*Original Article*

# Antidiabetic effect of the α-lipoic acid γ-cyclodextrin complex

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**In recent years, the number of patients suffering from diabetes mellitus has been increasing worldwide. In particular, type 2 diabetes mellitus, a lifestyle-related disease, is recognized as a serious disease with various complications. Many types of pharmaceutics or specific health foods have been used for the management of diabetes mellitus. At the same time, the relationship between diabetes mellitus and α-lipoic acid has been recognized for many years. In this study, we found that the α-lipoic acid γ-cyclodextrin complex exhibited an $HbA_{1c}$ lowering effect for treating type 2 diabetes mellitus in animal models. Moreover, in this study, we investigated the activation of phosphorylation of AMP-activated protein kinase, which plays a role in cellular energy homeostasis, in the liver of $KKA^{y}$ mice by using α-lipoic acid and the α-lipoic acid γ-cyclodextrin complex. Our results show that the α-lipoic acid γ-cyclodextrin complex strongly induced the phosphorylation of AMP-activated protein kinase. Thus, we concluded that intake of the α-lipoic acid γ-cyclodextrin complex exerted an antidiabetic effect by suppressing the elevation of postprandial hyperglycemia as well as doing exercise.**

*Key Words*: **α-lipoic acid, γCD complex, antidiabetic effect, type 2 diabetes mellitus**

Diabetes mellitus (DM) is a disease associated with absolute or relative insulin deficiency, affecting approximately 366 million people in 2011. Therefore, approaches based on novel concepts are needed. We focused on alpha-lipoic acid (αLA), which is known as a functional food ingredient in Japan. αLA functions as a cofactor for mitochondrial enzymes such as pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and the branched-chain alpha-keto acid dehydrogenase complex. αLA plays an essential role in glucose and energy metabolism.[1,2] αLA has a chiral center at its $C_6$ carbon, leading to two enantiomers, R- and S-αLA, of which R-αLA is the naturally occurring form.[3] Commercially available αLA is a racemate of R- and S-αLA. αLA is a powerful antioxidant with potent free radical scavenging activity.[4] Treatment with αLA has been shown to protect cultured endothelial cells against oxidative stress induced by high glucose[5] and to preserve cellular antioxidative defense mechanisms.[6] Furthermore, in diabetic animal models, αLA has been demonstrated to exhibit beneficial effects on vascular and endothelial function.[7,8] In addition, αLA is used as a treatment for age-associated diseases such as DM and neurodegenerative diseases.[9,10] Wang *et al.*[11] reported that administration of αLA supplementation improved body composition, glucose tolerance, and energy expenditure in the aged mice. αLA increased skeletal muscle mitochondrial biogenesis with increased phosphorylation of AMP-activated protein kinase (AMPK) and messenger RNA expression of PGC-1α and GLUT4.[11] Therefore they suggested that αLA may be a promising supplement for treatment of obesity and/or insulin resistance in older patients. Although αLA is widely used as anti-diabetic and anti-aging compound in supplemental foods, it is unstable when exposed to low pH, light, or heat.[12] Takahashi *et al.*[13] have shown that it is possible to stabilize racemic αLA through complex formation with γ-cyclodextrin (γCD), and we have also recently reported that γCD can stabilize R-αLA to yield the R-αLA/γCD complex.[14] γCD is a cyclic oligosaccharide that consists of eight α-1,4-linked glycopyranose units, and is capable of forming complexes with a variety of ionic and lipophilic substances by taking the entire molecule or part of them into its cavity. γCD is enzymatically broken down into monosaccharides and therefore functions as an energy source. αCD and βCD are also well-known cyclic oligosaccharides which consist of six and seven α-1,4-linked glycopyranose units, respectively. Among them, γCD has the highest association constant with sodium taurocholate (Na TCA), which is an important emulsifier in the bile of mammals, and can form a water-soluble Na TCA/γCD complex and enhance the bioavailability of lipophilic ingredients.[15] In this study, we focused on γCD and used the αLA/γCD complex for *in vivo* experiments.

