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Original Research Communication

Alpha-Lipoic Acid Modulates Heat Shock Factor-1 Expression in Streptozotocin-Induced Diabetic Rat Kidney

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ABSTRACT

Increased oxidative stress and impaired heat shock protein (HSP) synthesis may contribute to diabetic nephropathy. The question of whether 8-week thiol antioxidant alpha-lipoic acid (LA) supplementation modulates HSP response and oxidative stress was studied in the kidney of streptozotocin-induced diabetic (SID) and nondiabetic rats. SID caused a histological mesangial expansion, tubular dilatation, and increased levels of transforming growth factor-beta (TGF-β), a mediator of glomerulosclerosis. SID increased 4-hydroxynonenal (4-HNE) protein adduct formation, a marker of lipid peroxidation, and heme oxygenase-1 (HO-1), also a marker of oxidative stress. Moreover, SID increased the DNA-binding activity of heat shock factor-1 (HSF-1) and expression of heat shock protein 60 (HSP60). In contrast, LA supplementation partially reversed histological findings of glomerulosclerosis and decreased TGF-β. LA also increased HSF-1 and decreased HO-1 protein expression, without affecting 4-HNE protein adduct levels. At the mRNA level, LA increased expression of HSF-1, HSP90, and glucose-regulated protein (GRP75) in both control and diabetic animals and HSP72 in SID rats. However, LA supplementation did not affect these HSPs at the protein level. These findings suggest that in addition to its antiglomerulosclerotic effects, LA can induce cytoprotective response in SID. *Antioxid. Redox Signal.* 9, 497–506.

INTRODUCTION

Nephropathy is a major cause of morbidity in diabetes (58). Diabetes results in increased oxidative stress, alterations in lipid metabolism, oxidative modification of proteins and lipids, and perturbations in tissue antioxidant systems (3, 9, 14, 18, 24, 27).

Experimental diabetes induced by streptozotocin (STZ) is accompanied by oxidative stress and renal injury characterized by glomerulosclerosis (33). Glomerulosclerosis is in part a result of oxidative stress-induced mesangial cell dysfunction mediated by transforming tissue growth factor-beta (TGF-β), which results in excessive accumulation of mesangial matrix (11). Both short- and long-term supplementation with alpha-lipoic acid (LA) prevented or attenuated these pathological changes (32, 33). LA is a natural, potent thiol antioxidant that is also capable of regenerating major physiological antioxidants of the lipid and aqueous phases (25, 48, 49). In addition to its antioxidant properties, LA is a cofactor in oxidative metabolism (39), a substrate for reduction, modulating intracellular reducing equivalents (45), stimulates glucose uptake (26), and regulates redox-dependent gene expression (49). Therefore, LA has been considered to be a metabolic antioxidant and has been used widely as a thera-

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peutic agent in diseases associated with increased oxidative stress, including diabetes and its complications (39).

We have previously shown in rats that 8 weeks of LA supplementation provided protection against lipid peroxidation and favorably influenced antioxidant levels in the heart and skeletal muscle after exhaustive exercise (25). Moreover, our group has recently shown that LA is capable of compensating the deleterious effects of oxidative stress and upregulating heat shock protein (HSP) synthesis in experimental diabetes in the liver and heart tissue (37). HSPs are a class of stress-induced proteins that protect against tissue injury by maintaining protein synthesis, repairing damaged proteins, and promoting the healing of injured tissue (41, 42). Our group has recently demonstrated impaired HSP synthesis in skeletal muscle, liver, and heart in STZ–induced experimental diabetes (SID) in rats (4, 37). We suggested that these changes were modulated by transcriptional mechanisms, most probably via decreased activity of heat shock transcription factor-1 (HSF-1) (4).

Data from the few previous studies on the effects of LA on heat shock response are conflicting: LA supplementation *in vitro* did not alter HSP expression in endothelial cells and macrophages (15, 30), whereas LA was shown to normalize low levels of plasma HSPs in type 1 diabetic subjects (51). Biologically, LA exists as a lipoamide in mitochondrial proteins, where it is covalently linked to a lysyl residue. Interestingly, among all animal tissues, the highest concentrations of LA in the form of lipoyllysine have been detected in kidney (49). Because LA has the highest bioavailability in kidney, it is anticipated that renal tissue would be particularly responsive to LA supplementation. Nevertheless, to our knowledge the effect of LA on renal HSP expression has not been studied before.

We therefore tested our hypothesis that diabetes can modulate HSF-1 mediated HSP expression in the kidney of SID rats. We also examined whether LA supplementation could improve impaired renal tissue protection in experimental diabetes.

MATERIALS AND METHODS

Experimental protocol

The experimental protocol was approved by the Ethics Committee for the laboratory animal research of University of Kuopio, Finland. Animal care and experimental procedure were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985). Male outbred Wistar rats (n = 40) (National Laboratory Animal Center, Kuopio, Finland) were maintained at 22 ± 2°C with 12:12 h light:dark cycles and had free access to standard rat chow and water. Half of the rats were pair-matched according to their weight and randomly assigned to the diabetic group, which was induced by the injection of STZ as described below. The other half of the rats was kept as a control group. Rats with sustained diabetes (glucosuria of at least 20 m*M* 2 weeks after the STZ injection) and the nondiabetic control rats were further randomly divided into LA supplemented and nonsupplemented groups (n = 10 per group).

