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Protection against age-dependent renal injury in the F344xBrown Norway male rat is associated with maintained nitric oxide synthase

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Abstract

Age-dependent renal damage is influenced by genetic background and the Fisher344xBrown Norway (F344xBN) rat is resistant to glomerular injury. In vulnerable strains, a fall in renal nitric oxide synthase (NOS) contributes to age-dependent renal damage. Here, we investigated renal NOS in young (3 months) and old (30 months) male F344xBN to test the hypothesis that renal NOS is maintained in “protected” strains. We also examined if 6 months of renin-angiotensin system (RAS) blockade using angiotensin converting enzyme inhibition (ACEI) and angiotensin receptor blockade (ARB) provides further benefit in these “protected” old rats. Aging increased tubulointerstitial injury but glomerular sclerosis was minimal and NOS and superoxide dismutase abundance increased. There was no change in the NOS inhibitor, ADMA (asymmetric dimethylarginine) or its regulatory enzymes. RAS blockade with ARB protected against tubulointerstitial injury and increased nNOS α , but ACEI, which also increased nNOS α , had no protective effect on the tubulointerstitium. We conclude that the glomerular sclerosis-resistant aged male F344xBN rat maintains renal NOS, thus reinforcing our hypothesis that progressive glomerular injury is related to renal NOS deficiency. The tubulointerstitial injury seen with aging is reversed with 6 months of ARB but not ACEI and is not associated with renal NOS.

Keywords

rat strain difference; renin-angiotensin system blockade; asymmetric dimethylarginine; tubulointerstitial injury; oxidative stress

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1. Introduction

In man, the kidney develops structural damage with age that is associated with thickening of the glomerular basement membrane, expansion of glomerular mesangium, increases in extracellular matrix proteins and appearance of tubulointerstitial injury (Kasiske, 1987; McLachlan et al., 1977). In addition, glomerular filtration rate (GFR) falls secondary to both glomerular injury and to falls in renal plasma flow because of renal vasoconstriction (McLachlan et al., 1977). Even in the absence of primary kidney disease, a decline in renal function is expected although not inevitable, as demonstrated by the Baltimore Longitudinal Study on Aging (Lindeman et al., 1985). Age-dependent kidney damage and dysfunction are also seen in the aging rat, with some strains showing rapidly developing, age-dependent chronic kidney disease (CKD), while others maintain excellent renal function and structure even when very old (Baylis and Corman, 1998).

All forms of CKD are associated with nitric oxide (NO) deficiency, which is both a result of CKD and a contributing factor to progression (Baylis, 2008; Baylis, 2009b). In the Sprague-Dawley (SD) rat where renal disease progresses rapidly, age-dependent kidney damage is related to decreased abundance and activity of the NO synthesizing enzyme, NO synthase (NOS) in the kidney cortex (Erdely et al., 2003b). Plasma levels of the endogenous inhibitor of NOS, asymmetric dimethylarginine (ADMA) are also elevated in elderly humans and rats (Boger et al., 2000; Kielstein et al., 2003; Xiong et al., 2001), providing an additional mechanism of NO deficiency in aging.

It is evident that genetic background plays a critical role in how organ function changes with age. In fact, age-dependent changes in humans can be attributed to more than 600 genes, about 100 of which contain expression-associated single nucleotide polymorphisms (Wheeler et al., 2009). In contrast to the injury prone Sprague-Dawley, age dependent CKD develops slowly in the Munich-Wistar rat and is minimal in WAG/Rij (Baylis and Corman, 1998) and Fisher 344xBrown Norway (F344xBN) strains (Lipman et al., 1996). With a life span of ~36 months and relatively preserved renal function, the F344xBN is considered a model of “healthy aging” (Diz, 2008). When compared to another commonly utilized aging model, the F344, which interestingly has increased insulin resistance and glomerular nephropathy but no systemic hypertension with age, the F344xBN has fewer glomerular lesions and a greater mean age at which 50% mortality occurs (F344: 103 wks. vs. F344xBN: 145 wks; Lipman et al., 1996). It is clear that genetic differences dictate outcome of age-related declines so further characterization of the various aging models is critical for aging investigations.

