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Magnetic resonance elastography can monitor changes in medullary stiffness in response to treatment in the swine ischemic kidney

Xin Zhang, M.D.¹, Xiangyang Zhu, M.D., Ph.D.¹, Christopher M. Ferguson¹, Kai Jiang, Ph.D.¹, Tyson Burningham, M.D.¹, Amir Lerman, M.D.², and Lilach O. Lerman, M.D., Ph.D.^{1,2}

¹Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN, 55905, United States

²Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN, 55905, United States

Abstract

Object—Low-energy shockwave (SW) therapy attenuates damage in the stenotic kidney (STK) caused by atherosclerotic renal artery stenosis (ARAS). We hypothesized that magnetic resonance elastography (MRE) would detect attenuation of fibrosis following SW in unilateral ARAS kidneys.

Materials and Methods—Domestic pigs were randomized to control, unilateral ARAS, and ARAS treated with 6 sessions of SW over 3 consecutive weeks (n=7 each) starting after 3 weeks of ARAS or sham. Four weeks after SW treatment, renal fibrosis was evaluated with MRE in-vivo or trichrome staining ex-vivo. Blood pressure, single-kidney renal-blood-flow (RBF) and glomerular-filtration-rate (GFR) were assessed.

Results—MRE detected increased stiffness in the STK medulla (15.3 ± 2.1 vs. 10.1 ± 0.8 kPa, $p < 0.05$) that moderately correlated with severity of fibrosis ($R^2 = 0.501$, $p < 0.01$), but did not identify mild STK cortical or contralateral kidney fibrosis. Trichrome staining showed that medullary fibrosis was increased in ARAS and alleviated by SW ($10.4 \pm 1.8\%$ vs. $2.9 \pm 0.2\%$,

Correspondence: Lilach O. Lerman, MD, PhD, Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. Fax: (507)-266-9316, Phone: (507)-266-9376, lerman.lilach@mayo.edu.

Author contributions:

Zhang: Protocol, data collection, data analysis

Zhu: Data collection

Ferguson: Data collection/data analysis

Jiang: Data analysis

Burningham: Data analysis

Lerman A: Protocol

Lerman LO: Protocol/project development

Compliance with Ethical Standards

Authors have no conflict of interests.

All applicable international, national, and/or institutional guidelines for care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

This article does not contain any studies with human participants performed by any of the authors.

$p < 0.01$). SW slightly decreased blood pressure and normalized STK RBF and GFR in ARAS. In the contralateral kidney, SW reversed the increase in RBF and GFR.

Conclusion—MRE might be a tool for noninvasive monitoring of medullary fibrosis in response to treatment in kidney disease.

Keywords

magnetic resonance elastography; renal artery stenosis; stiffness; renal medulla; extracorporeal shockwave

Introduction

Atherosclerotic renal artery stenosis (ARAS) remains the leading cause of renovascular hypertension, and is increasing in prevalence due to aging of the population and increased prevalence of atherosclerosis risk factors. As it progresses, ARAS results in downstream renal pathological alterations with gradual functional loss [1].

Parenchymal damage may also change the mechanical properties of the tissue. As fibrosis develops in the kidney, excessive extracellular matrix deposition often increases renal stiffness, which might be detectable *in vivo* by imaging techniques such as magnetic resonance elastography (MRE) [2]. Previous studies have shown that magnetic resonance imaging (MRI) techniques provide measurements of fibrosis, such as diffusion-weighted magnetic resonance imaging (DWI) [3] and magnetization-transfer MRI [4, 5]. Although we have applied the intravoxel incoherent motion-derived parameters to improve the accuracy of DWI for evaluation of renal fibrosis [6], this method still needs standardization of protocols. Magnetization transfer MRI requires validation as well. On the other hand, the commercially available MRE technique has been standardized across major MR manufacturers, and allows rapid clinical translation, despite a number of remaining limitations. MRE also has higher diagnostic accuracy for staging of all liver fibrosis stages [7].

By passing acoustic shear waves through a target tissue and tracking their propagation using phase-contrast MRI, MRE measures the resulting transverse strain and derives quantitative values for tissue stiffness and visualization of elasticity in defined regions of interest (ROI) [8, 9]. The feasibility of using MRE in the kidney has been demonstrated in healthy [10] and transplant patients [11]. More recently, in large animals with ARAS [12] MRE has been shown capable of detecting increased fibrosis [12], thus supporting its utility in experimental studies in ischemic renal disease.

