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α-Lipoic acid protects kidney from oxidative stress and mitochondrial dysfunction associated to inflammatory conditions

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An adequate redox status is important for maintaining mitochondrial function in inflammatory conditions. The aim of this work was to evaluate the effects of α-lipoic acid (LA) in kidney oxidative metabolism and mitochondrial function in lipopolysaccharide (LPS) treated rats. Sprague-Dawley rats (female, 45 ± 5 days old) were treated with LPS (10 mg kg^{-1}) and/or LA (100 mg kg^{-1}). It was observed in LPS-treated animals that the LA prevented the increase in 1.2 fold of NO production, decreased (30–40%) mitochondrial complex I–III and IV activities, and decreased (26%) membrane potential and cardiolipin oxidation (76%). No differences were observed in mitochondrial O_2 consumption, mitochondrial complex II–III activity, and ATP production when LPS group was compared to LA + LPS group. Based on the improvement of mitochondrial function, the decreased production of mitochondrial NO and restoration of cardiolipin levels, this work provides a new evidence that α-lipoic acid protects kidney from oxidative stress and mitochondrial dysfunction.

Introduction

Maintenance of tissue redox status is essential for optimal physiological functions. Not only the endogenous production levels but also the dietary provision of sulfhydryl groups (RSH) is required for cellular redox homeostasis. α-Lipoic acid (LA) is a disulphide derivative of octanoic acid. Though the enantiomer *R* is biologically active as a prosthetic group of various enzymatic complexes, both *R* and *S* forms are sources of SH groups and contribute to tissue antioxidant defenses. Although endogenous synthesis appears to supply most of the necessary LA needed for intermediary metabolism, it can also be absorbed from the diet, and it is commercially available as a nutritional supplement.[1,2] LA is present in almost every food; it can be found in relatively high amounts in meat, spinach, broccoli, and yeasts.[3] Recently, LA has been shown to have beneficial effects in pathological conditions, where oxidative stress and mitochondrial dysfunction play a role in the disease mechanisms.[4–7] Among these conditions, inflammatory syndromes appear as relevant targets for LA and related compounds that act in the maintenance of an adequate redox status.[8,9]

Circulating LPS in blood vessels and its detrimental consequences (endotoxemia) constitute a paradigm of inflammatory syndrome, with massive increases of nitric oxide (NO) and inflammatory cytokines in biological fluids, and occurrence of oxidative stress.[10,11] Lipopolysaccharide (LPS) is a cell-wall component of gram-negative bacteria and a major cause of endotoxemia. LPS leads to the occurrence of oxidative stress and organ dysfunction, as previously reported.[12] Moreover, there has been growing interest in the investigation of the role of circulating LPS in the pathology of metabolic derangements associated with obesity because a relation between the elevated levels of circulating LPS and metabolic dysregulation has been observed (metabolic endotoxemia).[13,14] The development of acute renal failure (ARF) in endotoxemia has a prevalence of 40%, and its pathogenesis remains only partially understood. It has been suggested that kidney oxidative stress and bioenergetic derangement may be a major factor to cause the ARF during endotoxemia.[15,16] However, to date, no specific kidney-protective modality is available. The recent observation that endotoxemia caused a decrease in O_2 delivery to the kidney without changing tissue O_2 levels clearly suggests an alteration of mitochondrial function.[17] Moreover, the NO produced in this inflammatory condition is of significant interest because it competes with O_2 and regulates the primary functions of mitochondria, including respiration and transmembrane

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potential. Although the recent advances in understanding the pathophysiology of LPS-ARF have been achieved, its effective management is still lacking due to its complexity and multifactorial character.

Interestingly, beneficial effects of the restoration of RSH group levels in kidney[9] and LA supplementation in heart and muscle have been reported.[7] Results from our laboratory have shown that LA was able to reduce the oxidative stress and mitochondrial dysfunction in the heart, diaphragm and liver of endotoxemic animals.[7] Therefore, the aim of this work was to evaluate the effects of LA in kidney oxidative metabolism and mitochondrial function in lipopolysaccharide (LPS) treated rats, emphasizing the importance of an adequate redox status for maintaining mitochondrial function.

Materials and methods

Drugs and chemicals

D,L-α-Lipoic acid (LA, racemic mixture) and lipopolysaccharide (LPS, serotype 026:B6 from *Escherichia coli*) were purchased from Sigma-Aldrich (St Louis, MO, US). Mitochondrial fluorescent probes were provided by Molecular Probes (Eugene, OR, US). Other reagents, enzymes, and enzyme substrates were reagent grade and were also obtained from Sigma-Aldrich.

Experimental design

Rats (Sprague-Dawley, female, 45 ± 5 days old) from the animal facility of the University of Buenos Aires were used. The animals were housed under specific conditions of temperature and humidity controlled environment, and had unlimited access to water and food (pelleted rodent non purified diet). LPS was injected ip in a single dose of 10 mg kg^{-1} body weight. LA was injected ip in a single dose of 100 mg kg^{-1} body weight. Treatments were performed 6 h before sacrifice. The four groups studied were: (a) control group: animals were injected ip with saline solution (vehicle), (b) LA group: animals were injected only with LA (100 mg kg^{-1} body weight), (c) LPS group: animals were injected only with LPS (10 mg kg^{-1}), (d) LA + LPS group: animals were co-injected (at the same time in a single injection) with LA and LPS (100 mg kg^{-1} and 10 mg kg^{-1}, respectively). Animal treatment was carried out in accordance with the guidelines of the 6344/96 regulation of the Argentinian National Drug, Food and Medical Technology Administration (ANMAT).