Preparation of diabetic rats

Diabetes was induced by a single intraperitoneal injection of STZ at a dose of 60 mg/kg (prepared in 0.1 *M* citrate buffer, pH 4.5) to 12-week-old animals, as described earlier (4). STZ has been shown to destroy pancreatic beta cells, being a model of experimental type 1 diabetes (54). The state of diabetes was confirmed by glucosuria using glucose test strips (BM-Test-5L, Boehringer Mannheim, Mannheim, Germany) after 1 week of STZ injection. A dipstick urine test was repeated once a week during the study. Blood glucose levels were also measured at the end of the study in truncal blood collected immediately after decapitation using a commercial kit (Gluco-quant Glucose/HK, Boehringer Mannheim) based on a hexokinase/G6P-DH enzymatic method, as previously reported (18).

Supplementation protocol

A solution of racemic mixture of LA (Asta Medica, Frankfurt, Germany) was prepared in phosphate buffered saline (PBS, pH 7.4) and administered intragastrically to rats at doses of 150 mg per kg of body weight per day for 8 weeks. The corresponding control groups received PBS only.

Sample collection

After 8-week supplementation period, the animals were pair-matched between groups. LA-supplemented and nonsupplemented, SID and control rats were killed at rest by decapitation. Next, the kidneys were quickly removed, rinsed in ice-cold saline and blotted, cut into small pieces and snap-frozen in liquid nitrogen and stored at −70°C for later homogenization and biochemical determinations.

Histology

Kidney samples were fixed overnight with 4% formalin solution buffered with sodium-phosphate at +5°C. Routine paraffin-embedding and tissue processing were performed. Sections of 3 μm thickness were mounted on glass slides and stained with Hematoxylin–Eosin.

Western blot analysis

Samples were analyzed for HSP protein expression using standard Western blot techniques, as previously described (4, 37). Briefly, the samples were first pulverized under liquid nitrogen with a mortar and sonicated in a buffer containing 25% glycerol (wt/vol), 0.42 *M* NaCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* ethylenediaminetetraacetic acid, 20 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 5 μ*M* dithiotreitol, and 5 μ*M* phenylmethylsulphoxide at +4°C. Next, protein extracts (30 μg protein per lane) were electrophoresed together with molecular weight markers on 8 or 10% SDS/PAGE (sodium dodecyl sulfate/polyacrylamide gel electrophoresis) and transferred to a nitrocellulose membrane (Millipore, Bedford, MA). Equal transfer was checked and quantified by reversible protein staining of the nitrocellulose membrane with Ponceau S (Sigma, St. Louis, MO). After blocking with 5% (wt/vol) fat-free milk solution, at 37°C for 60 min, the membranes were treated with monoclonal antibodies (Ab) (all

from StressGen, Victoria, Canada) recognizing heme oxygenase-1 (HO-1), the 60 kDa HSP (HSP60), the 72 kDa inducible form of HSP (HSP72), the 90 kDa HSP (HSP90), and glucose-regulated protein 75 (GRP75). As secondary Ab, horseradish peroxidase (HRP)-conjugated anti-mouse (Santa-Cruz Biotechnology, Santa Cruz, CA) and anti-rat immunoglobulins (IGs) (Zymed Laboratories, San Francisco, CA) were used, respectively. The membranes were developed with enhanced chemiluminescence method (NEN Life Sciences, Boston, MA) and quantified using image-analysis software (ScionCorp, Frederick, MD).

For protein carbonyls (PCARB) measurements, tissue extracts were derivatized with 2,4-dinitrophenylhydrazine immediately before the electrophoresis, and subsequent Western blotting was performed as previously described (4). Rat monoclonal Ab against 2,4-dinitrophenyl and anti-rat IGs (Zymed Laboratories) were used as primary and secondary Ab, respectively. Measurement of 4-hydroxy-2-nonenal (4-HNE) protein adducts were performed as described (37) by using rabbit 4-HNE anti-serum (Alpha Diagnostics, San Antonio, TX) and HRP-conjugated anti-rabbit IGs (Santa-Cruz Biotechnology) as secondary antibody.

Analysis of TGF-β

TGF-β levels in the supernatants obtained from tissue homogenates were measured using a commercially available ELISA kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Results were normalized to the total protein concentration of tissue homogenates.

Analysis of gene expression

To analyze mRNA expression of HSP60, HSP72, HSP90, GRP75, HO-1, HSF-1, and beta actin (ACTB) in kidney tissue, a quantitative real-time RT-PCR was applied (20). Briefly, 100 mg of tissue was first homogenized with Ultra-Turrax and total cellular RNA was isolated using TRIzol reagent according to manufacturer's instructions (Life Technologies, Gaithersburg, MD). RNA concentrations were determined by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE) and their integrity was checked with gel electrophoresis. One microgram of total RNA from each sample was then converted to cDNA using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo(dT) primers (Promega, Madison, WI).

