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Mechanisms of antioxidant and pro-oxidant effects of α-lipoic acid in the diabetic and nondiabetic kidney

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Mechanisms of antioxidant and pro-oxidant effects of α-lipoic acid in the diabetic and nondiabetic kidney.

Background. α-Lipoic acid is a potent antioxidant that improves renal function in diabetes by lowering glycemia, however, the mechanisms by which α-lipoic acid exerts its antioxidant effects are not completely understood.

Methods. Metabolic parameters, renal function, and morphology, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and subunit expression were analyzed in nondiabetic and streptozotocin-induced diabetic rats fed normal rat chow (control) with or without α-lipoic acid (30 mg/kg body weight) for 12 weeks.

Results. Blood glucose was increased with diabetes (nondiabetic + control 89 ± 3 mg/dL and diabetic + control 336 ± 28 mg/dL) and was similar with α-lipoic acid treatment (diabetic + α-lipoic acid 351 ± 14 mg/dL). In contrast, α-lipoic acid attenuated albuminuria (nondiabetic + control 8.9 ± 1.3 mg/day; diabetic + control 28.1 ± 4.6 mg/day; and diabetic + α-lipoic acid 17.8 ± 1.2 mg/day) associated with diabetes. Similarly, α-lipoic acid attenuated glomerulosclerosis (nondiabetic + control 0.22 ± 0.01; diabetic + control 0.55 ± 0.04; diabetic + α-lipoic acid 0.36 ± 0.03), tubulointerstitial fibrosis (nondiabetic + control 0.42 ± 0.18; diabetic + control 1.52 ± 0.05; diabetic + α-lipoic acid 1.10 ± 0.05), superoxide anion ($O_2^{\cdot-}$) generation (nondiabetic +control 15.8 ± 1.7; diabetic +control 87.1 ± 3.5; diabetic + α-lipoic acid 25.5 ± 3.3 RLU/mg protein), and urine 8-isoprostane (8-iso) excretion (nondiabetic + control 7.4 ± 1.4; diabetic + control 26.0 ± 4.5; diabetic + α-lipoic acid 19.6 ± 5.6 ng/day) associated with diabetes. α-Lipoic acid also reduced kidney expression of NADPH oxidase subunits p22phox and p47phox. Surprisingly, α-lipoic acid appears to cause pro-oxidant effects in nondiabetic animals, resulting in increased albuminuria (nondiabetic + α-lipoic acid 14.2 ± 1.2 mg/day), increase in plasma creatinine levels (nondiabetic + control 59 ± 6; diabetic + control 68 ± 6; nondiabetic + α-lipoic acid 86 ± 9; diabetic + α-lipoic acid 69 ± 7 μmol/L), exacerbated glomerulosclerosis and tubulointerstitial fibrosis, increased $O_2^{\cdot-}$ generation, up-regulated p22phox and p47phox expression and increased 8-iso excretion.

Conclusion. We conclude that α-lipoic acid improves albuminuria and pathology in diabetes by reducing oxidative stress, while in healthy animals, α-lipoic acid may act as a pro-oxidant, contributing to renal dysfunction.

Key words: α-lipoic acid, diabetes, kidney, oxidative stress.

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Hyperglycemia-induced oxidative stress has been implicated in the pathophysiology of diabetic nephropathy [1, 2]. Recent findings have suggested that increased intracellular glucose levels, as a result of increased glucose uptake into the kidney, lead to the formation of advanced glycation end products (AGEs). AGEs in turn stimulate local production of oxygen free radicals leading to oxidative stress [1–4]. In uncontrolled diabetes, the levels of superoxide dismutase, the enzyme responsible for inactivating one such oxygen radical [i.e., superoxide anion ($O_2^{\cdot-}$)], are decreased in the kidney [5, 6]. Furthermore, other factors known to be up-regulated in diabetes, including angiotensin II (Ang II), have also been shown to increase formation of oxygen radicals, leading to oxidative tissue damage [7, 8]. Ang II up-regulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, whose product, $O_2^{\cdot-}$, can interact with nitric oxide to form peroxynitrite [7, 9–12]. Nitric oxide, a potent vasodilator and an antiproliferative agent, has been shown to be beneficial in preventing oxidative end-organ damage [7, 9–12]. These studies clearly indicate that preventing oxidative stress is beneficial in preventing abnormal regulation of cellular processes that contribute to the development and pathogenesis of diabetic renal disease.

Thus far, treatments with antioxidants, including vitamins E and C and taurine, have had limited success in preventing the progression of diabetic renal complications [13–15]. Both human and animal studies have shown that high doses of vitamin E fail to prevent albuminuria while lower doses exacerbate renal injury [16, 17]. These studies stress the importance of developing novel antioxidant

treatments for reducing the incidence and attenuating the progression of diabetic complications.

α-Lipoic acid is a sulfur-containing coenzyme involved in mitochondrial dehydrogenase reactions leading to adenosine triphosphate (ATP) formation [18, 19]. α-Lipoic acid, along with its major metabolite dihydrolipoic acid (DHLA), is a potent antioxidant via scavenging of oxygen free radicals, redox interaction with other antioxidants [20, 21], and inhibition of lipid peroxidation [6]. The beneficial effects of α-lipoic acid in diabetes have long been recognized, however, these beneficial effects have mainly been attributed to its glycemic control [22]. More recently, the effects of α-lipoic acid in the treatment of diabetic end-organ complications, including diabetic nephropathy, have been reported [6, 21, 23, 24]. In diabetic neuropathy, α-lipoic acid improves blood flow and reduces oxidative stress in the distal nerves [25]. In early diabetes, α-lipoic acid decreases vascular endothelial growth factor (VEGF) expression and hypoxia in the retina [26]. In diabetic nephropathy, α-lipoic acid has been shown to prevent renal insufficiency, glomerular mesangial matrix expansion, and glomerulosclerosis by restoring glutathione and reducing malondialdehyde levels [22]. The aim of our study was to further elucidate the mechanisms by which α-lipoic acid exerts its antioxidant effects and provides renoprotection in diabetic nephropathy.