Isolation of mitochondria and preparation of mitochondrial membranes

Rats were anesthetized [ketamine (50 mg kg^{-1}) plus xylazine (0.5 mg kg^{-1})] and kidneys were immediately excised. Renal cortex was separated from medulla and papilla. The kidney cortex was homogenized in a glass-Teflon homogenizer in a medium consisting of 250 mM sucrose, 5 mM Tris/HCl, 2 mM EGTA, 0.25% BSA (pH 7.4), at a ratio of 1 g kidney : 9 mL of medium. The homogenates were centrifuged at 700*g* for 10 min to discard nuclei and cell debris, and the supernatant was centrifuged at 7000*g* for 10 min to obtain mitochondria. The mitochondrial pellet (mitochondria able to carry out oxidative phosphorylation) was washed twice and resuspended in the same buffer. The whole procedure was carried out at 0–4 °C.[18] The purity of isolated mitochondria was assessed by determining lactate dehydrogenase activity; only mitochondria with less than 5% impurity were used. Mitochondrial membranes (MM) were obtained by freezing and thawing mitochondria three times, and homogenized by passing it through a 29G hypodermic needle.[18] Protein content was assayed with the Folin reagent, using bovine serum albumin as the standard.[19]

Oxygen uptake by mitochondria

A two-channel respirometer for high-resolution respirometry (Hansatech Oxygraph, Hansatech Instruments Ltd, Norfolk, England) was used. Kidney mitochondrial respiration was measured in a reaction medium that contained 120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 3 mM HEPES, 1 mg mL^{-1} BSA (pH 7.2), and 0.5 mg mL^{-1} of freshly isolated kidney mitochondria, at 25 °C. Malate and glutamate (6 mM) or succinate (8 mM) were used as substrates. In these conditions, state 4 respiration rate was measured; subsequently, ADP (1 mM) was added to establish state 3 respiration.[20] Results were expressed as ng-at O per min mg protein. Respiratory control ratio (RCR) was calculated as the ratio between state 3 : state 4 respiration rates.

Respiratory complexes activity

For the determination of NADH-cytochrome *c* reductase (complex I–III), 0.02 mg mL^{-1} MM was added to 100 mM KH_2PO_4–K_2HPO_4 (pH 7.4), 25 μM cytochrome c^{3+} and 0.5 mM KCN, and observed spectrophotometrically at 550 nm (ε = 19.6 mM^{-1} cm^{-1}) and 30 °C. Enzymatic activity was expressed as nmol reduced cytochrome c^{3+} per min mg protein. Succinate cytochrome *c* reductase activity (complex II–III) was similarly determined and expressed, except that NADH was substituted by 5 mM succinate. The activity was expressed as nmol reduced cytochrome c^{3+} per min mg protein. Cytochrome oxidase activity (complex IV) was assayed spectrophotometrically at 550 nm by following the oxidation rate of 50 mM cytochrome c^{2+} [21] in 0.1 M K_2HPO_4–KH_2PO_4 (pH 7.4), 50 μM cytochrome c^{2+} and 0.02 mg mL^{-1} MM. Results were expressed as k' (min^{-1}) mg^{-1} protein.

Nitric oxide production

Nitric oxide production was determined by the oxidation of oxyhemoglobin to methemoglobin, and observed spectrophotometrically at two wavelengths 577–591 nm (ε = 11.2 mM^{-1} cm^{-1}) in a Beckman DU 7400 diode array spectrophotometer at 30 °C.[22] Reaction medium consisted of 50 mM KH_2PO_4–K_2HPO_4 (pH 7.2), 0.1 mM $CaCl_2$, 0.2 mM L-arginine, 100 mM NADPH, 10 mM dithiothreitol, 4 mM Cu, Zn-SOD, 0.1 mM catalase, 1.0 mg protein mL^{-1} of MM, and 20 mM oxyhemoglobin. Control measurements in the presence of 2 mM N^G-methyl-L-arginine (L-NMMA) were performed to consider only the L-NMMA-sensitive hemoglobin oxidation, usually 90–95%,

due to NO formation. Results were expressed as nmol NO per min mg protein.

ATP production rate

A chemiluminescence assay based on the luciferin–luciferase system was used. Mitochondrial ATP production rate was determined in freshly purified mitochondria incubated in respiration buffer, consisting of 150 mM KCl, 25 mM Tris-HCl, 2 mM EDTA, 0.1% (w/v) BSA, 50 mM PBS and 100 μM $MgCl_2$ (pH 7.4), supplemented with 40 μM D-luciferin, 0.05 μg mL^{-1} luciferase and 150 μM di(adenosine) pentaphosphate, to inhibit the adenylate kinase, thus increasing assay specificity. ATP production was triggered by the addition of 6 mM malate and 6 mM glutamate or 8 mM succinate, and 125 μM ADP to the reaction well.[23] Measurements were carried out at 30 °C in a Labsystems Luminoskan RS Microplate Reader (Labsystem, Ramsey, Minnesota, USA). A negative control with 2 μM oligomycin was included to confirm that the emitted chemiluminescence is due to ATP synthesis by the F_o–F_1 ATP synthase. A calibration curve was obtained using ATP as the standard. Results were expressed as nmol ATP per min mg protein. In order to evaluate the efficiency of the oxidative phosphorylation process, the number of phosphorylated ADP molecules per oxygen atom (P/O ratio) was calculated as ATP production/state 3 O_2 consumption rates.

