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Effect of alkyl glycerophosphate on the activation of peroxisome proliferator-activated receptor gamma and glucose uptake in C2C12 cells

Tamotsu Tsukahara [a,*], Hisao Haniu [b], Yoshikazu Matsuda [c]

[a] Department of Integrative Physiology & Bio-System Control, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[b] Department of Orthopedic Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[c] Clinical Pharmacology Educational Center, Nihon Pharmaceutical University, Ina-machi, Saitama 362-0806, Japan

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ABSTRACT

Studies on the effects of lipids on skeletal muscle cells rarely examine the effects of lysophospholipids. Through our recent studies, we identified select forms of phospholipids, such as alkyl-LPA, as ligands for the intracellular receptor peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a nuclear hormone receptor implicated in many human diseases, including diabetes and obesity. We previously showed that alkyl-LPA is a specific agonist of PPARγ. However, the mechanism by which the alkyl-LPA–PPARγ axis affects skeletal muscle cells is poorly defined. Our objective in the present study was to determine whether alkyl-LPA and PPARγ activation promotes glucose uptake in skeletal muscle cells. Our findings indicate that PPARγ1 mRNA is more abundant than PPARγ2 mRNA in C2C12 cells. We showed that alkyl-LPA (3 μM) significantly activated PPARγ and increased intracellular glucose levels in skeletal muscle cells. We also showed that incubation of C2C12 cells with alkyl-LPA led to lipid accumulation in the cells. These findings suggest that alkyl-LPA activates PPARγ and stimulates glucose uptake in the absence of insulin in C2C12 cells. This may contribute to the plasma glucose-lowering effect in the treatment of insulin resistance.

1. Introduction

Excess fat is stored as triglycerides in adipose tissue, and fatty acids are released into the circulation in response to increased energy requirements. Fatty acids are transported in the blood bound to albumin or as a part of triacylglycerols in lipoprotein complexes [1]. If the lipid storage capacity of adipocytes is exceeded, adipocytes can no longer regulate the level of free fatty acids in the circulation, which results in an inappropriate deposition of lipids in tissues, the including skeletal muscle [2]. Excess fatty acids are metabolized to various molecules that may participate in or interfere with cellular signaling. Recently, the lipid-regulated transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) was reported to affect insulin sensitivity in muscle cells, which indicates the possibility of crosstalk between PPARγ and insulin in muscle cells [3]. Furthermore, PPARγ agonists have emerged as important pharmacological targets in the treatment of diabetes and insulin resistance [4]. Insulin resistance in skeletal muscles is a major factor in the development of type 2 diabetes. It is well established that insulin stimulation of glucose uptake in skeletal muscle cells is mediated through the translocation of glucose transporter type 4 (GLUT4) from intracellular vesicles to the cell surface [5]. GLUT4 expression in the C2C12 cell line is low, however [6,7]. Thus, this is an optimal cell line in which to examine the relationship between alkyl-LPA–PPARγ activation and glucose metabolism in the absence of insulin signaling. In addition, the results of our recent studies have shown that extracellular stimulation with select forms of lysophosphatidic acid (LPA) activates PPARγ [8,9]. The LPA analog alkyl-LPA is a high-affinity agonist of PPARγ. Binding studies using the PPARγ ligand-binding domain showed that the binding affinity of alkyl-LPA to PPARγ (60 nM) was similar to that of a synthetic agonist, rosiglitazone [8]. Alkyl-LPA is formed from alkyl dihydroxyacetone phosphate through an enzymatic reaction [10]. Alkyl-LPA has been detected in several biological fluids and tissues, including the human brain, ascitic fluid, and saliva [11]. The steady-state concentration of alkyl-LPA in the human brain is 0.44 nmol/g [12], which is sufficient to activate PPARγ. The exact mechanism and function of alkyl-LPA remain to be clarified, but PPARγ activation is thought to increase fatty acid flux to adipose tissue, thereby decreasing the levels of circulating free fatty acids and consequently reducing the availability of fatty acids to muscles. Thiazolidinediones (TZDs), including rosiglitazone and pioglitazone, are used clinically as insulin sensitizers to increase insulin-stimulated glucose uptake into skeletal muscles [13]. However, whether PPARγ expression plays any role in maintaining glucose disposal is unknown. Furthermore, synthetic agonists elicit a variety of side effects, including weight

* Corresponding author. Fax: +81 263 37 2599.
E-mail address: ttamotsu@shinshu-u.ac.jp (T. Tsukahara).

