

Prevention of Hypertension, Insulin Resistance, and Oxidative Stress by α -Lipoic Acid

Adil EL Midaoui, Jacques de Champlain

Abstract—The aim of the present study was to investigate whether a dietary supplementation of α -lipoic acid could prevent blood pressure elevation, insulin resistance, and the increase in aorta superoxide anion production in a new experimental model of hypertension associated with insulin resistance. Sprague-Dawley rats were given 10% D-glucose in their drinking water combined either with a normal chow diet or with an α -lipoic acid-supplemented diet and were compared with control rats during 3 weeks. Oxidative stress was evaluated by measuring the aortic superoxide anion production using the lucigenin chemiluminescence method. Increases in blood pressure, insulin resistance, and aorta superoxide production observed in glucose-fed rats were prevented by the supplementation of the diet with lipoic acid. Positive correlations were found between aortic superoxide production and blood pressure, between insulin resistance and blood pressure, or between superoxide production and insulin resistance. Moreover, a decrease in the activity of plasma glutathione peroxidase observed in the glucose-fed rats was prevented by lipoic acid treatment. These findings demonstrate that high-glucose feeding rapidly induced hypertension and insulin resistance in association with the induction of a vascular oxidative stress. The antihypertensive action and the prevention of insulin resistance by lipoic acid appears to be associated to its antioxidative properties because it prevented the increase in oxidative stress, as reflected by the normalization of superoxide anion production in aorta and the prevention of the fall in the activity of glutathione peroxidase in the glucose-fed rats. (*Hypertension*. 2002;39:303-307.)

Key Words: oxidative stress ■ insulin resistance ■ α -lipoic acid ■ hyperglycemia

Diabetes is recognized as an important cardiovascular risk factor. The association of diabetes and hypertension potentiates the degree of cardiovascular risk, so recent therapeutic guidelines recommend to lower blood pressure of hypertensive diabetic patients to levels below those recommended for other hypertensive patients. Indeed, the Hypertension Optimal Treatment Study revealed that lowering diastolic blood pressure in patients with diabetes to 80 mm Hg decreases the risk of major cardiovascular events and cardiovascular mortality compared with lowering the diastolic blood pressure to 90 mm Hg, as recommended for nondiabetic hypertensive patients.¹

Several hypotheses were suggested to explain the enhanced risks associated to diabetes; among these, one of the most plausible is an increase in oxidative stress.² Oxidative stress may result from either excessive production of reactive oxygen species (ROS), especially the superoxide anion (O_2^-), or from reduced antioxidant reserve. Several studies have demonstrated in normotensive animals that the membrane-bound NADH/NADPH oxidase pathway accounts for most of the vascular O_2^- production,³ whereas the Cu/Zn superoxide dismutase (SOD) contributes for most of the scavenging of the vascular-generated nonmitochondrial O_2^- .⁴ Previous stud-

ies have suggested that increased O_2^- production may be involved in the pathogenesis and complications of both diabetes and hypertension.^{5,6} In hypertensive patients, lower concentrations of antioxidants and SOD activity have been documented.⁷ Increased O_2^- generation and reduced NO production were also reported in neutrophils and platelets from essential hypertensive patients.⁸ Moreover, Hamilton et al⁹ have shown in vitro that the treatment with SOD potentiated the NO-dependent relaxation in human thoracic artery and saphenous vein. In animal studies, arterial tissue O_2^- levels were reported to be increased in spontaneously hypertensive rats (SHR)^{10,11} and in insulin-resistant rats.¹² Other studies have demonstrated that antioxidant treatment with α -tocopherol or tempol reduces the blood pressure in SHR.¹³ Moreover, the treatment of diabetic animals with probucol, which is a lipid-lowering drug with antioxidant properties, or with vitamin E reduced the oxidative stress and enhanced the insulin sensitivity.^{14,15}

The treatment with the thiol compound, α -lipoic acid (LA) was reported to lower blood pressure in SHR.¹⁶ LA, which is a potent antioxidant (with a redox potential E_0^+ of -290 mV compared with vitamin E, which has a redox potential E_0^+ of $+370$ mV), exists endogenously in tissues and acts as a

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From the Research group on Autonomic Nervous System, Department of Physiology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.