Although the physicochemical properties of the αLA/γCD complex were evaluated, the biological activity of αLA/γCD has not yet been investigated, and it remains unknown whether αLA/γCD regulates gene expression for glucose metabolism in *in vivo*. Therefore, in this study, we compared the effect of αLA/γCD with intact αLA in terms of DM-related biochemical parameters. Regarding the increased energy expenditure, the intracellular target is considered to be AMPK, the master regulator of cellular energy homeostasis. Moreover, we confirmed two factors related to glucose metabolism: (1) gene expression of the PPARγ2 mRNA in adipose tissue and (2) protein level of GLUT4 in skeletal muscle. We focused on commercially available racemic αLA complexed with γCD.

## Materials and Methods

**Reagents.** DL-Alpha lipoic acid was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). CAVAMAX® W8 FOOD (γCD) was purchased from Wacker Chemie AG (Munich,

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**Table 1.**

(a) Compositions of the experimental diet (Diets were adjusted for an effective αLA content of 0.5% for day 1 to day 5)

| Ingredient | Composition of 1,000 g of each experimental diet | | | |
|---|---|---|---|---|
| | HFD | HFD + γCD | HFD + αLA | HFD + αLA/γCD |
| Casein | 200 | 200 | 200 | 200 |
| Sucrose | 330 | 330 | 330 | 330 |
| Lard | 200 | 200 | 200 | 200 |
| Vitamin mix. AIN 93N | 10 | 10 | 10 | 10 |
| Mineral mix. AIN 93N | 35 | 35 | 35 | 35 |
| Cellulose | 50 | 50 | 50 | 50 |
| L-cystine | 1.8 | 1.8 | 1.8 | 1.8 |
| Choline bitartrtate | 2.5 | 2.5 | 2.5 | 2.5 |
| t-butilhydroquinone | 0.008 | 0.008 | 0.008 | 0.008 |
| Cornstarch | 170.7 | 125.7 | 165.7 | 120.7 |
| γCD | — | 45 | — | — |
| αLA | — | — | 5 | — |
| αLA/γCD | — | — | — | 50 |
| Total | 1,000 | 1,000 | 1,000 | 1,000 |

(b) Compositions of the experimental diet (Diets were adjusted for an effective αLA content of 0.25% for day 6 to day 31)

| Ingredient | Composition of 1,000 g of each experimental diet | | | |
|---|---|---|---|---|
| | HFD | HFD + γCD | HFD + αLA | HFD + αLA/γCD |
| Casein | 200 | 200 | 200 | 200 |
| Sucrose | 330 | 330 | 330 | 330 |
| Lard | 200 | 200 | 200 | 200 |
| Vitamin mix. AIN 93N | 10 | 10 | 10 | 10 |
| Mineral mix. AIN 93N | 35 | 35 | 35 | 35 |
| Cellulose | 50 | 50 | 50 | 50 |
| L-cystine | 1.8 | 1.8 | 1.8 | 1.8 |
| Choline bitartrtate | 2.5 | 2.5 | 2.5 | 2.5 |
| t-butilhydroquinone | 0.008 | 0.008 | 0.008 | 0.008 |
| Cornstarch | 170.7 | 148.2 | 168.2 | 145.7 |
| γCD | — | 22.5 | — | — |
| αLA | — | — | 2.5 | |
| αLA/γCD | — | — | — | 25 |
| Total | 1,000 | 1,000 | 1,000 | 1,000 |

Germany). All reagents used were purchased from Wako Pure Chemical Ind., Ltd. (Tokyo, Japan). An RNeasy Lipid Tissue Mini Kit was purchased from QIAGEN Inc. (Germantown, MD). Mini 4–15% Mini-PROTEAN® TGX™ precast gels were purchased from BIO-RAD (Hercules, CA). Specific antibodies against phospho-AMPKα (Thr172; 40H9) and AMPKα (23A3) were purchased from Cell Signaling Technologies (Beverly, MA). The antibody against GAPDH (6c5) was obtained from Santa Cruz (Dallas, TX). Immobilon™ Western Chemiluminescent HRP substrate was from Millipore (Billerica, MA).

**Preparation of the αLA/γCD complex.** We prepared the αLA/γCD complex as previously described.[13] αLA was dissolved in deionized water in which the pH was monitored and a corresponding molar amount of γCD was added for a 1:1 ratio with αLA. The solution was mixed with a mechanical stirrer at 300 rpm for 10 min before adding 1 M HCl for pH adjustment. Then, the suspension was continuously stirred in the dark for 18 h. All procedures were carried out at room temperature and the suspension temperature did not exceed 25°C. The freshly prepared suspension was frozen overnight and freeze-dried on the next day.