For PCR primer design, the annotated nucleotide sequences were retrieved from GenBank database (National Center for Biotechnology Information, Bethesda, MD) and BLAST searches (2) were performed to identify unique stretches of nucleotide sequence. The primers were designed not to amplify genomic DNA. The selected primer sequences were synthesized by Oligomer Oy (Helsinki, Finland) and were as follows: HSP60 forward primer (-F) 5′-AAAGCTGAACGAGCGACTTG-3′ and reverse primer (-R) 5′-ATCACTTGTCCCTCCAACCTTC-3′; HSP72-F 5′-CAACTGGCTTGACCGAAACC-3′ and HSP72-R 5′-AGCGCAAGCCTAGTCCACTTC-3′; HSP90-F 5′-GTACGAAACAGCACTCCTGTCTTC-3′ and HSP90-R 5′-ATCCTCATCAATACCTAGACCAAGC-3′; GRP75-F 5′-ACGAGGATGCCCAAGGTTC-3′ and GRP75-R 5′-TGAATGGCAGCTCCAATGG-3′; HO-1-F 5′-GGAAGGCTTTAAGCTGGTGATG-3′ and HO-1-R 5′-GGTTCTGCTTGTTTCGCTCTATC-3′; HSF-1-F 5′-GCCAGCATTCAGGAACTTCTATC-3′ and HSF-1-R 5′-CACCAGCTGCTTTCCTGAGT-3′; ACTB-F 5′- CATCCTGGCTCACACTGAATTC-3′ and ACTB-R 5′- TCCAGATGATTCAGAGCTCCATAG-3′.

The samples were amplified in duplicate using Brilliant SYBR Green Master Mix (Stratagene, La Jolla, CA) with 200 n*M* of gene-specific primers, and run on Mx3000P System (Stratagene) with the following program: a 10 min preincubation at 95°C, followed by 40 cycles of 15 s at 95°C, 20 s at 59°C, and 25 s at 72°C. The data were normalized relative to expression of ACTB by using the previously introduced algorithm (40). Unique amplification products and absence of primer–dimers was evaluated by melt curve analysis.

DNA-binding activity

For the measurement of HSF-1 DNA-binding activity, an electrophoretic mobility shift assay (EMSA) was performed as previously described (4). The protein extracts were prepared similar to Western blot analysis and mixed with isotope-labeled probes corresponding to the two overlapping heat shock elements. Protein–DNA complexes were resolved on a nondenaturing polyacrylamide gel. The EMSA gels were dried and the radioactivity was detected by autoradiography.

Statistics

Two-way ANOVA with Bonferroni correction and independent samples *t*-test were used to evaluate the effect of diabetes and LA supplementation. Values of $p < 0.05$ were considered statistically significant. Data are represented as means ± S.E.M. unless otherwise stated.

RESULTS

Effect of experimental diabetes

Induction of SID resulted in mesangial expansion and tubular dilatation (Fig. 1) and increased kidney TGF-β levels (Fig. 2), which is an indicator of diabetic glomerulosclerosis. SID also increased the levels of 4-HNE protein adducts and HO-1 (Figs. 2 and 3, respectively), markers of oxidative stress.

SID resulted in increased DNA-binding activity of HSF-1, which was not, however, paralleled with HSF-1 protein or mRNA (Figs. 4 and 5, respectively). A subsequent increase in the HSP72 mRNA (Fig. 6) and HSP60 protein (Fig. 7) and decreased expression of GRP75 mRNA were also observed in response to SID (Fig. 8).

Effect of LA supplementation

Supplementation with LA decreased the tubular dilatation and mesangial expansion, as observed by histological staining (Fig. 1), and reversed the SID-induced TGF-β (Fig. 2).

LA did not have any effect on 4-HNE protein adducts (Fig. 2), although it decreased the HO-1 protein in SID rats (Fig. 3). However, LA increased HSF-1 mRNA and protein in diabetic and nondiabetic rats, although the DNA-binding activity of HSF-1 remained unchanged (Figs. 4 and 5).

