# Calcium Mobilization Inhibits Lipid Accumulation During the Late Adipogenesis via Suppression of PPARγ and LXRα Signalings

Seung-Jin Kim, Hojung Choi, Chung-Hwan Jung, Sung-Soo Park,
Seung-Rye Cho, Sejong Oh[1], and Eungseok Kim*

*Dept. of Biological sciences, College of Natural sciences, Chonnam National University, Gwangju 500-757, Korea*
*[1]Division of Animal Science, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea*

## Abstract

Calcium plays a role as a signaling molecule in various cellular events. It has been reported that calcium suppresses adipocyte differentiation only in the early phase of adipogenesis. Herein, we demonstrate that treatment of A23187, a mobilizer of intracellular calcium, on day 4 post adipocyte differentiation could still reduce lipid accumulation in differentiating 3T3-L1 cells for 48 h. In addition, luciferase reporter gene and RT-Q-PCR assays demonstrate that A23187 can selectively inhibit transcriptional activities and expression of PPARγ and LXRα, suggesting that A23187 may reduce lipid accumulation in the late phase of adipogenesis via downregulation of PPARγ and LXRα expression and transactivation. Moreover, inhibition of HDAC activity by trichostatin A (TSA) partially blocked A23187-mediated downregulation of transcriptional activities of PPARγ and LXRα. Together, our data demonstrate that calcium mobilization inhibits expression and transcriptional activities of PPARγ and LXRα, resulting in reduced lipid accumulation in differentiating adipocytes, and thus, mobilization of intracellular calcium in adipocytes may serve as a new preventive and therapeutic approach for obesity.

**Key words:** Calcium, adipocyte, PPARγ, LXRα

## Introduction

Adipocytes are important cells for lipid homeostasis in our body by regulating flux of lipids into circulation in response to nutrition status (Guilherme *et al.*, 2008). In addition, adipocytes also play a role as highly specialized endocrine cells secreting a variety of adipocytokines such as TNFα, leptin, acdiponectin and resistin. These adipocytokines have been known to participate in various physiological processes including insulin sensitivity, cell proliferation, immune response and food intake (Kershaw and Flier, 2004; Rosen and Spiegelman, 2006). Increased lipid accumulation by dysregulated lipid metabolism in adipose tissues resulted in obesity and is frequently related with metabolic diseases including insulin resistance and type 2 diabetes (Fu *et al.*, 2005; Kershaw and Flier, 2004; Gregor and Hotamisligil, 2007; Shi and Burn, 2004).

Several transcription factors have been known to be involved in adipocyte differentiation (Gregoire *et al.*, 1998; Lane *et al.*, 1999; Rosen *et al.*, 2000). During adipogenesis, profile of a variety of genes has been changed and PPARγ and C/EBPα have been shown to play key roles in this process (Darlington *et al.*, 1998). PPARγ, the master regulator of adipocyte differentiation, belongs to the superfamily of nuclear receptors (NRs) (Chawla *et al.*, 1994). NR superfamily includes traditional endocrine receptors for steroids, thyroid hormones, vitamin D3, and retinoids, as well as a large number of orphan receptors of which ligands and physiological functions are initially unknown (Evans, 1988; Giguere, 1999). In addition to PPARγ, LXRα, a NR activated by oxysterol, has been known to function as a lipid sensor that responds to dietary lipids and their metabolic derivatives in liver and adipose tissues. Previous studies have demonstrated that LXRα enhances fatty acid synthesis by LXR-SREBP-1c-lipogenic gene pathway or direct upregulation of acetyl-CoA carboxylase and fatty acid synthase (FAS) expression (Chen *et al.*, 2004; Joseph *et al.*, 2002; Liang *et al.*, 2002).

Several studies have shown that high calcium intake from dairy products results in prevention of obesity and insulin resistance, implicating link between calcium and adiposity (Papakonstantinou *et al.*, 2003; Pilvi *et al.*,

*Corresponding author: Eungseok Kim, Dept. of Biological Sciences, College of Natural Sciences, Chonnam National University, Gwangju 500-757, Korea. Tel: 82-62-530-3402, Fax: 82-62-530-3409, E-mail: ekim@chonnam.ac.kr

2008). Recently, it was reported that mobilization of intracellular calcium ($[Ca^{2+}]_i$) resulted in complete inhibition of adipogenesis in the early phase of adipocyte differentiation (Draznin et al., 1988; Shi et al., 2000). However, several studies reported conflicting results about calcium effect on adipogenesis in the late phase of adipocyte differentiation (Jensen et al., 2004; Shi et al., 2000; Whitehead et al., 2001). Thus, calcium effect on lipid accumulation in differentiating adipocytes is still not established. In this study, we demonstrated that increase of $[Ca^{2+}]_i$ in the late phase of adipogenesis suppressed lipid accumulation in differentiating adipocytes and this inhibitory effect was due to decreased expression of lipogenic genes via reduced expression and transcriptional activities of PPARγ and LXRα. Suppressive effect of $[Ca^{2+}]_i$ on transcriptional activities of these nuclear receptors is partially blocked by a histone deacetylase (HDAC) inhibitor, suggesting that mobilization of $[Ca^{2+}]_i$ may increase HDAC activity on transcriptional machineries of lipogenic NRs.