**Component of diets with α-lipoic acids.** All animals had free access to water and semi-synthetic HFDs that were high in sugar and, therefore, hypercaloric (composition of the basal diet [%]: sucrose, 33.0; lard fat, 20.0; casein, 20.0; Kobe Women's University special diets, Japan). Diets were prepared for all groups using AIN-93N provided with mixture of the general diet (Oriental East Co., LTD., Tokyo, Japan), as shown in Table 1a and b. Before beginning the study, the stability of αLA contained in the test diets was confirmed by CycloChem Bio Co., LTD. The actual content in the supplemental diet was measured by using HPLC as reported before.[14] The residual αLA in the test diets after 4 weeks was higher than 90%.

**Animals.** Male type 2 diabetic KK$A^y$ mice (4 weeks old and weighing 22–25 g) were purchased from CLEA Japan Inc. (Tokyo, Japan) and used for *in vivo* studies when they were 8 weeks old. The animal studies were approved by the Experimental Animal Research Committee, Kyoto Pharmaceutical University (KPU), and were performed according to the Guidelines for Animal Experimentation at KPU.

**Administration of two types of αLAs in KK$A^y$ mice.** Type 2 diabetic KK$A^y$ mice with hyperinsulinemia, in which the $A^y$ allele at the agouti locus (initially from C57black/6J) was transferred to the inbred KK strain by repetitive back-crossing, were used as the congenic strain.[16] The introduction of the $A^y$ allele causes DM and massive hereditary obesity. The KK$A^y$ mice were allowed free access to a solid high-fat diet (HFD) with γCD, racemic αLA, or racemic αLA complexed by γCD (racemic αLA/γCD), and tap water. They were housed in an air-conditioned room with controlled temperature (23 ± 2°C) and humidity (60 ± 10%), with lights on from 8:00 to 20:00. The blood glucose levels and body mass of the KK$A^y$ mice were measured on every Monday, Wednesday, and Friday. The blood samples for glucose


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**Table 2.** Sequences of PCR Primers for quantification of mRNA

| Gene Name | Accession No. | Forward primer (5'-3') | Reverse primer (5'-3') |
|---|---|---|---|
| PPARγ2 | NM_011146.3 | GGTGAAACTCTGGGAGATTC | CAACCATTGGGTCAGCTCTTG |
| GAPDH | NM_008084.2 | AAATGGTGAAGGTCGGTGTGAAC | CAACAATCTCCACTTTGCCACTG |

PPARγ2, peroxisome proliferator-activated receptor γ2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**Table 3.** Changes of food intakes and body weights of untreated KKA$^y$ mice, and KKA$^y$ mice treated with γCD, αLA, and αLA/γCD at day 5 and day 29

| | Food intake (g) | | Body weight (g) | |
|---|---|---|---|---|
| | Day 5 | Day 29 | Day 5 | Day 29 |
| Untreated KKA$^y$ mice | 7.6 ± 0.5 | 6.2 ± 0.7 | 38.3 ± 3.4 | 46.7 ± 4.3 |
| KKA$^y$ mice treated with γCD | 7.9 ± 1.0 | 6.4 ± 0.8 | 40.7 ± 2.3 | 48.9 ± 3.7 |
| KKA$^y$ mice treated with αLA | 5.4 ± 0.3 | 6.1 ± 0.7 | 37.9 ± 2.0 | 45.4 ± 1.7 |
| KKA$^y$ mice treated with αLA/γCD | 4.7 ± 0.7 | 5.9 ± 0.6 | 36.8 ± 2.8 | 43.6 ± 2.6 |

level analysis were obtained from the tail vein of each mouse and measured using a Glucocard (Arkray, Kyoto, Japan). The intake of solid diet and drinking water in each mouse were monitored every Monday, Wednesday, and Friday, throughout the course of the experiments. After treatment, the levels of hemoglobin $A_{1c}$ ($HbA_{1c}$) in the blood, which had been obtained from the mouse tail vein, were measured using an immunoassay method with the DCA 2000 (Bayer-Sankyo Co., Ltd., Tokyo, Japan). The mice were then subjected to a 12-h fast, and blood samples, livers, adipose tissues, and skeletal muscle were collected. The blood samples were centrifuged at 650 × *g* for 10 min at 4°C, and the resultant plasma samples were used for the analysis of biochemical parameters. The blood urea nitrogen (BUN), triglyceride (TG), and total cholesterol (TCHO) levels were measured using a Fuji Dry-Chem system (Fuji Medical Co., Tokyo, Japan). The plasma levels of adiponectin were determined using an adiponectin immunoassay kit (R&D Systems Inc., Minneapolis, MN).

