

### Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study)

**OBJECTIVE:** To evaluate the efficacy and safety of oral treatment with the antioxidant alpha-lipoic acid (ALA) in NIDDM patients with cardiac autonomic neuropathy (CAN), assessed by heart rate variability (HRV). **RESEARCH DESIGN AND METHODS:** In a randomized, double-blind placebo-controlled multicenter trial (Deutsche Kardiale Autonome Neuropathie [DEKAN] Study), NIDDM patients with reduced HRV were randomly assigned to treatment with daily oral dose of 800 mg ALA (n = 39) or placebo (n = 34) for 4 months. Parameters of HRV at rest included the coefficient of variation (CV), root mean square successive difference (RMSSD), and spectral power in the low-frequency (LF; 0.05-0.15 Hz) and high-frequency (HF; 0.15-0.5 Hz) bands. In addition, cardiovascular autonomic symptoms were assessed. **RESULTS:** Seventeen patients dropped out of the study (ALA n = 10; placebo n = 7). Mean blood pressure and HbA1 levels did not differ between the groups at baseline and during the study, but heart rate at baseline was higher in the group treated with ALA (P < 0.05). RMSSD increased from baseline to 4 months by 1.5 ms (-37.6 to 77.1) [median (minimum-maximum)] in the group given ALA and decreased by -0.1 ms (-19.2 to 32.8) in the placebo group (P < 0.05 for ALA vs. placebo). Power spectrum in the LF band increased by 0.06 bpm<sup>2</sup> (-0.09 to 0.62) in ALA, whereas it declined by -0.01 bpm<sup>2</sup> (-0.48 to 1.86) in placebo (P < 0.05 for ALA vs. placebo). Furthermore, there was a trend toward a favorable effect of ALA versus placebo for the CV and HF band power spectrum (P = 0.097 and P = 0.094 for ALA vs. placebo). The changes in cardiovascular autonomic symptoms did not differ significantly between the groups during the period studied. No differences between the groups were noted regarding the rates of adverse events. **CONCLUSIONS:** These findings suggest that treatment with ALA using a well-tolerated oral dose of 800 mg/day for 4 months may slightly improve CAN in NIDDM patients. Ziegler D, Schatz H, Conrad F, Gries FA, Ulrich H, Reichel G. *Diabetes Care* 1997 Mar;20(3):369-73.

### Effect of alpha-lipoic acid on the progression of endothelial cell damage and albuminuria in patients with diabetes mellitus: an exploratory study

Oxidative stress plays a central role in the pathogenesis and progression of late microangiopathic complications (diabetic nephropathy) in diabetes mellitus. Previous studies suggested that treatment of diabetic patients with the antioxidant alpha-lipoic acid reduce oxidative stress and urinary albumin excretion. In this prospective, open and non-randomized study, the effect of alpha-lipoic acid on the progression of endothelial cell damage and the course of diabetic nephropathy, as assessed by measurement of plasma thrombomodulin and urinary albumin concentration (UAC), was evaluated in 84 patients with diabetes mellitus over 18 months. Forty-nine patients (34 with Type 1 diabetes, 15 with Type 2 diabetes) had no antioxidant treatment and served as a control group. Thirty-five patients (20 with Type 1 diabetes, 15 with Type 2 diabetes) were treated with 600 mg alpha-lipoic acid per day. Only patients with an urinary albumin concentration <200 mg/l were included into the study. After 18 months of follow up, the plasma thrombomodulin level increased from 35.9+/-9.5 to 39.7+/-9.9 ng/ml (P<0.05) in the control group. In the alpha-lipoic acid treated group the plasma thrombomodulin level decreased from 37.5+/-16.2 to 30.9+/-14.5 ng/ml (P<0.01). The UAC increased in patients without alpha-lipoic acid treatment from 21.2+/-29.5 to 36.9+/-60.6 ng/l (P<0.05), but was unchanged with alpha-lipoic acid. It is postulated that the significant decrease in plasma thrombomodulin and failure of UAC to increase observed in the alpha-lipoic acid treated group is due to antioxidative effects of alpha-lipoic acid, and if so that oxidative stress plays a central role in the pathogenesis of diabetic nephropathy. Furthermore, progression of the disease might be inhibited by antioxidant drugs. A placebo-controlled study is needed. Morcos M et al. *Diabetes Res Clin Pract* 2001 Jun;52(3):175-83.