In the present study, we investigated the impact of aging on various determinants of NO production in the F344xBN rat. Determinants included 1) abundance of the NO synthesizing protein, NOS; 2) NOS inhibitor levels; 3) abundance of enzymes that regulate NOS inhibitors; 4) abundance of anti-oxidant and oxidative markers. The primary goal was to test the hypothesis that in the absence of significant age-dependent damage, renal NOS abundance is maintained. We also investigated whether there would be any beneficial effect of chronic renin-angiotensin system (RAS) blockade in these “protected” rats.

2. Methods

2.1 Animal care

All animal procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (*Principles of Laboratory Animal Care*; NIH publication No. 86–23, revised 1985) and approved and monitored by the University of Florida Institutional Animal Care and Use Committee. Young (3 months; n=8) and old (24

months; n=24) male F344xBN rats were purchased from the National Institute of Aging colony (Harlan Sprague Dawley, Indianapolis, IN) and singly housed in a temperature/light-controlled environment and given access to standard rat chow/water *ad libitum*. Old rats were divided into three groups (n=8/group) and used to compare 6 months of placebo (normal aging) with RAS blockade with either an angiotensin converting enzyme inhibitor (ACEI; enalapril; 40 mg/kg body weight) or angiotensin II receptor blocker (ARB; candesartan; 10 mg/kg body weight). Bacon flavored tablets (BioServ #F05072) were given with or without the drug compounded into them, and old rats were sacrificed for study at 30 months of age by rapid decapitation. Young rats (n=8) were maintained on the same *ad lib* diet and daily bacon flavored tablets without drug for two weeks and then sacrificed at ~3 months of age under isoflurane anesthesia. Blood was taken either by aortic puncture or from the trunk in young and old, respectively, and then spun for collection of plasma. The kidneys were removed and while one kidney was prepared for histological analyses (see below), the other was separated into cortical and medullary sections, and then flash-frozen in liquid nitrogen. However, all analyses were conducted in cortical tissue only. All samples were stored at -80°C for further analyses. Plasma creatinine levels were measured by HPLC as previously described (Sasser et al., 2009).

2.2 Histological Analyses

One kidney was cut along the transverse axis and fixed in 10% buffered formalin for 48 hours at 4°C, paraffin wax embedded, cut into 5-micron thick sections and stained with periodic acid schiff, followed by a hematoxylin counterstain (Sigma). Sections were then examined, blind, for the level of glomerular sclerosis, glomerular ischemia/atrophy, tubular atrophy, and interstitial fibrosis. Each category was scored (0=none, 1=10%, 2=10–25%, 3=25–50%, 4=50–75%, 5=75–100%) based on the percentage of structures that displayed the described injury.

2.3 Western Blot

Relative protein abundances of endothelial nitric oxide synthase (eNOS; BD Transduction; 1:250), neuronal NOS α (nNOS α ; Santa Cruz; 1:50), nNOS β (ABR; 1:500), dimethyldiaminohydrolase (DDAH) isoforms (Santa Cruz; DDAH1 1:250 and DDAH2 1:100), protein methyltransferase (PRMT1; Millipore; 1:2000), superoxide dismutase (SOD) isoforms (Stressgen Reagents; EC SOD 1:250, CuZn SOD 1:2000, and Mn SOD 1:2000), and p22phox (Santa Cruz; 1:50) were measured by Western Blot. Homogenized samples of kidney cortex standardized by protein concentrations (50–200 ug) were separated by electrophoresis (7.5% or 12% acrylamide gel, 200 V, 65 min) and transferred onto nitrocellulose membranes (GE Healthcare) for 60 min at 0.18 A as previously described (Sasser et al., 2009). Membranes were stained with Ponceau Red (Sigma) to check for transfer efficiency/uniformity and equal loading, incubated in blocking solution for 60 min, and then washed in TBS + 0.05 % Tween before overnight primary antibody incubation at 4°C. Membranes were then incubated with the appropriate secondary antibody for one hour at room temperature, with a series of washes before and after, and developed with enhanced chemiluminescent reagents (Thermo Scientific). Bands were quantified by densitometry using the VersaDoc Imaging System and One Analysis Software (BioRad). Protein abundance was calculated as integrated optical density (IOD) of the protein of interest (after subtraction of background), factored for Ponceau Red stain (total protein loaded), and normalized with an internal positive control value. This allowed for quantitative comparisons between different membranes. The specific protein abundance is represented as IOD/Red Ponceau/Control relative to the appropriate control group.

