Chapter 4

Proteinuria associated endothelial dysfunction is strain dependent

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Abstract

Introduction: Proteinuria-associated endothelial dysfunction (ED) is assumed to play a main role in the cardiovascular morbidity in proteinuric patients. However, the connection between proteinuria and systemic endothelial function is not clear yet. Therefore, we studied aortic endothelial function in MWF and FHH inbred rat strains with genetic proteinuria to determine the specific impact of proteinuria on the development of ED.

Methods: Proteinuria, cardiac function, systemic blood pressure, plasma lipid profiles, aortic endothelial function, plasma levels of COX products and dimethylarginines were investigated in 26 week old inbred rat strains with (MWF and FHH) and without (LEW) proteinuric renal disease.

Results: The endothelium-dependent relaxation was significantly reduced in MWF ($P<0.05$ vs LEW or FHH). Plasma TxB_2, $PGF_{2\alpha}$, and PGE_2 levels were higher in MWF ($P<0.05$ vs LEW or FHH), whereas 6-keto-$PGF_{1\alpha}$ level was comparable in all groups. Arginine/ADMA ratio was highest in MWF.

Conclusions: This study differentiates common risk factors for ED in renal disease. Despite clear-cut proteinuria, FHH rats were devoid of changes in aortic endothelial function, indicating some other deleterious factors must accompany proteinuria in order for ED to ensue. Further exploration of this model may serve to dissect mechanistical pathways, and guide development of protective strategies in the vascular damage of renal disease.

Introduction

It is well documented both in clinical and in experimental studies that renal disease is an important risk factor for the development of cardiovascular (CV) disease.[1-10] Several candidate mechanisms for the vascular damage in renal disease have been put forward, including hypertension, endothelial dysfunction (ED), inflammation, involvement of the renin-angiotensin-aldosterone-system (RAAS), proteinuria and its systemic sequelae such as hypercholesterolemia.[11] Of these factors, proteinuria is by far the strongest and most consistent risk factor for CV damage.

Proteinuria-associated ED is assumed to play a main role in the CV morbidity in proteinuric patients, as strongly suggested by epidemiological data.[12] Nephrotic range proteinuria was shown to be associated with ED that is reversible upon remission of the nephrotic syndrome.[13] Comparison with endothelial function in subjects with primary dyslipidemia showed that proteinuria-associated ED, in non-uremic patients, was distinct from the ED in dyslipidemia, supporting its specificity for the proteinuric state. However, in spite of its large clinical impact, the direct effects of proteinuria and/or the nephrotic state on systemic endothelial function have been investigated in a small number of studies only[14] and moreover, the experimental studies were exclusively performed in the adriamycin-induced model of nephrosis. We recently showed that vascular function in the adriamycin model is strongly affected by the adriamycin exposure.[15] Whereas some vascular abnormalities specific for the nephrotic state could be dissociated from the effects of adriamycin exposure per se, the impairment of endothelium-dependent vasodilation could be fully attributed to the effects of adriamycin exposure as such. Studies in non-biased models of proteinuria, therefore are needed to better elucidate the endothelial abnormalities of proteinuric renal disease, and its CV consequences.

Fawn-hooded Hypertensive (FHH) rats are inbred and known to be a model of chronic kidney disease with initial hyperfiltration, in which spontaneous hypertension, proteinuria and severe glomerulosclerosis develops at a young age, eventually leading to end-stage renal failure-related death. Munich Wistar Fromter (MWF) inbred rat model is another well known genetic model of

spontaneous proteinuria, hypertension, focal segmental glomerulosclerosis, and renal failure. In the current study therefore, we studied two different proteinuric strains to determine the specific impact of proteinuria on the development of ED, independent of uremia, and to establish robustness across different genetic backgrounds. In addition to endothelial function, cardiac function was also investigated to be able to account for a possible effect of cardiac dysfunction on endothelial function as a confounder for proteinuria-associated ED. Non-hypertensive and non-proteinuric inbred Lewis (LEW) rats were selected as age-matched controls.

Materials and methods

Animals

Experiments were performed in thirty eight 26 week old male rats from 3 different inbred strains, namely, LEW (LEW/SsNHsd, n=12), FHH (FHH/EurMcwiCrl, n=14), and MWF (MWF/ZtmHsd, n=12) rats. Animals were housed under standard conditions until 26 weeks of age at the animal facilities of the University of Groningen. All animals had free access to food (standard rat chow; Hope Farms, Woerden, The Netherlands) and drinking water throughout the study. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the University of Groningen.

Functional Measurements

Urinary Measurements: Measurements of water and food intake as well as 24-h urine collections for determination of urinary total protein excretion and urine production were performed one week before the determination of cardiac function by placing the rats in metabolic cages. Urinary total protein excretion was determined by end-point measurement with TCA precipitation (Nephelometer Analyzer II; Dade Behring, Marburg, Germany) in 24-h urine samples.