Mitochondrial membrane potential ($\Delta\Psi_m$)

Freshly isolated kidney mitochondria (25 μg protein mL^{-1}) were incubated in 210 mM mannitol, 70 mM sucrose, 5 mM HEPES, 1 mM PBS (pH 7.4) and a potentiometric cationic probe 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) (30 nM). The procedure was performed in the dark at 37 °C for 20 min. After the incubation period, mitochondria were acquired by a Partec PAS-III flow cytometer (Partec GmbH, Münster, Germany) equipped with a 488 nm argon laser. To exclude debris, samples were gated based on light-scattering properties, and 30 000 events per sample within this gate (R1) were collected. To quantify the resulting changes in membrane potential after the addition of 2 mM malate and 5 mM glutamate or 8 mM succinate, $DiOC_6$ signal was analyzed in the FL-1 channel and median fluorescence intensities (MFI) were obtained. Autofluorescence (negative control) was evaluated in the samples without $DiOC_6$. Total depolarization induced by 2 μM *m*-CCCP was used as a positive control. Mitochondrial preparations that showed no changes in membrane potential under this condition were discarded.[24,25]

Evaluation of cardiolipin content

10-*N*-Nonyl acridine orange (NAO) selectively binds to cardiolipin at the inner mitochondrial membrane, indicating the mitochondrial cardiolipin content. Freshly isolated kidney mitochondria (25 μg protein mL^{-1}) were incubated in 210 mM mannitol, 70 mM sucrose, 5 mM HEPES, 1 mM PBS (pH 7.4), and 100 nM NAO. The procedure was performed in the dark at 37 °C for 20 min. Mitochondrial events were acquired by the cytometer and analyzed as previously described.[26]

Statistics

Results were expressed as mean values ± SEM, and represent the mean of six independent experiments. Six animals per treatment were used, and experiments were run in triplicate for each independent experiment. ANOVA followed by Student–Newman–Keuls test was used to analyze the differences between mean values of more than two groups. Statistical significance was considered at $p < 0.05$.

Results

Mitochondrial O_2 consumption is a classical approach to characterize mitochondrial function. Fig. 1 shows a representative trace of mitochondrial O_2 consumption measurement in control conditions, including the effect of 2 μM oligomycin and 2 μM *m*-CCCP on O_2 uptake. As shown in Table 1, no significant differences were observed in mitochondrial O_2 consumption in state 4 or state 3; after either malate and

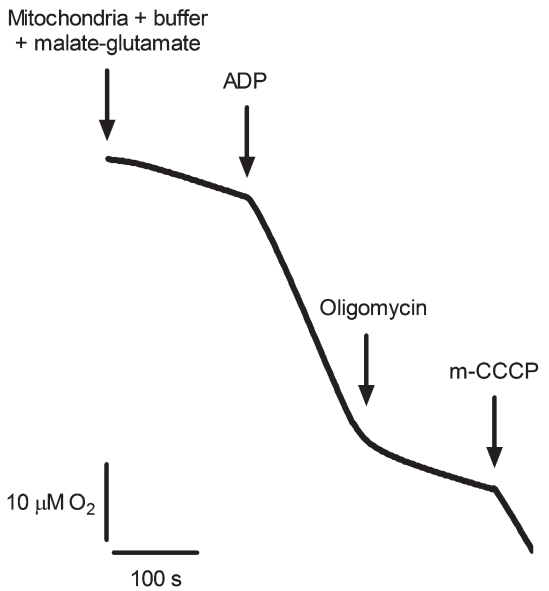

Fig. 1 Oxygen consumption of kidney mitochondria in state 4 and state 3, and after the addition of 2 μM oligomycin and 2 μM *m*-CCCP.

Table 1 Respiration of kidney mitochondria from control, LA, LPS and LA + LPS treated animals

Mitochondrial states (substrate)	O_2 consumption (ng-at O per min mg protein)			
	Control	LA	LPS	LA + LPS
Malate + glutamate				
State 4	14 ± 1	13 ± 2	12 ± 2	13 ± 2
State 3	101 ± 4	100 ± 7	96 ± 9	104 ± 11
Respiratory control	7.46	7.74	7.72	8.14
Succinate				
State 4	30 ± 2	32 ± 4	29 ± 4	34 ± 3
State 3	114 ± 7	117 ± 10	111 ± 14	123 ± 8
Respiratory control	3.77	3.63	3.86	3.61

glutamate or succinate addition as substrates (RCR: control (malate + glutamate): 7.46; control (succinate): 3.77).

To further analyze the mitochondrial function, respiratory complexes activities were measured. Complex I–III activity, shown in Table 2, was found to be decreased by 32% in LPS-treated animals compared with control and LA groups. Complex IV activity was observed to be significantly diminished by 32% with respect to the control group or LA group. Complex II–III activity showed no significant changes. These decreased values returned to the corresponding control values when animals were also treated with LA.