2.4 Plasma and Tissue L-Arg/ADMA/SDMA

ADMA concentrations in plasma and renal cortical tissue homogenates were measured by reverse-phase HPLC using the Waters AccQ-Fluor fluorescence method as previously described (Sasser et al., 2009). Renal cortical tissue ADMA concentrations (μM) were normalized to total protein (mg/ml) and therefore expressed as $\mu\text{mol/mg}$.

2.5 Hydrogen Peroxide

Renal cortical concentration of hydrogen peroxide was measured using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes) according to the manufacturer's instructions with the following modifications: Renal cortical tissue was homogenized with $1\times$ phosphate-buffered saline (PBS; MediaTech, Inc.; 0.1 g tissue: 400 μl PBS), diluted 1:3, incubated at room temperature with Amplex Red reagent and HRP for 45 minutes, and then read at a wavelength absorbance of 560 nm. Assay specificity was confirmed using 2000 units of catalase (Sigma). Renal cortical H_2O_2 concentrations (μM) were normalized to total protein (mg/ml) and therefore expressed as nmol/mg.

2.6 Statistical Analyses

Data are presented as mean $\pm$ SEM and analyzed by either one-way ANOVA followed by post-hoc analysis, or the non-parametric Kruskal-Wallis test (for histology only) using GraphPad Prism 4 software (San Diego, CA). Significance was defined as $p < 0.05$.

3. Results

3.1 Functional Measurements and Histological Analyses

Body weight (BW) increased with age (young: 337 ± 8 vs. old: 560 ± 24 g) and plasma creatinine fell (young: 0.11 ± 0.01 vs. old: 0.06 ± 0.00 mg/dl). Histological analyses revealed no difference in the percentage of glomeruli with sclerosis in old vs. young rats although there were increases in glomerular ischemia/atrophy, tubular atrophy, and interstitial fibrosis with age (Fig. 1). RAS blockade had no impact on the age-dependent rise in BW (old: 560 ± 24 vs. old-ACEI: 542 ± 17 vs. old-ARB: 546 ± 18 g) but plasma creatinine significantly rose with both ACEI and ARB treatment (old-ACEI: 0.09 ± 0.01 vs. old-ARB: 0.09 vs. 0.01 mg/dl) in comparison to untreated old rats. The only impact of RAS inhibition on histology was that ARB treatment significantly reduced both tubular atrophy and interstitial fibrosis compared to untreated old rats and those given ACEI (Fig. 1).

3.2 Determinants of NO

NO_x (stable metabolites of $\text{NO} = \text{NO}_3 + \text{NO}_2$) levels in the plasma were unchanged with age (young: 12 ± 1 vs. old: 12 ± 4 μM) and ARB treatment (9 ± 2 μM), although ACEI increased plasma NO_x (30 ± 1 μM). Both eNOS and nNOS α were present in increased abundance in the kidney cortex of old rats (Figs. 2A and 2B), whereas nNOS β abundance (Fig. 2C) remained unchanged. Neither ACEI nor ARB treatment in old rats affected kidney cortex eNOS or nNOS β abundance (Figs. 2A and 2C); however, ARB did increase nNOS β abundance compared to old untreated rats and marked increases in nNOS α were detected with both ACEI and ARB treatment (Fig. 2B).

