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Statins reverse renal inflammation and endothelial dysfunction induced by chronic high salt intake

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can also cause tissue injury independently of blood pressure. For instance, salt promotes kidney and myocardial fibrosis along with increased transforming growth factor (TGF)-β expression and abnormal microvascular function, all of these in the absence of changes in blood pressure (55). In addition, a HS diet impairs the vasodilator response to ACh, a marker of endothelial dysfunction. Under normal conditions, ACh-induced vasodilatation entails the expression of endothelial nitric oxide synthase (eNOS) and thereby nitric oxide (NO) activity. Acute HS intake causes abnormal vascular smooth muscle relaxation in the aorta and in skeletal, cerebral, and resistance arteries (56). Whether chronic HS intake, in the absence of blood pressure changes, causes similar effects on endothelial function in the kidney is unknown.

Interestingly, the lipid-lowering 3-hydroxy 3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) exert beneficial lipid-independent effects on endothelial function, increasing NO upregulation, and reducing oxidative stress and vascular inflammation (11, 21, 46, 58). Thus, in the kidney, statins could also counteract inflammation by rising eNOS activity and hence NO bioavailability.

Consequently, we hypothesized that HS intake impairs kidney structure and function in the absence of significant blood pressure changes and that these changes are reversed by HMG-CoA reductase inhibition. Accordingly, the aim of this study was to assess whether HS intake induces renal endothelial dysfunction and inflammation as reported in other vascular territories in the absence of hypertension and to evaluate the effects of statins on these salt-induced changes.

METHODS

Animals. Male Wistar-Hokkaido rats (~185 g, from the Faculty of Veterinary Science, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina) were housed individually and maintained on ad libitum standard rat chow and tap water in a 12:12-h light-dark cycle. After a 1-wk acclimation period, the rats were divided into 4 groups of 10 animals each: 1) untreated on a normal-salt (NS) diet, 2) untreated on a HS diet, 3) atorvastatin-treated on a NS diet (NS + Ator), and 4) atorvastatin-treated on a HS diet (HS + Ator). The NS rats were fed a diet containing 0.8% NaCl. The HS groups were fed a diet containing 4% NaCl. Atorvastatin was mixed into the rat chow to achieve a dose of 30 mg·kg−1·day−1 (58). After 1 wk of acclimation in the metabolic cages and after 6 wk on their respective regimens, we measured body weight, urinary sodium excretion, and systolic blood pressure by the tail-cuff method (16). Briefly, for 7 days the rats were acclimated to restraint and tail-cuff inflation. Each rat was placed in a plastic restraint maintained at 33–36°C, with its tail
passing through the optical sensor and the compression cuff and then taped to the platform. The cuff was connected to a blood pressure monitor. On inflation, the cuff stopped the blood flow through the tail, and upon deflation the sensor detected the reappearance of the blood flow. The pressure lecture at this point was used as a measure of systolic blood pressure. Five to eight readings were performed and averaged for a single session value. Then, blood was drawn from the tail to measure serum creatinine and cholesterol. Body weights were measured once a week. Twenty-four-hour urine was collected for the determination of urinary excretion of protein, creatinine, and sodium as described previously (16).

All experiments were approved by the Institutional Animal Care and Use Committee at the J. Robert Cade Foundation before performance of any procedures using animals. Housing and handling of the animals were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Renal vascular reactivity. At the end of the experimental period, animals were anesthetized with thiopental sodium (40 mg/kg ip) and placed on a heating pad to maintain rectal temperature at 37°C throughout the study. After tracheal intubation, mean blood pressure was measured through a polyethylene catheter (PE-50) inserted into the right carotid artery and recorded via a transducer connected to a multichannel polygraph (Tekmar). The right jugular vein was cannulated to measure renal blood flow (RBF; model T106, Transonic Systems). The left femoral artery was also cannulated with PE-50 tubing and advanced through the abdominal aorta up into the renal artery, with care taken not to disturb RBF according to previous readings. Through this tubing, we injected first increasing doses (10^{-10} through 10^{-5} mol/l) of ACh, then SNP, and finally ANG II while continuously measuring RBF. The left ureter was catheterized with PE-10 tubing for urine collection.

