ELSEVIER

European Journal of Pharmacology 422 (2001) 23–29

ejp

www.elsevier.nl/locate/ejphar

# Stage-specific effects of a thiazolidinedione on proliferation, differentiation and PPARγ mRNA expression in 3T3-L1 adipocytes

Toshinari Takamura*, Erika Nohara, Yukihiro Nagai, Ken-ichi Kobayashi

*First Department of Internal Medicine, School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8641, Japan*

Received 9 May 2001; accepted 15 May 2001

**Abstract**

To clarify the target phase of thiazolidinediones, which are ligands for peroxisome proliferator-activated receptor (PPAR)γ, during adipocyte differentiation, the effects of a thiazolidinedione, pioglitazone, on every stage during the course of adipocyte differentiation were investigated. Pioglitazone did not affect the cellular protein content and [$^3$H]thymidine incorporation into preconfluent 3T3-L1 preadipocytes. Induction of differentiation of confluent 3T3-L1 preadipocytes with insulin, dexamethasone and isomethylbutylxanthine for 48 h resulted in 30% inhibition of [$^3$H]thymidine incorporation into the cells and 354% increase in cellular protein content. Pioglitazone at 1 μM accelerated the increase in cellular protein content by 33% and the inhibition in the [$^3$H]thymidine incorporation by 12%. Pioglitazone, when added from the start of the induction stage, dose-dependently enhanced cellular triglyceride accumulation, and both basal and insulin-stimulated glucose transporting activity producing only a slight increase in the ratio of insulin stimulation to basal glucose transporting activity. In mature adipocytes, however, pioglitazone did not enhance either of the transporting activities. PPARγ messenger RNA (mRNA) levels estimated by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) increased during the course of adipocyte differentiation. Although pioglitazone dose-dependently up-regulated PPARγ mRNA levels in postconfluent preadipocytes without induction, it down-regulated them in mature adipocytes. Thus, a PPARγ agonist, pioglitazone, arrested the growth, and increased protein content and PPARγ mRNA levels in postconfluent preadipocytes, followed by commitment and hypertrophy of 3T3-L1 cells without changing insulin sensitivity, whereas it failed to stimulate glucose transporting activities and down-regulated PPARγ mRNA expression in mature adipocytes. 

*Keywords:* Adipocyte differentiation; Glucose transport; Pioglitazone; Peroxisome proliferator-activated receptor γ (PPARγ); Cell proliferation; Thiazolidinedione

## 1. Introduction

Insulin resistance plays a major role in the pathogenesis and exacerbation of type 2 diabetes (Kahn and Porte, 1997). Obesity is the most common cause of insulin resistance so that particularly in type 2 diabetes, which is associated with obesity, the alleviation of insulin resistance is the primary therapeutic target (Beck-Nielsen and Hother-Nielsen, 1996). Initiation and perpetuation of obesity occur not only as a cause of hypertrophy of adipose tissue but also of transformation of preadipocytes into adipocytes triggered by adipose tissue hyperplasia (Caro et al., 1989). Thiazolidinediones, a new class of antidiabetic agents, ameliorate hyperglycemia in type 2 diabetic patients, mainly by enhancing insulin action (Iwamoto et al., 1991; Hofmann and Colca, 1992). They were also demonstrated to be a direct ligand for peroxisome proliferator-activated receptor (PPAR)γ, a member of the nuclear receptor superfamily of ligand-dependent transcription factors (Forman et al., 1995; Kliewer et al., 1995). PPARγ was initially identified as a regulator for adipocyte differentiation through stimulation of target gene transcription (Tontonoz et al., 1994a,b). Forced expression of PPARγ in fibroblasts makes them differentiate into adipocytes (Tontonoz et al., 1994b). The binding affinity of thiazolidinediones to PPARγ correlates with their antidiabetic action, suggesting that PPARγ is the functional receptor of this class of compounds during their antidiabetic action (Willson et al., 1996; Berger et al., 1996). Although initial studies reported enhancement of adipocyte differentiation

* Corresponding author. Tel.: +81-76-265-2234; fax: +81-76-234-4250.