**mRNA expression analysis.** For mouse adipose tissue samples, total RNA was extracted using QIAzol (QIAGEN) and a RNeasy Lipid Tissue Mini Kit. The concentrations of RNA in the obtained samples were measured by NanoDrop and purified using amplification grade DNase I (Invitrogen, Carlsbad, CA). Reverse transcription (RT) was performed with 0.5 μg of total RNA by using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, IN). Real-time PCR was performed using the ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Tokyo, Japan) and SYBR-Green with the appropriate primers. The expression of target genes was detected by preparing primers as listed in Table 2. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as internal control. Relative mRNA expression was determined using the $2^{-\Delta\Delta CT}$ method.

**Immunoblotting analysis.** Isolated liver (150 mg) and muscle tissues (200 mg) from mice were homogenized with 1.5 mL buffer I (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $Na_3VO_4$, 5 mM NaF, 1 mM PMSF, and 5 μg/ml Leupeptin). The homogenate was centrifuged at 14,500 × *g* for 20 min at 4°C, and protein concentration was measured using the BCA reagent (Thermo Scientific, Waltham, MA) with BSA as the standard. The lysates (10 μg of total protein) were separated using a 4–15% Mini-PROTEAN® TGX™ precast gel. The resolved proteins were transferred onto a PVDF membrane. The membrane was blocked with 5% BSA in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBS/T), and incubated overnight at 4°C with primary antibodies in 5% BSA-TBS/T. Immunocomplexes on the membrane were incubated with an HRP-conjugated secondary antibody, visualized using an Immobilon™ Western Chemiluminescent HRP substrate, and exposed to Amersham Hyperfilm™ ECL (GE Healthcare UK Ltd., Buckinghamshire, UK). Specific immunoreaction products were quantitated using the NIH ImageJ software.

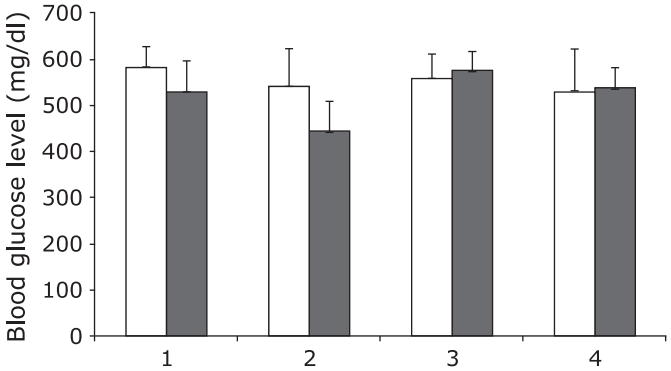


**Fig. 1.** Changes of blood glucose levels before (open column) and after (closed column) several treatments in untreated KKA$^y$ mice, KKA$^y$ mice treated with γCD, αLA, and αLA/γCD for 31 days ($n$ = 5–7). Data are expressed as means ± SD. Group 1: untreated, group 2: γCD, group 3: αLA, and group 4: αLA/γCD.

**Statistical analysis.** Data are expressed as the mean values ± SD. Statistical differences among the groups were detected by a Tukey's all pairwise comparison test.

