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α-Lipoic acid prevents diabetes mellitus in diabetes-prone obese rats

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Abstract

Several lines of evidence have suggested that triglyceride accumulation in skeletal muscle and pancreatic islets is causally related to type 2 diabetes mellitus. We recently showed that α-lipoic acid (ALA), a potent antioxidant and cofactor of mitochondrial respiratory enzymes, reduces body weight of rodents by suppressing food intake and increasing energy expenditure. We sought to determine if ALA can prevent the development of diabetes mellitus in obese Otsuka Long–Evans Tokushima Fatty (OLETF) rats. Most (78%) untreated OLETF rats showed glycosuria at 40 weeks of age, but this was completely prevented by ALA. Compared with untreated OLETF rats, ALA reduced body weight and protected pancreatic β-cells from destruction. ALA also reduced triglyceride accumulation in skeletal muscle and pancreatic islets. These results indicate that ALA prevents diabetes mellitus in obese diabetes-prone rats by reducing lipid accumulation in non-adipose tissue as well as in adipose tissue.

Keywords: α-Lipoic acid; Diabetes mellitus; Intracellular triglyceride; Skeletal muscle; Pancreatic islets

Obesity is the most important risk factor for type 2 diabetes mellitus [1]. Several lines of evidence have suggested that triglyceride accumulation in skeletal muscle and pancreatic islets is causally related to skeletal muscle insulin resistance and pancreatic β-cell dysfunction in obesity [2–4]. In addition, storage of excess triglycerides and long chain fatty acyl CoA (LCAC) is believed to increase the formation of oxygen free radicals in cells [4,5]. This has led to the hypothesis that, in obesity, increased lipid accumulation in skeletal muscle and pancreatic islets increases oxidative stress and causes functional defects in these tissues, leading to the development of type 2 diabetes mellitus [5,6].

α-Lipoic acid (ALA) is a naturally occurring short chain fatty acid with sulfhydryl groups that has potent antioxidative activity in a wide variety of experimental systems and is clinically used to treat diabetic neuropathy [7,8]. ALA is a unique antioxidant because it has beneficial effects on fuel metabolism. ALA is an essential cofactor of mitochondrial respiratory enzymes, including the pyruvate dehydrogenase (PDH) complex [7,8], and ALA supplementation in older rats has been found to improve mitochondrial function and enhance metabolic rate [9]. ALA has also been shown to protect against oxidative stress-induced insulin resistance in vitro [10–12] and to stimulate peripheral glucose utilization in pre-diabetic obese rats [13] and patients with type 2 diabetes mellitus [14]. In addition, we recently reported that ALA reduced the body weight of rodents by suppressing food intake and increasing energy expenditure [15]. The Otsuka Long–Evans Tokushima Fatty

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(OLETF) rat is an animal model of obesity and diabetes mellitus. Even though these rats are severely obese from the young ages, diabetes develops later in life [16]. Thus, pre-diabetic rats can be used to test the effects of various agents on the development of diabetes [17]. In the present study, we utilized this model to test potential preventive effects of ALA on the development of diabetes.

Materials and methods

Animals. Four-week-old diabetes-prone male OLETF rats and non-diabetic control Long–Evans Tokushima Otsuka (LETO) rats were obtained from the Otsuka Pharmaceutical Company (Tokushima, Japan). The rats were housed at ambient temperature (22 ± 1 °C), with 12:12-h light–dark cycles and free access to water and rat chow. All experiments were approved by the Institutional Animal Care and Use Committee at the Asan Institute for Life Sciences, Seoul, Korea.

Effects of ALA on the development of diabetes mellitus and β-cell mass. Thirty-six 9-week-old OLETF rats were divided randomly into two groups, which were fed standard rat chow with (treatment group, $n = 18$) or without (control group, $n = 18$) 0.5% (wt/wt, 200 mg/Bwt kg/day) racemic ALA (Sigma, St. Louis, Missouri, USA). Control LETO rats ($n = 18$) were fed standard rat chow without ALA. Body weight and urinary glucose (Diastix, Amex, Tokyo, Japan) were monitored weekly beginning at 12 weeks of age. Individual rats were classified as diabetic on the basis of positive glycosuria tests.