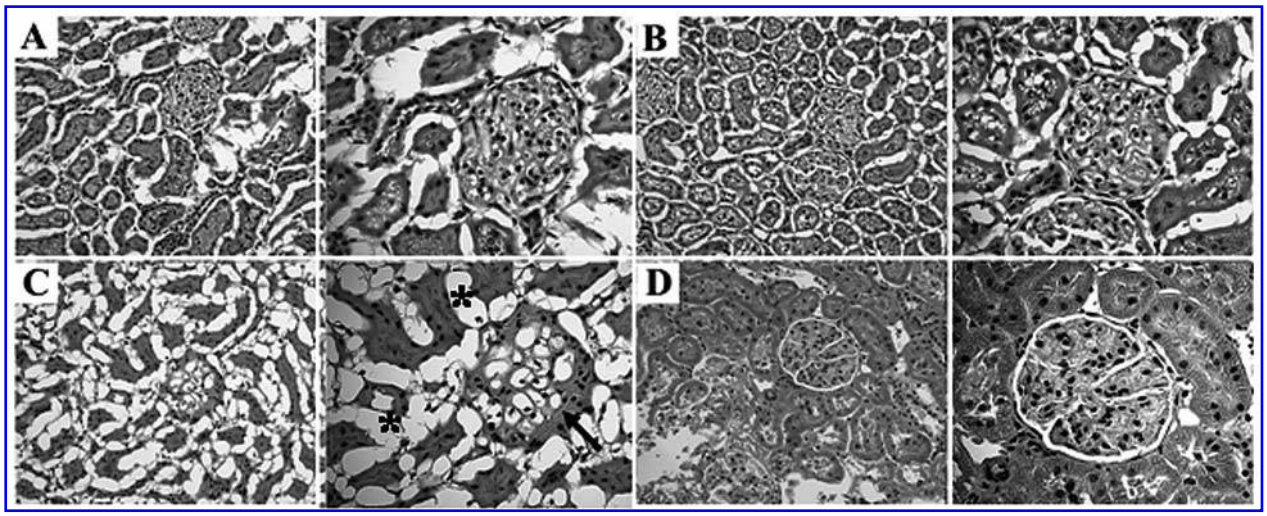

FIG. 1. Representative light microscopy images of the kidney tissue of nondiabetic control animals (A), nondiabetic control animals supplemented with alpha-lipoic acid (LA) for 8 weeks (B), streptozotocin-induced diabetic (SID) animals (C), and SID rats LA supplemented for 8 weeks (D). Specimens were stained by Hematoxylin–Eosin. Magnification 10 X and 20 X. Asterisks indicate tubular dilatation; arrows indicate mesangial expansion.

The mRNA for HSP72 was only induced in diabetic rats (Fig. 6), whereas the mRNA for GRP75 and HSP90 was upregulated in all LA-supplemented animals (Figs. 8 and 9, respectively). However, LA did not have any effect on these HSPs at the protein level.

DISCUSSION

Diabetic nephropathy induced by STZ is associated with disturbances in the protein turnover (55) and may therefore impair the function of molecular chaperones such as HSPs, which participate transiently in the folding of other proteins into oligomeric structures. In this study, we have tested for the first time the role of natural antioxidant supplementation (LA) on the HSP synthesis in the kidney of diabetic (SID) and nondiabetic rats.

SID was characterized histologically by glomerulosclerosis and tubular dilatation, and also by increased synthesis of TGF-β, which is a marker of glomerulosclerosis (11). In addition, we observed an increased SID-mediated oxidative stress, as indicated by elevated levels of 4-HNE protein adducts and upregulation of HO-1 mRNA. SID also increased HSF-1 DNA-binding activity and HSP60 protein content. LA supplementation increased levels of HSF-1 mRNA and protein and HSP72 mRNA, and reversed the induction of HO-1

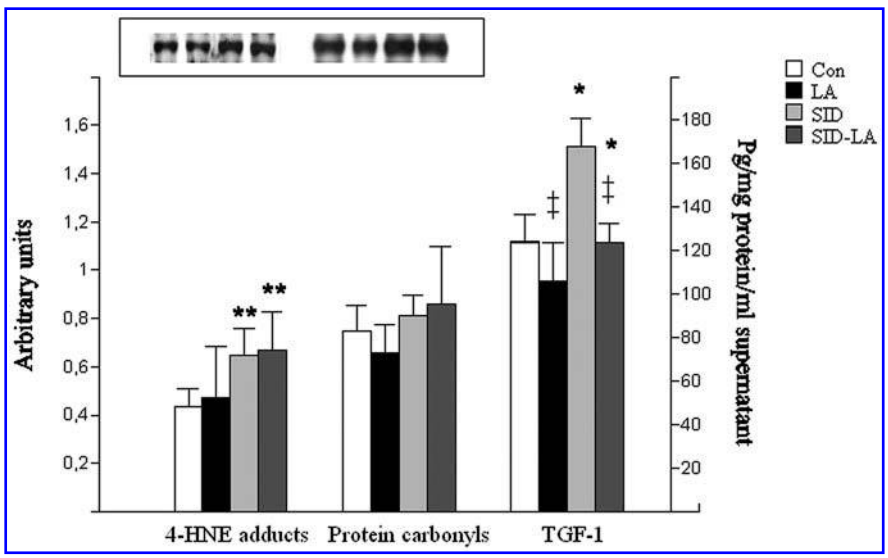

FIG. 2. Effect of streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid supplementation (LA) on the levels of 4-hydroxynonenal (4-HNE) protein adducts, protein carbonyls (PCARB), and transforming growth factor-beta1 (TGF-β1) in kidney tissue. *Open bars* represent nondiabetic control sedentary rats (CON); *closed bars* represent nondiabetic rats after LA supplementation (LA); *light gray bars* represent streptozotocin-induced diabetic rats (SID), *dark gray bars* represent diabetic rats after LA supplementation (SID-LA). Values for 4-HNE and PCARB are from densitometric measurements, and TGF-β1 represents concentration per mg per kidney tissue from ELISA measurement. Data are expressed as mean ± S.E.M. Difference due to SID: $*p < 0.05$; $**p < 0.01$. Difference due to LA supplementation: $‡p < 0.05$.