*E-mail address:* tt@medf.m.kanazawa-u.ac.jp (T. Takamura).

0014-2999/01/$ - see front matter 
PII: S0014-2999(01)01053-6

by thiazolidinediones (Sparks et al., 1991; Kletzien et al., 1992; Sandouk et al., 1993), their specific actions, especially on mature adipocytes and on the proliferation of preadipocytes, have remained unclear.

To better understand the effects of a thiazolidinedione on adipocyte differentiation, we investigated its action at every stage during the course of adipocyte differentiation, that is, proliferation of preadipocytes, commitment of preadipocytes to adipocytes, adipocyte hypertrophy as reflected by triglyceride accumulation, and glucose transporting activity as an adipocyte function. We also investigated the effect of a thiazolidinedione on PPARγ messenger RNA (mRNA) expression during the course of the differentiation.

## 2. Materials and methods

### 2.1. Cell lines and culture conditions

Mouse fibroblast line 3T3-L1 preadipocytes (Green and Kehinde, 1973) obtained from RIKEN Cell Bank (Ibaraki, Japan) were inoculated at a density of 5000 cells/$cm^2$, and grown in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Gibco). Confluent cells were induced to differentiate by treatment with an induction medium containing 0.5 mM dexamethasone (Sigma, St. Louis, MO), 0.25 mM isobutylmethylxanthine (Sigma) and 1 μg/ml insulin (Sigma) for 48 h. This commitment stage was defined as lasting from day 1 to day 2. The cells were then incubated for several more days with DMEM supplemented with 10% fetal bovine serum. The cultures were replenished every 2 days, and cell differentiation was monitored by evaluating cell morphology under phase-contrast microscopy. Cells were considered to be adipocytes when numerous lipid droplets were observed in the cytoplasm. The thiazolidinedione compound pioglitazone was supplied by Takeda Chemical Industries, Osaka, Japan. A concentrated stock of pioglitazone was prepared at 1 mM in ethanol.

### 2.2. Evaluation of proliferation of 3T3-L1 preadipocytes

Proliferation of the cells was evaluated by [$^3$H]thymidine incorporation into the cells and by quantitating cellular protein content. Cells were incubated in the presence or absence of pioglitazone for 24 h in 96-well culture plates, and then pulsed for 6 h with 1 μCi/ml of [$^3$H]thymidine (Amersham Pharmacia Biotech, Piscataway, NJ). Cells were washed with phosphate-buffered saline (PBS), trypsinized, harvested onto glass filters (Unifilter, Packard Japan, Tokyo, Japan), and the radioactivity was counted. Protein concentration of the cell homogenate was measured with the aid of Coomassie Protein Assay Reagent (Pierce, Rockford, IL).

### 2.3. Evaluation of cellular triglyceride accumulation

For quantitation of cellular triglyceride, the cells were washed with PBS, lysed with the buffer in a commercially available test kit (Sanassay TG-N; Sanko Pure Chemical, Tokyo, Japan), and disrupted by sonication. The triglyceride content of the homogenate was then determined with the above kit using the colorimetric method as per the manufacturer's protocol and expressed as mg per mg of protein.

### 2.4. Measurement of glucose transport

Basal and insulin-stimulated glucose transporting activities were estimated by measuring 2-deoxy-[$^3$H]glucose uptake (Traxinger and Marshall, 1990). 2-Deoxy-glucose is transported into the cytosol with the same kinetics as for glucose and phosphorylated by intracellular hexokinase but is not further metabolized (Betz et al., 1979). After preincubating adipocytes with Krebs–Ringer buffer in the absence or presence of 1 μg/ml insulin for 10 min at 37°C, 2-deoxy-glucose (final concentration: 100 μM) containing 2-deoxy-[$^3$H]glucose (final concentration: 0.25 μCi/ml, Amersham) was added. The uptake was stopped after 5 min by washing the cells three times with cold PBS containing 0.1 mM phloretin (Sigma), which is known to block the glucose transporter (Betz et al., 1975). Non-transporter-mediated uptake and cell surface binding measured in the presence of 1 mM phloretin were subtracted from the data. Glucose transporting activity was expressed as pmol per mg of protein per min.