Restoration of vessel patency by percutaneous transluminal renal angioplasty and stenting does not often lead to improvement of renal function compared to optimal medical therapy to control blood pressure and maintain renal function [13], likely because correction of an obstruction in the main renal artery alone cannot correct the irreversible downstream intrarenal damage [14, 15]. In addition to inflammation, chronic reduction of renal perfusion leads to permanent changes in microvascular structure (remodeling/regression) associated with inadequate renal angiogenic signaling [16], which may lead to development of tubulointerstitial fibrosis. Hence, the quest for targeted treatment modalities continues.

Low-energy extracorporeal shockwave (SW) therapy, at 10% energy of the traditional SW used for lithotripsy, evokes neovascularization, and improves regional blood flow and function in various ischemic tissues [17-19]. We have previously shown that SW improves renal regional blood flow and function in ARAS and alleviates renal fibrosis determined by histology [20]. The ability to detect improved renal structure *in vivo* may have therapeutic implications for monitoring disease progression. However, whether MRE could detect a change in fibrosis in SW-treated kidneys *in vivo* is unknown. In this study, we hypothesized that MRE would effectively detect a decrease in tissue scarring in treated ARAS kidneys 4 weeks after completion of a SW-treatment regimen.

Materials and Methods

Animal groups

This study was approved by the Institutional Animal Care and Use Committee. Twenty-one domestic female pigs (50-60 Kg) were studied during 16 weeks of observation. Pigs were randomized to Normal, ARAS, and ARAS treated with SW (ARAS+SW). Normal pigs were fed isocaloric diets of standard chow, and ARAS pigs with a high-fat diet containing 2% cholesterol (Harlan Teklad, Madison, WI) [21]. All animals had free access to water.

ARAS induction

ARAS was induced after 6 weeks of diet by placing a local irritant coil in the right main renal artery, leading to gradual development of unilateral ARAS, as previously described [22]. Six weeks later, the degrees of stenosis were determined by renal angiography.

SW treatment

The SW (Omnispec Vetspec Model, Medispec® LTD, Germantown, MD, USA; spark voltage 10-24 kV; energy density 0.09 mJ/mm²; frequency 120 pulse/minute) regimen was initiated 3 weeks after RAS induction, bi-weekly for 3 consecutive weeks (a total of 6 sessions), as previously described [20]. In brief, pigs were laid prone, the SW applicator placed perpendicularly above the stenotic kidney to distribute energy through the stenotic kidney along its short axis, and an Acuson SC2000 ultrasound system (Global Siemens Healthcare, Erlangen, Germany) used to guide SW localization on the kidney.

Four weeks after completion of SW, the animals underwent *in vivo* imaging studies to assess renal elasticity and function. Animals were euthanized three days after *in vivo* studies, using a lethal intravenous dose of sodium pentobarbital (100 mg/kg, Fatal Plus, Vortech Pharmaceuticals, Fort Washington, PA). The kidneys were removed using a retroperitoneal incision and immediately dissected and prepared in formalin for tissue studies.

MRE

Four weeks after completion of SW, tissue stiffness was evaluated by MRE (Signa TwinSpeed EXCITE 3T system, GE Healthcare, Waukesha, WI) [12]. Pigs were intubated to enable subsequent breath-hold, and placed on their dorsal surface within the bore of the MRI scanner (Signa TwinSpeed EXCITE 3T system, GE Healthcare, Waukesha, WI). Image acquisition was performed during 30 second breath-holds, achieved by pausing the