Glomerular volume. Upon completion of the renal vascular reactivity studies, the left kidney was excised to measure glomerular volume. For this, we followed a sieving technique modified from the procedure described by Beierwaltes et al. (3). After removal and mincing of the cortices, glomeruli were harvested by successive sieving through a 60-mesh (250 μm) stainless steel sieve and then through two Nitex monofilament screens, 48 (390 μm) and 72 mesh (250 μm). The filtered suspension contained glomeruli and remnants of severed tubuli that were then filtered through a 200-mesh (60 μm) silk bolting cloth. The recovered glomeruli were again suspended in modified Krebs solution, centrifuged at 750 g for 5 min, and the supernatants were decanted. This material was transferred to an observation/incubation chamber on an inverted microscope (Diaphot Nikon) containing the same solution used during the sieving procedure. Glomeruli were photographed, and then a grid was placed on a similar chamber containing the same solution and photographed at the same power previously used to photograph glomeruli (×140). This photographed grid contains premeasured squares that allow calibration of a digital image system, Summa Sketch II. Glomerular diameters were measured in no less than 30 glomeruli per kidney, and volumes were calculated by the Weibel-Gomez method (47).

Determination of expression of eNOS and TGF-β1 in kidney. After 6 wk on their respective regimens, the kidneys were perfused with cold heparinized PBS, and then cortices were dissected and placed in RIPA-DOC buffer with protease inhibitors (100 μg/ml PMSF, 5 μg/ml leupeptin, 2 μg/ml isoleucine, and 2 μg/ml orthovanadate). To obtain total cortex protein samples, an aliquot of each cortical sample was homogenized with 10 strokes with a Kontes homogenizer. Glomeruli were isolated as described above (Glomerular volume). Samples were clarified by centrifugation at 13,000 rpm for 5 min at 4°C, and the supernatant was stored at −70°C until use.

The protein concentration in each sample was determined by the Bradford method; further diluted in Laemmli sample buffer, a 100 μg protein/sample was electrophoresed in a 15% polyacrylamide gel and electrotransferred to a polyvinylidene difluoride membrane (Millipore). The membrane was blocked with 5% BSA for 1 h, then incubated overnight at 4°C with primary antibody followed with appropriate horseradish peroxidase-conjugated secondary antibody (JacksonImmunoresearch), visualized by chemiluminescence (ICN), and quantified by densitometry with Scion Image 4.0 software. The following antibodies were used: anti-eNOS (1:500 dilution; Santa Cruz Biotechnology); anti-TGF-β1 (1:500; Chemicon); and anti-β-actin (1:1,000; Santa Cruz Biotechnology).

Histological study. The right kidney was fixed before removal by a 4% paraformaldehyde infusion. The tissue was then embedded in paraffin for assessment by light microscopy and immunohistochemistry. Two- to three-micrometers sections were cut and stained with hematoxylin-eosin and periodic acid-Schiff. Glomerular damage (as revealed by fibrosis, adherence to the capsule, and mesangial expansion and proliferation), interstitial mononuclear cell infiltration, and arteriolar thickening were assessed. A minimum of 100 glomeruli were evaluated in each kidney. The pathologist was blind to the sample and used ×40 resolution to grade the severity in a scale from 0 to 3 as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. An average score was obtained for both glomerular and interstitial changes.

Statistical analysis. All values are expressed as means ± SD. The level of significance for the difference between means was evaluated by one-way analysis of variance followed by Tukey’s post hoc tests. Basal vs. final blood pressure, urinary protein excretion, and eNOS expression were compared using Kruskal-Wallis one-way analysis of variance by rank.

RESULTS

Physiological parameters and renal function. Mean body weight was similar in all groups at baseline and throughout the experimental period (Table 1). Atorvastatin had no effect on water or food ingestion (data not shown). The expected increase in body weight during the 6-wk study period was similar in all groups. The systolic blood pressures at baseline and at the end of the study were not different between groups (Table 1). All animals remained normotensive during the 6 wk of treatment.