## Materials and Methods

### Cell culture, Adipocyte differentiation and Oil Red O staining

3T3-L1 and HEK293T cells obtained American Type Culture Collection (ATCC). 3T3-L1 was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% bovine serum and 100 μg/mL penicillin-streptomycin and maintained on the petri-dishes. HEK293T was cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and same condition of antibiotics as described above. 3T3-L1 cells cultured to 90% confluence. Two days later, the medium was replaced with the differentiation induction medium (MDI) containing 5 μg/mL insulin, 0.25 mM 3-Isobutyl-1-methylxanthine (IBMX) and 0.25 μM dexamethasone in DMEM containing 10% FBS. The cells were treated with MDI during 72 h. The medium was replaced with MDI except 0.25 mM IBMX. Cells cultured mature adipocyte until 8 d. Cells were fixed with 10% formaldehyde in phosphate-buffered saline (PBS) and stained with Oil Red O (Sigma, USA).

### Reporter gene assay

Reporter genes pGL3-TK-Cyp7a-LXRE$_{x3}$-Luc, pGL3-TK-AcoI-PPRE$_{x3}$-Luc, pGL3-TK-HCR1-TR4RE$_{x3}$-Luc and pGL3-TK-HCR1 were previously described (Peet et al., 1998; Forman et al., 1995; Kim et al., 2003). Transfections were performed using SuperFect reagent (Qiagen) according to the manufacturer's instructions in HEK293T cells. Luciferase activities were measured in a luciferase reporter assay system (Berthold). Relative luciferase activity (fold) was expressed based on the induction fold relative to transfection of an empty vector (set as 1-fold) and the results were expressed as means ± SD of three separate experiments.

### RNA isolation, cDNA synthesis and RT-Q-PCR (Reverse transcriptase quantitative PCR)

Evaluation of adipogenic gene mRNA expression was

**Table 1. Primer sequence of genes for RT-Q-PCR**

| Gene | Sequence | Ref. |
|---|---|---|
| TR4 | CAGCAGTTCATCCTAACCAGCCC<br>CTGCTCCGACAGCTGTAGGTC | (Kim et al., 2003) |
| PPARγ | TGCTGTTATGGGTGAAACTCTGGG<br>CGCTTGATGTCAAAGGAATGCG | (Cariou et al., 2006) |
| FXRα | CCAACCTGGGCTTCTACCC<br>CACACAGCTCATCCCCTTT | (Cariou et al., 2006) |
| PEPCK | TCAACACCGACCTCCCTTAC<br>CCCTAGCCTGTTCTCTGTGC | (Kitamura et al., 2006) |
| aP2 | GAATTCGATGAAATCACCGCA<br>CTCTTTATTGTGGTCGACTTTCC | (Berger et al., 2001) |
| LXRα | TGCCATCAGCATCTTCTCTG<br>GGCTCACCAGCTTCATTAGC | (Volle et al., 2007) |
| SREBP-1c | GATCAAAGAGGAGCCAGTGC<br>TAGATGGTGGCTGCTGAGTG | (Cariou et al., 2006) |
| FAS | AGATCCTGGAACGAGAACACGAT<br>GAGACGTGTCACTCCTGGACTTG | (Schmid et al., 2005) |
| 36B4 | AGATGCAGCAGATCCGCAT<br>ATATGAGGCAGCAGTTTCTCCAG | (Moniotte et al., 2001) |

determined by RT-Q-PCR. Total RNA was isolated from 3T3-L1 cells using RiboEX (Geneall, Korea), and cDNA was synthesized using MMLV-RTase (Promega) and Oligo dT primers (Promega) according to the manufacturer's instructions. Primer sequences of mouse genes for RT-Q-PCR are shown in Table 1. Relative expression levels of Adipogenic genes were determined by relative to the internal control 36B4 transcripts using a Gel doc XR system (Bio-Rad).