### 2.5. Analysis of PPARγ mRNA

Total RNA was prepared from the cells by acid guanidium thiocyanate–phenol–chloroform extraction (Chomczynski and Sacchi, 1987) and used for analysis of PPARγ mRNA by reverse transcription-polymerase chain reaction (RT-PCR). First-strand cDNA was synthesized from total RNA by using random hexamers (Pharmacia Biotech, Buckinghamshire, England). PCR was performed with *TaKaRa Ex Taq* (Takara, Tokyo, Japan) for each set of specific primers: 5′-GGTGAAACTCTGGGAGATTC-3′ and 5′-CAACCATTGGGTCAGCTCTT-3′ for mouse PPARγ mRNA (Vidal-Puig et al., 1996), and 5′-ACAGCTGAGAGGGAAATCGTGCG-3′ and 5′-ACTTGCGCTCAGGAGGAGCAATG-3′ for the housekeeping gene β-actin mRNA (Tokunaga et al., 1986) as an internal control. The conditions for PCR were 94°C for 30 s, 54°C for 1 min and 72°C for 30 s (25 cycles) for PPARγ, and 94°C for 30 s, 68°C for 1 min and 72°C for 30 s (28 cycles) for β-actin using the TaKaRa PCR

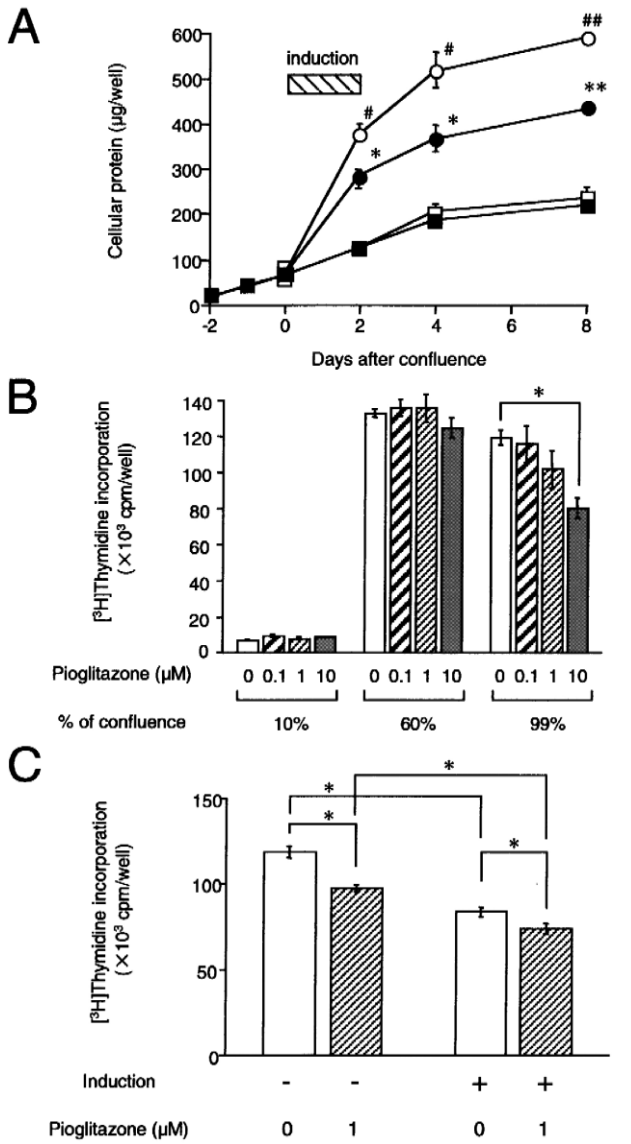


Fig. 1. Effects of pioglitazone on cellular protein content (A) and [$^3$H]thymidine incorporation into 3T3-L1 cells (B, C). (A) Confluent 3T3-L1 cells (day 0) with (○, ●) or without (□, ■) the induction of differentiation for 48 h were incubated in the presence (○, □) or absence (●, ■) of 1 μM of pioglitazone. The cellular protein content was determined as described in Section 2 and expressed as μg per well (12-well plate). Values represent means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. cells without the induction of differentiation; $\#P < 0.05$, $\#\#P < 0.01$ vs. cells incubated without pioglitazone. (B) Cell proliferation was evaluated by [$^3$H]thymidine incorporation into the cells. Preconfluent preadipocytes were incubated in the presence or absence of pioglitazone for 24 h in 96-well culture plates, and then pulsed for 6 h with 1 μCi/ml of [$^3$H]thymidine. (C) Confluent 3T3-L1 cells were incubated with or without the induction of differentiation in the presence or absence of 1 μM of pioglitazone for 24 h, and then pulsed for 6 h with 1 μCi/ml of [$^3$H]thymidine. Radioactivity incorporated into the cells was counted as described in Section 2 and expressed as cpm per well. Values represent means ± S.E.M. $^{*}P < 0.05$.