Table 2 Respiratory complexes I, II, IV activities in kidney mitochondrial membranes from control, LA, LPS and LA + LPS treated animals

Treatment	Complex I–III (nmol per min mg protein)	Complex II–III (nmol per min mg protein)	Complex IV (10^{-2} min^{-1} mg^{-1} protein)
Control	155 ± 9	82 ± 6	348 ± 31
LA	160 ± 12	77 ± 6	334 ± 30
LPS	106 ± 2**	74 ± 4	237 ± 30*
LA + LPS	146 ± 7	98 ± 10	310 ± 31

*$p < 0.05$ compared with the control group. **$p < 0.01$ compared with the control group.

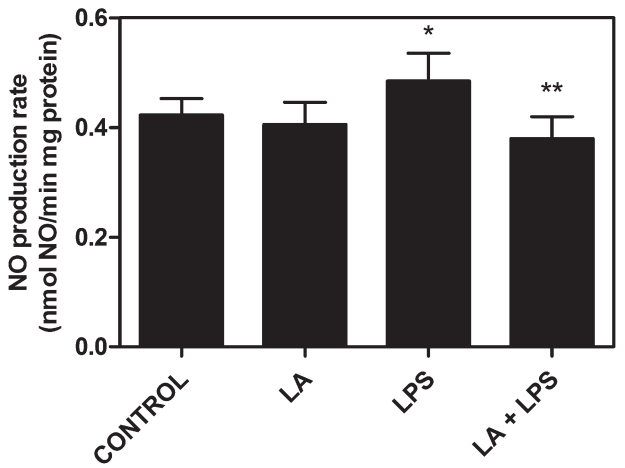

Fig. 2 Mitochondrial NO production in kidney from septic rats pretreated with α-lipoic acid. Nitric oxide production was determined by the oxidation of oxyhemoglobin to methemoglobin at 30 °C. The results are expressed as NO production rate sensitive to L-NMMA. *$p < 0.05$ compared with the control group. **$p < 0.01$ compared with the LPS group.

As NO plays an important regulatory role in mitochondrial respiration, mitochondrial NO production was analyzed. As shown in Fig. 2, NO production by mitochondrial membranes was significantly increased by 15% in endotoxemic animals compared to the control group (control: 0.42 ± 0.04 nmol NO per min mg protein, $p < 0.01$). The treatment with α-lipoic acid prevented the increase of NO production in kidney (Fig. 2).

Mitochondria, through a highly efficient process, convert chemical energy, derived from the reduction of O_2 to water, into the proton-motive force that is used to drive the endergonic synthesis of ATP. A chemiluminescence assay based on the luciferin–luciferase system was used to determine the mitochondrial ATP production rate. No significant differences were observed in ATP production rate after either malate and glutamate or succinate addition as the substrates (control (malate + glutamate): 192 ± 13 nmol per min mg protein; control (succinate): 193 ± 13 nmol per min mg protein). Results are shown in Fig. 3. Considering the state 3 O_2 consumption of mitochondria and mitochondrial ATP production rates, the number of phosphorylated ADP molecules per oxygen atom (P/O ratio) was calculated.[27] As it is shown in Table 3, no changes were observed in P/O ratio among the assessed groups.

For $\Delta\Psi_m$ measurement, mitochondria were selected from the background based on the light-scattering properties (SSC

Table 3 P/O ratio, number of phosphorylated ADP molecules per oxygen atom consumed during active respiration, in control, LA, LPS and LA + LPS treated animals

	Control	LA	LPS	LA + LPS
Malate + glutamate	1.90 ± 0.15	2.03 ± 0.22	1.85 ± 0.34	1.77 ± 0.22
Succinate	1.70 ± 0.15	1.53 ± 0.19	1.64 ± 0.29	1.30 ± 0.10

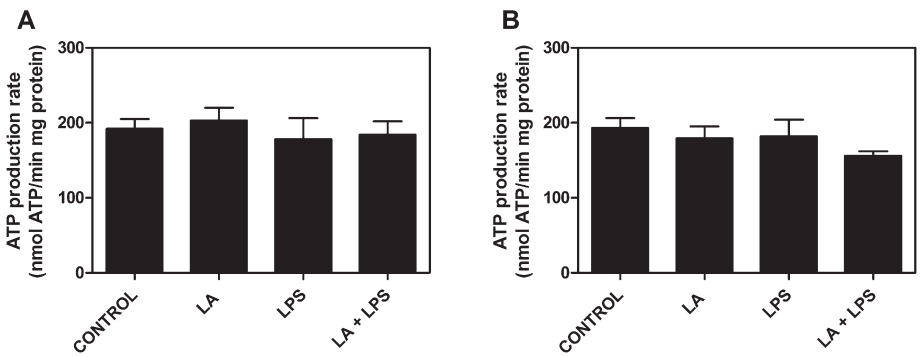

Fig. 3 (A) ATP production rate by isolated kidney mitochondria using malate and glutamate as substrates. (B) ATP production rate by isolated kidney mitochondria using succinate as substrate.