Correspondence to Jacques de Champlain MD, PhD, Department of Physiology, Faculty of Medicine, University of Montreal, CP 6128, Succursale Centre Ville, Montreal, Quebec, Canada H3C 3J7. E-mail dechamplj@physio.umontreal.ca

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cofactor of key mitochondrial enzymes, controlling glucose oxidation, such as the pyruvate dehydrogenase and the α -ketoglutarate dehydrogenase.¹⁷ In type 2 diabetics, LA treatment was found to increase insulin-stimulated glucose metabolism.¹⁸ The treatment of insulin-resistant fatty Zucker rats with LA was found to increase both oxidative and nonoxidative glucose metabolism and to enhance the insulin sensitivity.¹⁹ The administration of LA to mice also increased tissue levels of glutathione.²⁰ The present study was designed to investigate whether a chronic dietary supplementation with LA could prevent blood pressure elevation, insulin resistance, and vascular oxidative stress in a new experimental model of hypertension associated to chronic glucose feeding.

Methods

Animals and Protocols

Male Sprague-Dawley rats (Charles River Canada, Montreal, Quebec, Canada) weighing 230 to 260 g were used in the present study. A group of Sprague-Dawley ($n=8$) was given 10% D-glucose to drink in addition to a normal chow diet during 3 weeks; a group of Sprague-Dawley rats ($n=10$) was given 10% glucose to drink but received simultaneously an LA-supplemented diet (500 mg/kg feed), and another group of age-matched control Sprague-Dawley rats ($n=8$) received only tap water and normal chow diet during 3 weeks. Rat chow was purchased from Charles River. The LA-supplemented diet was obtained from Ren's Feed Supplies Limited. The body weight and blood pressure of all rats were recorded weekly. The systolic blood pressure, which was measured by tail-cuff photoplethysmography (Harvard Apparatus Ltd) weekly and 1 day before the end of the study in the conscious rat, was recorded on a system MacLab/8 (AD Instruments Pty). For each blood pressure measure, at least 3 blood pressure readings were averaged. At the end of the treatment, the rats were killed by decapitation after light anesthesia with CO₂. All blood samples were drawn in the morning after fasting overnight (16 hours). The aorta was cut into 2-mm ring segments and then washed at least 3 times with Krebs-Hepes buffer before O₂⁻ measurement.

Laboratory Analysis

Plasma glucose concentrations were measured with a glucometer (Elite, Bayer Inc). Insulin levels were determined by radioimmunoassay method (kit 07260102; ICN Pharmaceuticals Costa Mesa). Erythrocyte and plasma SOD activity was determined spectrophotometrically (kit, Randox Laboratories Canada Ltd). Glutathione peroxidase (GPx) activity in erythrocyte and plasma was evaluated as previously described.²¹ To estimate the degree of insulin resistance, we have used the Homeostasis Model Assessment (HOMA) as an index of insulin resistance, as calculated by the following formula: $[\text{insulin (in } \mu\text{U/mL})} \times \text{glucose (in mmol/L)}] / 22.5$.²²

O₂⁻ Measurement

The O₂⁻ production was measured using the lucigenin-enhanced chemiluminescence method as described previously.²³ Superoxide production was expressed as counts per minute per milligram fresh tissue (cpm/mg aortic tissue). In another study, the superoxide production was measured using low concentration of lucigenin (5 $\mu\text{mol/L}$) in control and glucose-fed rats.

Drugs

Chemical components of solutions and all drugs were purchased from Sigma Chemical Co.