Pancreatic morphology and immunohistochemical analysis. At 9, 18, 27, and 40 weeks of age, four rats ($n = 4$ in each group) were anesthetized by intraperitoneal injections of 40 mg/kg pentobarbital sodium and sacrificed. The entire pancreas was removed, processed for paraffin embedding, and stained with hematoxylin and eosin for analysis of islet morphology. For determination of β-cell mass, two longitudinal sections of each pancreas were stained with guinea pig anti-insulin polyclonal antibody (Biomeda, Foster, California, USA).

Assessment of β-cell mass. The β-cell mass of each pancreas was measured according to the method of Bonner-Weir [18]. Briefly, each pancreas was weighed, and four random cross-sections were taken. The entire area and the area occupied by β-cells were determined by planimetry using software Image Tool for Windows, version 1.28 (University of Texas Health Science Center, San Antonio, Texas, USA). The β-cell mass was determined from the formula, pancreatic weight × (insulin-positive area/area of pancreas)$^{3/2}$.

Effects of ALA on metabolic parameters and triglyceride content of skeletal muscle and islets. Fifteen-week-old OLETF rats were fed rat chow with ($n = 6$) or without ($n = 6$) 0.5% ALA for 3 weeks. After a 5-h fast, these rats and LETO rats of the same age were sacrificed under pentobarbital sodium anesthesia. Blood was drawn from the inferior vena cava, and the soleus muscles were frozen in situ, using aluminum tongs pre-cooled in liquid nitrogen, and stored at −70 °C. Pancreatic islets were isolated by collagenase digestion [19]. After two washes with Hanks' solution to remove collagenase, the islets were suspended in a basal incubation medium consisting of KRBB/10 mM Hepes, pH 7.4, 3 mM glucose, and 0.3% BSA at 0 °C.

Measurement of plasma metabolic parameters. Plasma glucose and lactate concentrations were determined using a glucose and lactate analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, Ohio, USA). Plasma free fatty acid (FFA) concentration was determined by an enzymatic assay using a kit from Wako Chemical (Osaka, Japan), and plasma triglyceride concentration was measured enzymatically (Sigma). Plasma insulin and leptin concentrations were determined by radioimmunoassay (Linco, St. Louis, Missouri, USA).

Measurement of triglyceride contents. Triglyceride contents of each muscle and islet sample were determined using the Sigma Triglyceride (GPO-Trinder) kit [20].

Statistical analysis. Data are expressed as means ± SEM. Statistical analysis was performed by two-tailed unpaired Student's t test or by one-way analysis of variance followed by post hoc Tukey's multiple comparison test. Statistical significance was defined as $P < 0.05$. All analyses were performed using Statistical Package for Social Science (SPSS), version 9.0 (SPSS, Chicago, Illinois, USA).

Results

Effects of ALA on body weight and the development of diabetes

Urinary glucose was detected in 14 of 18 (78%) untreated 40-week-old OLETF rats (Fig. 1A), but in none of the 18 ALA-treated OLETF rats (0%) at the same age. Body weight at 40 weeks of age was significantly lower in the ALA-treated than in the untreated OLETF rats (Fig. 1B and Table 1).

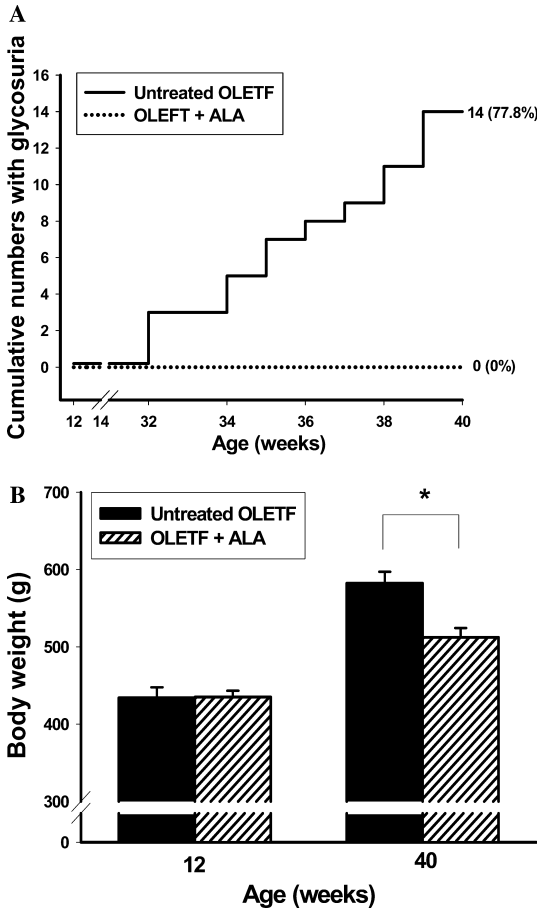

Fig. 1. Effects of ALA on the development of diabetes mellitus and body weight. (A) Cumulative numbers of untreated and ALA-treated OLETF rats showing glycosuria over time. (B) Mean body weights of untreated and ALA-treated OLETF rats at 12 and 40 weeks of age. Bars indicate means ± SEM. *$P < 0.01$ by unpaired, two-tailed Student's t test.