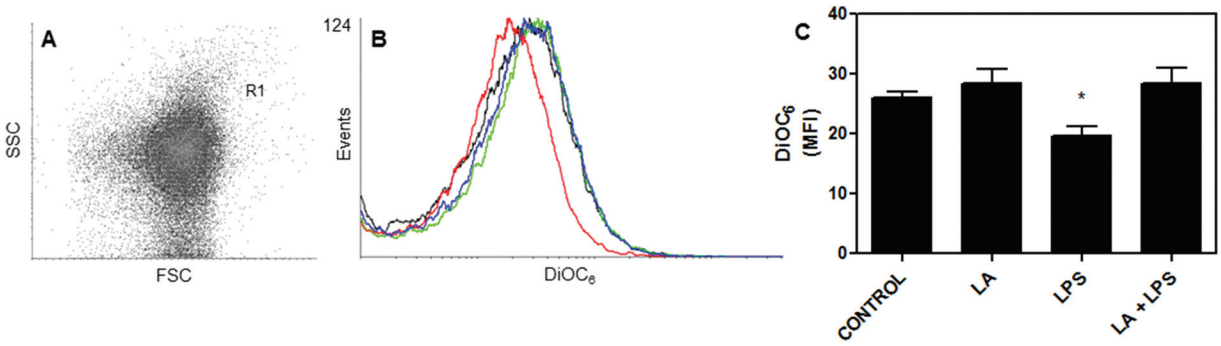

Fig. 4 Evaluation of kidney mitochondrial membrane potential by flow cytometry. (A) Mitochondria were selected based on light scattering properties and 30 000 events within R1 gate were collected. (B) Overlaid histograms of gated (R1) mitochondrial events *versus* $DiOC_6$ fluorescence intensity. Control (black), LA (blue), LPS (red), LA + LPS (green). (C) $DiOC_6$ fluorescence quantification of gated (R1) histograms, indicating kidney mitochondrial membrane potential. *$p < 0.05$ compared with the control group and with LA + LPS.

vs. FSC, Fig. 4A) and events within gate R1 were chosen for analysis. As it is shown in the overlaid histogram (Fig. 4B), LPS treatment induced a decrease in $DiOC_6$ signal in comparison with the control, when malate and glutamate were used as the respiratory substrate, indicating mitochondrial depolarization in this group. This depolarization was reverted by LA co-treatment. The quantification of $DiOC_6$ fluorescence as MFI (Fig. 4C) showed that LPS significantly decreased kidney mitochondrial membrane potential by 26%, which returned to the control values in LA + LPS group. No significant differences were observed among experimental groups when succinate was used as the substrate (data not shown).

LPS treatment led to kidney mitochondrial inner membrane depolarization despite no difference being observed in P/O ratio values; therefore, the intactness of membrane lipid constituents was analyzed. To evaluate mitochondrial cardiolipin levels, mitochondria were selected from the background based on light-scattering properties (SSC *vs.* FSC, Fig. 5A) and events within gate R1 were chosen for analysis. As it is shown in the overlaid histogram (Fig. 5B), LPS treatment induced a strong decrease in the NAO signal in comparison with the control group. This observation was reverted by LA pre-treatment. No significant changes were observed in mitochondrial volume (FSC) among the different experimental groups (data not shown), indicating that variations in NAO signal accounts for the changes in cardiolipin content. The quantification of NAO fluorescence as MFI (Fig. 5C) showed that LPS significantly decreased kidney mitochondrial cardiolipin content by 67%, which returned to control values for the LA + LPS group.

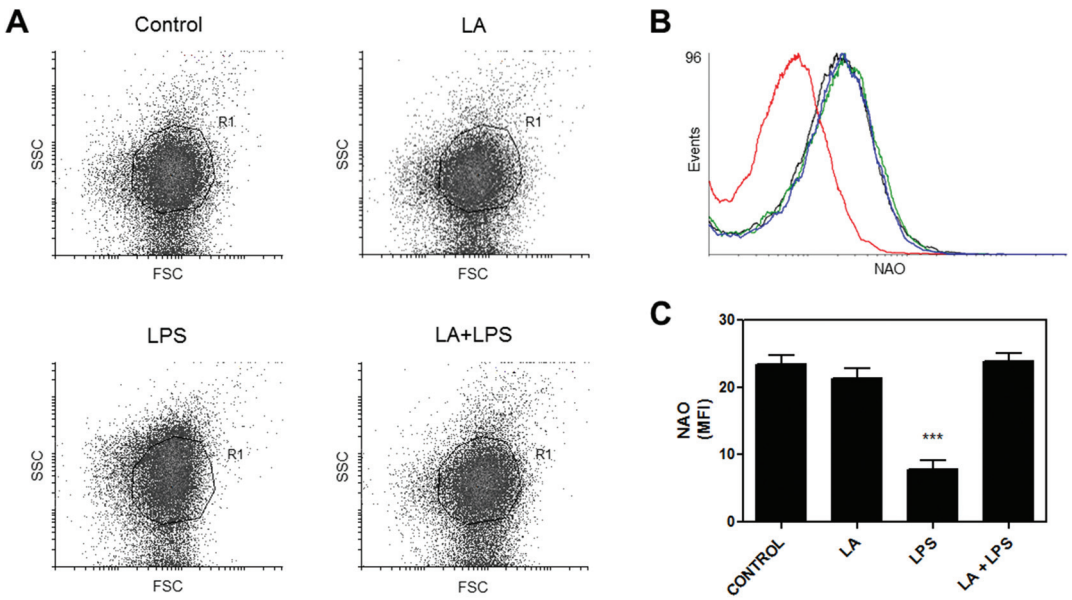

Fig. 5 Evaluation of kidney mitochondrial cardiolipin content by flow cytometry. (A) Mitochondria were selected based on light scattering properties and 30 000 events within R1 gate were collected. (B) Overlaid histograms of gated (R1) mitochondrial events *versus* NAO fluorescence intensity. Control (black), LA (blue), LPS (red), LA + LPS (green). (C) NAO fluorescence quantification of gated (R1) histograms, indicating kidney mitochondrial cardiolipin content. ***$p < 0.001$ compared with the control group and with LA + LPS.