## Results

**Anti-diabetic effects of α-lipoic acids *in vivo*.** The mice received the HFD, or HFD with γ-CD, αLA, or αLA/γCD for 31 days. Food intake decreased 35% by using 0.5% αLAs for the first 5 days. It may be bad taste for the mice because 0.5% of αLAs was too many. Thus we changed the ratio of αLAs. When we changed the diets from 0.5% αLAs to 0.25% αLAs on day 5, food intake was equal across all groups (Table 3). The body weights and glucose levels did not change in any group (Table 3 and Fig. 1). Serum BUN was identical in each treatment group, indicating that there was no kidney damage due to γCD or αLA treatment (Table 4). Furthermore, serum TG and TCHO levels did not significantly change among all groups (Table 4). In the untreated KKA$^y$ mice, the level of $HbA_{1c}$, which indicates the average blood glucose levels over a long period, was 10.7 ± 0.8%, whereas the level in KKA$^y$ mice treated with αLA/γCD was significantly lowered to 8.3 ± 1.3% (Fig. 2). Plasma adiponectin, a known indicator of insulin resistance, was measured after administration for 31 days. No significant difference was observed in the plasma adiponectin levels. However, treatment with αLAs, especially αLA/γCD, showed a tendency to improve hypoadiponectinemia (Fig. 3A).


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**Table 4.** Serum parameters of untreated KKA$^{y}$ mice, and KKA$^{y}$ mice treated with γCD, αLA, and αLA/γCD

| | BUN (mg/dl) | TCHO (mg/dl) | TG (mg/dl) |
|---|---|---|---|
| Untreated KKA$^{y}$ mice | 24.3 ± 3.0 | 200 ± 12 | 692 ± 320 |
| KKA$^{y}$ mice treated with γCD | 24.2 ± 3.8 | 212 ± 26 | 533 ± 186 |
| KKA$^{y}$ mice treated with αLA | 24.1 ± 2.9 | 208 ± 21 | 653 ± 199 |
| KKA$^{y}$ mice treated with αLA/γCD | 20.5 ± 2.8 | 196 ± 53 | 612 ± 195 |

**PPARγ2 mRNA expression in adipose tissue.** We investigated the expression level of PPARγ2 mRNA in adipose tissue. PPARγ is one of the key transcription factors that regulate adipogenesis and glucose and lipid metabolism.[17] PPARγ plays a significant role in the transcriptional activation of adiponectin via a functional PPAR-responsive element (PPRE) in its promoter.[18] Specifically, PPARγ2 is highly expressed in white adipose tissue.[19] The expression levels of PPARγ2 mRNA in most groups were the same as the control group. However, the αLA/γCD group showed a tendency of increased expression levels of PPARγ2 mRNA without a statistical significant difference (Fig. 3B).

**Effect of racemic αLAs on phosphorylation of AMP-activated protein kinase (AMPK) and the level of GLUT4 protein in liver or skeletal muscle.** After 31 days of treatment with αLAs, the livers were isolated and used for biochemical experiments. We analyzed the phosphorylation levels of the major signal transduction protein in livers and muscles. It is well known that AMPK is an enzyme that plays a role in cellular energy homeostasis. Results from the analysis of the phosphorylation of AMPK in the liver are shown in Fig. 4. The phosphorylation levels of AMPK in the liver increased in the αLAs groups, especially with significance in the αLA/γCD group. The oral feeding of αLA/γCD enhanced the phosphorylation level of AMPK, indicating that αLA activated AMPK.

The expression levels of GLUT4 protein were examined in the skeletal muscle of the KKA$^{y}$ mice. The results indicate no changes in the expression levels of GLUT4 among the tested groups (Fig. 5). Moreover, the expression levels of GLUT4 in both the cytosolic and total membrane fractions exhibited no differences among the groups. The results in this study indicated that αLAs did not stimulate GLUT4 translocation to the membrane.

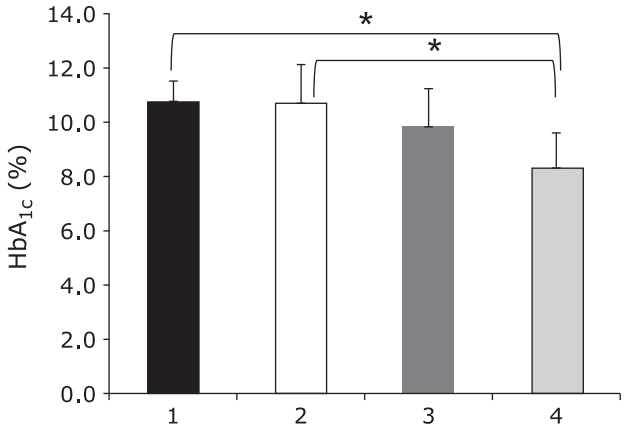


**Fig. 2.** Concentration of $HbA_{1c}$ levels of untreated KKA$^{y}$ mice, KKA$^{y}$ mice treated with γCD, αLA, and αLA/γCD ($n$ = 5–7). Data are expressed as means ± SD. Group 1: untreated, group 2: γCD, group 3: αLA, and group 4: αLA/γCD. Significance: *$p$<0.05.