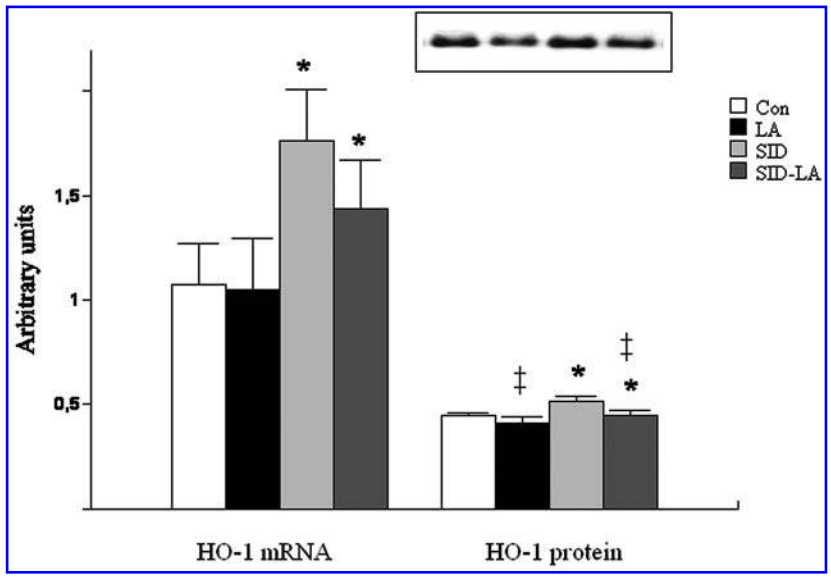

FIG. 3. Effect of streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid supplementation (LA) on the mRNA and protein expression of heme oxygenase-1 (HO-1) in rat kidney. Groups are as in Fig. 2. Densitometric values are mean ± S.E.M. Difference due to SID: *$p < 0.05$. Difference due to LA supplementation: #$p < 0.05$.

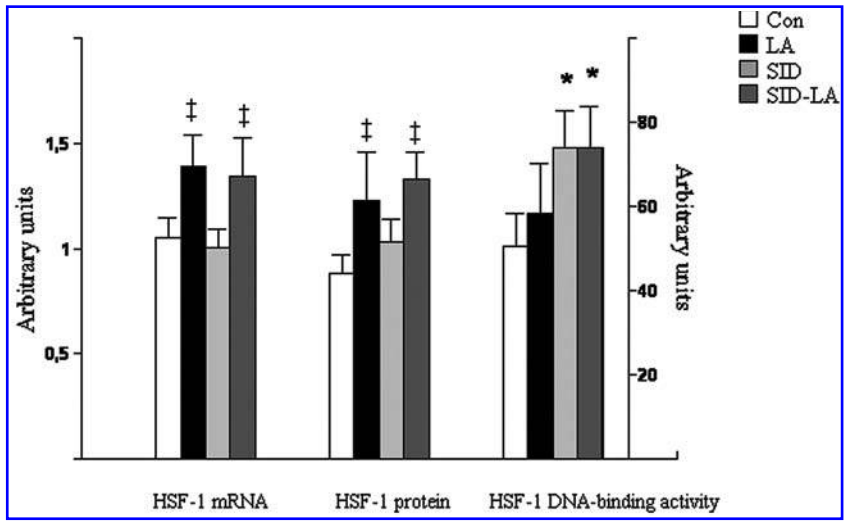

FIG. 4 Protein, mRNA expression, and DNA-binding activity of heat shock factor-1 (HSF-1) in kidney tissue in response to streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid (LA) supplementation. Groups are as in Fig. 2. Densitometric values are mean ± S.E.M. Difference due to SID: *$p < 0.05$. Difference due to LA supplementation: ‡$p < 0.05$.

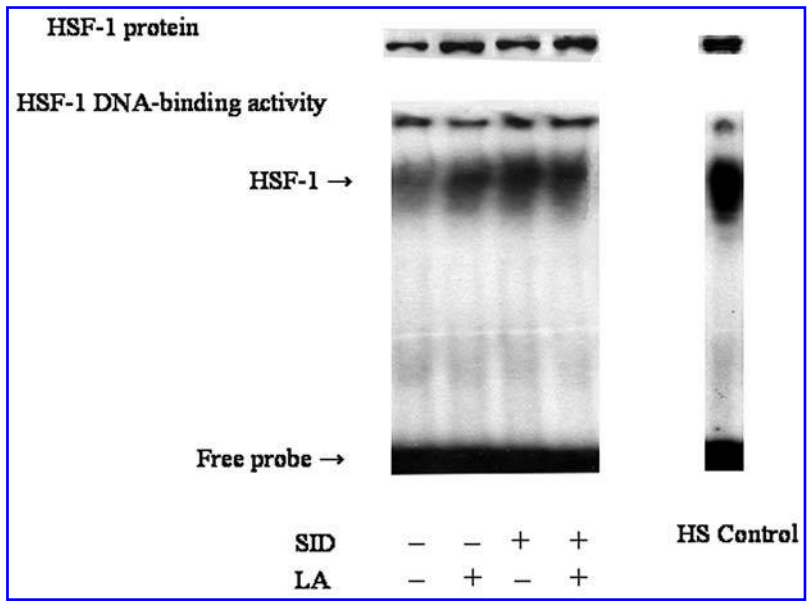

FIG. 5. Western blot and gel mobility shift images of heat shock factor-1 (HSF-1) binding activity in kidney tissue in response to streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid (LA) supplementation.