METHODS

Animal model

Male Sprague-Dawley rats (Harlan, ~200 g) were randomly divided into four treatment groups: nondiabetic on control (normal rat chow) diet, nondiabetic on α-lipoic acid (normal rat chow containing α-lipoic acid) diet, diabetic on control diet, and diabetic on α-lipoic acid diet, with $N = 4$ to 6 animals per group. α-Lipoic acid diet was custom prepared by Harlan Teklad and contained 400 mg/kg of α-lipoic acid. The rats were supplemented with α-lipoic acid at a dose of 30 mg α-lipoic acid/kg body weight per day. The diabetic rats were given additional chow without α-lipoic acid to make up the difference in the food intake. Following an overnight fast, the animals were either given a single intraperitoneal injection of 0.1 mol/L citrate buffer, pH 4.5 (nondiabetic) or 55 mg/kg streptozotocin in 0.1 mol/L citrate buffer (diabetic). The diabetic animals were given daily injections of insulin (4 to 6 U) (Lantus) (Aventis Pharmaceuticals Inc., Kansas City, MO, USA) to maintain blood glucose levels between 250 and 400 mg/dL. During the treatment period (12 weeks), the animals were placed in metabolic cages every 4 weeks and urine samples collected for the measurement of albumin excretion and creatinine clearance. After 12 weeks of treatment, the animals were anesthetized with sodium pentobarbital (40 mg/kg intraperitoneally) and their femoral vessels catheterized. Systemic blood pressure was monitored electronically using Cardiomax-II (Columbus Instruments, Columbus, OH, USA) blood pressure analyzer. Following blood pressure measurements, blood samples were collected by cardiac puncture for measurement of plasma creatinine. An abdominal incision was made, the right kidney was removed, dissected into cortex and medulla, and snap-frozen in liquid nitrogen for $O_2^{\cdot-}$ measurement and Western blot analysis. The left kidney was perfused first with ice-cold phosphate-buffered saline (PBS) until cleared of blood and then with 4% paraformaldehyde for morphologic analysis and immunohistochemistry.

Plasma creatinine

Plasma creatinine concentrations were determined using a Beckman Creatinine Analyzer II (Brea, CA, USA) with the modified Jaffé rate method, according to the manufacturer's instructions.

Urine albumin excretion (UAE)

Urine albumin concentration was determined using a Nephrat II Albumin Kit (Exocell, Inc., Philadelphia, PA, USA) according to the manufacturers' protocol.

Morphology

Four percent paraformaldehyde-fixed tissue was embedded in paraffin and sectioned at 4 μm for morphologic analysis. The sections were stained with periodic acid-Schiff (PAS) (for demonstration of glycogen content) and Masson's trichrome (for demonstration of collagen deposition).

Glomerulosclerotic index

PAS-stained sections were examined using a Nikon Eclipse E600 light microscope. Ten sections and 80 glomeruli per section were randomly selected and the degree of glomerulosclerosis determined using a semiquantitative scoring method as previously described [27].

Index of tubulointerstitial fibrosis

Masson's trichome-stained sections were examined using a Nikon Eclipse E600 light microscope. Forty sections were randomly selected, and the degree of tubulointerstitial fibrosis determined using a semiquantitative scoring method as previously described [28].

NADPH-stimulate $O_2^{\cdot-}$ generation

$O_2^{\cdot-}$ generation in response to stimulation with NADPH was assessed by lucigenin-enhanced chemiluminescence as previously described [29]. Briefly, cortical

and medullary tissues were homogenized in PBS then incubated with 5 μmol/L lucigenin and the oxidase reaction stimulated by addition of 1 mmol/L NADPH. The background-adjusted chemiluminescence signal was sampled every second for 10 minutes and recorded using a tube luminometer with automated injector (AutoLumat*Plus* LB 953) (EG&G Berthhold, Germany).

Renal excretion of 8-iso prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

8-iso $PGF_{2\alpha}$ was assessed as previously described [30]. Briefly, total 8-iso $PGF_{2\alpha}$ was purified from urine samples using a phenylboronic acid column (Varian Inc., Palo Alto, CA, USA) and eluted with ethyl acetate containing 1% methanol and assayed with an enzyme immunoassay procedure (Cayman Chemical, Ann Arbor, MI, USA).

Imunohistochemistry

Paraffin sections were incubated with 10% nonimmune goat serum, followed by incubation with monoclonal antibodies directed against p22phox [31] and p47phox [32] for 3 hours at room temperature. The positive immunoreaction was detected using the Envision Plus Peroxidase Kit (Dako Corporation, Carpentaria, CA, USA) and by counterstaining with Mayer's hematoxylin. Sections incubated with 10% nonimmune goat serum instead of the primary antiserum were used as negative controls.