### Alpha-lipoic acid reduces expression of vascular cell adhesion molecule-1 and endothelial adhesion of human monocytes after stimulation with advanced glycation end products

Advanced glycation end products (AGEs) have been identified as relevant mediators of late diabetic complications such as atherosclerotic disease. The endothelial migration of monocytes is one of the first steps in atherogenesis and monocyte-

endothelial interaction itself is linked to the expression of adhesion molecules like vascular cell adhesion molecule-1 (VCAM-1). Recently, stimulation of VCAM-1 by AGEs has been demonstrated. Since endothelial stimulation by AGEs is followed by generation of oxygen free radicals with subsequent activation of nuclear transcription factor kappaB, we investigated the influence of alpha-lipoic acid on the expression of VCAM-1 and monocyte adherence to endothelial cells in vitro by means of cell-associated chemiluminescence assays and quantitative reverse transcriptase polymerase chain reaction using a constructed recombinant RNA standard. We found that alpha-lipoic acid was able to decrease the number of VCAM-1 transcripts from 41.0 +/- 11.2 to 9.5 +/- 4.7 RNA copies per cell in AGE-stimulated cell cultures. Furthermore, expression of VCAM-1 was suppressed in a time- and dose-dependent manner by alpha-lipoic acid as shown by chemiluminescence endothelial cell assay. Pretreatment of endothelial cells with 0.5 mM or 5 mM alpha-lipoic acid reduced AGE-induced endothelial binding of monocytes from 22.5 +/- 2.9% to 18.3 +/- 1.9% and 13.8 +/- 1.8% respectively. Thus, we suggest that extracellularly administered alpha-lipoic acid reduces AGE-albumin-induced endothelial expression of VCAM-1 and monocyte binding to endothelium in vitro. These in vitro results may contribute to the understanding of a potential antioxidative treatment of atherosclerosis. Kunt T et al. *Clin Sci (Colch)* 1999 Jan;96(1):75-82.

## Effects of dietary supplementation of alpha-lipoic acid on early glomerular injury in diabetes mellitus

Antioxidants, in particular vitamin E (VE), have been reported to protect against diabetic renal injury. alpha-Lipoic acid (LA) has been found to attenuate diabetic peripheral neuropathy, but its effects on nephropathy have not been examined. In the present study, parameters of glomerular injury were examined in streptozotocin diabetic rats after 2 mo on unsupplemented diets and in diabetic rats that received the lowest daily dose of dietary LA (30 mg/kg body wt), VE (100 IU/kg body wt), or vitamin C (VC; 1 g/kg body wt), which detectably increased the renal cortical content of each antioxidant. Blood glucose values did not differ among the diabetic groups. At 2 mo, inulin clearance, urinary albumin excretion, fractional albumin clearance, glomerular volume, and glomerular content of immunoreactive transforming growth factor-beta (TGF-beta) and collagen alpha1 (IV) all were significantly increased in unsupplemented D compared with age-matched nondiabetic controls. With the exception of inulin clearance, LA prevented or significantly attenuated the increase in all of these glomerular parameters in D, as well as the increases in renal tubular cell TGF-beta seen in D. At the dose used, VE reduced inulin clearance in D to control levels but failed to alter any of the other indices of glomerular injury or to suppress renal tubular cell TGF-beta in D. VC suppressed urinary albumin excretion, fractional albumin clearance, and glomerular volume but not glomerular or tubular TGF-beta or glomerular collagen alpha1 (IV) content. LA but not VE or VC significantly increased renal cortical glutathione content in D. These data indicate that LA is effective in the prevention of early diabetic glomerular injury and suggest that this agent may have advantages over high doses of either VE or VC. Melhem MF, Craven PA, Derubertis FR. *J Am Soc Nephrol* 2001 Jan;12(1):124-33.