## Results

### Calcium mobilization inhibits lipid accumulation in the late phase of adipogenesis

To study the role of $[Ca^{2+}]_i$ in the adipocyte differentiation, a calcium ionophore A23187 were used to mimic physiological $[Ca^{2+}]_i$ mobilization in 3T3-L1 cells (Berridge, 1993; Campbell, 1983; Duffus and Patterson, 1974). We first treated A23187 to differentiating 3T3-L1 cells for 48 h at different time points and then determined lipid accumulation in 3T3-L1 adipocytes on day 8 post adipocyte differentiation by Oil-Red O staining. While treatment of A23187 on day 2 post adipocyte differentiation

completely blocked adipogensis, addition of A23187 on day 4 only partially suppressed lipid accumulation in differentiated 3T3-L1 adipocytes. In contrast, we were not able to see any suppressive effect of A23187 on lipid accumulation when A23187 was treated to differentiating 3T3-L1 cells on day 6 (Fig. 1A). Since Fig. 1A showed that addition of A23187 was still able to partially suppress adipogenesis even on day 4 post adipocyte differentiation on which PPARγ and C/EBPα are already expressed, we evaluated calcium mobilization effect on expression of lipogenic genes using RT-Q-PCR. As shown in Fig. 1B, 48 h treatment of 2 μM A23187 to differentiating 3T3-L1 cells on day 4 resulted in significant reduction of mRNA levels of aP2, FAS and PEPCK genes in day 8 adipocytes as compared with vehicle-treated adipocytes. Data from Fig. 1 suggests that suppressive effect of calcium mobilization on lipid accumulation in the late phase of adipocyte differentiation may be at least partially due to reduced expression of lipogenic genes.

### Calcium mobilization selectively downregulates PPARγ and LXRα signaling

Our studies demonstrated that $[Ca^{2+}]_i$ mobilization
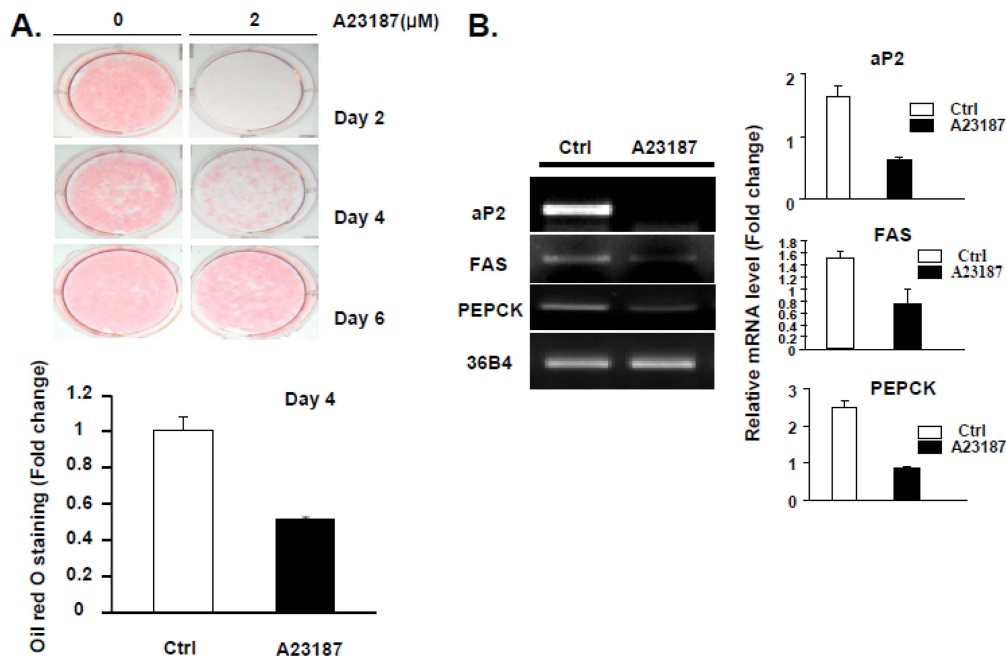


**Fig. 1. $[Ca^{2+}]_i$ inhibits lipid accumulation during the late phase of adipogenesis.** A. A23187 reduced lipid accumulation in 3T3-L1 adipocytes during the late phase of adipogenesis. Two μM A23187 was treated for 48 h to 3T3-L1 cells at different time points after MDI stimulation and mature adipocytes were stained with the triglyceride specific dye Oil Red O and photographed on day 8. Lower panel, Quantitative analysis of lipid accumulation in adipocytes by measuring $OD_{500\,nm}$ of Oil Red O eluted from A23187- or vehicle-treated adipocytes as indicated. B. A23187 suppressed expression of adipogenic marker genes. After treatment of vehicle or 2 μM A23187 for 48 h on day 4 after MDI stimulation, mRNA levels of adipogenic genes in vehicle- or A23187-treated adipocytes were determined by RT-Q-PCR. Representative RT-Q-PCR data shown here is from three independent experiments with similar results.