Western blotting

Homogenized samples from the renal cortex and medulla were separated on 18% sodium dodecyl sulfate (SDS-PAGE) gels and the proteins were transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat milk, followed by primary antibodies as described for immunohistochemistry at 4°C overnight. The membranes were washed and incubated with horseradish peroxidase-conjugated goat antimouse IgG, and the proteins were visualized by chemiluminescence (KPL, Gaithersburg, MD, USA). The densities of specific bands were quantitated by densitometry using Scion Image beta (version 4.02) software. Band densities were normalized to the total amount of protein loaded in each well, as determined by densitometric analysis of gels stained with Coomassie blue.

Statistical analysis

Data are expressed as mean ± SEM, except for albuminuria, which is expressed as geometric mean x/÷ tolerance factor, and were analyzed with a two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test, using the SigmaStat software (Jandel Scientific, San Rafael, CA, USA). Differences were considered statistically significant at $P < 0.05$.

Table 1. Effects of α-lipoic acid on body and kidney weight and blood glucose

	Control		α-Lipoic acid	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Body weight *g*	440 ± 12	347 ± 23[b]	472 ± 13[a]	386 ± 26[b]
Kidney weight *g*	1.2 ± 0.1	1.7 ± 0.1[b]	1.3 ± 0.1	1.4 ± 0.1[a]
Kidney/body weight	2.8 ± 0.1	4.9 ± 0.7[c]	2.6 ± 0.05	3.1 ± 0.2[a]
Blood glucose *mg/dL*	89 ± 3	336 ± 28[c]	92 ± 3	352 ± 14[c]
Mean arterial pressure *mm Hg*	119 ± 9	122 ± 14	117 ± 13	120 ± 11

Data are expressed as mean ± SEM.
[a] $P < 0.05$; [b] $P < 0.05$ vs. nondiabetic in same treatment group; [c] $P < 0.01$ vs. control in same metabolic state.

RESULTS

Metabolic parameters and blood pressure

Compared to nondiabetic + control animals, body weight in the diabetic + control group was decreased by 1.2-fold. Similarly, body weight in the diabetic + α-lipoic acid group decreased by 1.2-fold compared to nondiabetic + α-lipoic acid (Table 1). Kidney weight was increased by 1.4-fold in the diabetic + control group compared to nondiabetic + control but was not significantly changed in the diabetic + α-lipoic acid group, as compared to nondiabetic + α-lipoic acid (Table 1). Kidney to body weight ratio was also increased in the diabetic + control group compared to nondiabetic + control (1.8-fold), whereas kidney/body weight remained similar in nondiabetic + α-lipoic acid and diabetic + α-lipoic acid animals (Table 1). Overall, α-lipoic acid treatment resulted in significant reductions in kidney weight (1.2-fold) and kidney/body weight ratio (1.6-fold) in diabetic animals (Table 1).

Blood glucose was threefold higher in diabetic compared to the nondiabetic group (Table 1). In the diabetic group, no differences in blood glucose levels were observed between the control and α-lipoic acid–treated rats. No differences in mean arterial pressure (MAP) were observed between any of the treatment groups (Table 1).

UAE and plasma creatinine

UAE increased by threefold with no change in plasma creatinine in the diabetic + control group compared to nondiabetic + control (Table 2). Consistent with its protective properties, α-lipoic acid treatment reduced albumin excretion by 1.6-fold in diabetic animals. In contrast, α-lipoic acid actually increased albumin excretion (1.6-fold) and increased plasma creatinine (1.2-fold) in

Table 2. Effect of α-lipoic acid on renal function and structure

	Control		α-Lipoic acid	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Urine albumin excretion *mg/24 hours*	8.8 x/÷1.3	28.1 x/÷4.6[d]	14.2 x/÷1.2[a]	17.8 x/÷1.2[b]
Plasma creatinine *μmol/L*	59 ± 6	68 ± 6	86 ± 9.0[a]	69 ± 7[c]
Glomerulosclerotic index	0.22 ± 0.01	0.55 ± 0.04[d]	0.61 ± 0.04[b]	0.36 ± 0.03[b,d]
Cortical tubulointerstial fibrosis index	0.42 ± 0.2	1.52 ± 0.05[d]	1.29 ± 0.2[b]	1.10 ± 0.05[a]
Medullary tubulointerstitial fibrosis index	0.56 ± 0.2	2.0 ± 0.1[d]	0.72 ± 0.2	0.49 ± 0.07[a]

Data are expressed as mean ± SEM except for urine albumin excretion, which is expressed as geometric mean x/÷ tolerance factor.
[a] $P < 0.05$; [b] $P < 0.01$ vs. control in same metabolic state; [c] $P < 0.05$; [d] $P < 0.05$ vs. nondiabetic in same treatment group.