Plasma levels of ADMA, L-Arginine, and SDMA were unchanged with aging although the plasma L-Arginine:ADMA ratio increased, favoring increased NO production (Table 1). Similarly, in the kidney cortex there were also no differences in ADMA and L-Arginine levels in young vs. old, although the L-Arginine:ADMA ratio increased (Table 2). There was no effect of RAS blockade on plasma levels of ADMA, L-Arginine, L-Arginine:ADMA ratio, or SDMA compared to old untreated rats (Table 1). Kidney cortex PRMT-1, the

enzyme responsible for ADMA production was increased with age (Fig. 3A) whereas the ADMA-degrading enzyme, DDAH1 tended to increase in abundance (Fig. 3B) and there was no change in DDAH2 abundance in the kidney cortex (Fig. 3C). ACEI and ARB treatment reduced kidney cortex PRMT-1 abundance (Fig. 3A) but had no effect on abundance of either DDAH isoform compared to kidneys from old untreated rats (Figs. 3B and 3C).

3.3 Oxidative Stress markers

We also assessed indices of oxidative stress in the F344xBN rodent model of aging and found significant increases in p22phox abundance in the kidney cortex of old vs. young rats (Fig. 4A) but no change in the H₂O₂ content of the kidney cortex (Fig. 4B). Old rats showed marked increases in the kidney cortex abundance of EC SOD and Mn SOD with no change in CuZn SOD abundance (Fig. 5). RAS blockade had no impact on kidney cortex p22phox abundance or H₂O₂ content compared to old untreated rats (Figs. 4A and 4B), although ACEI reduced kidney cortex EC SOD abundance to levels that fell further with ARB (Fig. 5A). In contrast, ACEI increased CuZn SOD abundance whereas ARB decreased it (Fig. 5B). No statistically significant changes were detected for the Mn SOD abundance among any of the old groups (Fig. 5C).

4. Discussion

The main finding of this study is that the protection against age-dependent glomerular sclerosis seen in the F344xBN rat is associated with preserved eNOS and nNOS α protein abundance in kidney cortex and with no age-dependent increase in plasma ADMA. Also, the antioxidant SOD's in the kidney increase with age and apparently balance the increased oxidant-generating NADPH oxidase (indicated by increased p22phox). Although this rat strain does not develop glomerular sclerosis with age, tubulointerstitial injury increased, which was prevented by RAS inhibition with the ARB, whereas ACEI had no protective effect.

It is always difficult to determine what changes occur due to normal aging and what is due to increased susceptibility to acquired disease. The renal response to aging is extremely variable in humans and in rats (Baylis and Corman, 1998; Lindeman et al., 1985), and of note, the female of many strains, including the SD, show marked protection compared to the male (Baylis and Corman, 1998; Erdely et al., 2003b). In the aged male SD rat where glomerular sclerosis is severe (>60% of glomeruli damaged at 24 months), renal eNOS and nNOS protein abundance falls with age (Erdely et al., 2003b). In contrast, in the present study we report that in the aged male F344xBN, where only ~ 2.5% of glomeruli show sclerotic damage by 30 months of age, the renal eNOS and nNOS isoforms are preserved. We have suggested that falls in renal NOS protein abundance are both a consequence and a cause of progression of several forms of CKD (Baylis, 2009a). The present study expands this by showing that normal aging is not inevitably accompanied by loss of renal NOS, reinforcing our hypothesis that there is a causal association between kidney injury and loss of renal NOS protein (Baylis, 2009a).