Table 1
Effects of ALA on plasma concentrations of metabolic parameters and oxidative stress markers in OLETF and control (LETO) rats

	LETO	Untreated OLETF	OLETF + ALA
Mean food intake (g/day)	24 ± 0.3	33 ± 0.4	19 ± 5.1[f]
Body weight (g)			
At 12 weeks	352 ± 8	420 ± 9[a]	421 ± 9[a]
At 40 weeks	508 ± 10	593 ± 14[a]	506 ± 20[e]
Glucose (mmol/L)	7.7 ± 0.03	8.5 ± 0.23[a]	7.7 ± 0.23[d]
Insulin (pmol/L)	65.6 ± 5.2	141.1 ± 11.7[b]	80.2 ± 15.5[d]
Leptin (μg/L)	7.9 ± 0.4	20.1 ± 4.6[b]	7.2 ± 1.1[e]
FFA (mmol/L)	0.53 ± 0.05	0.82 ± 0.03[b]	0.59 ± 0.04[e]
TG (mmol/L)	0.78 ± 0.11	2.86 ± 0.42[c]	0.60 ± 0.09[f]
MDA (μmol/L)	1.04 ± 0.07	1.52 ± 0.07[b]	1.12 ± 0.10[d]
8-OHDG (ng/ml)	0.10 ± 0.01	0.34 ± 0.02[b]	0.16 ± 0.02[d]

Abbreviations: TG, triglyceride. All data are expressed as means ± SEM.
[a]$p < 0.05$; [b]$p < 0.01$; and [c]$p < 0.001$ vs. LETO.
[d]$p < 0.05$; [e]$p < 0.01$; and [f]$p < 0.001$ vs. untreated OLETF.

Effects of ALA on pancreatic islet morphology

The islets of 9-week-old OLETF rats appeared normal (data not shown), but by 18 weeks of age they were enlarged compared with those of LETO rats (Fig. 2A). In 27-week-old OLETF rats, the islets appeared disorganized, with extensions into the surrounding exocrine tissue. Hematoxylin and eosin staining disclosed focal regions of degeneration, destruction, fibrosis, and moderate infiltration of lymphocytes (data not shown). In 40-week-old OLETF rats, the insulin-positive cells were dispersed and low in number, and the contour of the islets was irregular and could not be easily delineated. In contrast, the islets of 18-, 27-, and 40-week-old ALA-treated OLETF rats appeared normal, showing no signs of islet cell hypertrophy, destruction, or uneven distribution of insulin-positive cells.

Effects of ALA on β-cell mass

β-Cell mass was significantly higher in untreated OLETF rats than in LETO rats at 9, 18, and 27 weeks of age (Fig. 2B). At 40 weeks of age, however, following the development of diabetes, β-cell mass decreased in OLETF rats, to a level significantly lower than that in LETO rats (Fig. 2B). In the ALA-treated OLETF rats, β-cell mass was relatively stable throughout the study period and was significantly lower than that of untreated