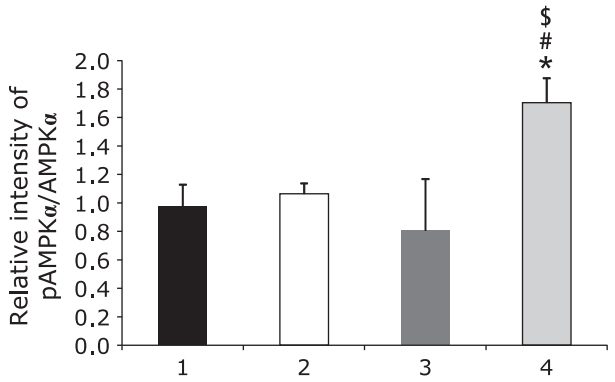


**Fig. 4.** Effect of αLAs on the phosphorylation of AMPKα in liver (n = 5–6). Ten μg of the total proteins was resolved on a 4–15% Mini-PROTEAN® TGX™ precast gel, transferred to a PVDF membrane, and immunoblotted with anti-phospho-AMPKα (Thr172) (40H9), anti-AMPKα (23A3), and anti-GAPDH (6c5) (data not shown). The intensity of immunoblots, which indicates the phosphorylation state, was measured using NIH ImageJ software. Data are expressed as means ± SD. Group 1: untreated, group 2: γCD, group 3: αLA, and group 4: αLA/γCD. Significance: *$p$<0.01 vs untreated KKA$^{y}$ mice, $^{\#}p$<0.01 vs γCD, $^{\$}p$<0.01 vs αLA.

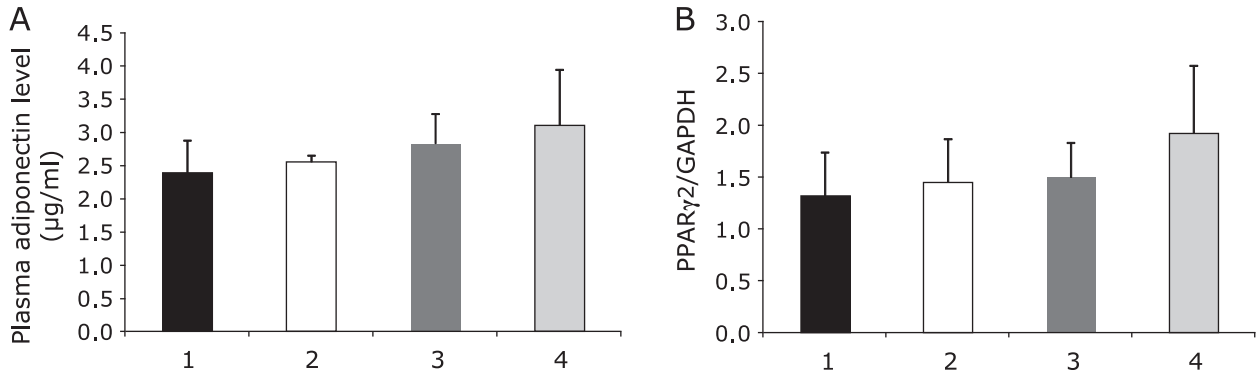


**Fig. 3.** Concentration of adiponectin in plasma (A) and expression levels of PPARγ2 mRNA in adipose tissue (B) ($n$ = 5–7). Data are expressed as means ± SD. Group 1: untreated, group 2: γCD, group 3: αLA, and group 4: αLA/γCD.