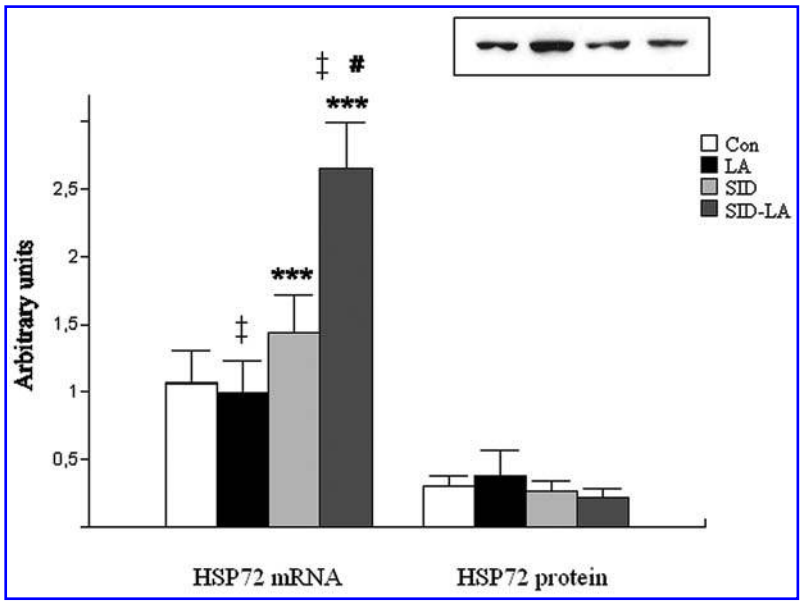

FIG. 6. Effect of streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid supplementation (LA) on the mRNA and protein expression of heat shock protein 72 (HSP72) in kidney tissue. HS represents control samples of heat shock exposed HeLa cells. Groups are as in Fig. 2. Densitometric values are mean ± S.E.M. Difference due to SID: ***$p < 0.001$. Difference due to LA supplementation: ‡$p < 0.05$. Interaction between SID and LA: #$p < 0.05$.

and TGF-β protein expression. LA also seemed to at least partly reverse the histological abnormalities associated with glomerulosclerosis.

In the kidney, chronic hyperglycemia results in the modification of cellular proteins that may accumulate in cells and accelerate further tissue injury (52). Furthermore, we (4, 37) and others (53) have previously shown that SID decreased levels of HSP72, HSP60, and GRP75 in the heart, liver, and skeletal muscle. It thus seems that alterations in kidney HSP synthesis differ from other tissues that may reflect diverse and tissue-specific patterns of HSP regulation.

Other HSPs are also likely to be affected in the diabetic kidney. For instance, HSP27 is normally present in the medulla, a region exposed to osmotic stress (35), and HSP27 has been suggested to provide protection against this type of stress (6). Whereas HSP47 has a crucial role in collagen biosynthesis (13), increased expression of HSP47 is associated with glomerulosclerosis and tubulointestinal fibrosis in human diabetic nephropathy (43). In rats with SID, HSP47 was mostly involved in the chronic phase of the diabetes (29). In this study we only measured TGF-β to demonstrate glomerulosclerosis. The renal TGF-β system is significantly upregulated in diabetic nephropathy and because TGF-β levels are causally related to the degree of glomerulosclerosis and procollagen synthesis (11), they will most probably correlate with increased levels of HSP47.

Oxidative stress and generation of reactive oxygen species (ROS) are critical factors in the development of diabetic nephropathy because they have shown to increase the levels of mediators such as PCARB (5). SID was shown to augment the generation of ROS in 8 weeks (46), and to induce oxidative damage in the kidney at 2 (17) and 4 (19, 38) weeks after

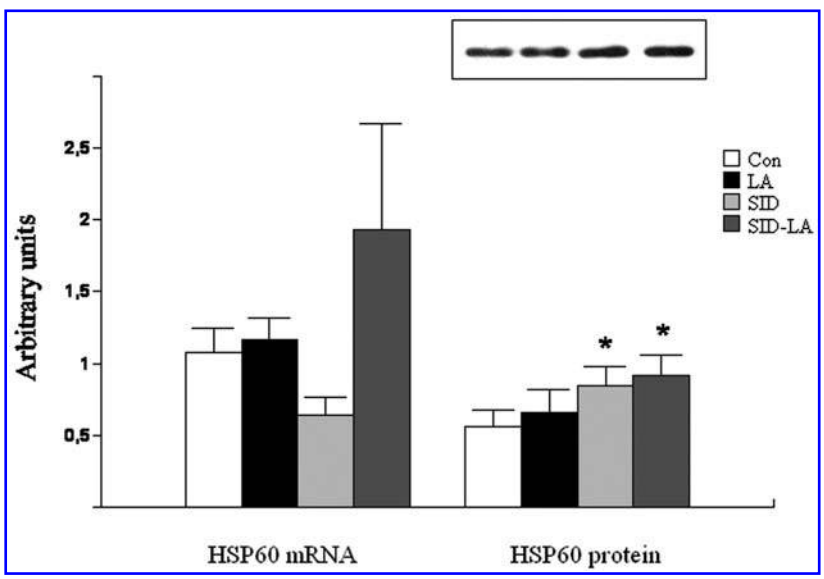

FIG. 7. Effect of streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid supplementation (LA) on the mRNA and protein expression of heat shock protein 60 (HSP60) in kidney tissue. Groups are as in Fig. 2. Densitometric values are mean ± S.E.M. Difference due to SID: *$p < 0.05$.