Thermal Cycler (Takara). The PCR products were separated by electrophoresis on a 1.2% agarose gel with ethidium bromide staining.

### 2.6. Statistical analysis

Values are presented as means ± S.E.M. Statistical differences between groups were evaluated with Student's *t*-test. Significance was determined at $P < 0.05$. All calculations were performed with the computer program Statview, version 4.0, for Macintosh (Abacus Concepts, Berkeley, CA).

## 3. Results

### 3.1. Effects of pioglitazone on proliferation of 3T3-L1 preadipocytes

3T3-L1 preadipocytes proliferated vigorously in the presence of DMEM supplemented with 10% fetal bovine serum. In preconfluent 3T3-L1 preadipocytes (10–60% confluent), pioglitazone did not affect cell proliferation as estimated from cellular protein content (Fig. 1A) and [$^3$H]thymidine incorporation into the cells (Fig. 1B). Morphologically, pioglitazone did not commit the preadipocytes to adipocytes before confluence. Pioglitazone tended to inhibit [$^3$H]thymidine incorporation into postconfluent 3T3-L1 preadipocytes (Fig. 1B,C). Confluent 3T3-L1 preadipocytes were efficiently committed to adipocytes by treating them with the induction medium including dexamethasone, isobutylmethylxanthine and insulin for 48 h (days 1–2). The induction of differentiation resulted in 354% increase in cellular protein content (Fig. 1A) and 30% inhibition of [$^3$H]thymidine incorporation into the cells (Fig. 1C). Pioglitazone, when added at 1 μM from the induction step, accelerated the increase in the cellular protein content by 33% (Fig. 1A) and the inhibition in

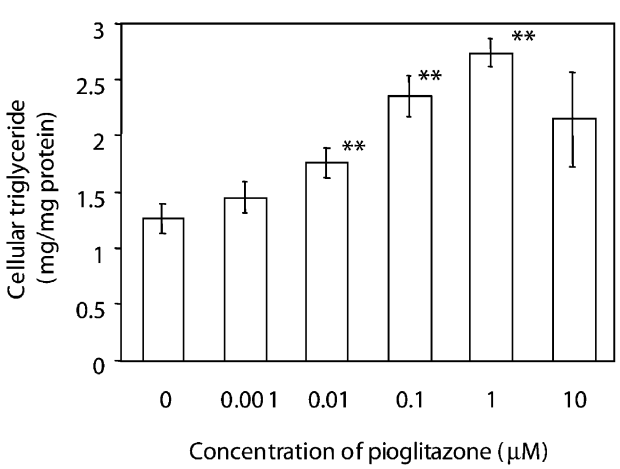


Fig. 2. Dose-dependent effect of pioglitazone on cellular triglyceride content on day 8. Various concentrations of pioglitazone were added from the induction stage. On day 8, the cellular triglyceride content was determined as described in Section 2 and expressed as mg per mg of protein. Values represent means ± S.E.M. $^{**}P < 0.01$ vs. control (0 μM).