inhibits lipid accumulation even in the late phase of adipogenesis via downregulation of lipogenic genes. Various NRs have been known to be involved in lipid metabolism via regulation of lipogenic gene expression. To test whether calcium inhibits lipid accumulation in differentiating 3T3-L1 cells via reduction of lipogenic NR expression, we first evaluated effect of A23187 on mRNA levels of several NRs including PPARγ and LXRα in differentiating adipocytes using RT-Q-PCR. As shown in Fig. 2A, 48 hour treatment of 2 μM A23187 to day 4 3T3-L1 cells resulted in reduction of mRNA levels of PPARγ and LXRα but not of FXRα and TR4 (Fig. 2A). Next, we tested whether addition of rosiglitazone, a PPARγ agoinst, could overcome A23187-mediated suppression of lipid accumulation in differentiating adipocytes during the late phase of adipogenesis by Oil Red O staining. As expected, 48 h treatment of A23187 on day 4 3T3-L1 cells reduced lipid accumulation in day 8 adipocytes (Fig. 2B). When rosiglitazone was co-treated with A23187, inhibitory effect of A23187 on lipid accumulation in differentiating adipocytes was partially derepressed, suggesting the possibility that during the late phase of adipogenesis, A23187 may suppress lipid accumulation in differentiating adipocytes by downregulation not only of expression but also of transcriptional activity of lipogenic NRs. In addition, pre-

vious report proposed that calreticulin, a major $Ca^{2+}$-buffering protein in the lumen of the ER which is induced by PPARγ, might inhibit PPARγ activity by negative feedback mechanism (Szabo *et al.*, 2008). Thus, by using reporter gene assay, we investigated if $[Ca^{2+}]_i$ mobilization could regulate transactivation of NRs implicated in lipid metabolism. Addition of A23187 resulted in suppression of ligand (1 μM GW3965 or 1 μM rosiglitazone) induced LXRα and PPARγ transcriptional activities in HEK293T cells (Fig. 3). However, we were not able to see any significant effect of A23187 on TR4- and FXRα-mediated transcriptional activities, suggesting that A23187 may inhibit lipid accumulation in differentiating 3T3-L1 cells via specific modulation of PPARγ and LXRα activities.

## Inhibition of HDAC activity reduces suppressive effect of A23187 on PPARγ and LXRα transcriptional activities

Previous studies have demonstrated that increased HDAC activity enforces corepressor complex and thus, blocks recruitment of coactivators to liganded NRs within their target gene promoter, resulting in repression of NR function in the regulation of their target gene expression.

To test whether HDACs mediate suppressive effect of
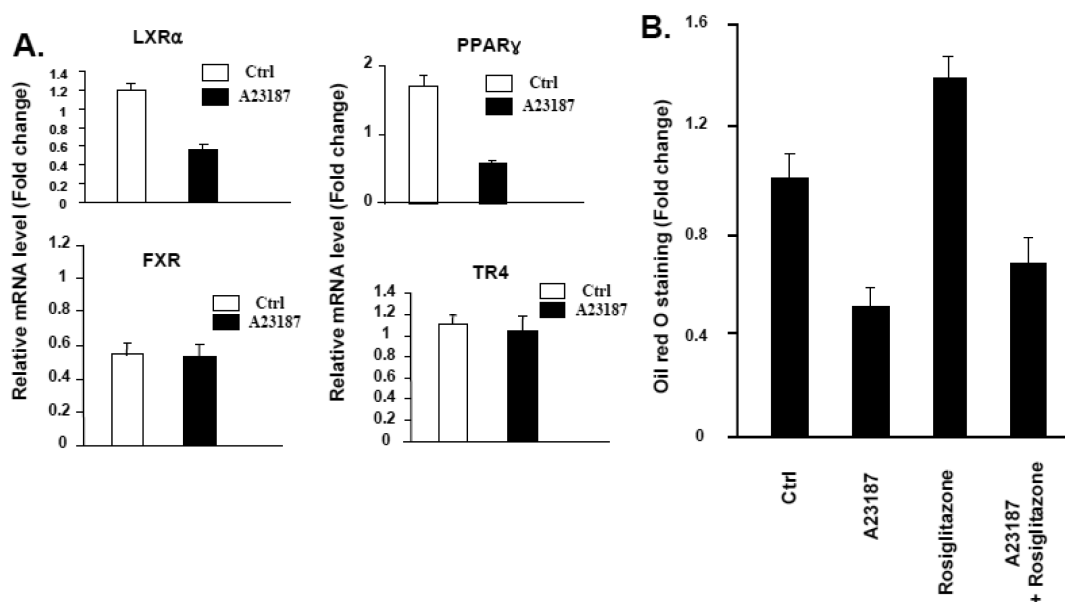


