Lipoic Acid Prevents Hypertension, Hyperglycemia, and the Increase in Heart Mitochondrial Superoxide Production

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**Background:** The present study was designed to investigate whether the effects of dietary supplementation with α-lipoic acid could prevent the increase in mitochondrial superoxide production in the heart as well as the enhanced formation of advanced glycation end-products (AGE) that are associated with the development of hypertension and insulin resistance in chronically glucose-fed rats.

**Methods:** Sprague Dawley rats were either given or not given a 10% D-glucose solution to drink during 4 weeks, combined either with a normal chow diet or with α-lipoic acid supplemented diet. The oxidative stress was evaluated by measuring the heart mitochondrial superoxide production using the lucigenin chemiluminescence method. The formation of AGE was also assessed in plasma and aorta.

**Results:** Chronic administration of glucose resulted in a 29% increase in blood pressure, 30% increase in glycemia, 286% increase in insulinemia, and 408% increase in insulin resistance index. Chronic glucose feeding also resulted in a 22% greater mitochondrial superoxide anion production in heart and in an increase of 63% in AGE content in aorta. Increases in blood pressure, aorta AGE content and heart mitochondrial superoxide production were prevented in the rats fed glucose supplemented with lipoic acid. The simultaneous treatment with lipoic acid also attenuated the rise in insulin levels as well as in insulin resistance in the glucose fed rats.

**Conclusion:** These findings demonstrate that α-lipoic acid supplementation prevents development of hypertension and hyperglycemia, presumably through its antioxidative properties, as reflected by prevention of an increase in heart mitochondrial superoxide anion production and in AGE formation in the aorta of chronically glucose treated rats. Am J Hypertens 2003;16:173–179 © 2003 American Journal of Hypertension, Ltd.

**Key Words:** Diabetes, hypertension, insulin resistance, oxidative stress, lipoic acid.

There is growing evidence that oxidative stress plays a major role in the development and progression of cardiovascular diseases. In fact, elevated free radicals have been postulated to participate in the development of complications in both diabetes and hypertension.\(^1,2\) Oxidative stress may result from either excessive production of reactive oxygen species (ROS) or from a reduced antioxidant reserve. It has been demonstrated that mitochondria may be one of the major sources of endogenous ROS.\(^3\) Indeed, Nishikawa et al\(^4\) have reported that in cultured bovine aortic endothelial cells, hyperglycemia resulted in an overproduction of superoxide anion in mitochondria. Others studies have also shown that oxidative stress impairs insulin internalization in endothelial cells.\(^5\) Moreover, it has been demonstrated that advanced glycation end-products (AGE) are implicated in the development of kidney and vascular damage in experimental diabetic animals.\(^6\)

On the other hand, Zhang et al\(^7\) have shown that the antioxidant treatment with α-tocopherol resulted in an inhibition of mitochondrial ROS production and lipid peroxidation. They suggested that α-tocopherol treatment...
may prove useful in preventing diseases associated with mitochondrially derived oxidative stress. It was also reported that treatment with antioxidants such as α-tocopherol and acetylsalicylic acid inhibited AGE formation. Recent studies from our laboratory have demonstrated that chronic treatment with aspirin prevented the development of hypertension and attenuated markedly insulin resistance in chronically glucose fed rats, mainly through its antioxidative properties.

α-Lipoic acid is a disulfide compound that is found naturally in mitochondria as the coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Lipoic acid has been used as therapy for many diseases, especially diabetes. Dietary supplementation with that substance was reported to increase unbound lipoic acid levels, which can act as a potent antioxidant and reduce oxidative stress both in vitro and in vivo. Other studies have demonstrated that lipoic acid improved the insulin sensitivity in patients with type 2 diabetes. Treatment of insulin resistant Zucker rats with α-lipoic acid was found to increase both oxidative and nonoxidative glucose metabolism and to reduce insulin resistance. α-lipoic acid dietary supplementation in aged rats was also reported to improve mitochondrial function and to decrease oxidative stress. In one recent study from our laboratory, we observed that α-lipoic acid prevented development of hypertension, enhanced superoxide anion production in aorta, and insulin resistance in glucose fed rats. Moreover, recent studies have demonstrated that normalization of mitochondrial superoxide production blocked the damage induced by hyperglycemia in vascular endothelial cells. In addition, it is well known that in comparison to other cardiovascular tissues, mitochondria are more abundant in the heart, as 40% of myocardial cellular content is occupied by mitochondria. The present study was designed to investigate whether dietary supplementation with α-lipoic acid could decrease cardiac mitochondrial superoxide production as well as formation of AGE in association with prevention of hypertension and insulin resistance in chronically glucose fed rats.