In addition to NOS protein abundance, NO production is controlled by the local and circulating concentrations of the endogenous NOS inhibitor, ADMA. Several studies report that plasma ADMA increases in normal aging humans and that an increase, although delayed, also occurs in aging women (Kielstein et al., 2003; Schulze et al., 2005). This may be associated with the age-dependent development of endothelial dysfunction (Bode-Boger et al., 2003). Increased plasma ADMA has also been reported in the aging male SD rat (Xiong et al., 2001). Control of plasma ADMA level is mainly by degradation by the DDAH1 enzyme, which is abundant in liver and kidney (Palm et al., 2007; Sasser et al.,

2009). ADMA is made by PRMT1, a class of enzymes that methylate amino acids (including arginine) while they are incorporated into intact proteins (Nicholson et al., 2009). The free (active) ADMA is released by proteolysis. In this study, we found that in the 30-month old male F344xBN, neither circulating nor renal cortical ADMA levels were changed with age. The renal abundance of the enzyme responsible for ADMA production, PRMT-1, was unchanged with age and the ADMA-degrading enzyme, DDAH1 (main renal enzyme) tended to increase and DDAH2 was unchanged. Thus, an increase in plasma ADMA with age is not inevitable and in fact we observed an *increase* in the ratio between L-Arginine/ADMA, an effect that favors NO production and enhanced endothelial function. This was due to a non-significant tendency for increased plasma L-Arginine with age, also reported by us previously in the aging male SD (Mistry et al., 2002).

Furthermore, in spite of a lifetime (30 months) of oxidative metabolism, the F344xBN shows no obvious signs of increased oxidative stress. DDAH activity and abundance is reported to be redox sensitive and it is widely believed that exposure to oxidative stress will inhibit DDAH and increase ADMA (Palm et al., 2007). Activity of PRMT1 is also increased by oxidative stress; however, as noted above, the abundance of these enzymes were not affected by aging in the F344xBN. Ang-II induces oxidative stress in the kidney (Gill and Wilcox, 2006; Modlinger et al., 2006) and when kidney injury develops with age, there is also activation of the intrarenal angiotensin II (Ang II) system; however in the F344/BN rat where no glomerular damage develops, there is no increase in intrarenal ANGII (Kasper 2008). We did observe an increased abundance of the renal NADPH oxidase subunit, p22phox, with age, which presumably implies increased superoxide production. However, we also found increases in EC SOD and Mn SOD, a likely compensatory response to scavenge the increased superoxide production. Since renal cortical H₂O₂ is also unchanged with age there is presumably also increased renal catalase activity.

Chronic RAS blockade is used for treatment of CKD and/or hypertension. Chronic ACEI protects against glomerular sclerosis, reduces proteinuria, and lowers blood pressure in aging male Munich-Wistar rats, treated from 3 to 30 months of age (Anderson et al., 1994). Lifetime treatment with ACEI in the male WAG/Rij rat also reduces blood pressure, urinary protein excretion, and expansion of the mesangial matrix (Heudes et al., 1994), although WAG/Rij rats show minimal age-dependent injury (Baylis and Corman, 1998). In the present study, male F344xBN rats show little evidence of glomerular sclerosis and yet some beneficial effects of RAS blockade were observed. It is interesting that even in the absence of glomerular sclerosis, significant tubulointerstitial injury develops in these rats by 30 months of age, and 6 months of treatment with the ARB protected against tubulointerstitial injury. Tubulointerstitial injury develops as peritubular capillaries are lost in various forms of CKD and is particularly prominent in slowly evolving age-dependent damage (Lombardi et al., 1999).

Although not reported in the present study, there is no age-dependent proteinuria in the F344xBN rat, and long term ACE inhibition with enalapril had no effect on urinary protein excretion (Kasper, 2008). Thus, tubulointerstitial injury, in the absence of glomerular injury, does not cause proteinuria in this model. In the aging Wistar rat, where substantial glomerular damage occurs, endothelin receptor blockade reversed proteinuria and prevented glomerulosclerosis, while tubulointerstitial injury remained (Ortmann et al., 2004). Thus, it is likely that the development of proteinuria in response to kidney damage is primarily glomerular, rather than tubular in origin.