Cardiac Function and Isolation of Organs: Under 2.5% isoflurane in O_2 anesthesia, cardiac performance was measured by a pressure transducer catheter

which was inserted through the right carotid artery (Micro-Tip 3French; Millar Instruments Inc., Houston, TX), connected to a personal computer that was equipped with an analog-to-digital converter and appropriate software (Millar Instruments). After a 10-min period of stabilization, left ventricular end diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), and heart rate were recorded. Thereafter, the catheter was withdrawn into the aortic root to measure central systolic and diastolic blood pressures. As a parameter of global myocardial contractility and relaxation, we determined the maximal rates of increase and decrease in left ventricular pressure (LVP) ($+dP/dt_{max}$ and $-dP/dt_{max}$), which were normalized to left ventricular pressure change (*i.e.*, LVSP – LVEDP) for individual rats.

After hemodynamic measurements, blood samples (2-3 mL) were taken for biochemical analyses. The heart was rapidly excised and placed in ice-cold saline for Langendorff perfusion and the thoracic aorta was isolated for contraction measurements. Both kidneys were excised and divided into 3 sections horizontally. Mid sections of right and left kidneys were fixed by immersion for 48 h in a 4% buffered formaldehyde solution (Klinipath, Duiven, The Netherlands) and subsequently embedded in paraffin according to standard procedures for histology.

Langendorff Perfusion: Retrograde perfusion of the aorta, as described by Langendorff,[16] was started with a modified Krebs' solution (pH 7.5) and equilibrated with 95% O_2 and 5% CO_2. Perfusion pressure was maintained at 80 mmHg, temperature was continuously measured in the aorta-cannula tip and kept at 37.0 °C. Hearts were paced at 360 Hz by a stimulator (Grass S88D, Grass Instrument CO., Quincy, MA USA). Coronary flow (CF) was measured by a microprocessor, which controlled the perfusion pressure by adjusting a peristaltic perfusion pump. After equilibrating for 10 min, baseline CF was recorded and 10^{-4} mol/L sodium nitroprusside (SN) was infused for 2 min through a tube which was inserted into a lateral branch of the perfusion cannula for the measurement of coronary reserve. Baseline CF and coronary reserve were corrected for heart weights of individual rats in order to obtain CF per min per g tissue.

Aortic rings measurements: As described previously,[17] aorta segments (approximately 2 mm) were cleaned of adherent tissue and mounted in an organ bath with Krebs solution (pH 7.5) which was kept at 37 °C and continuously bubbled with 95% O_2 and 5% CO_2. Rings were randomized to one of three protocols. Prior to isotonic measurements of vascular contractility, arteries were allowed to equilibrate for 40 min. To test for viability of smooth muscle cells and endothelium, arteries were preconstricted with phenylephrine (PE; 10^{-6} mol/L). After wash out and another 30 min of stabilization endothelial function was measured as endothelium-dependent relaxation to cumulative doses of acetylcholine (ACh; $3x10^{-8}$ mol/L – $3x10^{-4}$ mol/L) in absence and subsequently in presence of indomethacin (10^{-5} mol/L) and indomethacin+L-NMMA (N^{ω}-monomethyl-L-arginine, 10^{-4} mol/L) to investigate the contribution of prostaglandins (PGs) and nitric oxide (NO) to endothelium-mediated relaxation in the vessels precontracted submaximally by PE (10^{-6} mol/L). Finally SN (10^{-3} mol/L) was added to the organ baths. By analyzing the above mentioned protocols of endothelium-dependent relaxation, the contribution of all three mediators (PGs, NO, and endothelium-derived hyperpolarizing factor (EDHF)) was calculated as a difference between Area Under the Curve (AUC) of respective ACh-concentration-response curves.

Measurements of Plasma Lipid Profile: The total cholesterol and triglycerides were measured by enzymatic assays (Roche/Hitachi Moduler Analyser P800-E170, Roche Diagnostics, Indianapolis, USA).

Measurements of Creatinine Clearance: Plasma and urine creatinine were measured by means of a photometric assay with the Jaffé method without deproteinization (DiaSys Diagnostic Systems, Holzheim, Germany) and creatinine clearance was calculated as Creatinine Clearance = (Urine Creatinine x Urine flow) / (Plasma Creatinine x Body Weight) and presented as mL/min/100 g body weight.