**Materials and Methods**

**Animals and Protocols**

Studies were performed in male Sprague-Dawley (SD) rats weighing 230 to 250 g (Charles River Canada, Montreal, PQ, Canada). The rats were divided into four groups. Eight SD rats were given 10% D-glucose to drink in addition to a normal chow diet during 4 weeks; 10 SD rats were given 10% glucose to drink but received simultaneously an α-lipoic acid supplemented diet (500 mg/kg feed); eight SD rats were given ordinary water to drink but simultaneously received an α-lipoic acid (LA) supplemented diet, and the fourth group, considered as a control group, received drinking water and chow free of any drug. Rat chow was purchased from Charles River. The α-lipoic acid supplemented diet was obtained from Ren’s Feed Supplies Limited (Oakville, ON, Canada). At the end of the treatment, the rats were killed by decapitation after light anesthesia with CO₂. After opening the thorax, blood was withdrawn into a vacutainer tube for serum biochemistry. All blood samples were drawn early in the morning after fasting overnight (16 h). The heart was quickly excised and placed in a beaker containing cold isolation media (4°C) until isolation of mitochondria.

**Measurement of Enzyme Activities**

Blood glucose concentrations were measured with a glucometer (Elite, Bayer Inc., Toronto, ON, Canada). Insulin levels were determined by radioimmunoassay (RIA) method (Rat insulin RIA kit, Linco Research, St. Charles, MO). To evaluate the degree of insulin resistance, the Homeostasis Model Assessment (HOMA) was used as an index of insulin resistance and calculated by the following formula: insulin (µU/mL) × glucose (mmol/L)/22.5. One of the most important and potent endogenous antioxidant systems is the glutathione system. This system is critical for scavenging peroxides and other lipid derived oxidants, as well as for scavenging dicarbonyls. Important components of this system are glutathione peroxidase, glutathione reductase (GRred), and glutathione-S-transferase (GST) Using glutathione as the electron donor, glutathione peroxidase (GPx) scavenges peroxide radicals, including hydrogen peroxide and organic peroxides; this results in oxidation of glutathione to oxidized glutathione (GSSG). Glutathione reductase reduces GSSG to glutathione using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor. Glutathione-S-transferase efficiently scavenges many strong oxidants by forming glutathiyl adducts that are excreted. Plasma superoxide dismutase (SOD) activity was estimated using a kit from Randox Laboratories Canada Ltd. (Mississauga, ON, Canada). The GPx activity in plasma was measured spectrophotometrically as previously described. The activities of GRed and GST were measured in plasma and aorta according to methods described previously. The activity of GRed was determined by monitoring the decrease in absorbance at 340 nm based on the oxidation of NADPH, which is coupled to the reduction of one GSSG back to two glutathiones. The enzyme activity of GRed was expressed as mmole NADPH oxidized/min/mg protein. The activity of GST was evaluated by monitoring the increase in absorbance at 340 nm based on the formation of 1-glutathyl-2,4-nitrobenzene. Protein concentrations were measured by bicinchoninic acid procedure using bovine serum albumin as reference.

**Measurement of Advanced Glycation End-Products**

As described previously, formation of AGE in plasma and aorta was assessed by their fluorescence characteris-
tics, with excitation at 370 nm and emission at 440 nm, using a microplate fluorometer. Data are presented in terms of picomoles of sugar equivalents per milligram of protein.

**Drugs**

All chemicals used in experiments were purchased from Sigma Chemical Co. (St. Louis, MO).

**Mitochondrial Isolation**

The mitochondrial isolation procedure was performed according to McKee et al., with a few modifications. Briefly, hearts were removed and placed in beakers filled with cold (4°C) isolation medium containing (in mmol/L) 250 sucrose, 5 3-(N-morpholino) propanesulfonic acid (MOPS), and 2 ethylene-glycol-bis(1H,2H-ethane)-tetra-acetic acid (EGTA), pH 7.0 MOPS, sucrose, EGTA (MSE). All subsequent steps were performed on ice. Hearts were carefully trimmed of all nonventricular tissue, weighed, and minced. The ventricular mince was homogenized in MSE plus 0.4 mg/mL of subtilisin (10 mL/g wet wt, Sigma protease XXVII, previously referred to as naga-rase) in a Tek-Mar homogenizer (Fisher Scientific, Dyna-Mix, Indiana, Pennsylvania) adjusted by rheostat to 60% for two 10-sec bursts. The homogenate was diluted with MSE (10 mL/g wet wt) and centrifuged at 600 g for 5 min. The supernatant was carefully decanted into a clean cold tube and mitochondria pelleted at 8500 g for 10 min.