ventilator. MRE pulse sequences were set to the parameters: TR/TE/FOV/imaging matrix/thickness/number of averages/shots/parallel encoding factor = (2200 ms/64 ms/34 cm/96*96/3 mm/1/2/2) interpolated to 256×256 images by zero-filling. Both kidneys were included in each MRE acquisition, so that the number of slices varied based the size of the kidneys. Two-D straight-axial images with multi-slice, spin echo, flow compensated, echo planar imaging (EPI) in combination with motion sensitizing gradients (MEG) were synchronized with two passive pneumatic drivers producing 120 Hz vibrations, which produced a 3D stack of images. The two passive drivers were secured to the dorsal surface of the animal proximal to the kidneys, and connected by flexible plastic tubing to an active driver located adjacent to the MRI room. Sampling consisted of four time points per slice in all three directions. The 120 Hz trapezoidal MEG waveform was on each side of the spin-echo pulse, with 0th and 1st moment nulling. MEG alternated in polarity and amplitude during acquisition to measure the different tissue displacement components, and was harmonized with motion generated by the passive drivers. Motion sensitivity was 3.2 $\mu\text{m/radian}$, and motion was measured in all six orthogonal directions ($\pm x$, $\pm y$, $\pm z$). Post-processing and image analysis was performed using MRE/Wave© (MRI Research Lab, Mayo Clinic, Rochester, MN). Tissue displacement measurements were used to develop the speed of the shear wave, and finally shear modulus. Data processing included phase unwrapping, curl filtration that removes the compressional components by eliminating the longitudinal waves [23, 24]. Bulk motion in the background was removed using a Gaussian bandpass filter [25]. A local frequency estimate was used to estimate the local spatial frequency of the propagation pattern (shear waves) at 120 Hz. The algorithm is a mixture of local estimates of frequency over multiple scales. The resulting shear stiffness is produced from filters that are a derivative of directional and radial components. The formula used to calculate shear stiffness(s) is: (mechanical freq., squared)/(spatial freq., squared), and is based on a soft tissue density of 1000 kg/m³.

For elasticity analysis, we drew ROIs on T₂-weighted images or magnitude images, and then propagated those ROIs onto the elastograms for quantification [25]. While MRE maps are spatially not homogeneously distributed [26], to increase uniformity stiffness values are commonly averaged over large ROIs, that are in turn averaged across multiple slices. MRE has been previously shown to have good Inter-observer variability [12] and reproducibility over >24 hours intervals [27], suggesting that this approach is successful.

Our previous studies have shown good correlation between histological changes and stiffness in the medulla but not in cortex [12], which is more affected by hemodynamic variables such as renal blood flow (RBF) [25]. Therefore, cortical and medullary ROIs were individually selected on EPI magnitude images from both kidney, and verified with the aid of co-registered contrast-enhanced CT images subsequently obtained in the same pig during the vascular phase of contrast agent transit [28].

MDCT

A few days after MRE studies, blood pressure was measured in all animals using an arterial catheter during in-vivo studies, and bilateral single-kidney function, including RBF and glomerular filtration rate (GFR) were assessed using multi-detector computed tomography

(MDCT) using an MDCT scanner (Somatom Definition FLASH dual energy 128, Siemens Medical Solution, Forchheim, Germany) as we have shown [22, 29]. A central venous injection of contrast (iopamidol, 0.5ml/kg/2sec) was used to assess stenotic (STK) and contralateral kidney (CLK) RBF and GFR after acquisition of 140 consecutive scans. Another injection of iopamidol (1 ml/kg/5sec) was used to image 3D stacks of 5mm-thick kidney sections with 0.48mm pixel spacing for later volumetric analysis. Reconstruction and analysis of images was performed using AnalyzeTM (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN).

Planimetry of the helical volume obtained at the vascular phase of contrast transit in MDCT scans was used to calculate cortical and medullary volume. Siemens Syngo Applications Suite (Siemens, Germany) was used to quantify the degree of obstruction of the renal artery using 0.6 mm-thick MDCT scans with 0.48 mm pixel spacing. For assessment of renal function, ROIs were selected from tomographic images from the aorta, renal cortex, and medulla to generate time-attenuation curves in each region and fit them using curve-fitting algorithms (a Γ -variate model) obtain measures of renal function single-kidney function [29]. MDCT-based measurement of renal volume served to convert regional assessment (mL/min/cc tissue) to single-kidney RBF and GFR (mL/min).

Renal fibrosis

Cortical and medullary kidney tissue was embedded in paraffin and sectioned (5 μ m thick) for Masson's trichrome staining. The degree of fibrosis in the renal cortex and medulla was semi-automatically quantified in 10-15 fields using a masking algorithm based on color thresholding and edge detection in a computer-aided image-analysis program AxioVision[®] (Carl Zeiss MicroImaging, Thornwood, NY) and expressed as an average of percent fibrosis to total field area. Trichrome staining was performed to evaluate tissue fibrosis.