Measurements of Plasma Levels of Cyclooxygenase (COX) products: Plasma (100 µl) and urine (100 µl) samples were passed through Sep Pak C18 cartridges (Waters Ass., USA) and eluted with 2 ml of methanol. Samples were

stored at -20 °C in methanol. Because of the instability of prostacyclin and tromboxane in biological fluids, their stabile metabolites, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B2 (TxB_2), respectively, as well as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) were measured using radioimmunoassay. Eluates of 100 µl were dried under vacuum, dissolved in 100 µl ELISA assay buffer and pipette into the 96-well plates of the appropriate assay, resulting in a multiplication factor of 200 to obtain concentrations expressed as pg/ml. The used standard curves for 6-keto-$PGF_{1\alpha}$, TxB_2 and PGE_2 (Biotral assay, GE Health Care, UK) were 0.5-64 pg/ml, 0.5-64 pg/ml and 2.5-300 pg/ml respectively and for $PGF_{2\alpha}$ (Cayman, USA) 0.195-25 pg/ml. Further procedures were as described previously.[18]

Measurements of Plasma Arginine, ADMA, and SDMA: Plasma concentrations of arginine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) were measured by high-performance liquid chromatography with fluorescence detection as previously described,[19] with modified chromatographic separation conditions.[20] Intra- and inter-assay coefficients of variation were better than 1.5 and 3.5%, respectively. Because arginine is the substrate and ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), we also calculated the arginine/ADMA ratio.

Histology

Determination of Kidney Damage: Paraffin embedded mid sections of kidneys were cut in 3 µm thick slices and stained with Periodic Acid Schiff (PAS) and the incidence of focal glomerulosclerosis score (FGS) was microscopically evaluated by an examiner who was blinded for the groups according to standard procedures. The degree of mesangial matrix expansion (MME) and FGS were assessed in 50 glomeruli by scoring semi quantitatively on a scale of 0 to 4. FGS was scored positive when MME and adhesion to Bowman's capsule were present in the same quadrant. When one quadrant of the glomerulus was affected, a score of 1+ was assigned, two quadrants was scored as 2+, three quadrants as 3+, and four quadrants as 4+. Overall MME and FGS score is expressed in arbitrary units (AU) with a maximum of 200.

Solutions and Compounds

All compounds for Krebs solutions and all other drugs were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie, The Netherlands). The stock solution of indomethacin was prepared in 64 mmol/L $NaHCO_3$.

Statistical analysis

Data are expressed as mean±S.E.M.; *n* values represent the number of investigated rats. Concentration-response curves of ACh were compared by ANOVA for repeated measures followed by Bonferroni *post hoc* test for multiple comparisons. Each strain first was compared to LEW by performing One-Way ANOVA followed by Dunnet *post hoc* test. Tukey *post hoc* test was used for multiple strain comparisons. Differences were considered significant at $P<0.05$.

Results

Group Characteristics

The characteristics of male rats from the three different inbred strains are given in Table 1. Body weights were significantly higher in LEW rats than in FHH rats ($P<0.05$). FHH and MWF rats were mildly hypertensive ($P<0.05$ *versus* LEW). There was no difference in heart rate (data not shown) among the groups. As expected proteinuria was elevated in MWF and FHH ($P<0.05$ *versus* LEW). Accordingly, FGS was present in MWF and FHH rats ($P<0.05$ *versus* LEW). Total cholesterol and triglycerides were elevated in both MWF and FHH (Table 2) with the highest values in MWF ($P<0.05$ versus both LEW and FHH). Creatinine clearance however, was not impaired in the two proteinuric strains, indicating that, at least at the time point studied, these are relative pure models for proteinuria; actually creatinine clearance was elevated in the FHH, consistent with the hyperfiltration character of this strain.

Functional Measurements

Hemodynamic measurements: Hemodynamic data obtained from 26 week old LEW, FHH, and MWF rats are shown in figure 1. LVSP was elevated both in MWF and FHH compared to LEW ($P<0.05$).

Myocardial contractility ($+dP/dt_{max}$) in FHH and myocardial relaxation ($-dP/dt_{max}$) in MWF were significantly reduced when compared to LEW ($P<0.05$).

Table 1. *In vivo characteristics of LEW, FHH, and MWF rats.*

	LEW (n=12)	**FHH (n=14)**	**MWF (n=12)**
Body weight (g)	415±6	375±4*	398±7
HW/BW (mg/g)	3.0±0.1	3.7±0.1*#	3.4±0.1*
MABP (mmHg)	91±3	113±5*	110±4*
Urine output (mL/24 h)	4.6±1.1	32.0±1.6*#	15.7±3.2*
Proteinuria (mg/24 h)	12.1±1.2	168.2±17.4*#	201.3±14.2*
FGS (AU)	2.2±0.4	20.1±2.5*#	35.0±2.6*
Creatinine Clearance (mL/min/100 g)	1.13±0.05	1.71±0.26*#	0.95±0.04

Data are given as means±S.E.M. HW/BW: heart weight / body weight ratio, MABP: mean arterial blood pressure, FGS: focal glomerulosclerosis score for glomerular damage (in arbitrary units). *$P<0.05$ versus LEW, #$P<0.05$ versus MWF.