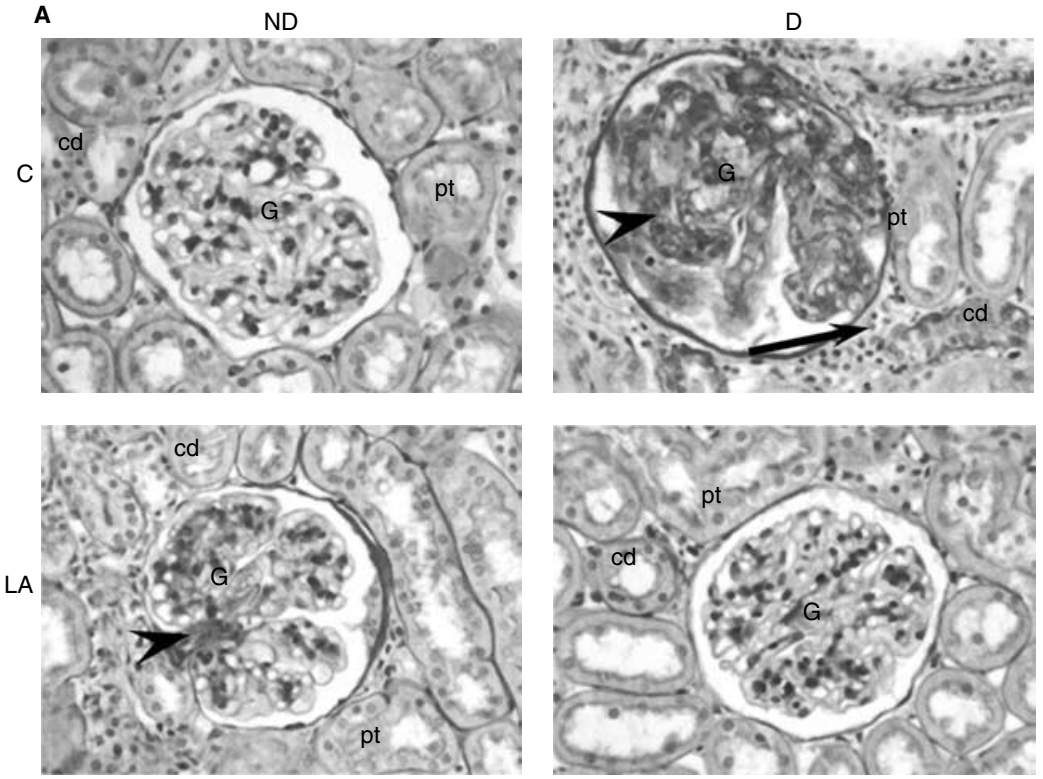

Fig. 1. Periodic acid-Schiff (PAS) and Masson's trichrome stains. (*A*) PAS-stained sections of the renal cortex of the nondiabetic (ND) and diabetic (D) kidneys treated with α-lipoic acid (LA). Glomerulosclerosis (G) with thickening of basement membranes of the proximal tubules (pt), mesangial expansion (arrow heads), and inflammatory infiltrates (arrows). Masson's trichrome-stained sections of the renal cortex (*B*) and medulla (*C*) of nondiabetic (ND) and diabetic (D) kidneys treated with α-lipoic acid (LA). Outlined are areas of tubulointerstitial fibrosis, accumulation of extracellular matrix (ECM) proteins, capillary occlusion, and increased proliferation of interstitial fibroblasts (original magnification ×400).

nondiabetic animals compared to nondiabetic + controls (Table 2).

Glomerulosclerosis and tubulointerstitial fibrosis

The kidneys of nondiabetic + control rats exhibited normal cortical (Fig. 1) and medullary (Fig. 1B) morphology. Mild glomerulosclerosis, characterized by glomerular basement membrane thickening and mesangial expansion was observed in diabetic + control animals, with a 2.6-fold greater glomerulosclerotic index compared to that of nondiabetic + control (Table 2). Treatment with α-lipoic acid reduced glomerulosclerosis in the diabetic group by 1.5-fold compared to diabetic + control; however, treatment of nondiabetic animals with α-lipoic acid was associated with moderate glomerulosclerosis, similar to that observed in the diabetic + control group (Fig. 1A). Mild tubulointerstitial fibrosis, characterized by accumulation of extracellular matrix (ECM), tubular dilatation, and atrophy was observed in the diabetic + control animals (Fig. 1B). The tubulointerstitial fibrosis index was 3.6-fold greater in both the renal cortex and medulla compared to that of nondiabetic + control (Table 2). Treatment with α-lipoic acid reduced tubulointerstitial fibrosis index in the renal cortex (1.3-fold) and medulla (1.4-fold) in the diabetic group compared to

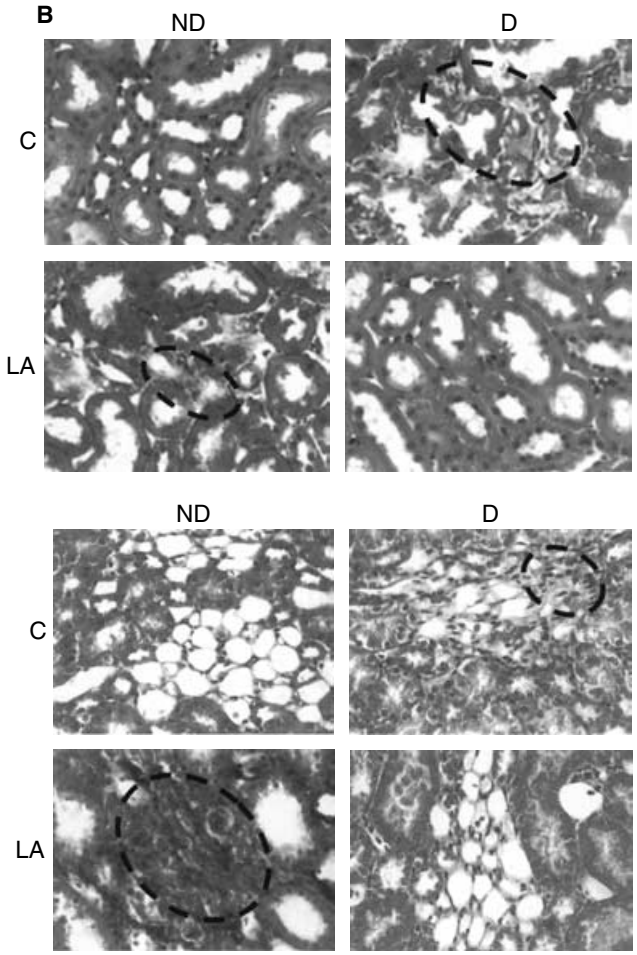

Fig. 1. (continued.)