Statistical Analysis

Statistical analysis was performed using JMP software package version 9.0, SAS Institute, Cary, NC. Results are shown as mean $\pm$ SEM. For data with normal distribution, one-way ANOVA was applied followed by unpaired Student's t-test. Wilcoxon test was performed for non-normal distribution data. Pearson correlation was performed to examine the correlation between stiffness and fibrosis in cortex and medulla in both STK and CLK. Results were considered significant for $p < 0.05$. The post-hoc Bonferroni correction was used for multiple comparisons by dividing this p value by the number of comparisons.

Results

General Characteristics

At 16 weeks, high fat diet elevated cholesterol and low-density lipoprotein levels in the plasma of both ARAS groups similarly, and RAS induction caused comparable degrees of stenosis. Mean arterial pressure (MAP) was elevated in both groups, and was lowered but not normalized by SW (Table 1).

MRE detects decreased medullary fibrosis in the treated stenotic kidney

ARAS decreased STK RBF and GFR, but both were improved by SW (Table 1). Trichrome staining showed that SW also alleviated the increased fibrosis in the STK cortex and medulla compared to ARAS ($1.4\pm0.5\%$ vs. $4.9\pm0.7\%$, $2.9\pm0.2\%$ vs. $10.4\pm1.8\%$, respectively, both $p<0.01$), but normalized it in cortex only, whereas medullary fibrosis remained higher than in normal kidneys (which was $1.7\pm0.2\%$, Figure 1, Table 2).

MRE-derived cortical tissue stiffness did not differ among the groups, but in the medulla MRE detected an increase in tissue stiffness compared to normal (15.3 ± 2.1 kPa vs. 10.1 ± 0.4 kPa, $p<0.05$), which was lowered by SW (to 10.1 ± 0.8 kPa, Figure 1, Table 2).

MRE does not detect subtle changes in the CLK fibrosis

ARAS increased RBF and GFR in the CLK, suggesting hyperfiltration to compensate for the function compromise of the STK. SW normalized both RBF and GFR in the CLK (Table 1). Cortical fibrosis did not change in the CLK, whereas medullary fibrosis increased in ARAS and was improved although not normalized after SW (Table 2, Figure S).

On the other hand, no change in stiffness was detected by MRE in either the cortex or medulla of the ARAS and ARAS+SW CLK (Table 2, Figure S).

Correlation analysis

Moderate correlation was found between tissue stiffness by MRE and fibrosis by trichrome in the STK medulla ($r^2=0.5$, $P<0.01$, Figure 2), but not in the cortex or in any CLK region.

Discussion

The present study shows that low-energy shockwave improves renal function in the stenotic ARAS swine kidney, accompanied by decreased fibrosis in both cortex and medulla, and attenuates hyperfiltration in the contralateral kidney. MRE was able to detect SW-induced changes in medullary stiffness after SW treatment in the stenotic ARAS kidney, which correlated with medullary tissue fibrosis, but did not detect STK cortical fibrosis or more subtle changes in the CLK. These observations suggest that MRE might be a useful tool to monitor medullary fibrosis in post-SW treatment individuals, but under the conditions applied in this study may have limited sensitivity to detect subtle changes.

We and others have demonstrated that in ARAS kidneys, prolonged damage to renal microvessels secondary to ischemia and oxidative stress in ARAS may lead to endothelial dysfunction and vascular loss [30, 31], and that co-existing inflammation [32, 33], may exacerbate microvascular dysfunction. Importantly, in ARAS kidneys the tissue remodeling process involves the development of both cortical and medullary fibrosis. RAS kidneys exhibit upregulated tubular and glomerular expression of pro-fibrotic factors like transforming growth factor- β , tissue inhibitor of metalloproteinase-1, and plasminogen activator inhibitor-1. Activation in the stenotic kidney of the renal-angiotensin-system may also stimulate collagen and extracellular matrix deposition [30]. Tissue fibrosis can be further accelerated in hypercholesterolemia, which increases the availability of oxidized low density lipoprotein, increasing intra- and extra-cellular matrix protein synthesis, and

decreasing its degradation [34]. Hypercholesterolemia also increases the expression of nuclear factor- κ B thereby enhancing tissue scarring [35]. Development of fibrosis further restricts expansion of the microcirculation to replace lost vessels and scar tissue, resulting in a vicious cycle of microvascular rarefaction [36] which characterizes parenchymal injury in ARAS kidney.