Table 2. *In vivo characteristics of 26 week old LEW, FHH, and MWF rats.*

	LEW (n=12)	FHH (n=14)	MWF (n=12)
Triglycerides (mg/dL)	173.0±9.5	162.2±13.1#	273.7±19.3*
Total Cholesterol (mg/dL)	77.5±1.5	115.6±4.6*#	132.0±5.7*
Arginine (μmol/l)	149±5	113±10*#	161±9
ADMA (μmol/l)	0.45±0.02	0.41±0.02	0.43±0.01
SDMA (μmol/l)	0.18±0.01	0.22±0.04	0.22±0.01
Arginine/ADMA ratio	339±15	278±25#	375±17

Data are given as means±S.E.M. ADMA: asymmetric dimethylarginine, SDMA: symmetric dimethylarginine. *P<0.05 versus LEW, #P<0.05 versus MWF.

Baseline coronary flow and coronary reserve: Absolute and corrected baseline and maximum CFs are shown in figure 2. Both corrected baseline and maximum CFs after 2 min infusion of 10^{-4} mol/L SN were similar in all groups.

Endothelium-dependent relaxation in the thoracic aorta: The cumulative dose-response curves of 26 week old LEW, FHH, and MWF rats to ACh in submaximally PE-precontracted isolated aortic rings are shown in figure 3. PE elicited similar contractile responses in all groups (data not shown). The endothelium-dependent relaxation response to ACh was markedly reduced in the rings from MWF (figure 3, P<0.01 *versus* both LEW and FHH). However, ACh induced relaxation was similar in the rings from LEW and FHH.

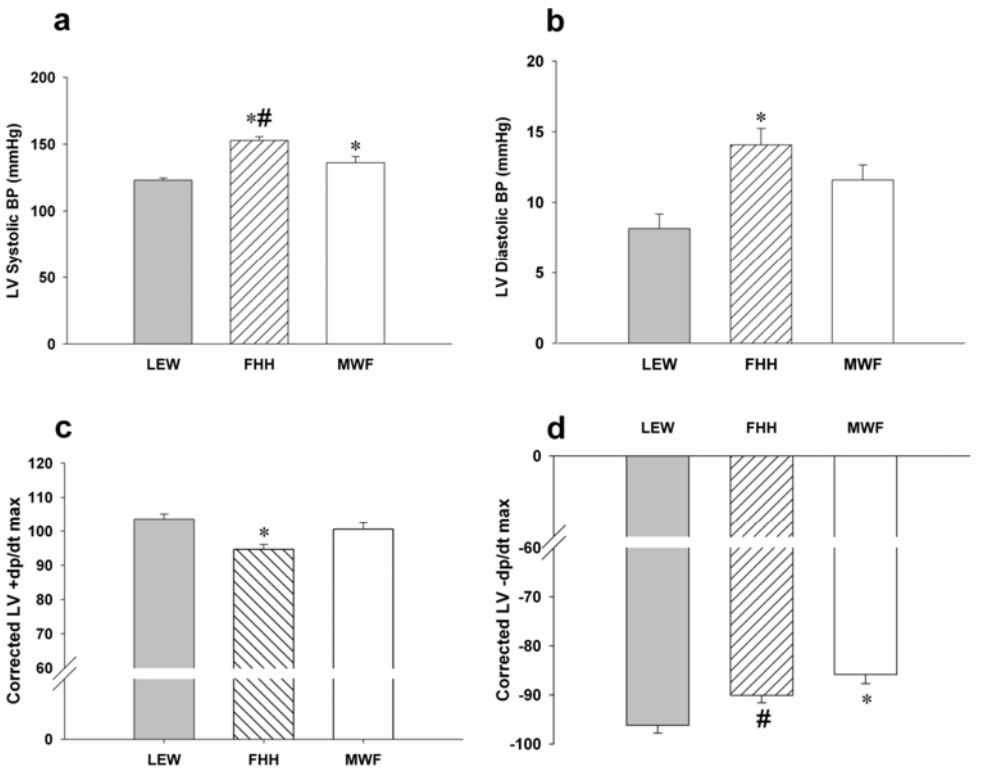

Figure 1. Hemodynamic data obtained from 26 week old LEW (n=12), FHH (n=14), and MWF (n=12) rats. Data are given as mean±S.E.M. LV Systolic BP: left ventricular systolic blood pressure, LV Diastolic BP: left ventricular end diastolic blood pressure, Corrected LV +dP/dtmax: maximal rate of increase of left ventricular pressure (myocardial contractility) which was normalized to left ventricular pressure change (left ventricular systolic blood pressure – left ventricular end diastolic blood pressure) for individual rats, Corrected LV -dP/dtmax: maximal rate of decrease of LV pressure (myocardial relaxation) which was normalized to left ventricular pressure change (left ventricular systolic blood pressure – left ventricular end diastolic blood pressure) for individual rats. *P<0.05 versus LEW, #P<0.05 versus MWF.