This supernatant was discarded and the pellet gently washed by swirling with 2 mL of MSE to remove a variable fluffy layer. The mitochondrial pellet was resuspended initially in 0.5 mL of MSE with an automatic pipette and diluted to 20 mL/g wet weight with MSE and both centrifugation steps repeated as above. The final mitochondrial pellet was also washed by swirling with 2 mL of MSE and no fluffy layer was observed. The final pellet was resuspended in respiration buffer (contained 250 mmol/L sucrose, 2 mmol/L HEPES, 2.5 mmol/L KH₂PO₄, 2.5 mmol/L MgCl₂, and 0.1% bovine serum albumin, pH 7.4 at 37°C; 0.5 mL/g original wet weight) and samples were removed to determine the protein concentration by the Bradford method.²³

**Chemiluminescence Measurement of O₂⁻ Production By Intact Mitochondria**

Mitochondrial superoxide anion production was evaluated using the lucigenin enhanced chemiluminescence method as described previously.²⁴ For measurement of lucigenin (bis-N-methyl-acridinium) derived chemiluminescence (LDCL) elicited with intact mitochondria, the reaction mixture contained 0.5 mg protein of mitochondria in 1.0 mL air saturated respiration buffer at 37°C in the presence or absence of exogeneous substrates. The mitochondrial substrates used included 6 mmol/L succinate. The LDCL was initiated with the addition of 5 μmol/L of lucigenin.

**Statistical Analysis**

Data are expressed as mean ± SEM (n). Significance was considered when P < .05. Our data were subjected to one-way analysis of variance (ANOVA), followed by the Bonferroni/Dunn multiple comparison test to estimate the significance of differences between groups. Simple regression analyses were used to evaluate the relationship between systolic blood pressure (BP) and mitochondrial heart superoxide anion production.

**Results**

**Blood Pressure**

Fig. 1 shows that 4 weeks of treatment with glucose resulted in a progressive increase in systolic (BP) (P < .01), which reached an average of 162 mm Hg. Supplementation of the diet with α-lipoic acid had no effect on systolic arterial pressure in control rats, whereas it totally prevented the increase in BP in glucose treated rats.
Body Weights and Metabolic Parameters

Body weights were not modified either by glucose feeding or by α-lipoic acid supplementation in all groups (388.8 ± 4.1 g in control; 389.3 ± 9.8 g in control + lipoic acid; 383.6 ± 8.1 g in glucose treated; 381.4 ± 13.5 g in glucose treated + lipoic acid). Glucose fed rats showed high levels of glucose (P < .01; Fig. 2A) in comparison to control rats. The 4-week treatment with α-lipoic acid completely prevented the hyperglycemia in glucose-fed rats so that glucose levels did not differ statistically from those in control rats. Insulin levels increased by 286% (Fig. 2B; P < .05) and the treatment with lipoic acid reduced this increase to 212% in glucose-fed rats, but those levels remained higher (P < .01) compared to those in control rats. A slight but not significant increase in insulin resistance index was observed in α-lipoic acid treated control rats.

Basal Heart Mitochondrial Superoxide Anion Production

Superoxide anion production in heart mitochondria obtained after 4 weeks of treatment, are shown in Fig. 3A. Basal heart mitochondrial superoxide production in chronically glucose fed rats was increased by 22% in comparison to those in control rats (P < .05). α-Lipoic acid supplementation prevented the rise in superoxide anion production in heart mitochondria of glucose treated rats.

Advanced Glycation End-Products Formation

The AGE formation in aorta and plasma, expressed as means of values obtained after 4 weeks of treatment, are presented in Fig. 3B and 3C, respectively. Aorta AGE formation of chronically glucose fed rats was increased by 63% in comparison to control rats (P < .01). Aorta AGE formation was not significantly modified
either by glucose feeding or by α-lipoic acid supplementation.

To evaluate the relationship between the heart mitochondrial superoxide anion production and systolic BP in control, glucose fed, and LA treated glucose fed rats, simple linear regression between these two parameters were calculated. As shown in Fig. 4, there was a statistically significant ($r = .540; P < .05$) positive correlation between the heart mitochondrial superoxide anion production and systolic BP within the combined groups of animals, but no correlation between the two parameters was found for any individual group of animals.