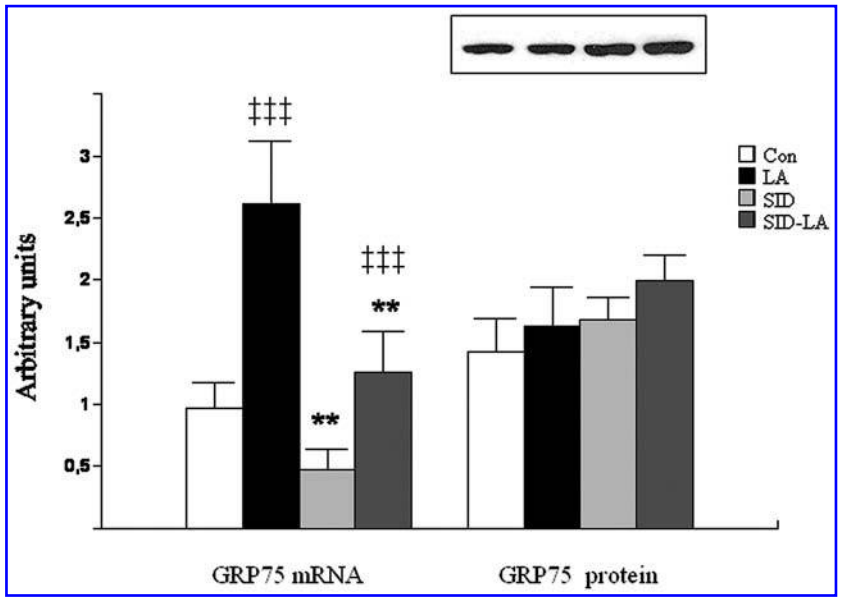

FIG. 8. Effect of streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid supplementation (LA) on the mRNA and protein expression of glucose-regulated protein 75 (GRP75) in kidney tissue. Groups are as in Fig. 2. Densitometric values are mean ± S.E.M. Difference due to SID: $^{**}p < 0.01$. Difference due to LA supplementation: ‡‡‡$p < 0.001$.

the induction. However, oxidative damage to kidney was shown to be reversed by insulin administration (57).

In our model, SID induced characteristic histological changes, including tubular dilatation and expansion of the mesangial matrix, which is in agreement with Melhem *et al*. (32, 33). In addition, we observed an increased production of TGF-β. Indeed, glomerular TGF-β has shown to be increased in both human and experimental diabetes mellitus, and this prosclerotic cytokine has been implicated as a major mediator of glomerular mesangial expansion in diabetic nephropathy (11, 50). Previous studies indicate that increased oxidative stress is one of the major inducer of TGF-β, and that LA and other antioxidants decreased TGF-β in the diabetic kidney (12, 32).

We observed an increased oxidative stress in the kidney of SID rats as demonstrated by increased lipid peroxidation (4-HNE protein adducts) and HO-1 expression. Indeed, high metabolic activity and vast blood flow predisposes kidney to oxidative stress, an inducer of HSP expression (7), although ROS have also been shown to participate in the homeostatic regulation of renal perfusion (47). However, in diabetic animals, we did not observe any enhancement in HSP response at the protein level, despite elevated oxidative stress and activation of HSF-1. HSP60 was the only exception.

In rats, SID leads to increased levels of IGF-1 in the glomeruli, which correlates with glomerular hypertrophy (16). It seems that HSP60 and IGF-1 cross-talk, because HSP60 is capable of upregulating functional IGF-1 receptors (IGF-1R) by inhibiting its ubiquitination (28). By itself, IGF-1R inhibits hyperglycemia-induced DNA damage and rescues mesangial cells by suppressing ROS and enhancing DNA repair, a possible auto-defense mechanism (56). Whether the amounts of HSP60 and IGF-1R correlate in our model, remains to be elucidated in future studies.