Table 1. Physiological parameters during high salt intake

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>SBP, mmHg</th>
<th>UαV, meq/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>6 wk</td>
<td>Basal</td>
</tr>
<tr>
<td>NS (n = 10)</td>
<td>187 ± 23</td>
<td>294 ± 26</td>
<td>113 ± 6</td>
</tr>
<tr>
<td>NS+Ator (n = 10)</td>
<td>190 ± 31</td>
<td>293 ± 30</td>
<td>111 ± 4</td>
</tr>
<tr>
<td>HS (n = 9)</td>
<td>190 ± 34</td>
<td>282 ± 22</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>HS+Ator (n = 13)</td>
<td>191 ± 27</td>
<td>282 ± 34</td>
<td>110 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals. SBP, systolic blood pressure; UαV, urinary sodium excretion rate; NS, normal salt; Ator, atorvastatin; HS, high salt. *P < 0.05 vs. control 6 wk.
As expected, rats on a HS diet increased their urinary sodium excretion rate (Table 1). Atorvastatin caused no changes in total serum cholesterol levels irrespective of the amount of salt in the diet (total serum cholesterol was 85.7 ± 10.4 mg/dl in the NS group, 85.2 ± 8.3 mg/dl in the NS+Ator group, 80.0 ± 11.1 mg/dl in the HS group, and 89.1 ± 14.9 mg/dl in the HS+Ator group). Similarly, atorvastatin did not change cholesterol in any group (data not shown). These data are consistent with other reports (7, 17, 30, 32, 35, 44, and 45) and is thought to result from a statins-induced compensatory increase in the hepatic 3-hydroxy-3-methylglutaryl-CoA reductase synthesis.

At the end of the 6-wk experimental period, the groups receiving a HS diet had higher mean creatinine clearance than NS groups (NS, 0.32 ± 0.09; NS+Ator, 0.31 ± 0.14; HS 0.47 ± 0.13, and HS+Ator, 0.49 ± 0.07 ml·min⁻¹·100 g body wt⁻¹, P < 0.014 vs. control). At 6 wk, the HS diet increased the urinary protein excretion rate 4.8-fold compared with basal levels (P < 0.05). This increase was prevented by atorvastatin (Fig. 1). In rats on NS intake, the protein excretion rate at 6 wk was unchanged from basal values.

Renal hemodynamic changes. Because proteinuria is often associated with endothelial dysfunction, we evaluated the effect of a HS diet on endothelium-dependent vasodilatation. Basal mean intra-arterial blood pressure was not different between the groups (NS, 86 ± 5; NS+Ator, 73 ± 4; HS 77 ± 3; and HS+Ator, 77 ± 5 mmHg, P > 0.20). Figure 2 shows that ACh-induced vasodilatation decreased by 40% in the HS group compared with the NS group (P < 0.05). This indicates that long-term HS intake causes endothelial dysfunction. Atorvastatin administration prevented this effect. SNP-induced vasodilatation and ANG II-induced vasoconstriction were unchanged by salt intake and atorvastatin treatment (data not shown). Because ACh-induced vasodilatation implies NO release, we evaluated whether the beneficial effects of atorvastatin on endothelial dysfunction were associated with changes in cortical and glomerular eNOS expression in the kidney. Figure 3 shows cortical eNOS expression as measured by Western blotting. The HS diet decreased cortical eNOS expression by 62% (P < 0.05). These effects were prevented by atorvastatin. In animals on a NS diet, atorvastatin had no effect on cortical eNOS expression. Figure 4 shows glomerular eNOS expression. The HS diet decreased glomerular eNOS expression by 48% (P < 0.05) while atorvastatin prevented this fall in eNOS expression. These findings demonstrate that atorvastatin prevents the endothelial dysfunction induced by a HS diet, at least in part, by increasing both cortical and glomerular eNOS expression.

Fig. 1. Effect of atorvastatin on urinary protein excretion during a high salt (HS) diet. NS, normal salt diet; NS+Ator, NS diet plus atorvastatin; HS+Ator, HS diet plus atorvastatin. *P < 0.05 vs. HS+Ator.

Fig. 2. Effect of atorvastatin on ACh-induced vasodilation during a HS diet. *P < 0.05 vs. other groups.