Statistics

Data are expressed as mean \pm SEM. Statistical analysis was performed by 1-way ANOVA. The statistical significances of the differences between groups were further established by the Bonfer-

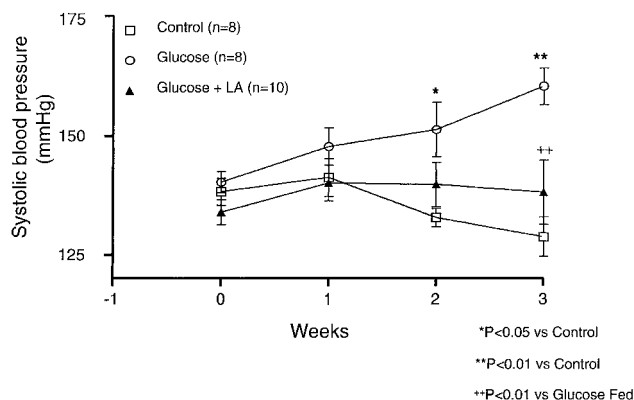


Figure 1. Evolution of systolic arterial pressure recorded in rats by tail-cuff photoplethysmography during 3 weeks in control rats and in 10% glucose-fed (glucose) rats given or not given LA supplementation. * $P<0.05$; ** $P<0.01$ vs control; ** $P<0.01$ vs glucose group.

roni/Dunn multiple comparison test. Significance was set at $P<0.05$, and P values are interpreted with the Bonferroni's correction when appropriate ($P<0.0167$), with 3 pairwise comparisons considered of interest: control rats versus the 2 other groups, and glucose-fed rats versus glucose and LA-treated rats. Simple regression analyses were used to examine the relations among blood pressure, HOMA, and aortic O₂⁻ production.

Results

Blood Pressure and Body Weight

As shown in Figure 1, the chronic administration of glucose in drinking water resulted in a progressive increase in systolic arterial pressure, which reached an average of 166 mm Hg after 3 weeks ($P<0.001$). The supplementation with LA prevented the rise in systolic blood pressure in D-glucose-treated rats, so their blood pressure did not statistically differ from that of control rats. As shown in Table 1, the final body weights were similar in all groups (359.4 \pm 7.3 in control, 360.6 \pm 7.8 in glucose, 362.0 \pm 5.4 in glucose+LA).

Plasma Glucose and Insulin Concentrations

The effects of chronic glucose feeding and LA-supplemented diet on plasma glucose and insulin levels are shown in Figure 2A and 2B. The plasma insulin was attenuated, although not significantly, but the plasma glucose was significantly diminished in D-glucose-treated animals given a LA-supplemented diet. However, after the treatment with LA, plasma insulin levels did not statistically differ from those in control rats. The development of insulin resistance, as reflected by a

TABLE 1. Body Weight and Systolic Blood Pressure

Characteristics	Control (n=8)	Glucose Fed (n=8)	Glucose Fed+LA (n=10)
Body weight, g			
Initial	240.4 \pm 7.8	244.6 \pm 7.5	242.5 \pm 6.2
Final	359.4 \pm 7.4	360.6 \pm 7.8	362.0 \pm 5.4
Blood pressure, mm Hg			
Initial	138.3 \pm 2.8	140.2 \pm 2.2	133.9 \pm 2.6
Final	128.8 \pm 4.1	166.3 \pm 3.8*	138.1 \pm 6.7†

Data are mean \pm SE. * $P<0.01$ vs control rats; † $P<0.01$ vs glucose-fed rats.