Discussion

The goal of this study was to analyze the recovery from oxidative stress and the restoration of kidney mitochondrial function by LA during an inflammatory situation triggered by LPS treatment in rats (acute endotoxemia). It is worth noting that this scenario was achieved by increasing RSH levels through an ip dose of LA 100 mg kg^{-1}, showing that an adequate redox state is required for optimal mitochondrial function in physiopathology. An adequate LA nutritional status and RSH levels were observed to be effective in restoring mitochondrial complex I and IV activities, inner membrane potential and cardiolipin content.

LA bioavailability in human tissues is low. Typical LA dietary sources include muscle meats, heart, kidney, and liver, and to a lesser degree, fruits and vegetables.[1] It is also available as a nutritional supplement. LA is rapidly absorbed and intracellularly reduced to dihydrolipoic acid, having an important role in mitochondrial energy metabolism.[5] Chemically, LA is able to scavenge active O_2 species, regenerate antioxidants and chelate metal ions, and has been used to attenuate inflammatory responses.[28] In this context, LA has been extensively used as a supplement to ameliorate pathological conditions involving oxidative stress.[29–31] For example, in a previously published work, we have shown that the administration of LA (100 mg kg^{-1} body weight) prevented muscle and heart mitochondrial dysfunction and oxidative stress in LPS-treated rats.[7] In this study, using the same dose of 100 mg kg^{-1} and in the same experimental model, we show that this antioxidant can also partially prevent kidney mitochondrial dysfunction and oxidative damage. Although this study was designed to study the effects of LA on mitochondria, it cannot be ruled out that the protective effects on kidney may be mediated by other mechanisms, for example the inhibition of NADPH oxidase, a key factor involved in oxidant production during inflammation.[32]

LPS treatment induces kidney inflammation[33] and endotoxemia, and as shown by the results presented here, occurs with decrease in complexes I and IV activities, mitochondrial depolarization, increased mitochondrial NO production and decreased cardiolipin content. Either mitochondrial O_2 consumption or ATP production remained unchanged. *In vivo* treatment with LA appears to contribute an ameliorating effect to the oxidation of lipids and mitochondrial dysfunction observed in this inflammatory condition. The LPS dose used in this study (10 mg kg^{-1} body weight) resembles an acute endotoxemia.[34] It is important to note that low levels of circulating LPS (1 mg kg^{-1}) also can affect the mitochondrial function,[35] showing the role of metabolic endotoxemia in the development of metabolic disease and the need to understand the role of acute *versus* chronic LPS exposure on mitochondrial function.

Nitric oxide has important implications for the metabolism of cellular energy. In physiological conditions, NO is an important regulator of mitochondrial respiration. In pathological conditions, namely inflammatory syndromes as endotoxemia, increased production of NO can lead to deleterious effects as oxidative stress and oxidative damage.[7] In this condition, the increased production of superoxide anion and NO, characteristic of inflammatory situations, leads to an increased formation rate of $ONOO^-$.[36] Peroxynitrite is a powerful oxidant that can yield the highly reactive hydroxyl radical ($HO^\bullet$) by homolysis, initiating free radical chain reactions (as lipid peroxidation).[37] In our model, LPS-treatment decreased complex I–III and complex IV activities and the co-treatment with LA prevented this inhibition. In a previous work, we calculated the mitochondrial steady-state $ONOO^-$ concentration in the heart and diaphragm of LPS-treated animals, which were 12 and 49 pM, respectively; this amount could allow the inhibition of complex I and IV activities.[36] Other effects of $ONOO^-$ relevant to this experimental model include: increase in NF-kB activity, lipid peroxidation in membranes, and inactivation of Mn-superoxide dismutase.[38,39]

Peroxidation of membrane lipid components has been hypothesized to be a major mechanism of oxygen-free radical toxicity and oxidative stress. Cardiolipin, a phospholipid of unusual structure exclusively localized almost within the inner mitochondrial membrane, is a target of oxygen-free radical attack either because of its high level of unsaturation or its location in the membrane.[40] Moreover, it has been suggested that cardiolipin plays an important role in the regulation of mitochondrial bioenergetics because it can interact with several membrane proteins, including anion carriers and respiratory complexes as complex I and IV.[40,41] The binding of NAO to the cardiolipin was reported to be decreased due to cardiolipin autoxidation. Consequently, a decrease in NAO fluorescence is observed because NAO is not able to bind with peroxidized cardiolipin. The previous observations conclude that low NAO fluorescence would be due to the oxidation/depletion of cardiolipin.[26] Oxidized cardiolipin may impact on various aspects of mitochondrial function: it has a weaker interaction with cytochrome *c*, participates in the permeabilization of mitochondria, and can generate 4-hydroxynonenal and other reactive lipid electrophiles.[41] It appears likely that increased mitochondrial NO and/or ROS production may lead to the oxidation of cardiolipin, which may in turn, affect the activities of respiratory chain complexes (complex I and IV) and membrane potential through the alteration of the normal lipid milieu, which is necessary for proper mitochondrial function. Although mitochondrial O_2 consumption was found slightly decreased in LPS-treated animals and returned to control values when pretreated with LA, these observations were not statistically significant. This situation may be explained by the reason that the observed changes are not critical enough to lead to impaired mitochondrial O_2 consumption, or that the impairment of mitochondrial O_2 consumption would develop as a late phenomenon in the bioenergetic dysfunction, which is finally present in organ failure.