Fig. 3. Endothelial nitric oxide synthase (eNOS) expression in renal cortex. A: graph representing cortical eNOS expression. AU, arbitrary units. *P < 0.05 vs. HS+Ator and vs. NS. B: representative Western blot analysis.
Glomerular volume and renal histological changes. Because glomerular changes are often associated with increased protein excretion rate, we evaluated the HS diet-induced changes in glomerular volume and histology.

HS intake increased glomerular volume by 33.33% (P < 0.001) (Table 2 and Fig. 5) while treatment with atorvastatin prevented the development of glomerular hypertrophy in this HS diet group. Atorvastatin did not decrease the glomerular volume in NS rats. The increase in glomerular volume observed in animals with the HS diet was positively correlated with the increase in protein excretion rate (r = 0.83).

Similarly, the HS diet caused a mild glomerular sclerosis together with interstitial mononuclear cell infiltration, all of which were prevented by atorvastatin (Table 2 and Fig. 6). These findings suggest that HS intake causes renal inflammation and that atorvastatin can prevent it even in the absence of changes in blood pressure or serum cholesterol levels.

The glomerular changes in the HS intake group were accompanied by a significant increase in TGF-β1 expression in the renal cortex. TGF-β1 expression as arbitrary units was 1.06 ± 0.39 for HS and 0.52 ± 0.11 for NS (P < 0.05) (Fig. 7). This change was reversed by atorvastatin (HS, 1.06 ± 0.39 vs. HS+Ator, 0.61 ± 0.09, P < 0.05).

DISCUSSION

Several lines of investigation suggest that salt plays an important role in the genesis of hypertension and in cardiovascular injury (55). However, harmful effects from HS intake have been also recognized in the absence of high blood pressure. Indeed, current evidence supports a direct effect of salt intake on vascular structures and although the mechanisms involved are largely unknown, it has been proposed that an anomalous endothelial response may bring about inadequate NO release and excessive TGF-β1 synthesis (50, 51). Indeed, in our study, after 6 wk of HS intake, we found higher urinary protein excretion, decreased renal endothelium-dependent vasodilatation, decreased glomerular eNOS expression, and increased renal TGF-β1 synthesis, all in the absence of hypertension. These changes were accompanied by increased glomerular volume and incipient renal interstitial inflammation. While changing neither serum cholesterol (30) nor blood pressure, atorvastatin prevented all of the salt-induced renal changes.

Endothelial function and glomerular eNOS expression. Our results must be viewed as taking place on a background of normal blood pressure. Indeed, hypertension did not follow HS intake, and yet endothelial-dependent vasodilatation in the kidney decreased in a dose-dependent fashion, varying from 42 to 57% compared with rats on NS intake. These findings were associated with diminished cortical and glomerular eNOS expression in the kidney, suggesting this could be in part responsible for the impaired endothelium-dependent vasodilatation. Indeed, glomerular eNOS deficiency hastens the progression of kidney disease (31) and correlates with vascular injury severity (22, 38). Thus our findings seem very relevant from a pathophysiological standpoint, as they could signal the onset of vascular lesions.

Our results are in line with studies showing that long-term HS intake leads to structural and functional changes in the microcirculation that are independent from changes in arterial pressure (4, 5, 25). Indeed, normotensive rats on a HS diet show impaired endothelium-dependent dilation in response to ACh or increased shear stress in several vascular territories. They also show that this impairment is due to selective loss of NO activity (4, 5). For instance, Li et al. (28) measured eNOS activity in living bovine aortic endothelial cells and showed that a 5-mmol/l increase in salt concentration (from 137 to 142 mmol/l) causes a 25% decrease in eNOS activity in a salt concentration-dependent manner. In fact, NO activity decreased by 25, 45, and 70%, with respective increments of 5, 10, and 20 mmol/l in NaCl concentration. They also showed that salt attenuated the NO-dependent proliferation of endothelial cells. Similarly, Banday et al. (1) found that a HS