## Lipoic acid reduces glycemia and increases muscle GLUT4 content in streptozotocin-diabetic rats

Alpha lipoic acid (lipoate [LA]), a cofactor of alpha-ketodehydrogenase, exhibits unique antioxidant properties. Recent studies suggest a direct effect of LA on glucose metabolism in both human and experimental diabetes. This study examines the possibility that LA positively affects glucose homeostasis in streptozotocin (STZ)-induced diabetic rats by altering skeletal muscle glucose utilization. Blood glucose concentration in STZ-diabetic rats following 10 days of intraperitoneal (i.p.) injection of LA 30 mg/kg was reduced compared with that in vehicle-treated diabetic rats (495 +/- 131 v 641 +/- 125 mg/dL in fed state, P = .003, and 189 +/- 48 v 341 +/- 36 mg/dL after 12-hour fast, P = .001). No effect of LA on plasma insulin was observed. Gastrocnemius muscle crude membrane GLUT4 protein was elevated both in control and in diabetic rats treated with LA by 1.5- and 2.8-fold, respectively, without significant changes in GLUT4 mRNA levels. Gastrocnemius lactic acid was increased in diabetic rats (19.9 +/- 5.5 v 10.4 +/- 2.8 mumol/g muscle, P < .05 v nondiabetic rats), and was normal in LA-treated diabetic rats (9.1 +/- 5.0 mumol/g muscle). Insulin-stimulated 2-deoxyglucose (2 DG) uptake into isolated soleus muscle was reduced in diabetic rats compared with the control group (474 +/- 15 v 568 +/- 52 pmol/mg muscle 30 min, respectively, P = .05). LA treatment prevented this reduction, resulting in insulin-stimulated glucose uptake comparable to that of nondiabetic animals. These results suggest that daily LA treatment may reduce blood glucose concentrations in STZ-diabetic rats by enhancing muscle GLUT4 protein content and by increasing muscle glucose utilization. Khamaisi M et al. *Metabolism* 1997 Jul;46(7):763-8.

## Lipoic acid improves nerve blood flow, reduces oxidative stress, and improves distal nerve conduction in experimental diabetic neuropathy

**OBJECTIVE**--To determine whether lipoic acid (LA) will reduce oxidative stress in diabetic peripheral nerves and improve neuropathy. **RESEARCH DESIGN AND METHODS**--We used the model of streptozotocin-induced diabetic neuropathy (SDN) and evaluated the efficacy of LA supplementation in improving nerve blood flow (NBF), electrophysiology, and indexes of oxidative stress in peripheral nerves affected by SDN, at 1 month after onset of diabetes and in age-matched control rats. LA, in doses of 20, 50, and 100 mg/kg, was administered intraperitoneally five times per week after onset of diabetes. **RESULTS**--NBF in SDN was reduced by 50%; LA did not affect the NBF of normal nerves but improved that of SDN in a dose-dependent manner. After 1 month of treatment, LA-supplemented rats (100 mg/kg) exhibited normal NBF. The most sensitive and reliable indicator of oxidative stress was reduction in reduced glutathione, which was significantly reduced in streptozotocin-induced diabetic and alpha-tocopherol-deficient nerves; it was improved in a dose-dependent manner in LA-supplemented rats. The conduction velocity of the digital nerve was reduced in SDN and was significantly improved by LA. **CONCLUSIONS**--These studies suggest that LA improves SDN, in significant part by reducing the effects of oxidative stress. The drug may have potential in the treatment of human diabetic neuropathy. Nagamatsu M et al. *Diabetes Care* 1995 Aug;18(8):1160-7.

## Effects of alpha-lipoic acid on microcirculation in patients with peripheral diabetic neuropathy

Diabetic polyneuropathy is a serious complication in patients with diabetes mellitus. In addition to the maintenance of a sufficient metabolic control, alpha-lipoic acid (ALA) (Thioctacid, Asta Medica) is known to have beneficial effects on diabetic polyneuropathy although the exact mechanism by which ALA exerts its effect is unknown. In order to study the effect of ALA on microcirculation in patients with diabetes mellitus and peripheral neuropathy one group of patients (4 female, 4 male, age 60+/-3 years, diabetes duration 19+/-4 years, BMI 24.8+/-1.3 kg/m<sup>2</sup>) received 1200 mg ALA orally per day over 6 weeks (trial 1). A second group of patients (5 female, 4 male, age 65+/-3 years, diabetes duration 14+/-4 years, BMI 23.6+/-0.7 kg/m<sup>2</sup>) was studied before and after they had received 600 mg ALA or placebo intravenously over 15 minutes in order to investigate whether ALA has an acute effect on microcirculation (trial 2). Patients were investigated by nailfold videocapillaroscopy. Capillary blood cell velocity was examined at rest and during postreactive hyperemia (occlusion of the wrist for 2 minutes, 200 mmHg) which is a parameter of the perfusion reserve on demand. The oral therapy with ALA resulted in a significant decrease in the time to peak capillary blood cell velocity (tpCBV) during postocclusive hyperemia (trial 1: 12.6+/-3.1 vs 35.4+/-10.9 s,  $p < 0.05$ ). The infusion of ALA also decreased the tpCBV in patients with diabetic neuropathy (trial 2: before: 20.8+/-4.5, ALA: 11.74+/-4.4, placebo: 21.9-5.0 s,  $p < 0.05$  ALA vs both placebo and before infusions) indicating that ALA has an acute effect on microcirculation. Capillary blood cell velocity at rest (rCBV), hemodynamic parameters, hemoglobinA1c and local skin temperature remained unchanged in both studies. These results demonstrate that in patients with diabetic polyneuropathy ALA improves microcirculation as indicated by an increased perfusion reserve on demand. The observed effects are apparently acute effects. With the restriction of the pilot character of this investigation the findings support the assumption that ALA might exert its beneficial effects at least partially by improving microcirculation which is likely to occur also at the level of the vasa nervorum. Haak E et al. *Exp Clin Endocrinol Diabetes* 2000;108(3):168-74.