**Antioxidant Reserve**

As shown in Table 1, the plasma activity of glutathione peroxidase of glucose treated rats was significantly diminished by 18% in comparison to control rats ($P < .05$). α-Lipoic acid had no effect either on glucose treated rats or on control treated rats. Chronic glucose feeding resulted in a significant increase in plasma SOD activity in comparison to control rats ($P < .01$). α-Lipoic acid did not modify this parameter in glucose fed rats or control rats. As shown in Table 1, chronic administration of glucose resulted in significant increases of 50% ($P < .05$) and 52% ($P < .05$) in plasma and aorta GRed activity, respectively, in comparison to control rats. α-Lipoic acid supplemented diet reduced, although not significantly so, those increases to 37% and 44% in plasma and aorta, respectively, in glucose fed rats. As shown in Table 1, the plasma and aorta GST activities were similar in all groups.

**Discussion**

In this study, it is reported for the first time that chronic glucose feeding for 4 weeks increased mitochondrial superoxide anion production in the rat heart. Furthermore, the hyperglycemia-induced mitochondrial $O_2^-$ production was associated with a progressive increase in systolic BP and with the development of insulin resistance. Moreover, the present study also demonstrated for the first time that α-lipoic acid supplementation prevented the increase in basal heart mitochondrial $O_2^-$ production and the rise in aortic AGE formation observed in chronically glucose fed rats. In addition, α-lipoic acid diet was found to prevent the rise in systolic BP and to attenuate the development of insulin resistance. Consequently, a significant correlation between the level of BP and the degree of heart mitochondrial superoxide anion production was observed in this study.

We have recently demonstrated that chronic glucose feeding increases arterial BP and superoxide production in aorta, suggesting that oxidative stress is implicated in the elevation of systolic arterial pressure in chronically glucose treated rats. The present study showed that the basal

**Table 1.** Glutathione peroxidase, superoxide dismutase, glutathione reductase and glutathione-s-transferase activities in plasma and aorta

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control ($n = 8$)</th>
<th>Control + LA ($n = 8$)</th>
<th>Glucose Fed ($n = 8$)</th>
<th>Glucose Fed + LA ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx activity plasma (mU/mL)</td>
<td>12.4 ± 0.7</td>
<td>12.1 ± 0.5</td>
<td>10.2 ± 0.6*</td>
<td>10.1 ± 0.4*</td>
</tr>
<tr>
<td>SOD activity plasma (U/mL)</td>
<td>0.76 ± 0.05</td>
<td>0.72 ± 0.05</td>
<td>1.02 ± 0.04*</td>
<td>0.96 ± 0.09</td>
</tr>
<tr>
<td>GRed activity (nmol/min/mg protein) Plasma</td>
<td>22.2 ± 2.1</td>
<td>25.1 ± 3.0</td>
<td>33.4 ± 3.3*</td>
<td>30.6 ± 2.3</td>
</tr>
<tr>
<td>Aorta</td>
<td>104.2 ± 5.6</td>
<td>118.9 ± 17.0</td>
<td>158.5 ± 14.0*</td>
<td>151.0 ± 13.0</td>
</tr>
<tr>
<td>GST activity (nmol/mg protein) Plasma</td>
<td>81.2 ± 1.7</td>
<td>74.3 ± 4.8</td>
<td>84.6 ± 8.6</td>
<td>78.4 ± 1.3</td>
</tr>
<tr>
<td>Aorta</td>
<td>364.6 ± 48.4</td>
<td>340.9 ± 52.2</td>
<td>364.2 ± 49.1</td>
<td>343.0 ± 45.7</td>
</tr>
</tbody>
</table>

LA = α-lipoic acid; GPx = glutathione peroxidase; SOD = superoxide dismutase; GRed = glutathione reductase; GST = glutathione-s-transferase.

Data are means ± SE.

* $P < .05$ v control rats.
O$_2^-$ production from heart mitochondria was increased by 22% in glucose fed rats. This is in agreement with previous in vitro studies that have demonstrated that hyperglycemia induced mitochondrial O$_2^-$ in bovine cultured aortic endothelial cells.\textsuperscript{4,25} Yamagishi et al\textsuperscript{26} have also previously reported that hyperglycemia increased reactive oxygen species generation in human platelets through overproduction of mitochondrial superoxide. The data presented in this article demonstrate that high glucose feeding induced mitochondrial O$_2^-$ overproduction in heart is associated to the higher BP levels in this model, as a significant positive correlation was observed between cardiac mitochondrial O$_2^-$ production and systolic BP ($r = 0.540, P < .05$). The antioxidant reserve in blood and aorta as measured by the activities of the SOD and GRed was elevated by chronic glucose feeding, whereas it was not modified by the supplementation diet with $\alpha$-lipoic acid. These results suggest that the insulin resistance state is associated with an increase in the antioxidant reserve in an attempt to antagonize the exaggerated production of ROS.