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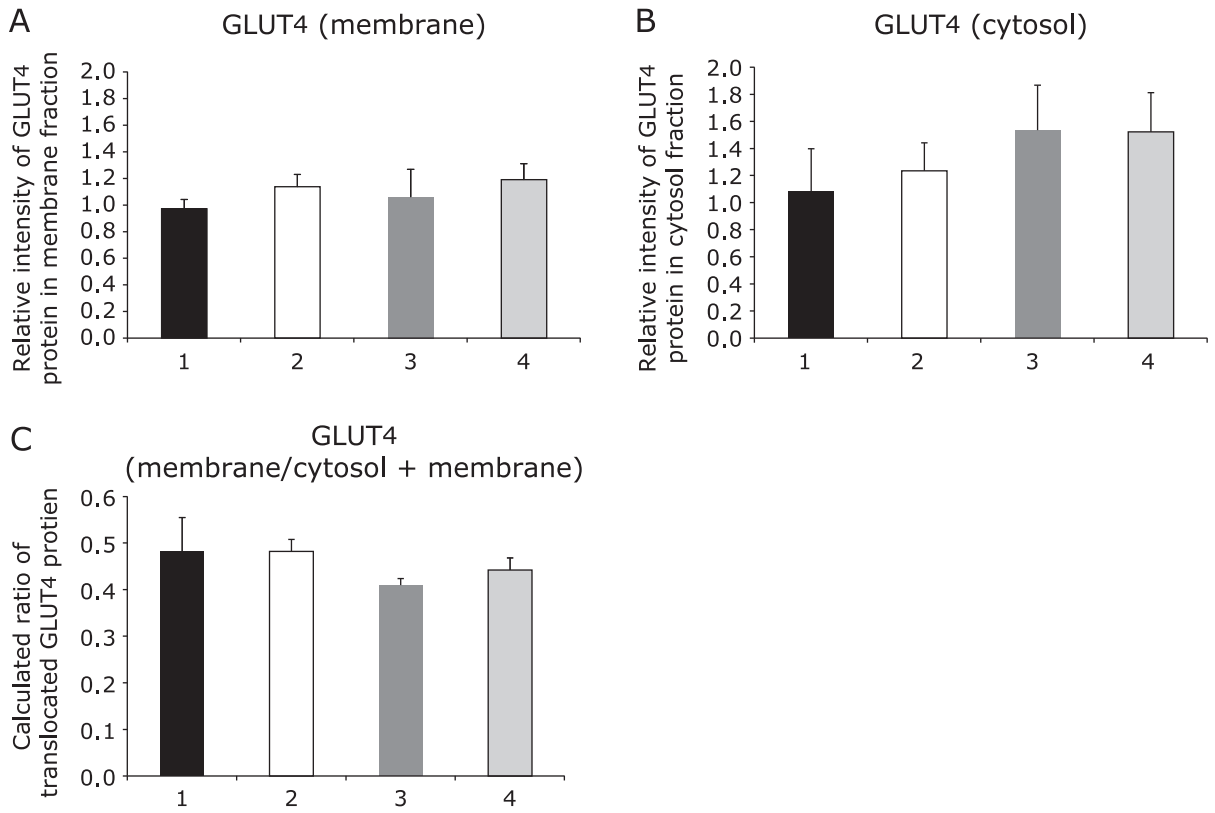


**Fig. 5.** Effect of αLA on the translocation level of GLUT4 protein in skeletal muscle ($n$ = 4–5). Each 10 μg of the total protein in both hind limb muscle cell membrane and cytoplasm crude were resolved on 4–15% SDS-PAGE gradient gel, transferred to a PVDF membrane, and immunoblotted with the anti-GLUT4 antibody. The intensity of immunoblots, which indicates the expression level, was measured using NIH ImageJ software. Data are expressed as means ± SD. Group 1: untreated, group 2: γCD, group 3: αLA, and group 4: αLA/γCD.

## Discussion

In this study, we examined the effect of racemic αLAs treatment on DM, and in particular, investigated the difference between αLA and αLA/γCD in terms of an antidiabetic effect. Previous studies reported the utility of racemic αLA in terms of the ameliorating insulin resistance of muscle glucose metabolism in animal models of obesity, hyperinsulinemia, and dyslipidemia.[20] From this report, it is clear that αLA could treat DM, and thus we aimed to research the antidiabetic efficacy mechanism of αLA in chronic administration experiments. However, αLA was not stable with respect to acid, ultraviolet light, or oxygen. Thus, we prepared the more stable αLA/γCD.[12–14] First, we evaluated the different antidiabetic effects between αLA and αLA/γCD in *in vivo* experiments using KKA$^y$ mice, a type 2 DM model animal. The effect of αLA/γCD was followed by analysis of $HbA_{1c}$ levels, plasma adiponectin levels, mRNA expression of adiponectin in adipose tissue, and the phosphorylation levels of AMPK in the liver.