**Fig. 2. Effect of increased $[Ca^{2+}]_i$ on expression of lipogenic NRs during the late phase of adipogenesis A.** Increased $[Ca^{2+}]_i$ reduced expression of PPARγ and LXRα but not of FXR and TR4. After treatment of vehicle or 2 μM A23187 for 48 h on at day 4 after MDI stimulation, mRNA levels of lipogenic NR genes in vehicle- or A23187-treated adipocytes were determined by RT-Q-PCR. 36B4 fragments were amplified as a loading control and mRNA level of each gene was quantified relative to transcript of the internal control, 36B4. B. Vehicle, 2 μM A23187 and 1 μM rosiglitazone were treated to differentiating 3T3-L1 cells for 48 h on day 4 after MDI stimulation as indicated. Relative lipid accumulation was determined by measuring Oil Red O eluted from adipocytes treated with vehicle, 2 μM A23187 or/and 1 μM rosiglitazone.
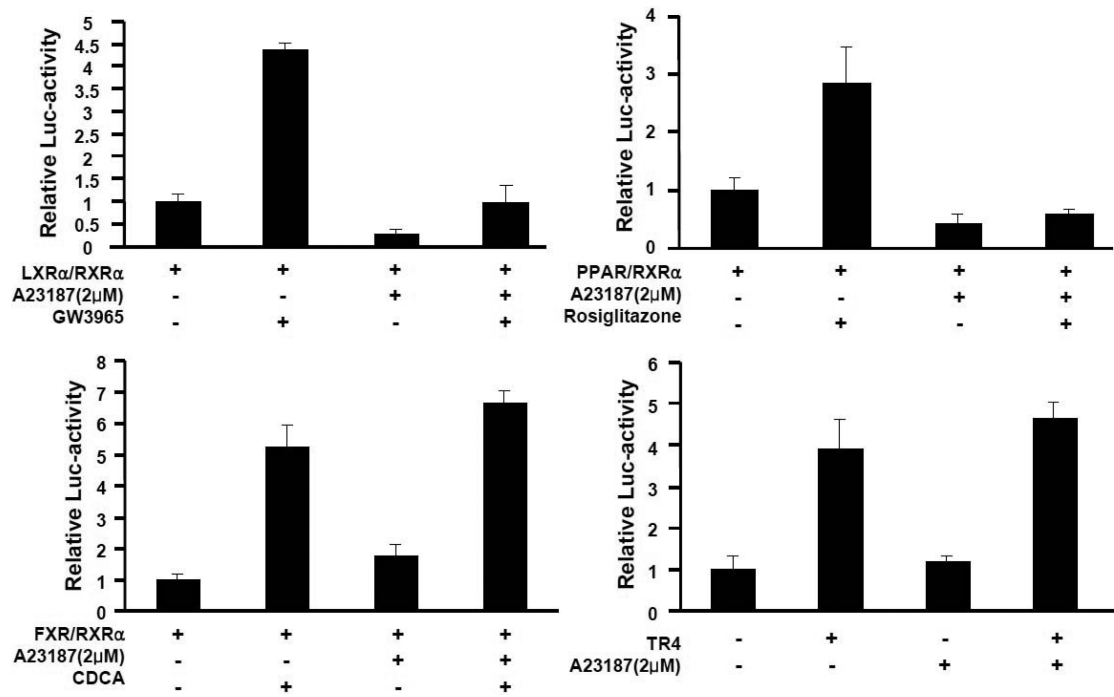
**Fig. 3. Increased [Ca²⁺]ᵢ selectively suppressed transcriptional activities of PPARγ and LXRα.** HEK293T cells were transfected with expression vectors for NRs (50 ng pCMX-LXRα, 50 ng pCMX-RXRα, 50 ng pSG5-PPARγ, 50 ng pSG5-FXRα or 100 ng pCMX-TR4) and the reporter genes containing appropriate response element for corresponding NR (pGL3-TK-Cyp7a-LXRE$_{x3}$-Luc, pGL3-TK-AcoI-PPRE$_{x3}$-Luc, pGL3-TK-HCR1-TR4RE$_{x3}$-Luc or pGL3-TK-HCR1 for FXR, 300 ng of each). Cells were then treated with 1 μM GW3965, 1 μM rosiglitazone or 100 μM CDCA for 24 h in the presence or absence of 2 μM A23187 as indicated and assayed for luciferase activity.
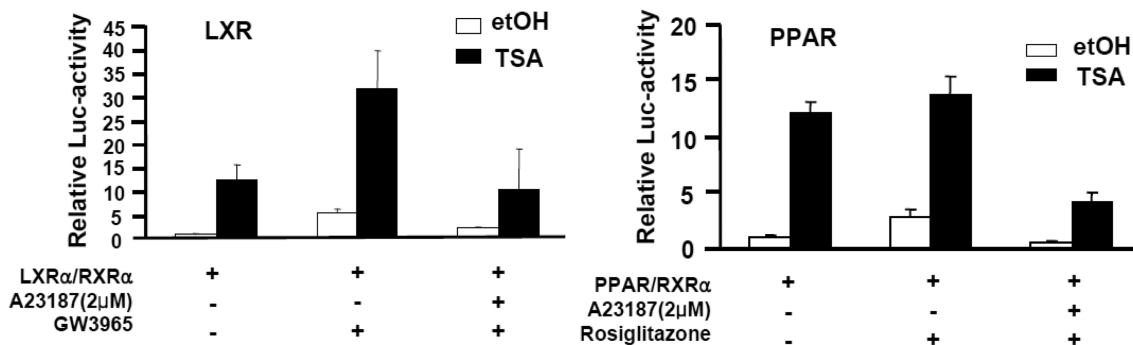