Table 2. Glomerular volume and renal histology

<table>
<thead>
<tr>
<th>Group</th>
<th>NS Diet</th>
<th>Atorvastatin</th>
<th>HS Diet</th>
<th>Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular volume, μm³</td>
<td>3.30 × 10⁶ ± 0.1</td>
<td>3.32 × 10⁶ ± 0.2</td>
<td>4.40 × 10⁶ ± 0.2*</td>
<td>3.60 × 10⁶ ± 0.2†</td>
</tr>
<tr>
<td>Renal interstitial infiltration (0-4+)</td>
<td>0</td>
<td>0</td>
<td>1.4 ± 0.06†</td>
<td>0</td>
</tr>
<tr>
<td>Glomerular sclerosis (0-4+)</td>
<td>0</td>
<td>0</td>
<td>1.25 ± 0.5†</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P = 0.0012. †P < 0.05 and ‡not significant vs. control.
diet decreases in vivo the eNOS protein expression in thoracic aortic tissue, while Zhu et al. (56), using aortic strips isolated from normotensive rats on a normal or HS diet, reported that methacholine-induced relaxation was significantly reduced in the HS group. Other investigators found that a HS diet impairs endothelium-dependent relaxation (1) only when combined with a pro-oxidant like l-buthionine sulfoximine. In this case, the impaired endothelial dysfunction is prevented by maneuvers that decrease oxidative stress.

These studies demonstrating salt-induced endothelial dysfunction contrast with reports that show increased, not decreased NO availability in animals on a HS diet. For instance, Wei-Zhong et al. (52) reported that HS intake (8.0%) for 7 days did not affect blood pressure but increased steady-state mRNA and protein levels of eNOS in the arterial wall. Also, Ortiz et al. (33) showed that a HS diet for 7 days increases eNOS in the thick ascending limb of Henle, suggesting a compensatory mechanism to prevent volume overload. Other investigators have reported that increased salt intake enhances NO produc-

Fig. 5. Representatives images of glomerular volume (×40).

Fig. 6. A: normal glomeruli and tubules from a rat on a NS diet. The juxtaglomerular apparatus, the urinary space, and the capillary lumen are preserved. B: 2 glomeruli from a rat on HS intake showing mesangial matrix expansion with mild increase in cellularity. The capillary lumens are obturated. C: outer region of renal medulla in a rat on a NS diet. D: outer region of renal medulla in a rat on a HS diet. An enlarged interstitium surrounds the tubuli with areas of mononuclear cell infiltration. Hematoxylin and eosin (×400).
tion in salt-resistant rats (8) and in healthy humans (2). In these settings, renal plasma flow, glomerular filtration rate and pressure-natriuresis improve while blood pressure returns to basal values (36). In addition, enhanced NO synthesis limits the production of TGF-β1. Other studies also report increased eNOS expression and preserved endothelial function in short-term HS diet experiments (37). A distinctive feature in these studies is the acute setting (a few days of oral salt loading) and the measurements taken at a time when regulatory mechanisms are still fully active laboring to excrete the ingested sodium. Viewed in this context, increased eNOS activity after an acute NaCl load seems a coherent response considering the well-known diuretic effects of NO. Nonetheless, because eNOS undergoes posttranslational changes that regulate its activity, decreased eNOS expression does not necessarily entail decreased NO production. Because we did not measure NO production in these animals, this might be a potential limitation of our study.

The apparent discrepancy between the latter findings and our results may reflect different experimental designs, namely, time of exposure to HS intake and the amount of salt in the diet. Specifically, studies failing to show endothelial dysfunction used diets containing 1–4% salt and lasting for <16 days (37). We believe eNOS expression may not hold during long-term HS intake, particular in the kidney. More precisely, solving this question was our goal and for this we assessed the long-term effects of a HS diet on renal vascular reactivity and cortical and glomerular eNOS expression. Six weeks of treatment is a rather extended period in the lifespan of a rat, and thus the impairment in renal vascular reactivity observed after 6 wk of HS intake could be the forerunner of further hemodynamic and structural changes.

**HS intake increases TGF-β1 expression and proteinuria and causes tissue changes.** We found that HS intake enhances TGF-β1 expression. This is in agreement with studies by Ying et al. (50) showing that dietary salt increases TGF-β1 expression in a dose-dependent fashion. Volume expansion during HS intake may increase shear stress and intracellular calcium and thus TGF-β1 expression via protein tyrosine kinase 2 (26, 43, 54). Moreover, HS intake increases TGF-β1 expression by enhancing the gene expression of phosphorylated p38 MAPK and p42/44 MAPK. Inhibition of these kinases decreases TGF-β1 production (53). Be that as it may, the higher TGF-β1 expression during HS intake may have played a role in the development of glomerular hypertrophy and sclerosis and interstitial mononuclear cell infiltration (58). All of these conditions are known to increase urinary protein excretion rate.