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gain, edema, increased fat mass, and tumor formation in rodents [14]. Thus, alkyl-LPA is a novel therapeutic agent that can be used for the treatment of obesity and diabetes in order to reduce the unwanted side effects associated with TZD therapy.

2. Materials and methods

2.1. Reagents

Alkyl glycerophosphate (18:1) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Anti-PPARγ rabbit polyclonal antibody (sc-7196) and anti-β-actin mouse monoclonal antibody (sc-47,778) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Rosiglitazone was purchased from Alexis Biochemicals, Inc., (Farmingdale, NY, USA).

2.2. Cell culture

Mouse myoblast C2C12 cells were purchased from the European Collection of Cell Cultures (ECACC). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) (Sigma–Aldrich), penicillin (100 U/mL), and streptomycin (100 μg/mL). The cells were grown and maintained on 100 mm culture plates (Iwaki, Tokyo, Japan) at 37 °C in a 5% CO_2 incubator. To induce myoblast differentiation, C2C12 cells were grown in DMEM supplemented with 2% horse serum (Nichirei Bio-science Inc., Tokyo, Japan). For each assay, differentiated C2C12 cells were incubated with the indicated dosage of compounds.

2.3. Western blot analysis

C2C12 cells were seeded onto 6-well plates (Iwaki, Tokyo, Japan) at a density of 1×10^5 cells/well. After the indicated treatments, cells were lysed on ice for 30 min in cell lysis buffer (20 mM Tris–HCl [pH 7.4], 10% [v/v] glycerol, 100 mM NaCl, 1% [v/v] Triton ×-100, 1/100 protease inhibitor cocktail [Sigma], and 1 mM dithiothreitol) and centrifuged at 16,000*g* for 20 min at 4 °C. The supernatants were collected as cell lysates and assayed for protein content with the Bradford method by using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA). Then, the cell lysates were separated on 5%–20% sodium dodecyl sulfate (SDS)–polyacrylamide gels (e-PAGEL; ATTO, Tokyo, Japan) and electrotransferred to Immobilon-P membranes (Millipore, Billerica, MA, USA). The membranes were blocked by Block Ace (DS Parma Biomedical Co. Ltd., Osaka, Japan) for 1 h and incubated with a primary antibody in Tris-buffered saline-Tween (TBS-T) with 5% Block Ace for 12 h at 4 °C. Bands were visualized with EzWestLumi plus (ATTO).

2.4. Reporter gene assay

To determine endogenous PPARγ activation, C2C12 cells were cotransfected with pGL3b-PPAR-responsive element (PPRE) (ACO)-Fluc and pSV40-β-galactosidase plasmids and subjected to the reporter gene assay, as described earlier. Twenty-four hours after transfection, the cells were treated with the specific compounds and cultured for an additional 20 h. Luciferase activity was measured with the Steady-Glo luciferase assay system (Promega, WI, USA) using the SpectraMax plate reader (Molecular Devices, CA, USA).

2.5. Determination of intracellular glucose levels

Before harvesting, alkyl-LPA-treated cells in DMEM containing 1 mg/mL glucose were washed twice with cold phosphate-buffered saline (PBS) and lysed with ion-free H_2O for 5 min on ice. To prevent the destruction of glucose during cell extraction, cells were handled on ice at all times. Intracellular concentrations of glucose were determined using a glucose assay kit (BioVision Inc., Milpitas, CA, USA) according to the manufacturer's instructions. Briefly, this is a coupled enzyme assay that relies on the oxidation of glucose to generate a product that reacts with a dye to generate color (570 nm) and fluorescence (*E*x/*E*m = 535/587 nm). In our study, glucose concentrations were determined by monitoring the absorbance at 570 nm and comparing it to that of a standard curve of known concentrations.