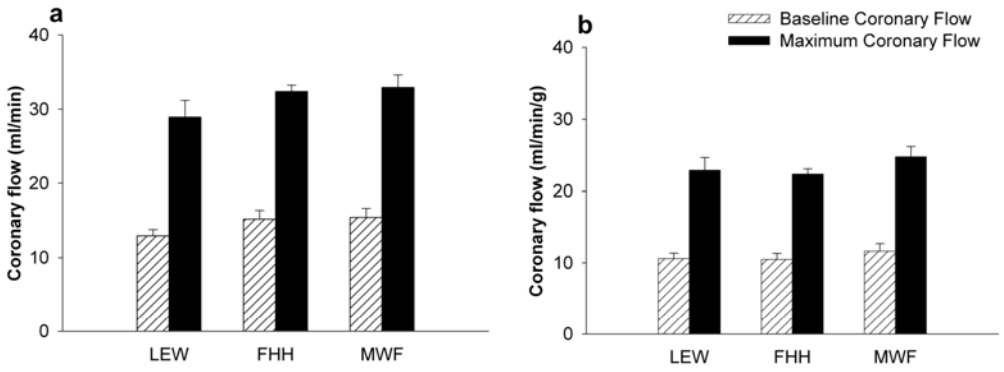

Figure 2. The Langendorff perfusion data on absolute **(a)** and corrected **(b)** baseline and maximum coronary flows with sodium nitroprusside obtained from 26 week old LEW (n=12), FHH (n=14), and MWF (n=12) rats. Maximum coronary flows were corrected for heart weights of individual rats. Data are given as mean±S.E.M. **P*<0.05 versus LEW, #*P*<0.05 versus MWF.

The contribution of prostaglandins, nitric oxide, and endothelium-derived hyperpolarizing factor on endothelium-dependent relaxation: The relative contributions of principal endothelial mediators (PGs, NO, and EDHF) to endothelial function was calculated as a difference between Area Under the Curve (AUC) of respective ACh-concentration-response curves and presented in figure 4. It shows that in both MWF (figure 4c) and FHH (figure 4b) rats the release of constrictive COX products play an important role in endothelial function in the thoracic aorta since only in these two groups COX inhibition resulted in a further dilation. In order to further investigate this finding; we measured the plasma levels of dilatory and constrictive COX products.

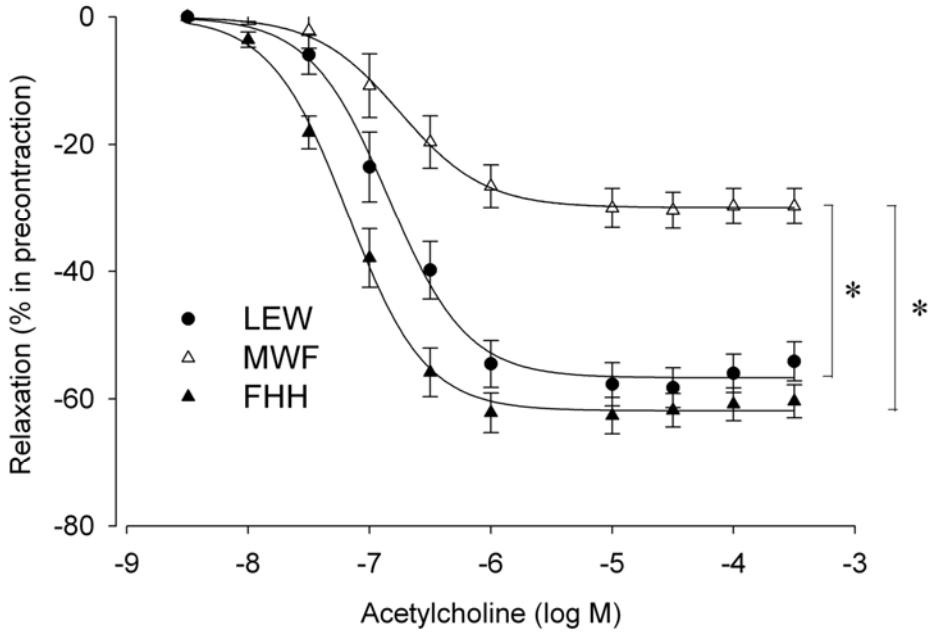

Figure 3. Acetylcholine-mediated endothelium-dependent relaxation curves of phenylephrine precontracted aortic rings obtained from LEW (n=12), FHH (n=14), and MWF (n=12) rats. *P<0.01

Plasma Levels of COX products: Plasma levels of constrictive COX products, TxB_2, $PGF_{2\alpha}$, and PGE_2 were significantly higher in MWF rats when compared to LEW and FHH rats, whereas the prostacyclin metabolite 6-keto-$PGF_{1\alpha}$ level was comparable in all groups (figure 5).