$HbA_{1c}$ levels in the αLA/γCD treatment group decreased considerably compared to those in the untreated KKA$^y$ mice group. Moreover, $HbA_{1c}$ levels of αLA/γCD treatment group tend to decrease compared with αLA treatment group ($p$<0.33). These results indicate that αLA/γCD has an effective hypoglycemic action. The plasma adiponectin levels with αLAs intake, especially with αLA/γCD, tended to rise, but exhibited no significant difference. Adiponectin, one of the adipocytokines, is known to decrease in the plasma and mRNA expression in both the obese humans and insulin resistant type 2 DM patients with high adipose mass.[21–23] It is also known that PPARγ plays significant roles in the transcriptional activation of the adiponectin gene via the PPRE in the promoter of adiponectin.[18] For this reason, we investigated whether αLAs would induce the expression level of PPARγ2 mRNA in adipose tissue.

In the real-time RT-PCR analysis, αLAs was found to induce PPARγ2 mRNA expression. The mRNA expression level with αLA/γCD was higher than that with αLA without γCD. This result suggests that αLA is more stable when it forms complex with γCD, compared to αLA without γCD.

It is reported that the one of the action mechanisms of adiponectin is the suppression of gluconeogenesis in the liver and the enhancement of fatty acid metabolism in the skeletal muscle.[24,25] It is also known that adiponectin activates AMPK in the liver.[26] It is well known that activated AMPK enhance glycolysis, proteolysis, and fatty acid oxidation, and inhibit the synthesis of sugar, fatty acid, and protein in reverse. This means the inducing AMPK phosphorylation could obtain the same effects as doing exercise, because doing exercise induces the AMPK phosphorylation.[27,28] It is reported that αLA was increased the phosphorylation of AMPK in the muscle. However, only a few report was reported the investigation of phosphorylation of AMPK in the liver. We expected that αLAs may induce the phosphorylation of AMPK in the liver; therefore, we investigated its phosphorylation level in this organ. Treatment with αLA/γCD significantly elevated the phosphorylation level of AMPKα. The reason comes from the fact that the αLA/γCD intake tended to rise the plasma adiponectin levels and PPARγ2 mRNA in adipose tissue in this study. Previous reports revealed phosphorylation and activation of AMPK are stimulated with full-length adiponectin in liver and stimulation of glucose utilization and fatty acid oxidation by adiponectin occurs through activation of AMPK.[26] Targeted disruption of AdipoR1, which is one of


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the adiponectin receptor, resulted in the abrogation of adiponectin-induced AMPK activation.[29] On the other hand, it is reported that αLA has the phosphorylation increased effect of AMPK in the HepG2 cell.[30] αLA has a potential impact on phosphorylation increased effect of AMPK without adiponectin.

From the results in this study, it was shown that the antidiabetic effects of αLA/γCD were stronger than αLA without γCD. This difference in antidiabetic effects results from γCD complexation with αLA. αLA complexed with γCD could exist more stably in the living body than αLA without γCD. A Japanese report showed that the bioavailability of αLA/γCD complex was ca. 10% higher than αLA in both of the fasted and fed healthy volunteers.[31] And our group recently presented in the 27th Pharmaceutical Science and Technology Symposium that the absorption of R-αLA/γCD was very quick and AUC of R-αLA/γCD was much higher than that of R-αLA itself. We suggest that the complexation with γCD provides an advantage for αLA to exert an antidiabetic effect. In particular, by inducing AMPK phosphorylation in the liver, we assumed that αLA/γCD intake will likely exert antidiabetic effects by suppressing the elevation of postprandial hyperglycemia as well as by exercise and reduction in the fasting glucose level.

In the future, we will take particular note of the anti-diabetic effects of αLA on the liver, especially by αLA/γCD, and will investigate the mechanism of activation of AMPK.

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## Conflicts of Interest

No potential conflicts of interest were disclosed.

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