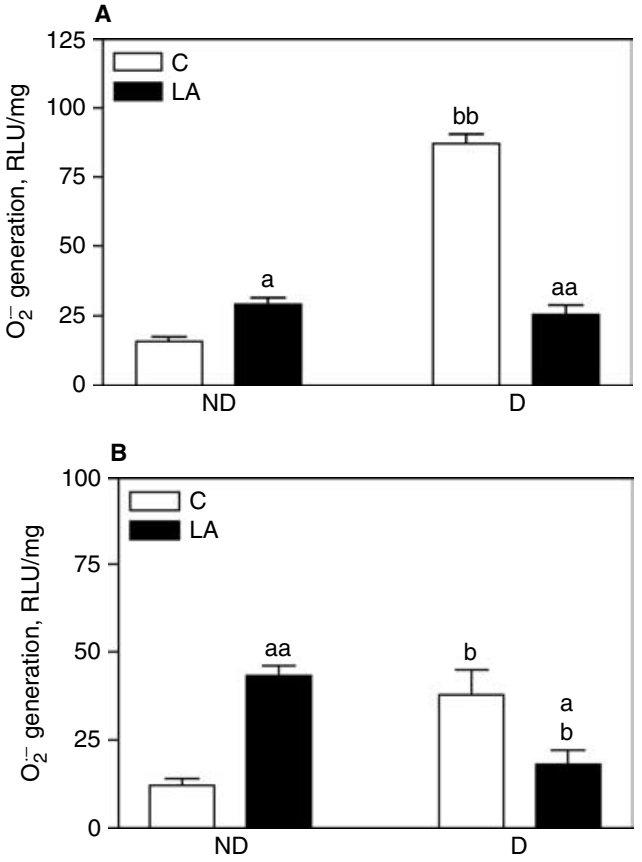

Fig. 2. Nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated superoxide anion ($O_2^{\cdot-}$) generation in the renal cortex and medulla in nondiabetic (ND) and diabetic (D) kidneys treated with α-lipoic acid (LA). (*A*) Cortical NADPH-stimulated $O_2^{\cdot-}$ levels. (*B*) Medullary NADPH-stimulated $O_2^{\cdot-}$ levels. Data are expressed as mean ± SEM ($N = 5$ per group). $^{a}P < 0.05$; $^{aa}P < 0.01$ vs. control in same metabolic state; $^{b}P < 0.05$; $^{bb}P < 0.01$ vs. nondiabetic in same treatment group.

diabetic + control. However, in the nondiabetic group, treatment with α-lipoic acid increased tubulointerstitial fibrosis in the renal cortex by threefold and the medulla by 1.3-fold compared to nondiabetic + control group.

NADPH-stimulated superoxide ($O_2^{\cdot-}$) generation

NADPH-stimulated $O_2^{\cdot-}$ generation was increased in both the renal cortex (5.5-fold) and medulla (threefold) in the diabetic + control group compared to nondiabetic + control (Fig. 2). While treatment with α-lipoic acid reduced NADPH-stimulated $O_2^{\cdot-}$ generation in the cortex by 3.4-fold and in the medulla by twofold of diabetic kidneys, α-lipoic acid increased NADPH-stimulated $O_2^{\cdot-}$ generation in the renal cortex (1.8-fold) and medulla (3.6-fold) of nondiabetic kidneys compared to nondiabetic + control (Fig. 2).

Renal excretion of 8-iso

Renal excretion of 8-iso increased by 3.5-fold in the diabetic + control group compared to nondiabetic + control (Fig. 3). While treatment with α-lipoic acid did not affect 8-iso excretion levels in the diabetic group, α-lipoic acid increased 8-iso excretion in the nondiabetic group by 2.6-fold.

NADPH oxidase subunit immunolocalization and protein expression

p22phox protein was immunolocalized to the macula densa and distal tubule (Fig. 4), which is similar in distribution to that previously observed in the normal rat kidney [33]. Consistent with our $O_2^{\cdot-}$ results, the intensity of p22phox immunostaining was increased in diabetes and attenuated with α-lipoic acid treatment. Western blot analysis confirmed the immunohistochemical observation, showing that p22phox protein expression was upregulated in the cortex (fourfold) and medulla (twofold) in diabetes, and this increase was attenuated by α-lipoic acid treatment (Fig. 5).