2.6. Quantitative real-time polymerase chain reaction analysis

Total RNA was prepared from C2C12 cells using NucleoSpin RNA II (Takara, Shiga, Japan). Total RNA (0.5 μg) was used for the subsequent synthesis of cDNA with the ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan), as recommended by the manufacturer. The mRNA levels were measured using an ECO real-time PCR system (Illumina Inc., San Diego, CA, USA) and a SYBR Green real-time PCR Master Mix-Plus (Toyobo). The following primer pairs were used in the analysis: mPPARγ1, 5′-TCT CTC CGT AAT GGA AGA CC-3′ (Forward [F]) and 5′-GCA TTA TGA GAC ATC CCC AC-3′ (Reverse [R]); mPPARγ2, 5′-GCT GTT ATG GGT GAA ACT CTG-3′ (F) and 5′-ATA AGG TGG AGA TGC AGG TTC-3′ (R); mPPARα, 5′-GAT ACC ACT ATG GAG TCC ACG CA-3′ (F) and 5′-GCC GAA AGA AGC CCT TGC-3′ (R); mPPAR δ/β, 5′-AGA TGG TGG CAG AGC TAT GAC C-3′ (F) and 5′-TCT CCT CCT GTG GCT GTT CC-3′ (R); mGLUT4, 5′-GTG ACT GGA ACA CTG GTC CTA-3′ (F) and 5′-CCA GCC ACG TTG CAT TGT AG-3′ (R); mMyoD, 5′-GAC CTG CGC TTT TTT GAG GAC C-3′ (F) and 5′-CAG GCC CAC AGC AAG CAG CGA C-3′ (R); mFABP4, 5′-CTT CGA TGA TTA CAT GAA AGA AGT G-3′ (F) and 5′-ACG CCC AGT TTG AAG GAA AT-3′ (R) and 18S rRNA, 5′-CAG CCA CCC GAG ATT GAG CA-3′ (F) and 5′-TAG TAG CGA CGG GCG GTG TG-3′ (R). All polymerase chain reactions (PCRs) were performed in a 10 μL volume by using 48-well PCR plates (Illumina). The cycling conditions were 95 °C for 10 min (enzyme activation) followed by 40 cycles at 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s. After amplification, the samples were slowly heated from 55 °C to 95 °C, and fluorescence was continuously monitored to obtain a melting curve. The relative mRNA level was calculated using the arithmetic formula $2^{-\Delta\Delta Cq}$, where ΔCq is the difference between the threshold cycle of a given target cDNA and that of an endogenous reference cDNA. Derivation of the formulas and validation tests has been described in Applied Biosystems User Bulletin No. 2.

2.7. Oil red O staining

The amount of lipid accumulated after 10 days was determined using a Lipid Staining Kit (Zen-Bio Inc., Research Triangle Park, NC, USA). Oil red O staining was performed as previously described [9]. Briefly, cells were incubated in a fixative solution for 1 h, stained with 0.6% oil red O solution in isopropyl alcohol/H_2O (60:40, v/v) for 1 h at room temperature, and washed 4 times with distilled water. Differentiation was examined by visual inspection and quantified by elution with isopropyl alcohol. The optical density was measured at 590 nm (Beckman DU-640).

2.8. Statistical analysis

Student's *t*-test was used for statistical comparisons. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Expression of PPARγ mRNA and protein in C2C12 cells

PPARγ has 2 isoforms: PPARγ1 and PPARγ2. Because of differential promoter usage and alternative splicing [15,16], PPARγ2 has 30 additional amino acids at it N-terminus. To evaluate the function of PPARγ in differentiated C2C12 cells, we first examined the expression of PPARγ1 and PPARγ2 mRNA and protein. As shown by the results of real-time PCR using isoform-specific primers, PPARγ1 mRNA was expressed at high levels in C2C12 cells, whereas the expression of PPARγ2 was very weak (Fig. 1B). Interestingly, compared to the levels of PPARγ2, PPARα, and PPARβ/δ mRNA, the levels of PPARγ1 mRNA increased significantly in alkyl-LPA-treated cells in a time-dependent manner. The expression levels of PPARγ1 and γ2 protein were in agreement with those of the respective mRNA.

3.2. Alkyl-LPA specifically interacts with PPARγ and can activate the PPRE

Next, we determined whether endogenous alkyl-LPA or rosiglitazone (10 μM) activate PPARγ. Administration of these agonists for 48 h to differentiated C2C12 cells transiently transfected with a pGL3b-PPRE (ACO)-Fluc plasmid resulted in a dose-dependent increase in PPARγ activation (Fig. 2A). Agonist-induced PPARγ activation was abolished by the specific PPARγ inhibitor GW9662 (Fig. 2B). The luciferase activity in C2C12 cells treated with alkyl-LPA (10 μM) for 48 h was approximately two-fold that in the vehicle (dimethyl sulfoxide [DMSO])-treated cells. Taken together, these results suggest that alkyl-LPA specifically interacts with PPARγ and can activate the PPRE reporter gene.