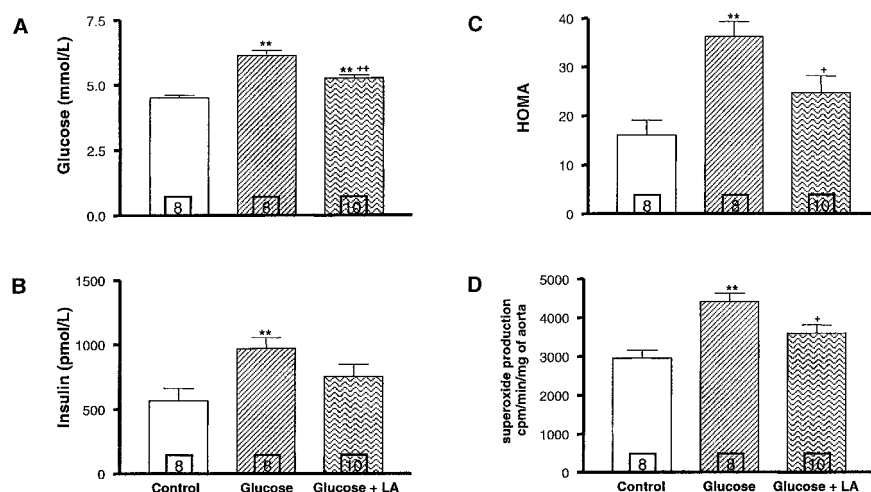


Figure 2. Effects of chronic glucose feeding combined or not with LA supplementation on plasma glucose levels expressed in mmol/L (A), on plasma insulin levels expressed in pmol/L (B), on HOMA (plasma glucose \times insulin/22.5) (C), and on O_2^- production in aorta expressed in cpm/min per mg of aorta (D). Values are mean \pm SE. Number of rats in each group is shown in each column. ** $P < 0.01$ vs control; *** $P < 0.01$, + $P < 0.05$ vs glucose group

higher HOMA, in glucose-fed rats ($P < 0.01$) (Figure 2C) was prevented by the LA diet in glucose-fed rats, so the index of insulin resistance did not differ from that in control rats.

Basal Aortic O_2^- Production

The effects of chronic glucose feeding and LA-supplemented diet on basal aortic O_2^- production are shown in Figure 2D. The chronic glucose feeding resulted in an increase of 52% in basal O_2^- production in the aorta ($P < 0.01$). The treatment with LA-supplemented diet prevented the rise in basal O_2^- production in aorta of D-glucose-treated rats ($P < 0.05$). When the superoxide production was measured using 5 μ mol/L of lucigenin, the following levels were found in aorta: 3008 ± 158 versus 5914 ± 479 cpm/min per mg ($P < 0.01$) in control and in glucose-fed rats, respectively. Therefore, the use of lower concentrations of lucigenin has revealed an even higher superoxide basal production in the aorta of glucose-fed rats.

To evaluate the relationships among the aortic O_2^- production, insulin resistance index, and systolic blood pressure in control, glucose-fed, and LA-treated glucose-fed rats, simple linear regressions between these parameters were calculated. As shown in Figure 3A, there was a statistically significant ($r = 0.543$, $P < 0.01$) positive correlation between the aortic O_2^- production and systolic blood pressure. There were also statistically significant positive correlations between the aortic O_2^- production and insulin resistance (HOMA; $r = 0.511$, $P < 0.05$; Figure 3B) as well as between HOMA and systolic blood pressure ($r = 0.609$, $P < 0.01$; Figure 3C).

Antioxidant Reserve

As shown in Table 2, the chronic administration of glucose combined with or not combined with LA had no effect on the activity of GPx in the red blood cells, but the administration of glucose induced a significant decrease of 16% ($P < 0.05$) in the activity of GPx in plasma. LA-supplemented diet prevented the decrease in GPx activity in the plasma of D-glucose-treated rats. As shown, the erythrocyte SOD activity was similar in all groups but in the plasma; significant increases in the SOD activity were observed in glucose-fed and in LA-supplemented glucose-fed rats compared with control.

Discussion

The major findings of the present study are as follows: (1) chronic glucose feeding for 3 weeks resulted in non-insulin-dependent diabetes as reflected by an increase in both blood glucose and insulin levels; (2) chronic administration of glucose was associated to a progressive increase in systolic arterial pressure, to an increased aortic basal O_2^- production and to lower plasma GPx activity; (3) supplementation of LA in the diet of chronically glucose-fed rats prevented the rise in blood pressure, the increase in aortic basal O_2^- production, the decrease in plasma GPx, and the development of insulin resistance; and (4) significant correlations were found between the level of blood pressure and the degree of basal superoxide production or the insulin resistance.