Tubulointerstitial injury has also been suggested to be an NO deficiency-mediated event in the aging kidney (Lombardi et al., 1999). Although there is no evidence of renal NO deficiency in the 30-month old F344xBN male, both ARB and ACEI lead to marked

increases in renal cortical nNOS α in the present study. However, in contrast to the beneficial effect of ARB, 6 months of ACEI treatment gave no reduction in tubulointerstitial injury, suggesting that the protection against tubulointerstitial injury produced by the ARB is not due to nNOS α stimulation. It is interesting that in this setting the ARB is superior to ACEI, whereas ACEI outperforms ARB in heart failure (Berry et al., 2001). ARB and ACEI are also thought to have equivalent efficacy in treating patients with a wide range of cardiovascular risk (Baumhakel and Bohm, 2009; Schindler, 2008).

The fall in plasma creatinine in the old untreated rat most likely relates to the well-known loss of lean body mass that occurs with age (Griffiths, 1996). It is interesting that both methods for RAS blockade raised plasma creatinine to the young (still very low) value. This may reflect RAS blockade-induced changes in body composition and/or activity level. It is unlikely to reflect loss of renal function since chronic intrarenal RAS blockade is associated with beneficial effects. Further, the plasma SDMA is unaffected by aging or RAS blockade and SDMA normally increases as GFR falls (Marcovecchio et al.).

In summary, we conclude that in the aged male F344xBN rat, in contrast to our previous findings in the aged male SD rat, renal NOS is preserved and there is minimal age-dependent glomerular sclerosis (Erdely et al., 2003a). Both anti-oxidant and oxidant systems in the kidney are activated with age and there is no net effect on circulating or renal cortical ADMA concentrations. The tubulointerstitial injury seen with aging is reversed with 6 months of ARB but not ACEI, and is not associated with renal NOS. Our data highlight the complexity of the aging process in that factors such as genetic background can dictate histological outcomes that are associated with the renal NO system.

Research Highlights

- Age-dependent glomerular injury is related to renal NOS deficiency
- Old F344XBN do not get glomerular sclerosis and maintain their kidney cortex NOS
- Protection associates with increases in antioxidants and no change in plasma ADMA
- In old F344XBN, 6m ARB, not ACEI, reversed tubulointerstitial injury

Abbreviations

CKD	chronic kidney disease
F344xBN	Fisher344xBrown Norway
ACEI	angiotensin converting enzyme inhibition
ARB	angiotensin receptor blockade
eNOS	endothelial nitric oxide synthase
nNOSα	neuronal NOS isoform α
nNOSβ	neuronal NOS isoform β
ADMA	asymmetric dimethylarginine
SDMA	symmetric dimethylarginine
DDAH1/DDAH2	dimethyldiaminohydrolase isoform 1 and 2

PRMT1	protein methyltransferase
EC/CuZn/Mn SOD	extracellular/copper zinc/manganese superoxide dismutase

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Figure 1.

Representative images of periodic acid Schiff stained sections that were examined, blind, for the level of glomerular sclerosis, glomerular ischemia/atrophy, tubular atrophy, and interstitial fibrosis. All images were taken at 400 $\times$ magnification. (A) A healthy, normal cortex in the young rat (~3 months). (B) A 30-month old, untreated rat, with mild glomerular sclerosis (left glomerulus) and tubular atrophy. (C) A 30-month old rat given 6 months of angiotensin converting enzyme inhibition (old-ACEI) with focal interstitial fibrosis (center of image). (D) A 30-month old rat given 6 months of angiotensin receptor blockade (old-ARB) showing some mesangial matrix expansion but otherwise with preserved glomerular and tubular architecture. (E) Quantification of the histological data. Each category was

scored (0=none, 1=10%, 2=10–25%, 3=25–50%, 4=50–75%, 5=75–100%) based on the percentage of structures that displayed the described injury. Note: Glomerular sclerosis was zero in all young, old-ACEI, and old-ARB. Also, tubular atrophy and interstitial fibrosis scores for old were identical. * $p<0.05$ vs. Young; + $p<0.05$ vs. Old; ** $p<0.05$ vs. Old-ACEI; n=8/group for all.