## Effect of alpha-lipoic acid on the progression of endothelial cell damage and albuminuria in patients with diabetes mellitus: an exploratory study

Oxidative stress plays a central role in the pathogenesis and progression of late microangiopathic complications (diabetic nephropathy) in diabetes mellitus. Previous studies suggested that treatment of diabetic patients with the antioxidant alpha-lipoic acid reduce oxidative stress and urinary albumin excretion. In this prospective, open and non-randomized study, the effect of alpha-lipoic acid on the progression of endothelial cell damage and the course of diabetic nephropathy, as assessed by measurement of plasma thrombomodulin and urinary albumin concentration (UAC), was evaluated in 84 patients with diabetes mellitus over 18 months. Forty-nine patients (34 with Type 1 diabetes, 15 with Type 2 diabetes) had no antioxidant treatment and served as a control group. Thirty-five patients (20 with Type 1 diabetes, 15 with Type 2 diabetes) were treated with 600 mg alpha-lipoic acid per day. Only patients with an urinary albumin concentration <200 mg/l were included into the study. After 18 months of follow up, the plasma thrombomodulin level increased from 35.9+/-9.5 to 39.7+/-9.9 ng/ml ( $P < 0.05$ ) in the control group. In the alpha-lipoic acid treated group the plasma thrombomodulin level decreased from 37.5+/-16.2 to 30.9+/-14.5 ng/ml ( $P < 0.01$ ). The UAC increased in patients without alpha-lipoic acid treatment from 21.2+/-29.5 to 36.9+/-60.6 ng/l ( $P < 0.05$ ), but was unchanged with alpha-lipoic acid. It is postulated that the significant decrease in plasma thrombomodulin and failure of UAC to increase observed in the alpha-lipoic acid treated group is due to antioxidative effects of alpha-lipoic acid, and if so that oxidative stress plays a central role in the pathogenesis of diabetic nephropathy. Furthermore, progression of the disease might be inhibited by antioxidant drugs. A placebo-controlled study is needed. Morcos M et al. *Diabetes Res Clin Pract* 2001 Jun;52(3):175-83.

## alpha-Lipoic acid treatment decreases serum lactate and pyruvate concentrations and improves glucose effectiveness in lean and obese patients with type 2 diabetes

**OBJECTIVE:** We examined the effect of lipoic acid (LA), a cofactor of the pyruvate dehydrogenase complex (PDH), on insulin sensitivity (SI) and glucose effectiveness (SG) and on serum lactate and pyruvate levels after oral glucose tolerance tests (OGTTs) and modified frequently sampled intravenous glucose tolerance tests (FSIGTTs) in lean (n = 10) and obese (n = 10) patients with type 2 diabetes. **RESEARCH DESIGN AND METHODS:** FSIGTT data were analyzed by minimal modeling technique to determine SI and SG before and after oral treatment (600 mg, twice a day, for 4 weeks). Serum lactate and pyruvate levels of diabetic patients after glucose loading were compared with those of lean (n = 10) and obese (n = 10) healthy control subjects in which SI and SG were also determined from FSIGTT data. **RESULTS:** Fasting lactate and pyruvate levels were significantly increased in patients with type 2 diabetes. These metabolites did not exceed elevated fasting concentrations after glucose loading in lean patients with type 2 diabetes. However, a twofold increase of lactate and pyruvate levels was measured in obese diabetic patients. LA treatment was associated with increased SG in both diabetic groups (lean 1.28 +/- 0.14 to 1.93 +/- 0.13; obese 1.07 +/- 0.11 to 1.53 +/- 0.08 x 10<sup>(-2)</sup> min<sup>-1</sup>, P < 0.05). Higher SI and lower fasting glucose were measured in lean diabetic patients only (P < 0.05). Lactate and pyruvate before and after glucose loading were approximately 45% lower in lean and obese diabetic patients after LA treatment. **CONCLUSIONS:** Treatment of lean and obese diabetic patients with LA prevents hyperglycemia-induced increments of serum lactate and pyruvate levels and increases SG. Konrad T et. al. *Diabetes Care* 1999 Feb;22(2):280-7.