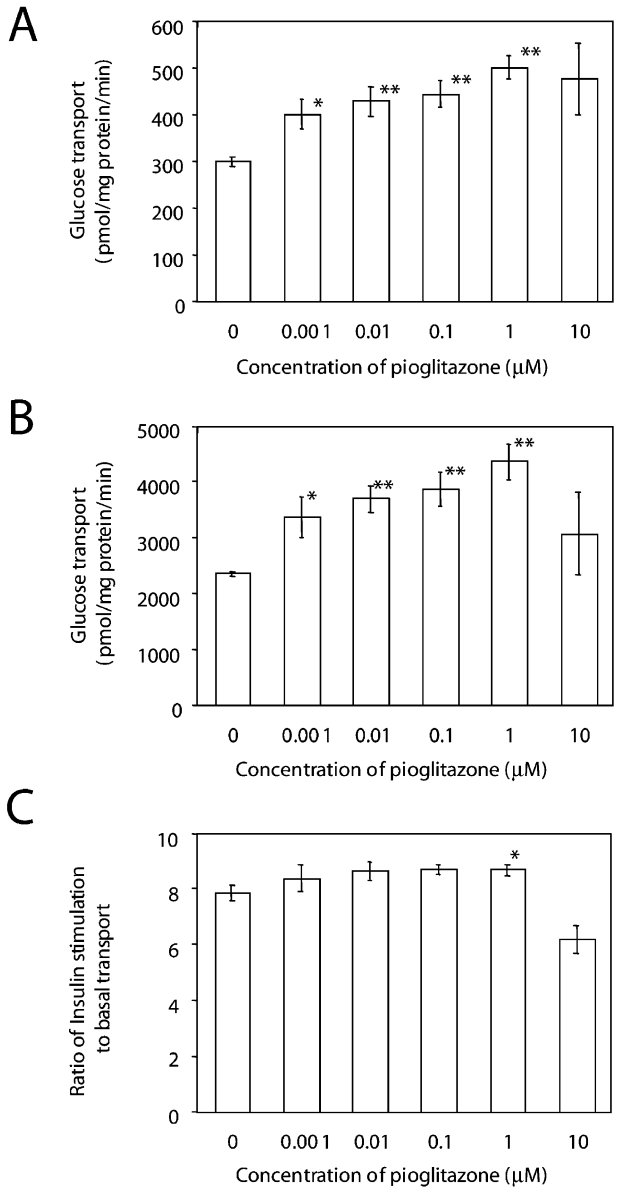


Fig. 3. Dose-dependent effect of pioglitazone on basal (A) and insulin-stimulated (B) glucose transporting activity, and on ratios of insulin stimulation to basal glucose transport (C) on day 8. Various concentrations of pioglitazone were added from the induction stage. On day 8, glucose transport into the cells was evaluated by using 2-deoxy-[$^3$H]glucose as described in Section 2 and expressed as pmol per mg of protein per min. Values represent means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control (0 μM).

[$^3$H]thymidine incorporation by 12% (Fig. 1C). Such inhibitory effect of pioglitazone on [$^3$H]thymidine incorporation was also observed in postconfluent preadipocytes without the induction (Fig. 1C).

### 3.2. Effects of pioglitazone on commitment of preadipocytes to adipocytes

After the induction of differentiation to adipocytes by treating them with the induction medium for 48 h, additional incubation with DMEM supplemented with 10% fetal bovine serum resulted in conversion of the cells into lipid accumulating adipocytes as assessed by quantitation of the cellular triglyceride content (Fig. 2). Pioglitazone, when added from the commitment stage (from day 1), dose-dependently enhanced the cellular triglyceride accumulation on day 8. Maximal triglyceride accumulation, approximately twice that of control, was observed after the addition of 1 μM of pioglitazone.