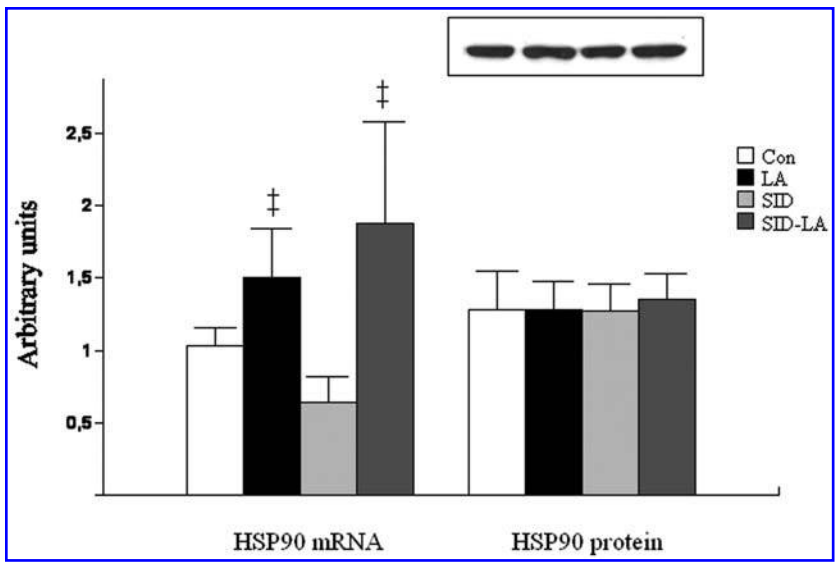

FIG. 9. Effect of streptozotocin-induced diabetes (SID) and 8-week alpha-lipoic acid supplementation (LA) on the mRNA and protein expression of heat shock protein 90 (HSP90) in kidney tissue. Groups are as in Fig. 2. Densitometric values are mean ± S.E.M. Difference due to LA supplementation: ‡$p < 0.05$.

In our study, the HSP72 protein expression tended to decrease in diabetic rats. Recently, we observed an overall impaired HSP protection in skeletal muscle, heart, and liver in SID rats (4). A key role for HSP70 in renal function is provided by the finding that HSP70 is required to stabilize kidney Na-K-ATPase during ATP depletion in LLC-PK1 cells (44), and that increased expression of HSP70 protects rat kidney mesangial cells against oxidative injury (10). In diabetes, kidneys are subjected to metabolic load as a consequence of alterations in pH, blood glucose, osmolarity, and altered fatty acid turnover. Therefore, in SID, when enhanced HSP response does not compensate for the metabolic changes, including oxidative stress, there may be a high predisposition for tissue injury.

We observed increased expression of HSF-1 in SID rats after 8 weeks of LA supplementation. Although LA has shown to have beneficial effects on oxidative stress in SID in the heart (37) and kidney (33, 36) and HSP60 synthesis in the liver (37), the effects of LA on HSP synthesis in kidney have been unknown. Earlier, it was demonstrated that 8-week supplementation with LA was successful for prevention of early glomerular injury in SID (32). In the current study, we used higher doses of LA, which we expected to provide similar or even better antioxidative effect. Previously, no effect of LA was found on the heat shock response in lipopolysaccharide-stimulated macrophages *in vitro* (15), whereas LA was found to recover lower plasma content of HSP72 in type 1 diabetic patients with polyneuropathy (51). LA supplementation induced HSF-1 mRNA and protein expression in the present study, but it did not affect HSP levels. Consistent with our findings, McCarthy suggested previously that high-dose LA may enhance HSP expression by activating HSF-1 via induction of disulfide formation in certain target proteins (31).

Prior to our study, there has been very little evidence to support this hypothesis. The major discrepancy that we observed in our study, that LA supplementation did not alter HSP expression, despite upregulation of HSF-1 at both mRNA and protein levels, could be explained by the metabolic conditions in diabetes that have shown to repress HSP induction and overall protein synthesis (4). In addition, to fully activate heat shock genes, HSF-1 hyperphosphorylation is required (23), which may, however, be impaired in SID resulting in unchanged HSP levels. Another possibility is that the HSP response can also be modulated at the post-transcriptional level (*i.e.*, via regulation of mRNA stabilization (23). HSF-1 is also a regulator of target genes other than HSPs, including cytokines and cell cycle regulators (8, 21). It is important to note that increased synthesis of HSPs is not always the direct outcome of increased HSF-1 DNA-binding activity, because heat shock response may only be partially activated (22). Furthermore, as HSP72 has shown to downregulate HSF-1 activation (1), an impaired translation of HSP72 mRNA may also decrease this negative feedback, and hence may increase the DNA-binding activity of HSF-1 without preceding changes in HSP72 levels.

The effect of LA on blunting HO-1 expression may be attributed to its antioxidant effects. Indeed, acute HO-1 induction has often been interpreted as a marker of increased oxidative stress, although overexpression of HO-1 has been shown to provide protection against oxidative damage (7, 34).

In conclusion, SID resulted in glomerulosclerosis and increased lipid peroxidation in rat kidney without compensatory upregulation of HSPs at the protein level. On the other hand, 8 weeks of LA supplementation decreased TGF-β, a key inducer of glomerulosclerosis, and HO-1, a marker of oxidative stress in diabetic rat kidney. We therefore suggest that impaired HSP synthesis may contribute to diabetic nephropathy, and that LA supplementation may act as a potential tool in the future to decrease diabetic renal complications, alone or together, with other HSP inducers.

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ABBREVIATIONS

EMSA, electrophoretic mobility shift assay; GRP75, glucose-regulated protein; 4-HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; HSF-1, heat shock factor-1; HSP60, heat shock protein 60; LA, alpha lipoic acid; PCARB, protein carbonyls; SID, streptozotocin-induced diabetes; TGF-β, transforming growth factor-beta.

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