**Fig. 4. Inhibition of HDAC activity reduced suppressive effect of A23187 on PPARγ and LXRα transcriptional activities.** HEK293T cells were transfected with NRs (LXRα or PPARγ, 50 ng each) and appropriate reporter genes (pGL3-TK-Cyp7a-LXRE$_{x3}$-Luc or pGL3-TK-AcoI-PPRE$_{x3}$-Luc, 300 ng each). Cells were then treated with 1 μM GW3965 or 1 μM rosiglitazone for 24 h in the presence or absence of 100nM TSA or/and 2 μM A23187 as indicated and assayed for luciferase activity.

A23187 on PPARγ and LXRα transactivation, we evaluated effect of TSA, a HDAC inhibitor, on A23187-mediated inhibition of PPARγ and LXRα activities. As shown in Fig. 4, ligand treatment (1 μM GW3965 or 1 μM rosiglitazone) to HEK293T cells transfected with LXRα or PPARγ induced LXRα or PPARγ transcriptional activities, respectively. These ligand effects on LXRα and PPARγ activities disappeared when A23187 was co-treated with their ligands (1 μM GW3965 or 1 μM rosiglitazone).

However, TSA treatment together with A23187 reduced A23187-mediated suppression of LXRα and PPARγ activities. This result suggests that A23187 inhibits lipid accumulation at least partially via inhibition of LXRα and PPARγ transactivation by increase of HDAC activity. Together, these data indicate that A23187 plays a role as a functional modulator of PPARγ and LXRα transcriptional activities in differentiating adipcytes.

## Discussion

Calcium participates in cell signaling pathway by regulation of signaling molecules such as PKC, MAPK and calmodulin-dependent enzymes and calcium mobilization has been known to affect differentiation program of various cell types including adipocytes and osteoclast (Zayzafoon, 2006). Previous studies have shown that calcium mobilization inhibits adipogenic program in the early phase of adipocyte differentiation via suppression of adipogenic transcription factor expression (Shi et al., 2000). However, role of $[Ca^{2+}]_i$ in the late phase of adipocyte differentiation remains controversial (Jensen et al., 2004; Whitehead et al., 2001). Several studies reported that $[Ca^{2+}]_i$ has its negative effect only in the early phase but not in the late phase of adipogenesis (Shi et al., 2000). In contrast to these studies, Zemel et al. have reported that increased $[Ca^{2+}]_i$ suppressed lipolysis in the late phase of adipogenesis, resulting in increased lipid accumulation in adipocytes. In this study, by using 3T3-L1 preadipocytes, we demonstrated that calcium mobilization by A23187 on day 4 post adipocyte differentiation resulted in partial reduction of lipid accumulation and this calcium effect may at least partially go through PPARγ and LXRα to reduce lipid accumulation during adipogenesis. Adipocyte differentiation is facilitated by serial induction of various transcription factors. Several lines of evidence have established that adipogenic program triggers C/EBPâ and ä expression followed by induction of C/EBPα and PPARγ, which in turn, orchestrate adipocyte differentiation and lipid accumulation by induction of adipogenic gene expression (Darlington et al., 1998). Our results showed that A23187 clearly inhibited transcriptional activities of PPARγ and LXRα. PPARγ has been well known to be the master regulator of adipocyte differentiation. While LXRα is highly expressed in adipocytes and its expression is regulated by PPARγ, role of LXRα in adipogenesis is not well defined. Several reports have shown that activation of LXRα facilitates lipid accumulation in adipocytes via induction of lipogenic genes (Juvet et al., 2003; Laffitte et al., 2001). In support of suggested function of LXRα in lipid accumulation, LXRα/â deficiency resulted in reduced adipose tissues in older mice. In contrast, in other studies, LXRα did not significantly contribute to adipocyte differentiation and lipid accumulation although it could induce SREBP-1c, a well-known lipogenic transcription factor (Sekiya et al., 2007). Additional studies will be needed to clearly determine the role of LXRα in adipogenesis. Recent study has proposed that

calreticulin is induced by PPARγ and inhibits PPARγ activity by negative feedback mechanism, leading to suppression of adipocyte differentiation (Szabo et al., 2008). Our findings showed that calcium selectively suppress activities of PPARγ and LXRα but not of TR4 and FXRα. Liganded NRs induces target gene expression by direct binding to their response elements within target gene promoters and recruitment of various coactivators are involved in these activities of liganded NRs. In contrast, corepressors inhibit NR function and corepressor effect on NR activity is associated with HDAC activity. A23187 has been reported to increase HDAC activity with reduction of histone H3 acetylation in adiponectin promoter (Kim et al., 2006). In agreement with previous report, reduced transcriptional activities of PPARγ and LXRα by A23187 were partially recovered when TSA was co-treated with A23187. Based on our findings, mobilization of $[Ca^{2+}]_i$ in the late phase of adipogenesis resulted in reduced lipid accumulation by suppression of PPARγ and LXRα expression and transcription activities and this suppressive effect of calcium on these NR activities may be at least partially through increase of HDAC activity.