Plasma Arginine, ADMA, and SDMA Levels: Plasma concentrations of arginine, ADMA, and SDMA are shown in Table 2. It shows that the arginine/ADMA ratio reflecting the amount of substrate relative to endogenous inhibitor of NOS was highest in MWF rats.

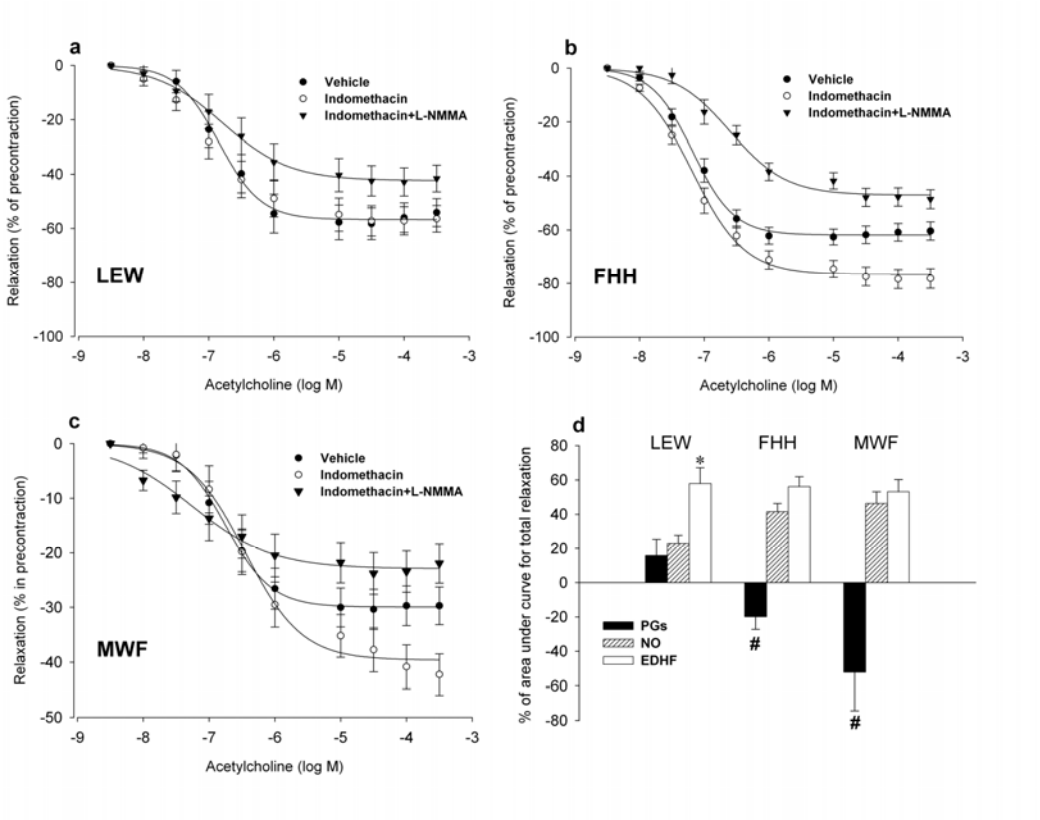

Figure 4. The contribution of prostaglandins (PGs), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) on endothelium-dependent relaxation curves of phenylephrine precontracted aortic rings obtained from LEW (4a; n=12), FHH (4b; n=14), and MWF (4c; n=12) rats. Endothelial function was calculated as a difference between Area Under the Curve (AUC; 4d) of respective ACh-concentration-response curves. Data are given as mean±S.E.M. **P*<0.05 EDHF versus both PGs and NO, #*P*<0.05 PGs versus both NO and EDHF.

Discussion

Our data on endothelial function in different proteinuric rat strains show that overall aortic endothelium-dependent relaxation capacity was impaired in MWF animals, which was in line with prior human studies.[13] Remarkably, however, aortic endothelium-derived vasorelaxation was preserved in proteinuric FHH rats. This difference could not be explained by differences in blood pressure or renal function. Neither could it be explained by possible confounders such as

cardiac function. To our knowledge this is the first study which shows the protection of endothelial dilatory function in relatively old rats with proteinuria.