The mechanical properties of the tissue often change as a result of parenchymal injury. As fibrosis progresses, the rigor or stiffness of the tissue often increases, thus creating changes in properties that can be visualized and measured by MRE. SW converts mechanical stimulus into cell signaling by upregulation of canonical mechanotransducers. This in turn elicits angiogenesis, improves the microvasculature, and decreases inflammation, which may indirectly contribute to decreased fibrosis and thereby stiffness in the ARAS kidney [20]. A direct effect of inflammation on renal stiffness is likely small relative to fibrosis. In our study, microvascular proliferation might have also attenuated renal vascular resistance distal to the stenosis, which may account for the improvement of RBF and GFR despite sustained renovascular hypertension and similar degree of stenosis [37]. Abrogation of pro-fibrotic signaling confers protection against development of fibrosis and atrophy in murine renovascular disease [38], demonstrating that renal scarring and tissue remodeling modulate progression to renal dysfunction. Therefore, in the current study, direct amelioration of fibrosis by SW might have improved function in the ARAS kidney. Given similar kidney volumes in the two ARAS groups, shrinkage was unlikely to impact our stiffness measurements.

We found that MRE effectively detected the corresponding change of tissue stiffness in the stenotic renal medulla in both treated and untreated ARAS pigs. We also found that the correlation between fibrosis and tissue stiffness was only significant in the medulla of the stenotic kidney, but not in the cortex or the contralateral kidney. This might be linked to the different structure and hemodynamics in the renal cortex and medulla. In general, cortical stiffness is more dependent than medullary stiffness on perfusion pressure, which directly modulates vascular filling and rigor. In ARAS, a fall in STK-RBF decreases the perfusion and filtration pressures in the microvessels and glomerulus. As a result, the stiffness of the cortex may correspondingly decrease, and consequently, its stiffness. Therefore, tissue stiffness is determined by both mechanical (e.g. fibrosis), as well as hemodynamic properties, and hemodynamic changes in the renal cortex might hamper the ability of MRE to identify an increase in stiffness secondary to accumulation of fibrotic tissues [12, 25, 39]. In addition, glomerulosclerosis is usually less prominent than tubulointerstitial fibrosis at an early stage of ARAS [40], which might also limit the ability of MRE to detect increased stiffness in the cortex of a stenotic kidney, at least using our acquisition parameters.

Improved blood and nutrient supply in the STK after SW halts progression of stenotic fibrosis and at the same time might ameliorate it in the CLK by lowering blood pressure. The more prominent fibrosis in ARAS-CLK indicates a greater sensitivity of medulla to hypertensive injury than the cortex. Notably, while the increased filtration and perfusion pressure in the CLK cortex could have increased its stiffness, MRE detected no significant difference from normal kidneys, suggesting that such change might be mild at this stage. After SW in the STK, despite a fall in blood pressure, trichrome staining showed that the

CLK medullary fibrosis remained elevated in ARAS+SW. This finding may reflect the continuous and prolonged effects of hypercholesterolemia-induced tissue fibrosis, and emphasizes the need for lipid lowering management in ARAS.

Indeed, the sensitivity and specificity for MRE to detect tissue stiffness may vary in the same organ and possibly depend on the severity of fibrosis [41, 42]. Accordingly, in the present study, MRE identified only increases in kidney stiffness associated with marked fibrosis. Additional studies are needed to define the cutoff values for identifying different levels of stiffness *in vivo*. Nevertheless, our results support the notion that measuring medullary stiffness alone may be useful for monitoring and staging renal fibrosis or evaluating the effectiveness of treatment.