Such cellular differentiation was associated with a markedly increased capacity for glucose transport as shown in Fig. 3. Pioglitazone enhanced both basal (Fig. 3A) and insulin-stimulated (Fig. 3B) glucose transport into 3T3-L1 adipocytes on day 8. Maximal glucose transporting activity, approximately twice that of control, was observed after the addition of 1 μM of pioglitazone, thus showing a strong similarity to the effects of pioglitazone on adipocyte hypertrophy (Fig. 2). Pioglitazone did not seem to enhance the action of insulin on glucose transport because it only

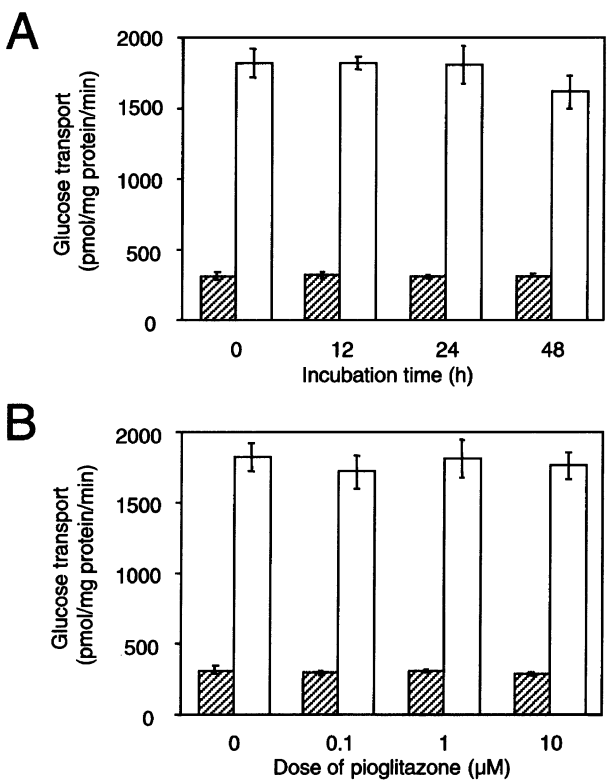


Fig. 4. Effects of pioglitazone on glucose transporting activity in mature 3T3-L1 adipocytes. Confluent 3T3-L1 preadipocytes were induced to differentiate to adipocytes by treatment with dexamethasone, isobutylmethylxanthine and insulin for 48 h. The cells were fully differentiated by additional incubation for 6 days with DMEM supplemented with 10% fetal bovine serum. After incubation in the presence of 1 μM of pioglitazone for 2 to 48 h (A) or in the presence of 0.1 to 10 μM of pioglitazone for 48 h (B), basal (hatched bar) and insulin-stimulated (open bar) glucose transporting activities were evaluated by using 2-deoxy-[$^3$H]glucose as described in Section 2 and expressed as pmol per mg of protein per min. Values represent means ± S.E.M.

slightly increased the ratio of insulin stimulation to basal glucose transport (Fig. 3C).

To clarify the specific effect of pioglitazone on the commitment stage, we examined contact inhibition-induced cell differentiation in the absence of the induction medium. When 3T3-L1 preadipocytes reached confluence, some proportion of the cells spontaneously underwent adipose conversion and accumulated triglyceride. Pioglitazone enhanced such contact inhibition-induced adipocyte differentiation in a dose-dependent manner from 0.01 to 1 μM (data not shown), suggesting that it enhances the commitment stage of adipocyte differentiation without interacting with other differentiation-inducing factors.

### 3.3. Effects of pioglitazone on mature 3T3-L1 adipocytes

To investigate whether a thiazolidinedione directly affects glucose transporting activity and insulin sensitivity, we tested the effect of pioglitazone on mature adipocytes. In fully differentiated mature 3T3-L1 adipocytes at day 8, pioglitazone at 1 μM enhanced neither basal nor insulin-stimulated glucose transporting activity during 2–48-h incubation (Fig. 4A). Similarly, pioglitazone added at doses from 0.1 to 10 μM for 48-h incubation did not affect the glucose transporting activity (Fig. 4B).