The control of vascular tone is a result of a delicate balance of vasoconstrictive and vasodilative substances which are released by the healthy endothelium. Once this endothelium-dependent balance is broken an increased peripheral vascular resistance and subsequently CV complications may appear. Therefore ED is a major promoter of both atherogenesis and thrombosis and, consequently, CV events.[21]

The mechanisms of ED in renal disease are complex and multiple pathogenic factors are likely to be involved, including proteinuria, hypertension as well as uremia. Moroever, once cardiac damage ensues, this itself could elicit ED as well.[22,23] In the current study we attempted to dissociate these by studying two different non-uremic proteinuric models. We had anticipated endothelial function, and the pathways involved to be similar in MWF and FHH as proteinuric models. However, it turned out that endothelial function dissociated between MWF and FHH. Moreover, we observed differences in contributions of NO, EDHF and PGs to the ACh-mediated endothelium dependent dilatation between rat strains, as demonstrated by the effects of L-NMMA and indomethacin, respectively, on endothelium-dependent vasodilation, supporting a role for increased vasoconstrictor COX products in the proteinuric models, in particular in the MWF. The differences in aortic endothelial function and in contribution of the different pathways could not be attributed to differences in cardiac function, as this was similar in both proteinuric strains.

The lack of difference in effect of L-NMMA on the vasodilator curves for FHH and MWF argues against a role for differences in the NO pathway as underlying mechanisms for the difference in aortic endothelial function between the strains. Additionally, we investigated the NO pathway by measuring plasma arginine and dimethylarginine concentrations, and hence arginine/ADMA ratio. A low arginine/ADMA ratio may result in a reduced NO production by lack of substrate and/or inhibition of NOS which may eventually cause ED. However, first, plasma ADMA levels were similar between the studied strains, and moreover, not FHH but MWF had the highest Arginine/ADMA ratio, is consistent with the

functional data in refuting a role for a lower NO availability in MWF as a mechanism underlying the difference with FHH. Although, it should be noted that plasma arginine and dimethylarginine levels may not correlate with the endothelial cell levels, we consider it likely that mechanisms other than NO pathway might be involved in the development of ED in MWF rat strain. At variance with our findings, elevated plasma ADMA levels were detected in chronic kidney disease patients either with normal or impaired renal function,[24] and accumulation of ADMA was shown to cause ED in patients with chronic kidney disease in whom renal function was normal.[25] Although elevated plasma ADMA levels in renal disease originally were attributed to impaired renal clearance, recent data have shown high levels of ADMA in chronic kidney disease patients with normal glomerular filtration[26] supporting the rationale for investigating the impact of additional characteristics of renal disease, such as proteinuria, on plasma dimethylarginines and the NO pathway. However, our data in rat aorta do not provide support for proteinuria-associated changes in the NO pathway in either proteinuric strain, and moreover, do not explain the difference in endothelial function between the strains.

The indomethacin-sensitive contribution to endothelium-dependent relaxation was apparently specific or at least a common denominator for endothelial function in the two proteinuric strains. Moreover, the contribution of constrictor COX products was less prominent in the FHH: this might therefore represent an underlying mechanism for the difference between the strains. A connection between contractile COX products and renal[27] and aortic[28] ED was shown in SHR rats previously. Furthermore above findings were also supported by a study in which chronic treatment with aldosterone was able to produce ED through COX-2 activation in normotensive and hypertensive conditions.[29] It is believed that these contractile COX products contract vascular smooth muscle and, thereby oppose the vasorelaxant effects of NO. Additionally, therefore we measured systemic COX products in the different rat strains. In line with aforementioned studies, and in support of our functional data, significantly elevated plasma levels of contractile COX products in MWF rats were associated with an aortic ED in contrast to FHH rats in which those COX products were

significantly lower. There was no difference in plasma 6-keto-$PGF_{1\alpha}$ levels among the groups, which is a reflection of similar dilatory PGs synthesis. Nevertheless, considering the consistency with the functional data, it is plausible that the lack of increase in contractile COX products, as opposed to the increase in contractile COX products in MWF critically contributes to the protection of proteinuric FHH rats against developing ED.