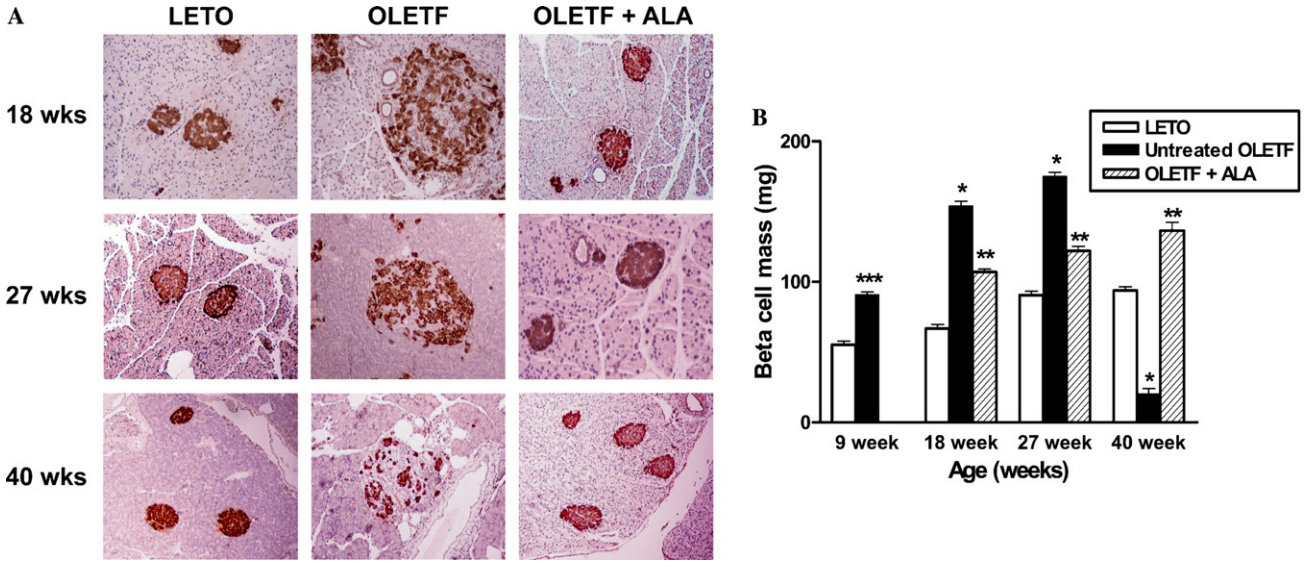

Fig. 2. ALA prevents β-cell deterioration and preserves β-cell mass. (A) Photomicrographs of pancreatic islets evaluated by insulin immunohistochemistry in LETO rats and untreated and ALA-treated OLETF rats at 18, 27, and 40 weeks of age. Brown-colored areas represent insulin-positive cells. (B) Mean β-cell mass in the three groups of rats. Bars indicate means ± SEM. *$P < 0.05$ versus LETO group and ALA group, **$P < 0.05$ versus untreated OLETF group by ANOVA, and ***$P < 0.05$ vs. LETO group by unpaired, two-tailed Student's t test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

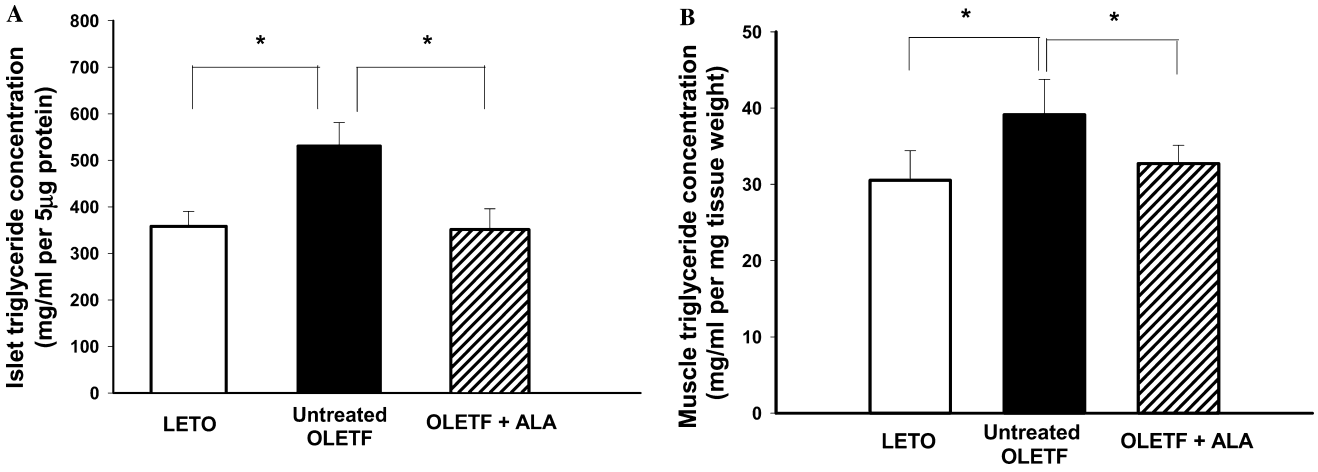

Fig. 3. ALA decreases triglyceride contents in non-adipose tissues. Triglyceride contents were measured in the islets (A) and soleus muscles (B) of LETO and untreated and ALA-treated OLETF rats. Bars indicate means ± SEM. $*P < 0.05$ by ANOVA.