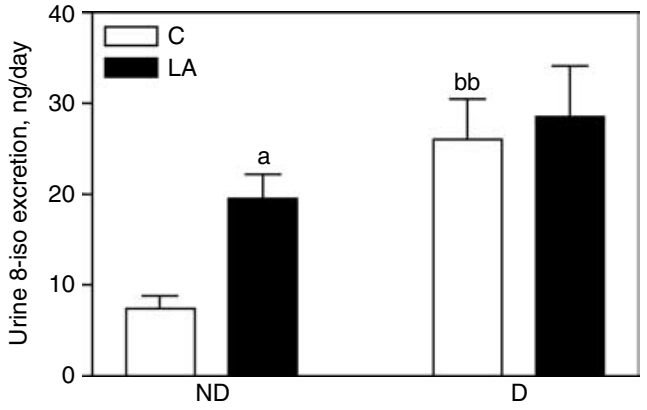

Fig. 3. Excretion of 8-isoprostane (8-iso) prostaglandin $F_{2\alpha}$ in nondiabetic (ND) and diabetic (D) kidneys treated with α-lipoic acid (LA). Data are expressed as mean ± SEM ($N = 5$ per group). $^{a}P < 0.05$ vs. control in same metabolic state; $^{bb}P < 0.01$ vs. nondiabetic in the same treatment group.

p47phox protein was immunolocalized to podocytes and distal tubule (Fig. 4), consistent with previous observations in the normal kidney [33]. While the overall intensity of p47phox immunostaining increased in diabetes, p47phox also stained to proximal tubules in diabetic kidneys, and this was attenuated with α-lipoic acid treatment (Fig. 4). Consistent with our immunolocalization studies, immunoblot analysis showed the p47phox protein expression was increased in the cortex (1.4-fold) and medulla (1.7-fold) in diabetes, and this was attenuated with α-lipoic acid treatment (Fig. 5). Expression of both p22phox and p47phox was increased in nondiabetic + α-lipoic acid compared to the nondiabetic + control kidneys.

DISCUSSION

This study demonstrates that dietary supplementation with 30 mg/kg α-lipoic acid for 12 weeks prevents the increase in albuminuria and development of glomerulosclerosis and tubulointerstitial fibrosis associated with diabetic nephropathy. Our studies indicate that one of the mechanisms by which α-lipoic acid exerts this renoprotective effect is via decreasing oxidative stress, specifically, by reducing NADPH-induced generation of $O_2^{\cdot-}$ and regulating the expression of NADPH oxidase subunits. Most interestingly, our study shows that the dietary supplementation with the same dose of α-lipoic acid is associated with a decline in renal function and development of glomerulosclerosis and tubulointerstitial fibrosis in the nondiabetic kidney. Thus, these findings indicate that, although α-lipoic acid is renoprotective in diabetic nephropathy, it has detrimental effects to the healthy kidney.

Our study confirms the previously reported findings that dietary supplementation with α-lipoic acid attenuates albuminuria and renal pathology associated with diabetic nephropathy in the streptozotocin-induced diabetic rat [24, 34, 35]. Studies in other animal models have reported that the beneficial effects of α-lipoic acid are mainly exerted through reducing glycemia and blood pressure [36, 37]. However, our study clearly demonstrates that α-lipoic acid is renoprotective through reducing oxidative tissue damage, rather than control of glucose or blood pressure (no differences in glucose levels or blood pressure were observed between the diabetic animals on control or α-lipoic acid–supplemented diet). An antioxidant effect of α-lipoic acid in diabetic nephropathy has also been suggested by others [35], and our study extends these findings to provide a mechanistic insight to explain at least part of the antioxidant effects of α-lipoic acid.

In diabetes, reactive oxygen species are generated from an excess shunting of glucose into the polyol and glucosamine pathways [38], which leads to the formation and activation of protein kinase C, formation of AGEs and glycation [39, 40]. This leads to tissue damage by increased synthesis of ECM proteins and enhanced expression of inflammatory mediators, thereby contributing to glomerulosclerosis and tubulointerstitial fibrosis in the kidney [41]. Our study shows that diabetes is associated with increased oxidative stress in both the renal cortex and medulla, as measured by NADPH-induced $O_2^{\cdot-}$ generation. Previous studies have demonstrated that α-lipoic acid also improves other markers of oxidative stress in the diabetic kidney, including glutathione and malondialdehyde [35, 42]. Although our study shows that α-lipoic acid exerts its renal antioxidant effects through decreasing NADPH-induced $O_2^{\cdot-}$ generation, no effects of α-lipoic acid on 8-iso excretion were observed in diabetic rats. This is most likely due to extrarenal production of $O_2^{\cdot-}$ by other oxidases not affected by α-lipoic acid. Our data also demonstrate that α-lipoic acid decreases the diabetes-associated up-regulation of p22phox and p47phox expression, the two NADPH oxidase subunits. Previous studies have demonstrated that one of the mechanisms contributing to increased oxidative stress in the diabetic kidney is increased expression of NADPH oxidase subunits, namely p47phox, p67phox, and NOX4 [2, 43]. In the spontaneously hypertensive rat, treatment with antioxidants, including α-tocopherol and ascorbic acid, reduces the expression of these subunits [44]. To date, there are no reports on the effects of antioxidants on the regulation of NADPH oxidase subunits in diabetic nephropathy. Our study clearly demonstrates that α-lipoic acid reduces oxidative stress by regulating the expression of enzymes involved in formation of reactive oxygen species.