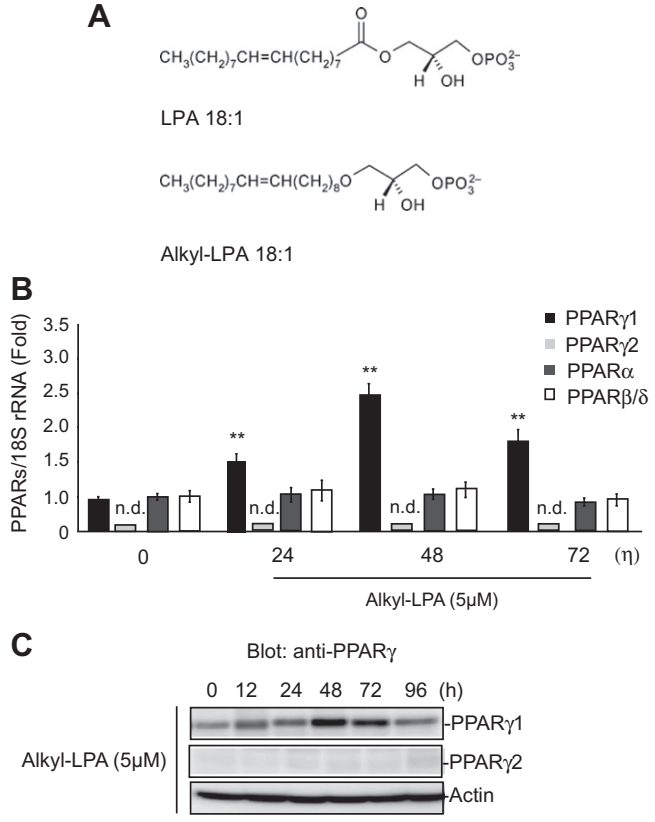

Fig. 1. (A) Structural formulas of lysophosphatidic acid (LPA) and alkyl-LPA. (B) Real-time polymerase chain reaction (PCR) measurement of the expression of mPPARγ1, mPPARγ2, mPPARα, and mPPAR β/δ mRNA in C2C12 cells. C2C12 cells were treated with 5 μM alkyl-LPA and incubated for specific periods. The relative mPPARγ1, mPPARγ2, mPPARα, and mPPAR δ/β mRNA levels, normalized to 18S rRNA, are expressed as mean ± standard error of the mean (SEM, $n = 3$); $^{**}p < 0.01$. (C) Serum-starved C2C12 cells were harvested 12, 24, 48, 72, and 96 h after treatment with alkyl-LPA. Whole-cell lysates were used for Western blotting with anti-peroxisome proliferator-activated receptor γ (PPARγ) antibody. β-Actin was used as an internal loading control and was detected using mouse anti-β-actin antibody.

3.3. Alkyl-LPA treatment induces lipid accumulation in C2C12 cells

We observed that incubation of differentiated C2C12 cells (10 days in culture) with alkyl-LPA (5 μM) led to lipid accumulation in cells, as indicated by the increase in oil red O staining (Fig. 2C). The morphology of the growing C2C12 cells was typical of myotubes, and conversion of C2C12 myoblasts into adipose-like cells [17] was not observed. After 10 days in culture, the cells were filled with lipid, which was positively stained by oil red O. Lipid droplets were located not only in single C2C12 cells, but also in fused myotubes. To establish the role of PPARγ in the alkyl-LPA-induced lipid accumulation, we treated cells with GW9662, a high-affinity, selective PPARγ inhibitor that abrogates PPARγ activation and signaling. GW9662 inhibited intracellular lipid accumulation in C2C12 cells treated with alkyl-LPA (Fig. 2D). As shown in (Fig. 2E), when cells were incubated for 10 days in medium containing alkyl-LPA, the mRNA level of MyoD, a specific myogenic marker, was markedly increased compared with the level in vehicle control. We also investigated the expression of a specific adipocyte differentiation marker (FABP4) after 7 days of exposure to alky-LPA. The FABP4 mRNA level was not significantly changed. These data suggest that the significant increase in lipid accumulation in C2C12 cells treated with alkyl-LPA is dependent on PPARγ activation.