Obesity is leading cause of various metabolic diseases including diabetes via insulin resistance. Thus, our study may provide the new strategy for the development of calcium milk products which can prevent obesity via modulation of lipid accumulation in adipocytes.

## Acknowledgements

## References

1. Berger, J., Tanen, M., Elbrecht, A., Hermanowski-Vosatka, A., Moller, D. E., Wright, S. D., and Thieringer, R. (2001) Peroxisome proliferator-activated receptor-gamma ligands inhibit adipocyte 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. J. Biol. Chem. **276**, 12629-12635.
2. Berridge, M. J. (1993) Inositol trisphosphate and calcium signalling. Nature **361**, 315-325.
3. Campbell, A. K. (1983) Intracellular calcium: Its universal role as regulator. John Wiley & Sons, Inc. Chichester; New York.
4. Cariou, B., van Harmelen, K., Duran-Sandoval, D., van Dijk, T. H., Grefhorst, A., Abdelkarim, M., Caron, S., Torpier, G., Fruchart, J. C., Gonzalez, F. J., Kuipers, F., and Staels, B.

(2006) The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J. Biol. Chem.* **281**, 11039-11049.

5. Chawla, A., Schwarz, E. J., Dimaculangan, D. D., and Lazar, M. A. (1994) Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* **135**, 798-800.

6. Chen, G., Liang, G., Ou, J., Goldstein, J. L., and Brown, M. S. (2004) Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. *Proc. Natl. Acad Sci. USA* **101**, 11245-11250.

7. Darlington, G. J., Ross, S. E., and MacDougald, O. A. (1998) The role of C/EBP genes in adipocyte differentiation. *J. Biol. Chem.* **273**, 30057-30060.

8. Draznin, B., Sussman, K. E., Eckel, R. H., Kao, M., Yost, T., and Sherman, N. A. (1988) Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. *J. Clin. Invest.* **82**, 1848-1852.

9. Duffus, J. H. and Patterson, L. J. (1974) Control of cell division in yeast using the ionophore, A23187 with calcium and magnesium. *Nature* **251**, 626-627.

10. Evans, R. M. (1988) The steroid and thyroid hormone receptor superfamily. *Science* **240**, 889-895.

11. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* **83**, 803-812.

12. Fu, M., Sun, T., Bookout, A. L., Downes, M., Yu, R. T., Evans, R. M., and Mangelsdorf, D. J. (2005) A Nuclear Receptor Atlas: 3T3-L1 adipogenesis. *Mol. Endocrinol.* **19**, 2437-2450.

13. Giguere, V. (1999) Orphan nuclear receptors: from gene to function. *Endocr. Rev.* **20**, 689-725.

14. Gregoire, F. M., Smas, C. M., and Sul, H. S. (1998) Understanding adipocyte differentiation. *Physiol. Rev.* **78**, 783-809

15. Gregor, M. F. and Hotamisligil, G. S. (2007) Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. *J. Lipid. Res.* **48**, 1905-1914.

16. Guilherme, A., Virbasius, J. V., Puri, V., and Czech, M. P. (2008) Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.* **9**, 367-377.

17. Jensen, B., Farach-Carson, M. C., Kenaley, E., and Akanbi, K. A. (2004) High extracellular calcium attenuates adipogenesis in 3T3-L1 preadipocytes. *Exp. Cell Res.* **301**, 280-292.

18. Joseph, S. B., Laffitte, B. A., Patel, P. H., Watson, M. A., Matsukuma, K. E., Walczak, R., Collins, J. L., Osborne, T. F., and Tontonoz, P. (2002) Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J. Biol. Chem.* **277**, 11019-11025.

19. Juvet, L. K., Andresen, S. M., Schuster, G. U., Dalen, K. T., Tobin, K. A., Hollung, K., Haugen, F., Jacinto, S., Ulven, S.

M., Bamberg, K., Gustafsson, J. A., and Nebb, H. I. (2003) On the role of liver X receptors in lipid accumulation in adipocytes. *Mol. Endocrinol.* **17**, 172-182.

20. Kershaw, E. E. and Flier, J. S. (2004) Adipose tissue as an endocrine organ. *J. Clin. Endocrinol. Metab.* **89**, 2548-2556

21. Kim, E., Xie, S., Yeh, S. D., Lee, Y. F., Collins, L. L., Hu, Y. C., Shyr, C. R., Mu, X. M., Liu, N. C., Chen, Y. T., Wang, P. H., and Chang, C. (2003) Disruption of TR4 orphan nuclear receptor reduces the expression of liver apolipoprotein E/C-I/C-II gene cluster. *J. Biol. Chem.* **278**, 46919-46926.