An unexpected and startling finding in our study was that dietary supplementation with α-lipoic acid, at doses that prevent the decline in renal function and pathology in

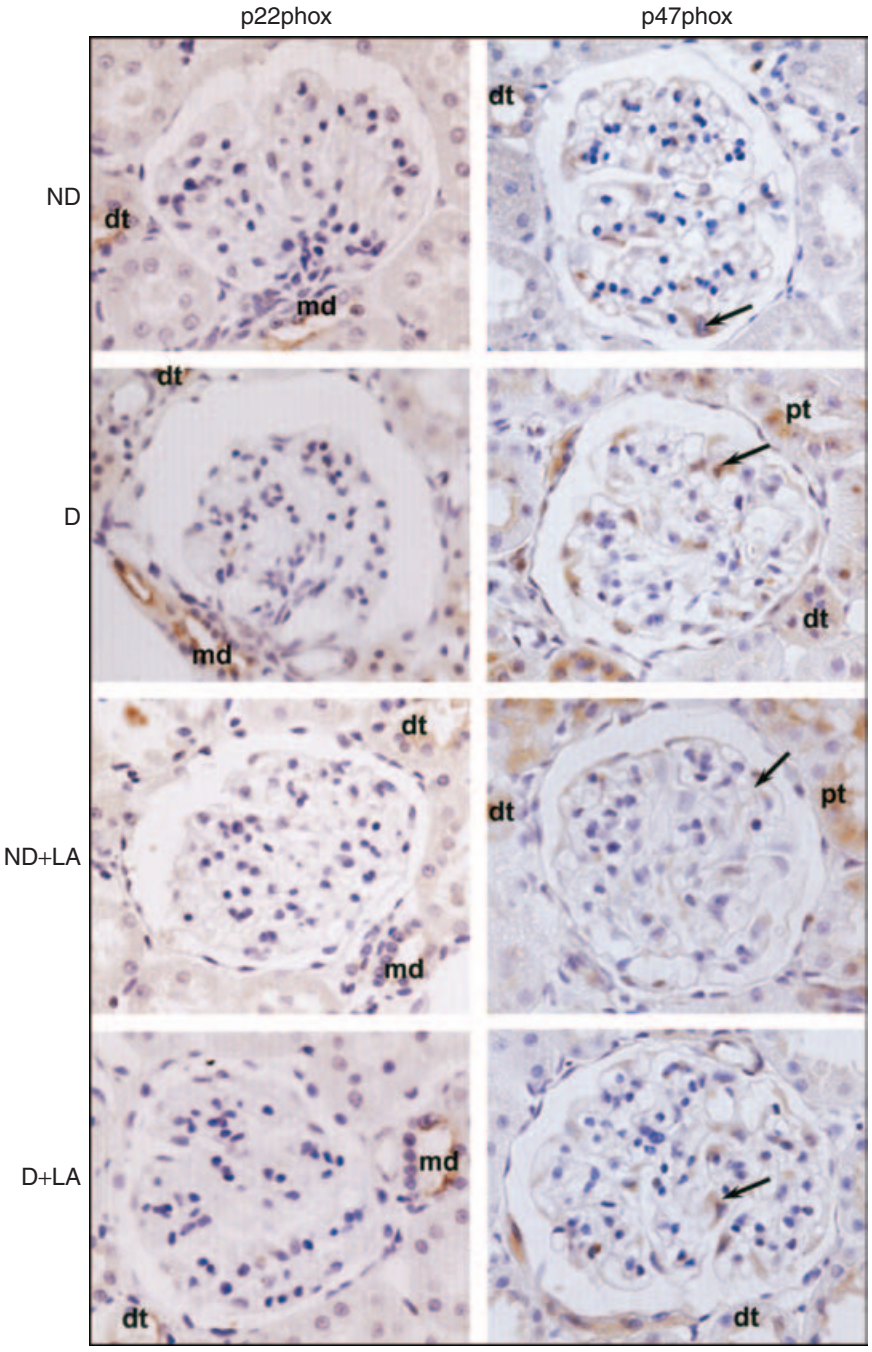

Fig. 4. Immunohistochemical localization of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits in nondiabetic (ND) and diabetic (D) kidneys treated with α-lipoic acid (LA). Abbreviations are: Md, macula densa; pt, proximal tubule; dt, distal tubule; arrows, podocytes (original magnification ×400).

diabetic nephropathy, are detrimental to the healthy kidney. In nondiabetic rats, treatment with α-lipoic acid is associated with a decline in creatinine clearance, increases in albuminuria, glomerulosclerosis, tubulointerstitial fibrosis, and increases in NADPH-induced $O_2^{\cdot-}$ generation and 8-iso excretion. These changes are similar to those observed in the kidneys of untreated diabetic rats. It is quite surprising that none of the previous studies examining the effects of α-lipoic acid in diabetic nephropathy have ever reported the effects of α-lipoic acid in the nondiabetic kidney, and, according to our findings, in contrast to the diabetic animals where α-lipoic acid exerts a protective effect, α-lipoic acid appears to have a deleterious effect in nondiabetic animals. Scott et al [45] reported that DHLA, the reduced metabolite of α-lipoic acid, may have pro-oxidant effects, via its ability to reduce iron and generate reactive sulfur-containing radicals that can damage proteins such as alpha 1-antiproteinase and creatine kinase [45]. α-lipoic acid and DHLA have also been shown to stimulate $O_2^{\cdot-}$ production in rat liver