3.4. Alkyl-LPA enhanced glucose uptake in C2C12 cells

We hypothesized that alkyl-LPA might stimulate PPARγ activation in differentiated C2C12 cells by increasing the intracellular glucose levels. To test this, C2C12 cells were stimulated with alkyl-LPA (5 μM) for specific periods in serum-free DMEM, which contained 4.5 g/L of glucose. Alkyl-LPA increased the intracellular glucose level in a time- and dose-dependent manner (Fig. 3A and B). Compared to untreated control cells, cells treated with alkyl-LPA showed a significant increase in the intracellular glucose level after 180 min of treatment. The intracellular glucose level gradually decreased over 12 h (Fig. 3A).

3.5. Alkyl-LPA-treated C2C12 cells express increased amounts of GLUT4 mRNA

Alkyl-LPA regulates gene expression through activation of its intracellular target, PPARγ. To determine whether alkyl-LPA induces GLUT4 expression via PPARγ, we examined differentiated C2C12 cells in the presence or absence of alkyl-LPA. Then, the expression of GLUT4 was determined via real-time PCR analysis (Fig. 4). Alkyl-LPA-treated C2C12 cells showed increased levels of GLUT4 mRNA beginning on day 3 (72 h). Alkyl-LPA-induced GLUT4 mRNA expression was abolished by the specific PPARγ inhibitor GW9662. These results indicate that alkyl-LPA acts as a transcriptional regulator of GLUT4 via the PPARγ signaling pathway in C2C12 cells.