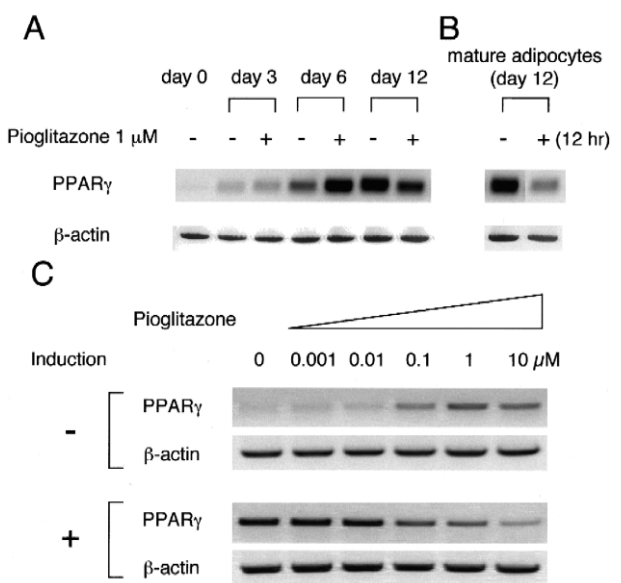


Fig. 5. PPARγ mRNA expression during adipocyte differentiation and its dual mode regulation by pioglitazone. Confluent 3T3-L1 cells were induced to differentiate and incubated in the presence (+) or absence (−) of 1 μM of pioglitazone for 12 days. Semi-quantitative RT-PCR for PPARγ mRNA and β-actin mRNA were performed as described in Section 2 during the course of adipocyte differentiation (A), and after 12 h incubation with pioglitazone in mature adipocytes at day 12 (B). (C) Confluent 3T3-L1 cells with or without the induction of differentiation for 48 h were incubated in the presence of various concentrations of pioglitazone (0.001–10 μM) for 12 days. Semi-quantitative RT-PCR for PPARγ mRNA and β-actin mRNA were performed at day 12. The PCR products were separated by electrophoresis on a 1.2% agarose gel.

### 3.4. Dual mode regulation of PPARγ mRNA levels by pioglitazone

PPARγ mRNA levels estimated by a semi-quantitative RT-PCR method increased during the course of adipocyte differentiation (Fig. 5A). Pioglitazone, when added from the induction stage, up-regulated PPARγ mRNA levels initially and down-regulated them at the later phase during adipocyte differentiation. PPARγ mRNA levels in mature adipocytes were also down-regulated by 12-h incubation with pioglitazone (Fig. 5B). Pioglitazone up-regulated PPARγ mRNA levels in postconfluent preadipocytes without the induction (Fig. 5C). Such dual mode regulations of pioglitazone on PPARγ mRNA expression were dose-dependent (Fig. 5C).

## 4. Discussion

The fact that forced expression of the PPARγ gene converts fibroblasts (Tontonoz et al., 1994b) and even myoblasts (Hu et al., 1995) to adipocytes clearly indicates that PPARγ plays a critical role in determining whether cells differentiate to adipocytes. However, after the commitment of cells to adipocytes, the role of PPARγ and its ligands, the thiazolidinediones, in the maintenance of hyperplasia and hypertrophy of adipocytes is still unclear. Cultured 3T3-L1 preadipocytes isolated from the established mouse fibroblast line 3T3 undergo adipose conversion and accumulate triglyceride when they reach a state of confluence (Green and Kehinde, 1973). They are known to be useful for the study of the basis for cellular lipid accumulation and obesity since the lipid accumulation responds to lipogenic or lipolytic agents (Green and Kehinde, 1975). In vivo, adipocyte precursor cells including preadipocytes are thought to be present throughout life (Ailhaud, 1992). Initiation and perpetuation of obesity occur not only as a result of hypertrophy of adipose tissue but also of transformation of preadipocytes into adipocytes triggered by adipose tissue hyperplasia (Caro et al., 1989). The processes of both hypertrophy and hyperplasia can be investigated in cultured 3T3-L1 preadipocytes, whereas only the process of hypertrophy of already differentiated adipocytes can be examined in the primary culture of isolated adipocytes. Therefore, 3T3-L1 preadipocytes are thought to constitute a more suitable model than isolated adipocytes for analyzing the proliferation stage, commitment of preadipocytes to adipocyte (induction of differentiation), and hypertrophy (lipid accumulation) during adipocyte differentiation.