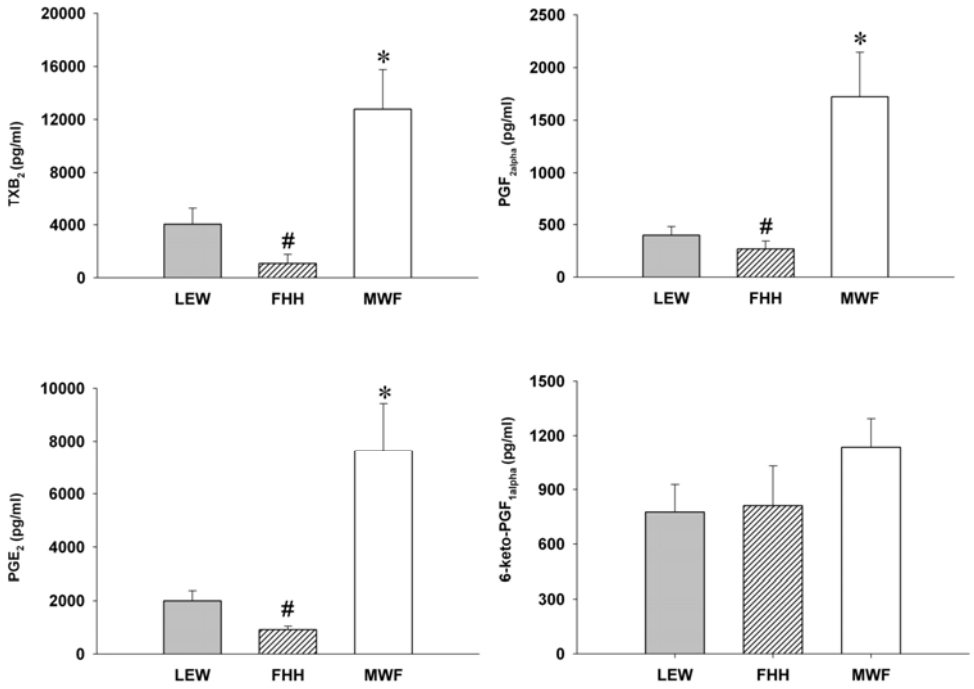

Figure 5. Plasma levels of COX products, 6-keto-prostaglandin F_{1alpha} (6-keto-PGF_{1alpha}), thromboxane B2 (TxB_2), prostaglandin F_{2alpha} (PGF_{2alpha}) and prostaglandin E_2 (PGE_2) in 26 week old LEW (n=12), FHH (n=14), and MWF (n=12) rats. Data are given as mean±S.E.M. **P*<0.05 versus LEW, #*P*<0.05 versus MWF.

As mentioned above proteinuria is associated with an extremely high CV morbidity and mortality.[9,30] The exact mechanism by which urinary protein excretion is associated with an increased risk for coronary heart disease is unclear, but it is likely multifactorial.[31] In addition to local and systemic ED due to the proteinuria-induced inflammatory response[13,32] the state of systemic nephrosis, characterized by dyslipidemia with a particularly atherogenic profile[33] might also be a factor in its pathogenesis.[15] Recently in MWF rats high proteinuria was shown to be caused by alterations in the components of the podocyte slit diaphragm or of the glomerular basement membrane.[34] In line with the literature proteinuric MWF and FHH rats in our study had significantly higher total plasma cholesterol levels when compared to non-proteinuric LEW rats, demonstrating the overt nephrotic state in these animals. This was associated with aortic ED in MWF only. Cardiac abnormalities were mild (lower myocardial contractility or relaxation) in either strain, with normal CF in both strains, which might well be due to the mildly hypertensive state in these nephrotic models. Although we did not measure RAAS activity, the current literature provides some information regarding the status of RAAS in FHH and MWF rats. Kuijpers and de Jong showed in their studies that plasma renin activity was significantly associated with high blood pressure levels in FHH rats.[35] Remuzzi et al. observed that RAAS blockade (between 25-40 weeks) normalized proteinuria, eliminated inflammatory cell infiltration, and ameliorated glomerular and tubular structural changes in MWF rats.[36] Two above mentioned studies give indirect evidence of higher RAAS activity in these proteinuric strains. Nevertheless, we cannot fully exclude the effects of proteinuria or systemic nephrosis on partial deterioration of cardiac function in proteinuric animals, since our previous work showed a significant loss in coronary endothelial function in MWF rats at 24 weeks of age.[37]

The main limitation of our study is that endothelial function was measured in the isolated aorta only. Whereas this is a commonly used experimental setting, the heterogeneity of the vascular bed is well-established, and it should be taken in mind that straightforward extrapolation of our data to other vascular beds is not warranted. The renal functional outcomes such as proteinuria, and FGS were all significantly worse in the endothelial dysfunctional MWF rats as a

normofiltration model at the age of 25 weeks when compared to the FHH rats that were in a state of hyperfiltration at the time of study. Further studies would be needed to establish the role of ED in renal and CV outcome in these strains.

This study differentiates common risk factors for ED in renal disease. FHH rats appear devoid of changes on the aortic endothelial dependent relaxation capacity in spite of overt proteinuria and systemic nephrosis indicating some other deleterious change must accompany proteinuria in order for ED to ensue. Our data suggest that lack of increase in contractile COX products may be involved in the lack of large conduit artery ED in the FHH. Therefore, studies on aortic endothelial function in the FHH strain may serve as a read out system to further dissect possible endogenous protective mechanisms for the vascular damage in renal disease, and perhaps even guide the development of therapeutic strategies for vasculoprotection in proteinuric patients.

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