. Median (range) indicated for variables not normally distributed. n, number of patients in the groups. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; hsCRP, high-sensitivity C-reactive protein; IL-6, Interleukin-6; GFR, glomerular filtration rate; β-blockers, beta-adrenergic antagonists; Ca-blockers, calcium channel antagonists; ACE-inhibitors, inhibitors of angiotensin-converting enzyme; ADMA, asymmetrical dimethyl L-arginine; NS, not significant.

P values indicate comparison between ESRD group vs. Controls and ESRD sub-group vs. Controls

* $P < 0.05$: ESRD group vs. Controls / NS: ESRD sub-group vs. Controls.

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Table 2. Baseline characteristics of pressurized resistance arteries from end-stage renal disease (ESRD) patients with cardiovascular disease (CVD) and/or diabetes mellitus (DM) (ESRD group), ESRD patients without CVD and/or DM (ESRD sub-group) and matched controls.

Parameters	ESRD group (n = 27)	ESRD sub-group (n = 14)	Controls (n = 27)	*P*-value
Internal Diameter (μm)	178 ± 8	175 ± 12	177 ± 10	NS
Passive diameter in Ca^{++} free PSS (μm)	186 ± 8	186 ± 12	186 ± 11	NS
Wall thickness (μm)	47 ± 2	44 ± 2	54 ± 2	NS
Wall lumen ratio	0.29 ± 0.02	0.12 ± 0.02	0.37 ± 0.04	NS
Cross-sectional area (μm^2)	14 952 ± 1 160	14 130 ± 1 417	17 417 ± 1 383	NS

Data are expressed as the mean ± standard error of the mean.
NS, not significant.

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Table 3. Potency of vasoconstrictor agonists and maximum constriction of resistance arteries from ESRD patients and controls.

Agonist	ESRD			Controls		
	ID	pEC_{50}	Max.response	ID	pEC_{50}	Max.response
NE	215 ± 13	6.7 ± 0.2	84 ± 6 (7)	199 ± 11	6.6 ± 0.2	77 ± 7 (11)
NE, L-NAME	195 ± 17	6.8 ± 0.4	89 ± 5 (6)	214 ± 17	7.1 ± 0.3	73 ± 10 (7)
PE	216 ± 15	5.7 ± 0.1	71 ± 13 (8)	214 ± 15	5.9 ± 0.2	79 ± 11 (10)
PE, L-NAME	184 ± 18	5.5 ± 0.3	78 ± 6 (6)	215 ± 27	5.8 ± 0.2	74 ± 6 (5)
Ang II	226 ± 18	8.7 ± 0.2	61 ± 9 (8)	199 ± 11	9.2 ± 0.2	79 ± 8 (10)
Ang II, L-NAME	178 ± 20	8.2 ± 0.5	70 ± 10 (5)	186 ± 16	9.0 ± 0.1	72 ± 8 (6)
ET-1	197 ± 16	8.7 ± 0.2	110 ± 5 (8)	192 ± 16	8.7 ± 0.2	103 ± 7 (10)
ET-1, L-NAME	187 ± 14	8.7 ± 0.1	118 ± 5 (6)	195 ± 11	8.9 ± 0.3	106 ± 8 (9)

Data are expressed as the mean ± standard error of the mean (SEM). The number of patients in the groups is indicated in parenthesis. ID, internal diameter (μm) of relaxed arteries at pressure 100 mmHg; pEC_{50}, negative logarithm of molar concentration of agonist, which is required to induce 50% of the maximum response; Max. response, contractile response of arteries to maximally used concentration of agonists expressed as a percentage of constriction to high K^+, 124 mmol/l; L-NAME, responses after NO synthase inhibition (N^{ω}-nitro-L-arginine-methyl ester, 300 μmol/l); NE, Norepinephrine; PE, Phenylephrine; Ang II, Angiotensin II; ET-1, Endothelin-1.

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Table 4. Staining levels of heat shock protein and nitrotyrosine in resistance arteries from end-stage renal disease (ESRD) patients and controls.

		Semi-quantitative assessment of staining (a.u.)	*P*-value	% of staining (i.a.s.)	*P*-value
Heat shock protein 90	Control	0.9 (0.3–1.7) [c]	NS	0.6 (0–21) [c]	NS
	ESRD	1.1 (0.5–2.2) [a]		2.5 (0–30) [a]	
Heat shock protein 70	Control	1.2 (0.2–2.9) [c]	0.02	1.8 (0–5) [c]	0.008
	ESRD	2.2 (1.7–3.0) [a]		6.9 (1–38) [a]	
Heat shock protein 27	Control	1.2 (0.7–2.0) [c]	0.04	0.1 (0–16) [c]	0.006
	ESRD	2.2 (0.9–3.0) [b]		15.6 (0–54) [b]	
Nitrotyrosine	Control	0.7 (0.0–2.0) [d]	0.01	0.6 (0–9) [d]	0.01
	ESRD	1.2 (0.5–2.5) [a]		2.3 (0–30) [a]	

[a;] n = 12, [b;] n = 13, [c;] n = 5, [d;] n = 6; n, number of participants in the groups.
NS, not significant; a.u., arbitrary unit; i.a.s., quantified by image analysis software.