We cannot exclude increased glomerular pressure (via different arteriole constriction) as the cause of increased proteinuria. This is suggested by the higher creatinine clearance in the HS intake group while blood pressure remained unchanged.

Finally, a HS diet may have induced proteinuria by disturbing podocyte permeability via increased TGF-β1 expression. Indeed, podocyte TGF-β1 receptor activation increases podocyte albumin permeability (50). Thus HS intake may increase proteinuria by hemodynamic and humoral mechanisms.

**Statins decrease HS diet-induced proteinuria and endothelial dysfunction.** Because HMG-CoA reductase inhibition has been shown to exert serum cholesterol-independent beneficial effects on endothelial dysfunction and proteinuria in several experimental models (39), we investigated whether the HMG-CoA reductase inhibitor atorvastatin could reverse the effects of a HS salt diet on these parameters. In effect, 6-wk treatment with atorvastatin fully corrected the renal vascular response to ACh and the increased protein excretion rate in rats on HS intake. These effects were independent of serum cholesterol or blood pressure levels.

As shown in several experimental models of renal injury, statins may attain their beneficial effects in more than one manner. For instance, statins block the synthesis of mevalonate, a precursor of isoprenoids-farnesylpyrophosphate (FPP) and geranylpyrophosphate (G-PP), which normally attach posttranslationally to intracellular signaling proteins. Thus, by blocking the synthesis of F-PP and G-PP, statins prevent the anchoring of growth factors to the cell membrane and cytoskeleton, hence hindering signal transductions to the nucleus, activation of transcription factors, and cell proliferation in the vascular endothelium (10). These mevalonate-dependent effects of statins seem unrelated to eNOS. Indeed, Yagi et al. (30) showed that pivastatin prevents ANG II-enhanced proteinuria in eNOS−/− mice (30).

**Atorvastatin preserves eNOS expression during salt loading.** The second relevant aspect of atorvastatin treatment is the reversion of endothelial dysfunction and the correction of eNOS expression in animals on HS intake. In this particular, atorvastatin could have reversed harmful HS diet effects on 1) eNOS synthesis and NO bioavailability; 2) increased levels of oxygen free radicals, leading to enhanced breakdown of NO (13); or 3) alterations in signal transduction pathways such as receptor G protein coupling, as shown for cAMP-mediated vasodilator responses in animals on a HS diet (15, 29).

Atorvastatin could have reversed the HS-induced eNOS inhibition by several means. First, statins prevent Rac1 from migrating to the cell membrane to activate NADH-oxidase and generate reactive oxygen species (12, 20, 48). Second, statins...
inhibit pro-oxidant enzyme systems (NADPH oxidase, xanthine oxidase, etc.) by blocking the expression of protein subunits of G proteins (p22phox and NOX2) (20). Third, statins prolong eNOS mRNA half-life and upregulate eNOS expression (49). Fourth, by blocking geranylgeranylation of Rho GTPase, statins also decrease the levels of the surface protein endothelin-1, a potent vasoconstrictor and mitogen (6). All these effects lead to decreased superoxide anion generation and enhanced eNOS activity and half-life. Thus any one of these mechanisms could have prevented the decreased eNOS expression during the HS diet.

In addition, atorvastatin reversed the increase in TGF-β1 expression induced by HS intake. This effect could be related to atorvastatin’s ability to inhibit p38MAPK, JNK, and ROCK (40).

In conclusion, long-term HS intake induces injurious effects on the kidney as evidenced by the increase protein excretion rate, glomerular hypertrophy, interstitial cell infiltration, and endothelium dysfunction. These inflammatory and hemodynamic changes were not related to hypertension. Atorvastatin corrected all of the changes, suggesting that NO-oxidative stress balance plays a significant role in the early stages of salt-induced kidney damage.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES
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