We have demonstrated that there was a significant elevation in blood pressure in rat chronically fed with glucose for 3 weeks. These findings are in agreement with previous observations that have shown that fructose feeding induced hypertension in rats.²⁴ Moreover, an increase of 52% in the aortic basal tissue O_2^- production was observed in glucose-fed rats. In previous studies, a similar enhancement of basal O_2^- production was also reported in aortas from insulin-resistant fructose-fed rats.²⁵ Kashiwagi et al²⁵ have suggested that the O_2^- production in the aorta of high fructose-fed rats was mediated through activation of NADH/NADPH oxidase. Similarly, in recent studies from our laboratory, it was reported that the enhanced O_2^- formation in aortic tissue resulted mainly from an increased NADH oxidase activity in SHR and deoxycorticosterone acetate-salt hypertensive rats.²⁶ In addition, the present study suggests an involvement of the vascular oxidative stress in the elevation of systolic arterial pressure induced by chronic glucose intake, because a significant positive relationship was observed between aortic O_2^- production and systolic blood pressure. A causal relationship between those parameters still remains to be clarified.

Many of the complications induced by diabetes are suspected to be mediated by oxygen free radical generation.²⁷ Moreover, the treatment with LA has been shown to prevent hyperglycemia, ketonemia, ketonuria, reduced glycogen in tissues, and a reduced rate of hepatic fatty acid synthesis in

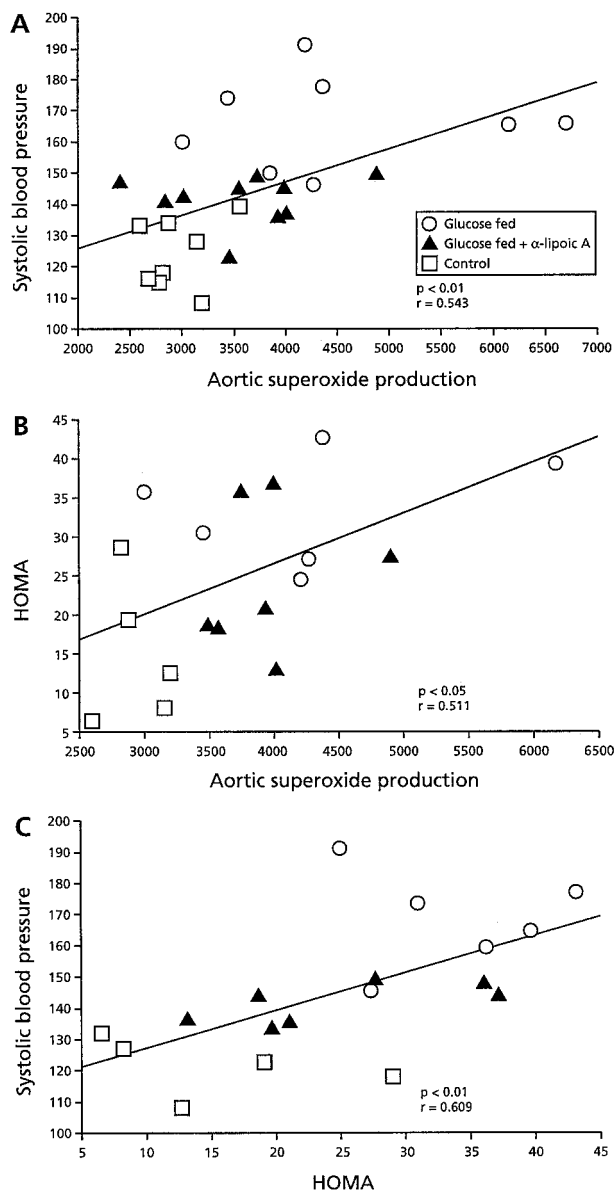


Figure 3. Relationships between the basal aortic superoxide production expressed in cpm/min per mg of aorta and the systolic blood pressure expressed in mm Hg (A); between insulin resistance index and the basal aortic superoxide production (B); and between the systolic blood pressure and insulin resistance index (C) in control (\square), glucose-fed (\circ) and glucose-fed treated with LA (\blacktriangle) rats.