Study Limitations

Our study is limited by the short duration of the disease, yet the similarity of renal structure and function in our swine model to human kidneys increases the translational potential of our results. Under our study conditions, artifacts such as blood flow and susceptibility artifacts challenge quantification of stiffness in the cortex, and not in the medulla. The sensitivity of MRE needs to be defined in additional studies. Future studies may develop and test tools, such as optimizing the EPI sequence or reconstruction software to reduce noise and improve homogeneity of MRE stiffness maps, or testing different slice thicknesses, to increase the sensitivity and specificity of MRE to detect fibrosis. In addition, redesigning the flat passive drivers to a better form-fitting concave shape might also improve the accuracy of targeting the kidneys with shear waves. The impact of smaller interpolated matrices on elastograms also warrants exploration in future studies.

Conclusions

MRE may be a useful noninvasive technique for evaluation of marked changes in medullary fibrosis in stenotic kidney following SW treatment. Further studies are needed to establish its sensitivity to detect mild fibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Acronyms

SW	shockwave
MRI	magnetic resonance imaging
MRE	magnetic resonance elastography

DWI	diffusion-weighted imaging
EPI	echo-planar imaging
MEG	motion encoding gradients
ROI	region of interest
MDCT	multi-detector computed tomography
ARAS	atherosclerotic renal artery stenosis
STK	stenotic kidney
CLK	contralateral kidney
RBF	renal blood flow
GFR	glomerular filtration rate
MAP	mean arterial pressure

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Figure 1.

Top: Representative images and quantification of trichrome staining from the stenotic (STK) cortex and medulla. SW therapy alleviated increased fibrosis in ARAS STK. Bottom: Representative magnetic resonance elastography images (yellow/red color indicates greater stiffness) from the STK cortex and medulla and quantification. The elasticity variation at the boundary between cortex and medulla is rather sharp. ARAS increased tissue stiffness in the medulla that was improved by SW.

Figure 2.
Correlation between tissue stiffness and fibrosis in the STK and CLK renal medulla and cortex.

Table 1

Characteristics of Normal and atherosclerotic renal artery stenosis (ARAS) pigs treated or untreated with shockwave (SW).

Characteristics	Normal (n=7)	ARAS (n=7)	ARAS+SW (n=7)
Degree of stenosis (%)	0	84±5	81±4
Mean arterial pressure (mmHg)	95.8±2.9	148.4±10.9 *	122.5±6.9 *#
Renal blood flow STK (ml/min)	494.3±53.2	268.2±15.8 *	426.7±74.9
Renal blood flow CLK (ml/min)	525.6 ±89.3	705.0±83.9 *	502.7±111.3
Glomerular filtration rate STK	72.9±6.8	50.9±6.1 *	71.5±10.1
Glomerular filtration rate CLK	78.8±6.7	102.6±12.1 *	80.1±23.2
LDL (mg/dl)	50±26	199±45 *	192±44 *
Total cholesterol (mg/dl)	100±32	312±60 *	300±84 *

LDL: low density lipoprotein; STK stenotic kidney; CLK contralateral kidney; Data are expressed as mean±SEM.

* Bonferroni-corrected p<0.05 vs. Normal.

Bonferroni-corrected p<0.05 vs. ARAS

Table 2

Renal fibrosis estimated with trichrome staining and magnetic resonance elastography in normal and atherosclerotic renal artery stenosis (ARAS) pigs treated or untreated with shockwave (SW).

Renal fibrosis	Normal (n=7)	ARAS (n=7)	ARAS+SW (n=7)
Trichrome STK Cortex (%)	1.2±0.5	4.9±0.7 [*]	1.5±0.5 [#]
STK Medulla (%)	1.7±0.2	10.4±1.8 [*]	2.9±0.2 ^{*#}
Trichrome CLK Cortex (%)	1.3±0.4	1.2±0.3	1.0±0.4
CLK Medulla (%)	1.6±0.3	6.8±0.9 [*]	3.7±0.6 ^{*#}
MRE STK Cortex (kPa)	12.0±0.7	12.9±0.9	11.7±1.7
STK Medulla (kPa)	10.2±0.4	15.3±2.1 [*]	10.4±0.8 [#]
MRE CLK Cortex (kPa)	12.0±0.8	12.8±0.9	11.2±1.0
CLK Medulla (kPa)	10.7±0.2	11.3±0.8	11.3±1.6

Data are expressed as mean±SEM. STK stenotic kidney; CLK contralateral kidney;

^{*} Bonferroni-corrected p<0.05 vs. Normal.

[#] Bonferroni-corrected p<0.05 vs. ARAS