22. Kim, H. B., Kong, M., Kim, T. M., Suh, Y. H., Kim, W. H., Lim, J. H., Song, J. H., and Jung, M. H. (2006) NFATc4 and ATF3 negatively regulate adiponectin gene expression in 3T3-L1 adipocytes. *Diabetes.* **55**, 1342-1352.

23. Kitamura, N., Wong, P., and Matsumura, F. (2006) Mechanistic investigation on the cause for reduced toxicity of TCDD in wa-1 homozygous TGFalpha mutant strain of mice as compared its matching wild-type counterpart, C57BL/6J mice. *J. Biochem. Mol. Toxicol.* **20**, 151-158.

24. Laffitte, B. A., Joseph, S. B., Walczak, R., Pei, L., Wilpitz, D. C., Collins, J. L., and Tontonoz, P. (2001) Autoregulation of the human liver X receptor alpha promoter. *Mol. Cell. Biol.* **21**, 7558-7568.

25. Lane, M. D., Tang, Q. Q., and Jiang, M. S. (1999) Role of the CCAAT enhancer binding proteins (C/EBPs) in adipocyte differentiation. *Biochem. Biophys. Res. Commun.* **266**, 677-683.

26. Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., and Brown, M. S. (2002) Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J. Biol. Chem.* **277**, 9520-9528.

27. Moniotte, S., Kobzik, L., Feron, O., Trochu, J. N., Gauthier, C., and Balligand, J. L. (2001) Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. *Circulation.* **103**, 1649-1655.

28. Papakonstantinou, E., Flatt, W. P., Huth, P. J., and Harris, R. B. (2003) High dietary calcium reduces body fat content, digestibility of fat, and serum vitamin D in rats. *Obes. Res.* **11**, 387-394.

29. Peet, D. J., Turley, S. D., Ma,W., Janowski, B. A., Lobaccaro, J. M., Hammer, R. E., and Mangelsdorf, D. J. (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRα. *Cell.* **93**, 693-704**.**

30. Pilvi, T.K., Storvik, M., Louhelainen, M., Merasto, S., Korpela, R., and Mervaala, E. M. (2008) Effect of dietary calcium and dairy proteins on the adipose tissue gene expression profile in diet-induced obesity. *J Nutrigenet Nutrigenomics.* **1**, 240-251.

31. Rosen, E. D. and Spiegelman, B. M. (2006) Adipocytes as regulators of energy balance and glucose homeostasis. *Nature.* **444**, 847-853.

32. Rosen, E. D., Walkey, C. J., Puigserver, P., and Spiegelman, B. M. (2000) Transcriptional regulation of adipogenesis.

*Genes. Dev.* **14**, 1293-1307.

33. Schmid, B., Rippmann, J. F., Tadayyon, M., and Hamilton, B. S. (2005) Inhibition of fatty acid synthase prevents preadipocyte differentiation. *Biochem. Biophys. Res. Commun.* **328**, 1073-1082.

34. Sekiya, M., Yahagi, N., Matsuzaka, T., Takeuchi, Y., Nakagawa, Y., Takahashi, H., Okazaki, H., Iizuka, Y., Ohashi, K., Gotoda, T., Ishibashi, S., Nagai, R., Yamazaki, T., Kadowaki, T., Yamada, N., Osuga, J., and Shimano, H. (2007) SREBP-1-independent regulation of lipogenic gene expression in adipocytes. *J. Lipid. Res.* **48**, 1581-1591.

35. Shi, H., Halvorsen, Y. D., Ellis, P. N., Wilkison, W. O., and Zemel, M. B. (2000) Role of intracellular calcium in human adipocyte differentiation. *Physiol Genomics.* **3**, 75-82.

36. Shi, Y. and Burn, P. (2004) Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. *Nat. Rev. Drug Discov.* **3**, 695-710.

37. Szabo, E., Qiu, Y., Baksh, S., Michalak, M., and Opas, M. (2008) Calreticulin inhibits commitment to adipocyte differentiation. *J. Cell Biol.* **182**, 103-116.

38. Volle, D. H., Mouzat, K., Duggavathi, R., Siddeek, B., Dechelotte, P., Sion, B., Veyssiere, G., Benahmed, M., and Lobaccaro, J. M. (2007) Multiple roles of the nuclear receptors for oxysterols liver X receptor to maintain male fertility. *Mol. Endocrinol.* **21**, 1014-1027.

39. Whitehead, J. P., Molero, J. C., Clark, S., Martin, S., Meneilly, G., and James, D. E. (2001) The role of Ca2+ in insulin-stimulated glucose transport in 3T3-L1 cells. *J. Biol. Chem.* **276**, 27816-27824.

40. Zayzafoon, M. (2006) Calcium/calmodulin signaling controls osteoblast growth and differentiation. *J. Cell Biochem.* **97**, 56-70.