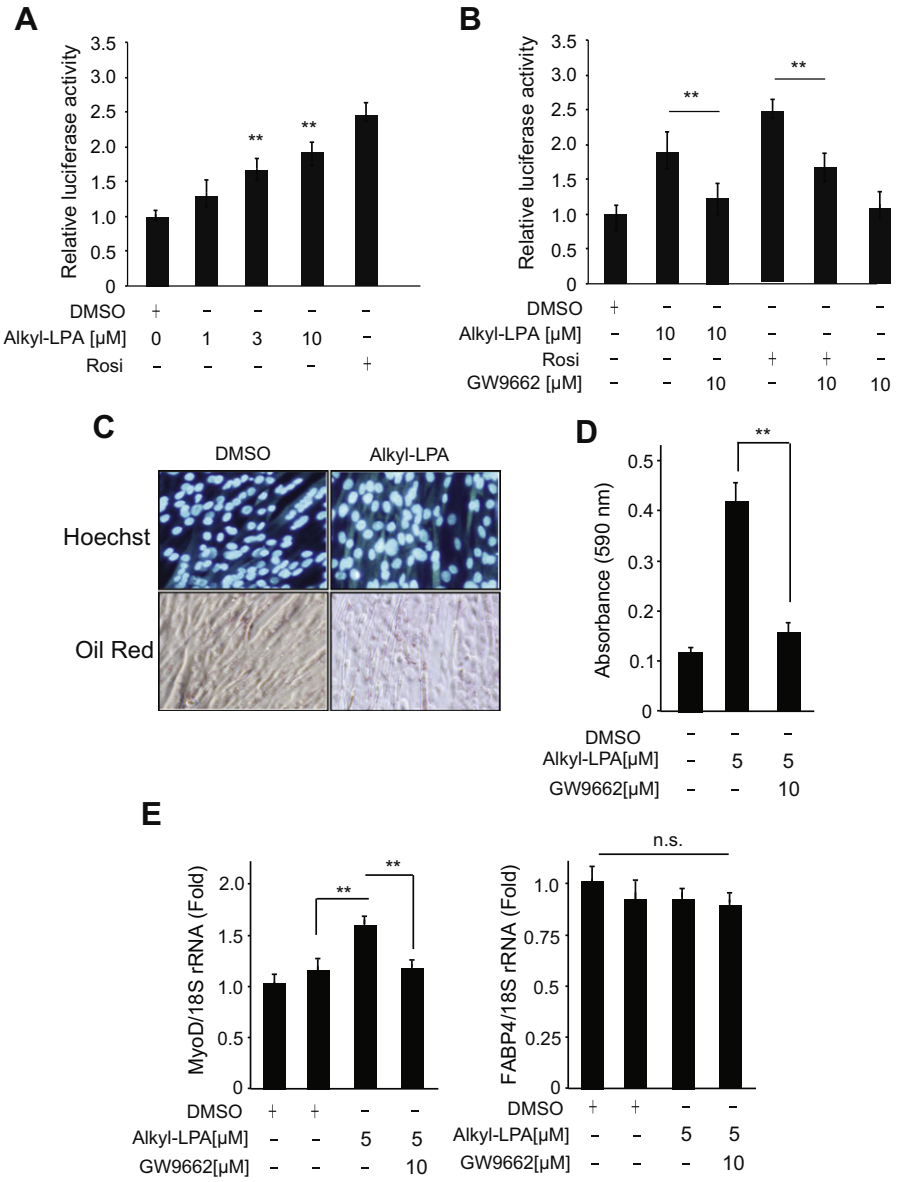

Fig. 2. C2C12 cells express functional peroxisome proliferator-activated receptor gamma (PPARγ). (A) C2C12 cells were transfected with the PPAR-responsive element (PPRE)-Luc and cytomegalovirus (CMV)-β-galactosidase plasmids and were treated with vehicle (dimethyl sulfoxide [DMSO]), alkyl-LPA (5 μM), or rosiglitazone (5 μM) for 48 h. The luciferase activity was WY-14,643 (PPARα specific agonist) and L-165,041 (PPAR δ/β specific agonist) measured in the lysates of treated cells and was normalized to β-galactosidase activity. (B) Data are presented as mean ± standard error of the mean (SEM, $n = 3$); $^{**}p < 0.01$. Alkyl-lysophosphatidic acid (LPA) treatment induces intracellular lipid accumulation. (C) Serum-starved C2C12 cells were treated with alkyl-LPA (5 μM) in serum-free Dulbecco's Modified Eagle's Medium (DMEM) for 7 days. Cellular morphology was visualized using a light microscope. (D) Quantitative analysis of lipid accumulation was assayed with a lipid-staining reagent to evaluate the lipid content. Oil red staining was performed after 7 days of exposure to 5 μM alkyl-LPA. Data are presented as mean ± SEM ($n = 3$), $^{**}p < 0.01$. (E) Effects of alkyl-LPA on the expression of myogenic and adipose differentiation markers in C2C12 cells. Serum-starved C2C12 cells were exposed to alkyl-LPA (5 μM) for 7 days, and RNA was isolated. The mRNA levels of a myogenic specific marker (MyoD) and an adipogenesis specific marker (FABP4) were determined via real-time PCR. The mRNA levels were normalized to 18S rRNA (mean ± SEM ($n = 3$), $^{**}p < 0.01$).

4. Discussion

The present study showed that the endogenous PPARγ agonist, alkyl-LPA, induces PPARγ transcriptional activity in skeletal muscle cells. The effect of alkyl-LPA depends on its interaction with PPARγ because alkyl-LPA stimulated PPARγ activation in a ligand-dependent manner. Furthermore, we observed a significant increase in PPARγ1 mRNA and protein levels. Alkyl-LPA induced PPARγ1 but not PPARγ2 expression in the C2C12 mouse myoblast cell line in a time-dependent manner. Under physiological conditions, the expression of PPARγ2 is limited to adipose tissues [15,16]. In addition, we showed that alkyl-LPA treatment induces intracellular lipid accumulation in C2C12 cells. Previous studies suggest that excessive intracellular lipid accumulation in muscles correlates with insulin resistance, which may lead to type 2 diabetes [18]. PPARγ agonists such as long-chain fatty acids (LCFA) and TZD drugs have also been reported to convert the differentiation pathway of myoblasts into that of adipocytes [17]. Our results suggest that exogenous factors such as alkyl-LPA could provide crucial information on the process of lipid deposition in muscle tissue.

We demonstrated that alkyl-LPA treatment increased the glucose concentration in C2C12 cells. C2C12 cells do not show insulin-sensitive glucose uptake and lack insulin-sensitive vesicles containing GLUT4 [19]. Regulation of GLUT4 has been a major