diabetes.²⁸ Borcea et al²⁹ have shown that treatment with LA improves significantly the imbalance between increased oxidative stress and depleted antioxidant defense, even in diabetic patients with poor glycemic control. In the present study, we have shown for the first time that supplementation of LA in the diet prevented simultaneously the development of hypertension, the development of insulin resistance, and the increase in aortic O_2^- production in glucose-fed rats. Although the activities of glutathione peroxidase and superoxide dismutase in erythrocytes were not affected by LA in glucose-fed rats, it was demonstrated that the decrease in the activity of plasma glutathione peroxidase was prevented by LA during chronic glucose feeding. The present study thus

TABLE 2. Glutathione Peroxidase and Superoxide Dismutase Activities in Erythrocytes and Plasma of Control, Glucose-Fed, and Glucose-Fed with α -LA Rats

Characteristics	Control (n=8)	Glucose Fed (n=8)	Glucose Fed+LA (n=10)
Glutathione peroxidase activity			
Erythrocytes, U/mL	46.3 \pm 1.8	47.3 \pm 1.5	50.5 \pm 1.2
Plasma, mU/mL	18.2 \pm 0.7	15.8 \pm 0.8*	18.7 \pm 0.5†
Superoxide dismutase activity			
Erythrocytes, U/mL	236 \pm 8	222 \pm 8	210 \pm 13
Plasma, mU/mL	0.78 \pm 0.04	1.04 \pm 0.07*	1.09 \pm 0.06*

Data are mean \pm SE. * P <0.05 vs control rats; † P <0.05 vs glucose-fed rats.

suggests that the antihypertensive and hypoglycemic effects of LA are associated to an attenuation of the oxidative stress as reflected by the decrease in the basal O_2^- production in aortic vessel and by the preservation of the activity of GPx in the plasma of chronically glucose-treated rats.

Previous studies have suggested that the vascular resistance to insulin³⁰ may contribute to hypertension in one genetic model of insulin resistance, the obese Zucker rat.³¹ In support of this hypothesis, several studies have suggested that insulin resistance and hyperinsulinemia play a pathogenic role in the development of high blood pressure hypertension.³² This suggestion is supported by the observation that drugs that specifically counter insulin resistance (and attenuate hyperinsulinemia) also exhibit antihypertensive effects. Recent studies have shown that LA improves insulin sensitivity in patients with type 2 diabetes.³³ Other studies have demonstrated that LA improves the effects of insulin on skeletal muscle glucose transport in animal models of insulin resistance.³⁴ Yasunari et al³⁵ have demonstrated that treatment with antioxidants improves impaired insulin-mediated glucose uptake in high glucose-fed rabbit. More importantly, in the present study, LA was found to counter the development of insulin resistance but also to simultaneously prevent the rise in the blood pressure in glucose-fed rats. This study suggests that the antihypertensive effect of LA in glucose-fed rats may also be associated to the improvement of insulin resistance and/or to the attenuation in insulin and glucose levels. Although these findings strongly support the contributing role of oxidative stress in the development of hypertension in glucose-fed animals, they also suggest the participation of oxidative stress in the development of insulin resistance. These conclusions are supported by the highly significant correlations that were found between aortic superoxide production and blood pressure (P <0.01), between superoxide production and HOMA (P <0.05), and between insulin resistance index and blood pressure (P <0.01).

Glycation of proteins may constitute an underlying factor in certain pathologies associated to diabetes, and free radicals may be involved in this process.³⁶ Although several mechanisms have been postulated for the pathogenesis of chronic diabetic complications, protein glycation and oxidation by glucose (glycoxidation) represent plausible mechanisms.³⁷ Interestingly, recent studies have shown that LA decreases

lipid peroxidation and protein glycosylation in high glucose-treated human erythrocytes.³⁷ From those observations, it is possible to postulate that LA, by decreasing oxidative stress, would be effective in preventing protein glycation thus reducing the development of diabetic complications. However, further work is needed to verify and test this hypothesis.

The present study therefore demonstrates that LA supplementation can attenuate the elevation of blood pressure and the development of insulin resistance in chronically glucose-fed rats. The antihypertensive and the hypoglycemic effects of LA seem to be associated to its antioxidative properties because it was found to prevent an increase in the oxidative stress as reflected by the normal O_2^- production in aorta and the fall in the activity of plasma glutathione peroxidase in the chronically glucose-fed rats.

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