In addition to the effects of LA on mitochondrial function, it may be analyzed as a critical component of cellular redox homeostasis because of its ability to regenerate other antioxi-

dants, increase intracellular GSH levels, and provide redox regulation of proteins and transcription factors.[42] LA can increase GSH levels through different mechanisms: reducing cystine to cysteine which is the limiting substrate for GSH synthesis,[1] directly reducing GSSG to GSH,[43] and recently, it has been shown that LA can induce *de novo* synthesis of GSH through the activation of transcription factor Nrf2.[44] Moreover, LA has been proposed to modulate other transcription factors, namely ERK and JNK; thus reducing mitochondrial oxidative stress.[45]

Overall, this work provides new evidence that *in vivo* treatment with α-lipoic acid may be considered as an strategy for maintaining cellular redox homeostasis in an inflammatory syndrome, based on the improvement of mitochondrial function, decreased production of mitochondrial NO and prevention of cardiolipin oxidation. Although this work is mainly focused on the effect of this compound on mitochondrial function and the oxidation of mitochondrial lipids, it is worth noting that the mechanism of the action of LA acid in cells is complex. The modulation of glutathione and other thiol levels, regeneration of antioxidants, and regulation of various signaling pathways, must be taken into consideration when discussing the overall effect of α-lipoic acid supplementation in pathology.

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References

1 K. P. Shay, R. F. Moreau, E. J. Smith, A. R. Smith and T. M. Hagen, *Biochim. Biophys. Acta*, 2009, **1790**(10), 1149–1160.
2 D. J. Keith, J. A. Butler, B. Bemer, B. Dixon, S. Johnson, M. Garrard, D. L. Sudakin, J. M. Christensen, C. Pereira and T. M. Hagen, *Pharmacol. Res.*, 2012, **66**(3), 199–206.
3 L. J. Reed, *J. Biol. Chem.*, 2001, **276**(42), 38329–38336.
4 A. J. Hobbs, A. Higgs and S. Moncada, *Annu. Rev. Pharmacol. Toxicol.*, 1999, **39**, 191–220.
5 L. Packer, E. H. Witt and H. J. Tritschler, *Free Radicals Biol. Med.*, 1995, **19**(2), 227–250.
6 M. P. Valdecantos, P. Perez-Matute, P. Gonzalez-Muniesa, P. L. Prieto-Hontoria, M. J. Moreno-Aliaga and J. A. Martinez, *J. Nutr. Biochem.*, 2012, **23**(12), 1676–1684.
7 V. Vanasco, M. C. Cimolai, P. Evelson and S. Alvarez, *Free Radical Res.*, 2008, **42**(9), 815–823.
8 A. Bilska and L. Wlodek, *Pharmacol. Rep.*, 2005, **57**(5), 570–577.
9 D. A. Lowes, N. R. Webster, M. P. Murphy and H. F. Galley, *Br. J. Anaesth.*, 2013, **110**(3), 472–480.
10 M. R. Pinsky, *Contrib. Nephrol.*, 2001, **132**, 354–366.
11 C. Szabó, Pathophysiological roles of nitric oxide in inflammation, in *Nitric Oxide: Biology and Pathobiology*, ed. L. J. Ignarro, Academic Press, San Diego, 2000, pp. 841–872.
12 V. Vanasco, P. Evelson, A. Boveris and S. Alvarez, *Chem. Biol. Interact.*, 2010, **184**(3), 313–318.
13 P. D. Cani, R. Bibiloni, C. Knauf, A. Waget, A. M. Neyrinck, N. M. Delzenne and R. Burcelin, *Diabetes*, 2008, **57**(6), 1470–1481.
14 P. D. Cani, J. Amar, M. A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A. M. Neyrinck, F. Fava, K. M. Tuohy, C. Chabo, A. Waget, E. Delmee, B. Cousin, T. Sulpice, B. Chamontin, J. Ferrieres, J. F. Tanti, G. R. Gibson, L. Casteilla, N. M. Delzenne, M. C. Alessi and R. Burcelin, *Diabetes*, 2007, **56**(7), 1761–1772.
15 A. Boveris, L. B. Valdez, S. Alvarez, T. Zaobornyj, A. D. Boveris and A. Navarro, *Antioxid. Redox Signaling*, 2003, **5**(3), 265–271.
16 H. M. Choi, S. K. Jo, S. H. Kim, J. W. Lee, E. Cho, Y. Y. Hyun, J. J. Cha, Y. S. Kang, D. R. Cha, W. Y. Cho and H. K. Kim, *Biochem. Biophys. Res. Commun.*, 2013, **435**(4), 678–684.
17 M. Tran, D. Tam, A. Bardia, M. Bhasin, G. C. Rowe, A. Kher, Z. K. Zsengeller, M. R. Akhavan-Sharif, E. V. Khankin, M. Saintgeniez, S. David, D. Burstein, S. A. Karumanchi, I. E. Stillman, Z. Arany and S. M. Parikh, *J. Clin. Invest.*, 2011, **121**(10), 4003–4014.
18 E. Cadenas and A. Boveris, *Biochem. J.*, 1980, **188**(1), 31–37.
19 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 1951, **193**(1), 265–275.
20 S. Alvarez and A. Boveris, Mitochondrial NO in endotoxemia, in Proceedings of the XII Biennial Meeting of the Society for Free radical research Internacional, ed. S. Puntarulo and A. Boveris, Medimond SRL, Italy, 2004, pp. 133–135.
21 C. L. Lisdero, M. C. Carreras, A. Meulemans, M. Melani, M. Aubier, J. Boczkowski and J. J. Poderoso, *Methods Enzymol.*, 2004, **382**, 67–81.
22 A. Boveris, S. L. Arnaiz, J. Bustamante, S. Alvarez, L. Valdez, A. D. Boveris and A. Navarro, *Methods Enzymol.*, 2002, **359**, 328–339.
23 B. Drew and C. Leeuwenburgh, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2003, **285**(5), R1259–R1267.
24 A. Czerniczyniec, A. G. Karadayian, J. Bustamante, R. A. Cutrera and S. Lores-Arnaiz, *Free Radicals Biol. Med.*, 2011, **51**(7), 1428–1436.
25 T. Marchini, N. Magnani, V. D'Annunzio, D. Tasat, R. J. Gelpi, S. Alvarez and P. Evelson, *Biochim. Biophys. Acta*, 2013, **1830**(3), 2545–2552.
26 A. Czerniczyniec, S. Lores-Arnaiz and J. Bustamante, *Free Radical Res.*, 2013, **47**(8), 614–623.
27 M. D. Brand and D. G. Nicholls, *Biochem. J.*, 2011, **435**(2), 297–312.
28 W. J. Zhang, H. Wei, T. Hagen and B. Frei, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**(10), 4077–4082.
29 A. R. Smith, S. V. Shenvi, M. Widlansky, J. H. Suh and T. M. Hagen, *Curr. Med. Chem.*, 2004, **11**(9), 1135–1146.