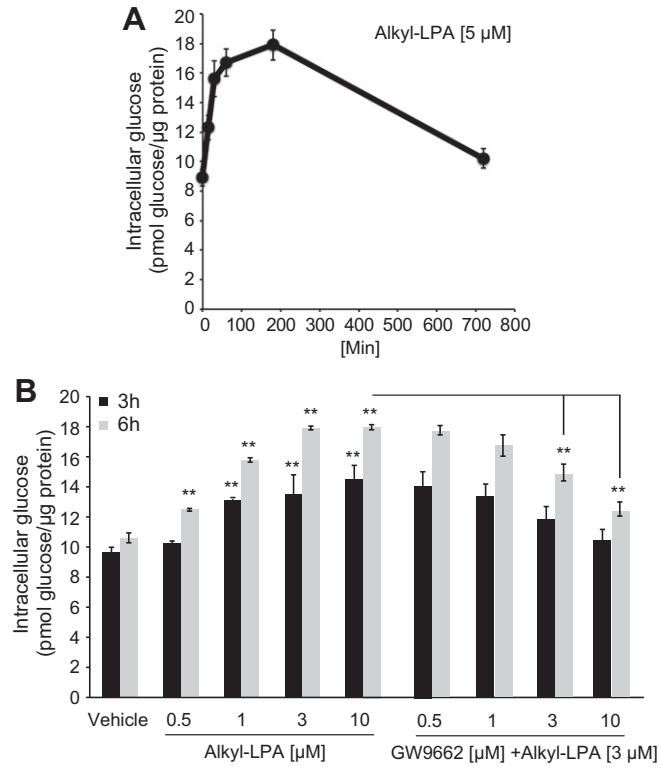

Fig. 3. Alkyl-lysophosphatidic acid (LPA) increases the intracellular glucose level of serum-starved C2C12 cells in a time- and dose-dependent manner. (A) and (B) Before harvesting, alkyl-LPA-treated cells in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 mg/mL glucose were washed twice with cold phosphate-buffered saline (PBS). They were lysed with ion-free H_2O for 5 min on ice. To prevent the destruction of glucose during cell extraction, cells were handled on ice at all times. Intracellular concentrations of glucose were determined using a glucose assay kit (BioVision Inc., Milpitas, CA, USA) according to the manufacturer's instructions. Briefly, this is a coupled enzyme assay that relies on the oxidation of glucose to generate a product, which reacts with a dye to generate color (570 nm) and fluorescence (*Ex*/*Em* = 535/587 nm). We measured glucose concentrations by monitoring the absorbance at 570 nm and comparing it to a standard curve of known concentrations.

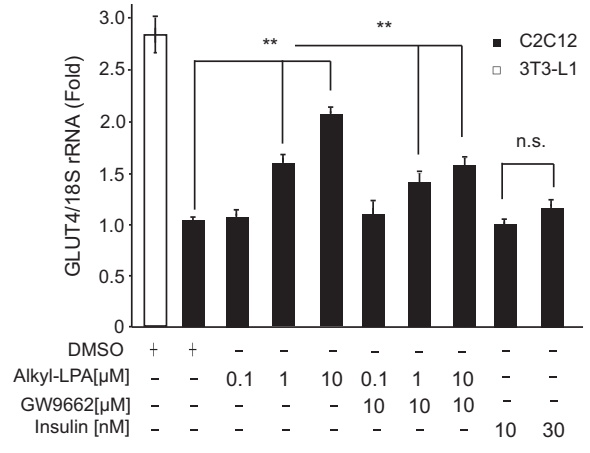

Fig. 4. Alkyl-LPA-treated C2C12 cells express increased levels of glucose transporter 4 (GLUT4) mRNA. Real-time polymerase chain reaction (PCR) measurement of GLUT4 mRNA expression in serum-starved C2C12 cells. Differentiated C2C12 cells were treated with alkyl-LPA or insulin and incubated for 24 h. The relative GLUT4 levels, normalized to 18S rRNA, are expressed as mean ± standard error of the mean (SEM, $n = 3$); $^{**}p < 0.01$. 3T3-L1 cells were used as a positive control.

focus of studies on type 2 diabetes. We also showed that alkyl-LPA-treated C2C12 cells have increased levels of GLUT4 mRNA, indicating that alkyl-LPA can regulate GLUT4 expression and glucose uptake. Taken together, our results indicates that regulation of PPARγ by alkyl-LPA and glucose partitioning to skeletal muscle may reduce plasma glucose levels and prevent the development of metabolic diseases, including hyperinsulinemia. A well-designed prospective study of the effects of other, more specific alkyl-LPA analogs using C2C12 cells would help clarify the mechanisms linking alkyl-LPA and muscle glucose metabolism.