Figure 2.

We assessed the kidney cortex protein abundance of the nitric oxide synthase (NOS) enzymes in young (~3 month), old (30 month), old-ARB, and old-ACEI male F344xBN rats: (A) endothelial (e)NOS (A), (B) neuronal (n) NOS isoform α (nNOS α), and (C) nNOS isoform β (nNOS β). * $p < 0.05$ vs. young; + $p < 0.05$ vs. old; $n = 8$ /group for all. Relative densities in protein abundance were determined by Western blot and normalized to Red Ponceau staining and an internal positive control.

Figure 3.

We assessed the kidney cortex protein abundance of the enzymes that regulate asymmetric dimethylarginine (ADMA) synthesis and catabolism in young (~3 month), old (30 month), old-ARB, and old-ACEI male F344xBN rats: (A) Protein methyltransferase-1 (PRMT-1), (B) dimethyldiaminohydrolase1 (DDAH1), and (C) DDAH2. * $p < 0.05$ vs. young; + $p < 0.05$ vs. old; $n = 8$ /group for all. Relative densities in protein abundance were determined by Western blot and normalized to Red Ponceau staining and an internal positive control.

Figure 4.

Oxidative stress in the kidney cortex as assessed by determining the protein abundance of p22phox (A) and content H₂O₂ (B) in young (~3 month), old (30 month), old-ARB and old-ACEI male F344xBN rats. *p<0.05 vs. Young; n=8/group for all. Relative densities in protein abundance were determined by Western blot and normalized to Red Ponceau staining and an internal positive control. Kidney cortex H₂O₂ content was confirmed with catalase and normalized to mg of protein.

Figure 5.

We assessed the kidney cortex protein abundance of the antioxidant enzymes in young (~3 month), old (30 month), old-ARB, and old-ACEI male F344xBN rats: (A) Extracellular superoxide dismutase (EC SOD), (B) cytosolic-located CuZn SOD, and (C) mitochondrial-located Mn SOD (Mn SOD). * $p < 0.05$ vs. Young; + $p < 0.05$ vs. Old; ** $p < 0.05$ vs. Old-ACEI; $n = 8$ /group for all. Relative densities in protein abundance were determined by Western blot and normalized to Red Ponceau staining and an internal positive control.

Table 1

The effect of aging and chronic RAS blockade on plasma levels of ADMA, L-Arginine, L-Arginine:ADMA, and SDMA in young (~3 month), old (30 month), old-ACEI, and old-ARB male F344xBN rats.

Group	ADMA (μM)	L-Arginine (μM)	L-Arginine:ADMA	SDMA (μM)
Plasma -Young	0.26±0.02	89±7	346±14	0.18±0.02
Plasma - Old	0.26±0.04	102±9.0	430±34 *	0.20±0.02
Plasma – Old ACEI	0.30±0.13	106±30	442±30	0.22±0.07
Plasma – Old ARB	0.21±0.03	94±26	374±33	0.19±0.04

RAS, renin angiotensin system; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker. ACEI and ARB treatment lasted for 6 months in old only.

*
p<0.05 vs. young.

Table 2

The effect of aging on kidney cortex levels of ADMA, L-Arginine, L-Arginine: ADMA, and SDMA in young (~3 month) and old (30 month) male F344xBN rats.

Group	ADMA (nmol/g)	L-Arginine (nmol/mg)	L-Arginine/ADMA	SDMA (nmol/g)
Kidney Cortex -Young	9±1	2.00±0.32	216±25	28±2
Kidney Cortex - Old	8±1	2.00±0.18	298±24 [*]	22±2 [*]

RAS, renin angiotensin system; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker. ACEI and ARB treatment lasted for 6 months in old only.

*
p<0.05 vs. young.