One of the most dangerous consequences of diabetes associated glucose toxicity is mediated through the oxidative stress resulting from increased production of ROS.\textsuperscript{27} The treatment with $\alpha$-lipoic acid has been shown to improve metabolic parameters, BP, vascular reactivity, and morphology of vessels already damaged by experimental diabetes.\textsuperscript{28} Rudich et al\textsuperscript{29} have shown that the treatment with $\alpha$-lipoic acid protected against oxidative stress induced impairment in insulin stimulated glucose transport in adipocyte cells. Other studies have demonstrated that lipoic acid reduced oxidative stress even in diabetic patients with poor glycemic control.\textsuperscript{30} Moreover, recent studies have shown that normalizing mitochondrial superoxide production blocked the pathways whereby hyperglycemia induced complications in vascular endothelial cells.\textsuperscript{4} In the present study, it was shown for the first time that supplementation of $\alpha$-lipoic acid in the diet blunted the rise in heart mitochondrial superoxide anion production in glucose fed rats. Moreover, treatment with lipoic acid simultaneously prevented the development of hypertension and insulin resistance in that model. The present study thus suggests that the antihypertensive and hypoin-\sulimemic effects of $\alpha$-lipoic acid may be linked to reduction in oxidative stress, as shown by the decrease in the basal heart mitochondrial superoxide anion production in chronically glucose fed rats. These findings are in accordance with our recent studies,\textsuperscript{15} in which it was demonstrated that prevention of the development of hypertension and insulin resistance with $\alpha$-lipoic acid was associated with its antioxidant vascular properties in that model.

Gopaul et al\textsuperscript{31} have suggested that oxidative stress is an early event in the evolution of type 2 diabetes and could precede the development of endothelial dysfunction and insulin resistance. Alternatively, several studies have reported that chronic and acute parenteral administration of lipoic acid in insulin-resistant type 2 diabetic patients improved insulin mediated glucose disposal by 30% and 55%, respectively.\textsuperscript{13, 32} Recent studies have indicated that lipoic acid administration to obeses Zucker rats improves insulin stimulated glucose uptake in muscle.\textsuperscript{33} Moreover, Maddux et al\textsuperscript{34} have shown that lipoic acid protected muscle cells from oxidative stress induced insulin resistance. Our findings are in agreement with those observations, given that in the present study, the chronic treatment with $\alpha$-lipoic acid was found to attenuate the development of insulin resistance and also to prevent an increase in superoxide anion production in heart mitochondria. Therefore, these findings strongly support the involvement of oxidative stress in the development of insulin resistance.

Glycation of proteins may constitute an underlying factor in certain pathologies associated with diabetes, and free radicals may be involved in this process.\textsuperscript{35} The presence of AGE is closely related to hyperglycemia, and their presence could explain many of the changes observed in complications of diabetes. Three pathways have been proposed to explain the mechanisms whereby hyperglycemia leads to cardiovascular complications through the activation of protein kinase C isofoms,\textsuperscript{36} through the aldose reductase pathway\textsuperscript{37} as well as through enhanced AGE formation.\textsuperscript{38} These three pathways have recently been shown to be the consequence of a single common mechanism constituted by hyperglycemia induced mitochondrial superoxide overproduction.\textsuperscript{4} Interestingly, in the present study, the chronic glucose feeding resulted in a significant increase ($P < .05$) in aortic AGE formation in comparison to control rats. More importantly, the supplementation of $\alpha$-lipoic acid in the diet prevented the rise in aorta AGE formation in chronically glucose fed rats. Thus, the present study suggested that $\alpha$-lipoic acid, by decreasing superoxide production, might prevent an increase in AGE, thus reducing the development of diabetic complications.

In conclusion, our data suggest that $\alpha$-lipoic acid supplementation prevented the development of hypertension and hyperglycemia through its antioxidative properties, as it was found to prevent both an increase in heart mitochondrial superoxide anion production and a rise in advanced glycated end-product formation in the aorta of chronically glucose treated rats. Still, the precise sequential relationship between those effects needs to be established before developing new therapeutic strategies for the prevention or regression of insulin resistance in humans.

**Acknowledgment**

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**References**