Acknowledgments

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References

[1] A.A. Spector, Fatty acid binding to plasma albumin, J. Lipid Res. 16 (1975) 165–179.
[2] M.P. Corcoran, S. Lamon-Fava, R.A. Fielding, Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise, Am. J. Clin. Nutr. 85 (2007) 662–677.
[3] A. Leonardini, L. Laviola, S. Perrini, A. Natalicchio, F. Giorgino, Cross-talk between PPARgamma and insulin signaling and modulation of insulin sensitivity, PPAR Res. 2009 (2009) 818945.
[4] S.M. Rangwala, M.A. Lazar, Peroxisome proliferator-activated receptor gamma in diabetes and metabolism, Trends Pharmacol. Sci. 25 (2004) 331–336.
[5] N.J. Bryant, R. Govers, D.E. James, Regulated transport of the glucose transporter GLUT4, Nat. Rev. Mol. Cell Biol. 3 (2002) 267–277.
[6] N. Kotliar, P.F. Pilch, Expression of the glucose transporter isoform GLUT 4 is insufficient to confer insulin-regulatable hexose uptake to cultured muscle cells, Mol. Endocrinol. 6 (1992) 337–345.
[7] L.L. Tortorella, P.F. Pilch, C2C12 myocytes lack an insulin-responsive vesicular compartment despite dexamethasone-induced GLUT4 expression, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E514–524.
[8] T. Tsukahara, R. Tsukahara, S. Yasuda, N. Makarova, W.J. Valentine, P. Allison, H. Yuan, D.L. Baker, Z. Li, R. Bittman, A. Parrill, G. Tigyi, Different residues mediate recognition of 1-*O*-oleyllysophosphatidic acid and rosiglitazone in the ligand binding domain of peroxisome proliferator-activated receptor gamma, J. Biol. Chem. 281 (2006) 3398–3407.
[9] T. Tsukahara, R. Tsukahara, Y. Fujiwara, J. Yue, Y. Cheng, H. Guo, A. Bolen, C. Zhang, L. Balazs, F. Re, G. Du, M.A. Frohman, D.L. Baker, A.L. Parrill, A. Uchiyama, T. Kobayashi, K. Murakami-Murofushi, G. Tigyi, Phospholipase D2-dependent inhibition of the nuclear hormone receptor PPARgamma by cyclic phosphatidic acid, Mol. Cell 39 (2010) 421–432.
[10] F. Gaits, O. Fourcade, F. Le Balle, G. Gueguen, B. Gaige, A. Gassama-Diagne, J. Fauvel, J.P. Salles, G. Mauco, M.F. Simon, H. Chap, Lysophosphatidic acid as a phospholipid mediator: pathways of synthesis, FEBS Lett. 410 (1997) 54–58.
[11] T. Sugiura, S. Nakane, S. Kishimoto, K. Waku, Y. Yoshioka, A. Tokumura, Lysophosphatidic acid, a growth factor-like lipid, in the saliva, J. Lipid Res. 43 (2002) 2049–2055.
[12] T. Sugiura, S. Nakane, S. Kishimoto, K. Waku, Y. Yoshioka, A. Tokumura, D.J. Hanahan, Occurrence of lysophosphatidic acid and its alkyl ether-linked analog in rat brain and comparison of their biological activities toward cultured neural cells, Biochim. Biophys. Acta 1440 (1999) 194–204.
[13] J.R. Zierath, J.W. Ryder, T. Doebber, J. Woods, M. Wu, J. Ventre, Z. Li, C. McCrary, J. Berger, B. Zhang, D.E. Moller, Role of skeletal muscle in thiazolidinedione insulin sensitizer (PPARgamma agonist) action, Endocrinology 139 (1998) 5034–5041.
[14] R.W. Nesto, D. Bell, R.O. Bonow, V. Fonseca, S.M. Grundy, E.S. Horton, M. Le Winter, D. Porte, C.F. Semenkovich, S. Smith, L.H. Young, R. Kahn, Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association, Diabetes Care 27 (2004) 256–263.
[15] P. Tontonoz, E. Hu, B.M. Spiegelman, Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor, Cell 79 (1994) 1147–1156.
[16] P. Tontonoz, E. Hu, R.A. Graves, A.I. Budavari, B.M. Spiegelman, MPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer, Genes Dev. 8 (1994) 1224–1234.
[17] P.A. Grimaldi, L. Teboul, H. Inadera, D. Gaillard, E.Z. Amri, Trans-differentiation of myoblasts to adipoblasts: triggering effects of fatty acids and thiazolidinediones, Prostaglandins Leukot. Essent. Fatty Acids 57 (1997) 71–75.
[18] B.H. Goodpaster, J. He, S. Watkins, D.E. Kelley, Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes, J. Clin. Endocrinol. Metab. 86 (2001) 5755–5761.
[19] D.N. Gross, S.R. Farmer, P.F. Pilch, Glut4 storage vesicles without Glut4: transcriptional regulation of insulin-dependent vesicular traffic, Mol. Cell. Biol. 24 (2004) 7151–7162.