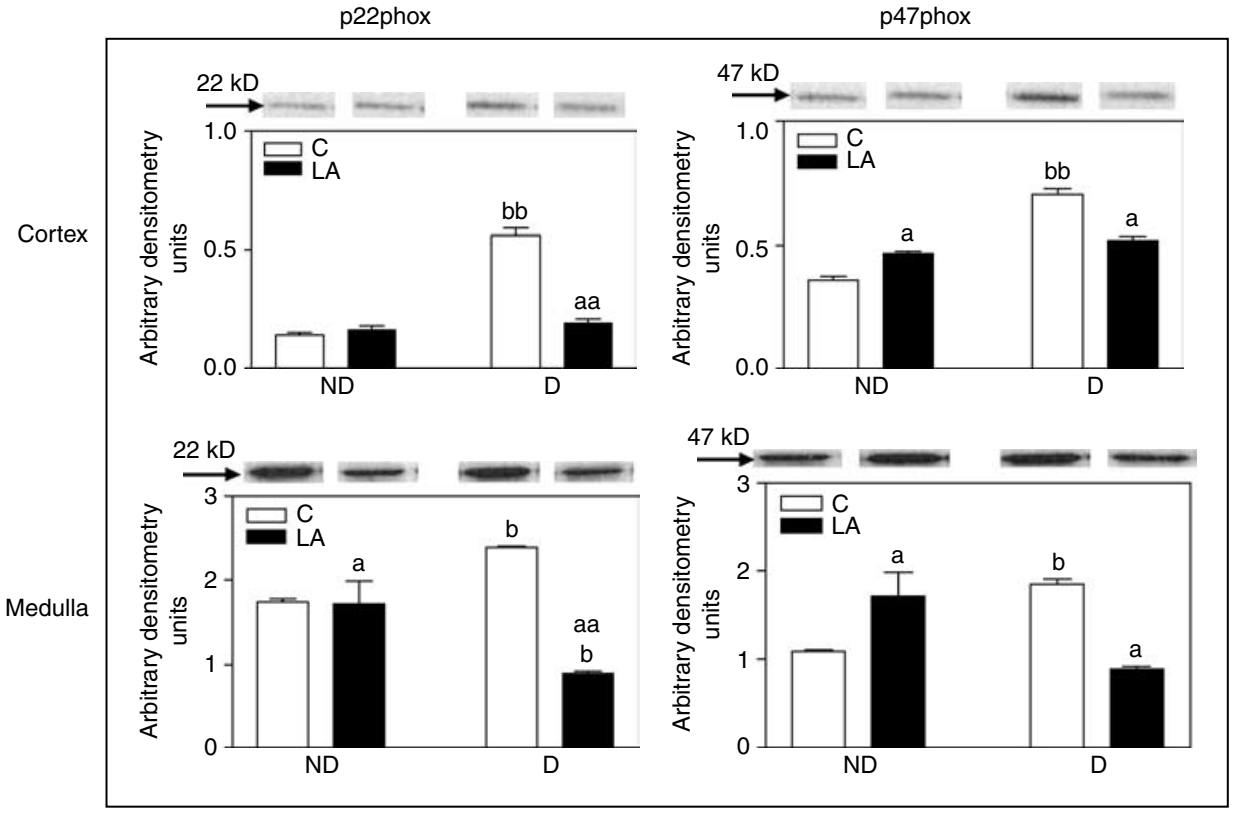

Fig. 5. Renal expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit protein in nondiabetic (ND) and diabetic (D) kidneys treated with α-lipoic acid (LA). Top panels in each graph are representative Western blots. Bottom panels in each graph are densitometric analyses of the blots shown above. Data are expressed as mean ± SEM ($N = 6$ per group). $^{a}P < 0.05$; $^{aa}P < 0.01$ vs. control in same metabolic state; $^{b}P < 0.05$; $^{bb}P < 0.01$ vs. nondiabetic in the same treatment group.

mitochondria and submitochondrial particles and in cultured 3T3-L1 adipocytes in response to high glucose stimuli [46]. Consistent with these findings, we observed that α-lipoic acid increases NADPH-induced $O_2^{\cdot-}$ and expression of p47phox in the nondiabetic kidney and thus by acting as a pro-oxidant, causes deleterious effects in the healthy kidney.

The detrimental effects of α-lipoic acid on renal function and pathology may be dose-related. We have chosen a dose of α-lipoic acid previously reported to have beneficial effects in the diabetic kidney [34]. Although our studies confirm these findings, we also show that this same dose of α-lipoic acid (30 mg/kg body weight) compromises renal structure and function under nondiabetic conditions. A wide range of daily recommended doses of α-lipoic acid have been reported and used in human trials, ranging from 100 to 1800 mg/day [47–49]; although little attention has been paid to this dose relative to body weight, duration of the trial or underlying physiologic state, especially relating to renal function. In a trial using dietary supplementation with α-lipoic acid at a dose of 600 mg/day for 3 months, a reduction of UAE was observed in type 2 diabetic patients [48]; however, no effects of α-lipoic acid in healthy individuals were reported in this study. Another human trial reported that α-lipoic acid at a dose of 600mg/day for 8 weeks in healthy volunteers (age group ∼38 years of age) reduced 8-iso excretion [50]; however, no other markers of oxidative stress or renal function were reported. In experimental animal models, α-lipoic acid has been used in the range of 25 to 100 mg/kg/day [24, 34, 35]. It is quite intriguing, however, that none of these animal studies report the findings in the control, healthy animals. Thus, our results stress the importance of monitoring the dose of α-lipoic acid supplementation, duration of treatment and its potential harmful effects in the healthy kidney.

Based on the findings of our study, we conclude that α-lipoic acid may exert both pro- and antioxidant effects, depending on the underlying physiologic and metabolic state. While α-lipoic acid is renoprotective in diabetic nephropathy, it may have potential harmful effects in an otherwise healthy kidney.

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