30 Y. Mythili, P. T. Sudharsan, E. Selvakumar and P. Varalakshmi, *Chem. Biol. Interact.*, 2004, **151**(1), 13–19.
31 A. Boveris, E. J. Roldan and S. Alvarez, Effect of lipioc acid on short-term ischemia-reperfusion in rat intestine, in *Biological oxidants and antioxidants*, ed. L. Packer and E. Cadenas, Hoppokrates Verlag, Stuttgart, 1994, pp. 77–86.
32 C. C. Yang, M. C. Ma, C. T. Chien, M. S. Wu, W. K. Sun and C. F. Chen, *J. Physiol.*, 2007, **582**(Pt 1), 407–419.
33 A. R. Nair, G. S. Masson, P. J. Ebenezer, F. Del Piero and J. Francis, *Free Radicals Biol. Med.*, 2014, **71**, 16–25.
34 M. S. Park, Y. R. Lee, S. Choi, H. K. Joo, E. J. Cho, C. S. Kim, J. B. Park, E. K. Jo and B. H. Jeon, *Biochem. Biophys. Res. Commun.*, 2013, **435**(4), 621–626.
35 M. I. Frisard, R. P. McMillan, J. Marchand, K. A. Wahlberg, Y. Wu, K. A. Voelker, L. Heilbronn, K. Haynie, B. Muoio, L. Li and M. W. Hulver, *Am. J. Physiol. Endocrinol. Metab.*, 2010, **298**(5), E988–E998.
36 S. Alvarez and A. Boveris, *Free Radicals Biol. Med.*, 2004, **37**(9), 1472–1478.
37 K. Stadler, M. G. Bonini, S. Dallas, J. Jiang, R. Radi, R. P. Mason and M. B. Kadiiska, *Free Radicals Biol. Med.*, 2008, **45**(6), 866–874.
38 M. L. Pall, *Int. J. Mol. Sci.*, 2013, **14**(11), 22274–22330.
39 H. Rubbo, A. Trostchansky and V. B. O'Donnell, *Arch. Biochem. Biophys.*, 2009, **484**(2), 167–172.
40 G. Petrosillo, P. Portincasa, I. Grattagliano, G. Casanova, M. Matera, F. M. Ruggiero, D. Ferri and G. Paradies, *Biochim. Biophys. Acta*, 2007, **1767**(10), 1260–1267.
41 H. Yin and M. Zhu, *Free Radical Res.*, 2012, **46**(8), 959–974.
42 R. Banerjee, *J. Biol. Chem.*, 2012, **287**(7), 4397–4402.
43 H. Moini, L. Packer and N. E. Saris, *Toxicol. Appl. Pharmacol.*, 2002, **182**(1), 84–90.
44 J. H. Suh, S. V. Shenvi, B. M. Dixon, H. Liu, A. K. Jaiswal, R. M. Liu and T. M. Hagen, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**(10), 3381–3386.
45 S. H. Suh, K. E. Lee, I. J. Kim, O. Kim, C. S. Kim, J. S. Choi, H. I. Choi, E. H. Bae, S. K. Ma, J. U. Lee and S. W. Kim, *Clin. Exp. Nephrol.*, 2014, DOI: 10.1007/s10157-014-0960-7.