OLETF rats through 27 weeks of age (Fig. 2B). In addition, the decrease in β-cell mass observed in untreated OLETF rats at 40 weeks was completely prevented by ALA treatment (Fig. 2B).

Basal metabolic parameters and oxidative stress markers

In agreement with our previous study [15] ALA significantly reduced body weight and food intake (Table 1). At 18 weeks of age, plasma glucose, insulin, leptin, FFA, and triglyceride levels were significantly higher in untreated OLETF rats than in LETO rats (Table 1). ALA treatment for 3 weeks decreased all these parameters in OLETF rats (Table 1). In addition, ALA decreased plasma levels of the oxidative stress markers, MDA and 8-OHDG, in OLETF rats that were significantly higher compared to LETO rats (Table 1).

Triglyceride contents in islets and skeletal muscle of ALA-treated rats

The triglyceride contents were significantly higher in the islets and soleus muscles of untreated OLETF rats than in LETO rats (Figs. 3A and B). ALA treatment decreased islet and muscle triglyceride contents to levels similar to those observed in LETO rats (Figs. 3A and B).

Discussion

We have shown here that chronic administration of ALA to pre-diabetic OLETF rats completely prevented the development of diabetes mellitus. ALA has been shown to protect against oxidative stress-induced insulin resistance in vitro [9–11] and to improve insulin sensitivity in diabetic patients and in experimental animals [12–14,21–23]. In addition, dihydrolipoic acid (DHLA), a reduced form of ALA, has been shown to protect pancreatic islets from damage by macrophages [24]. Our findings, that ALA prevents diabetes mellitus in OLETF rats by improving insulin action on skeletal muscle and exerting beneficial effects on pancreatic β-cells, are thus in good agreement with these previous studies.

The mechanism by which ALA prevents diabetes mellitus in OLETF rats may be multi-factorial. The ALA-associated prevention of diabetes could be due to antioxidant actions of this drug. Recent studies have suggested that oxidative stress plays a major role in the genesis of insulin resistance [25,26] and β-cell damage [4,5]. Treatment of diabetic C57BL/KsJ-db/db mice with the antioxidant *N*-acetyl-L-cysteine was shown to improve glycemic control and preserve pancreatic β-cell function [27]. ALA and DHLA have been shown to scavenge hydroxyl radicals, peroxynitrite, and singlet oxygen, and to regenerate thioredoxin, vitamin C, and glutathione [7,8]. Our finding that administration of ALA markedly reduced plasma levels of oxidative stress markers is thus in good agreement with this concept.

Chronic ALA treatment significantly reduced body weight gain and visceral fat mass in OLETF rats [15]. The decrease in adipose tissue observed during ALA treatment was associated with significant decreases in plasma FFA and triglyceride concentrations and tissue triglyceride contents. Decreased plasma FFA and triglyceride concentrations may attenuate lipid accumulation in skeletal muscle and pancreatic β-cells by decreasing fatty acid flux into these tissues. Increasing evidence suggests that lipid accumulation in skeletal muscle and pancreatic islets is causally related to the development of type 2 diabetes mellitus. Excess storage of triglycerides and LCAC in skeletal muscle increases the formation of oxygen free radicals in the cell [4,5], and has been linked to insulin resistance in humans

and in animal models [2–5]. In addition, animal models of obesity-related diabetes exhibit increased accumulation of triglycerides in the pancreatic islets. Accumulation of LCAC may induce apoptosis of pancreatic β-cells by sequentially activating the ceramide and inducible nitric oxide synthase pathways [4]. Recently, elevation of plasma FFA by lipid-heparin infusion for 4 days was shown to increase β-cell mass in rats [28]. Taken together, these findings suggest that elevated plasma FFA levels and triglyceride accumulation in pancreatic islets may be responsible for the entire course of β-cell changes, from early hypertrophy to late atrophy, in OLETF rats, and that ALA may prevent β-cell changes by reducing body adiposity and/or plasma FFA.

In conclusion, we have shown that treatment of prediabetic OLETF rats with ALA prevents the development of diabetes mellitus in these animals. ALA reduced body weight and plasma concentrations of FFA and triglyceride. As a consequence, ALA reduced triglyceride accumulation in skeletal muscle and pancreatic islets, leading to improvements in skeletal muscle insulin sensitivity and the prevention of β-cell destruction.

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