In this study, we used a cultured 3T3-L1 cell system to analyze the effects of a PPARγ ligand, pioglitazone, on each stage during adipocyte differentiation. Only one study has reported the inhibitory effect of thiazolidinediones on

the proliferation of fibroblasts (Sparks et al., 1991). However, we could not identify any specific effects of pioglitazone on the proliferation of preadipocytes before confluence. Our finding seems to be reasonable because 3T3-L1 preadipocytes did not express PPARγ mRNA as a target of pioglitazone before the induction of differentiation.

In 3T3-L1 cells, proliferation and differentiation are not thought to occur simultaneously in the course of cell differentiation because differentiation of preadipocytes to adipocytes occurs after confluence followed by growth arrest (Green and Meuth, 1974; Green and Kehinde, 1975). Our findings that decreased [$^3$H]thymidine incorporation into the cells and increased cellular protein content after the induction of differentiation with dexamethasone, isobutylmethylxanthine and insulin are thought to be consistent with the concept. Cells arrest the growth, increase biosynthesis of the proteins required for differentiation, and activate cellular metabolism. Actually, metabolic activity as reflected by LDH content and dehydrogenase activity that produces NADPH and NADH increased during the induction stage of differentiation (Takamura, unpublished data). We found that pioglitazone enhances decreased [$^3$H]thymidine incorporation into the cells and increased cellular protein content during the induction of differentiation. Thus, pioglitazone promotes adipocyte differentiation by accelerating the changes during the induction. Taking into consideration the fact that pioglitazone did not affect proliferation of preadipocytes, which do not express PPARγ mRNA, the promoting effect of pioglitazone on growth arrest and protein biosynthesis might be mediated by PPARγ. How pioglitazone affects the cell cycle status remains to be investigated.

In the commitment stage, pioglitazone stimulated adipocyte differentiation as reflected by increased cellular triglyceride content and enhanced glucose transport. Pioglitazone itself induced commitment of preadipocytes to adipocytes because it enhanced spontaneous adipocyte differentiation after confluence in the absence of the induction medium. Pioglitazone, when added from the induction stage, enhanced both basal and insulin-stimulated glucose transport without affecting the ratio of insulin stimulation to basal glucose transporting activity. This finding suggests that pioglitazone increases the activity and/or number of functional glucose transporters per cell. In fact, Tafuri (1996) observed that another thiazolidinedione compound, troglitazone, increased glucose transporter (Glut)1 protein levels in 3T3-L1 adipocytes without affecting Glut4 translocation. Such enhancement of glucose transporting activity by pioglitazone is thought to result not from direct activation of glucose transporter per se but from enhanced differentiation because, in fully differentiated mature 3T3-L1 adipocytes, pioglitazone could not stimulate either basal or insulin-stimulated glucose transport into the cells. Although an initial study has suggested that thiazolidinedione may act at a post-insulin receptor step (Kobayashi et al., 1983), this hypothesis is still controversial (Sparks et al., 1991; Tafuri, 1996; Camp et al., 1999). Kletzien et al. (1992) also observed that acute (4 h) treatment with pioglitazone had no effect on glucose transport in mature adipocytes. Whether such a weak effect of thiazolidinediones on mature adipocytes is related to the finding that thiazolidinediones down-regulate the expression of PPARγ in mature adipocytes remains to be investigated. Considering the failure of pioglitazone to enhance the ratio of insulin stimulation to basal glucose transporting activity as well as insulin action in mature adipocytes, the insulin sensitizing effect of thiazolidinediones (Iwamoto et al., 1991; Hofmann and Colca, 1992), at least in adipose tissues, seems to be mainly due to differentiation-dependent enhancement of basal glucose transport and not due to enhancement of insulin actions including Glut4 translocation.

As reported previously (Tontonoz et al., 1994a,b), PPARγ mRNA levels increased during the course of adipocyte differentiation. In postconfluent preadipocytes and in early phase adipocytes after the induction, pioglitazone up-regulated PPARγ mRNA levels concordantly with enhancement of adipocyte differentiation. However, in later phase or in mature adipocytes, pioglitazone down-regulated them while it still enhanced adipocyte differentiation. Previous reports indicating inhibition of PPARγ mRNA expression by the specific ligands (Camp et al., 1999; Hauser et al., 2000) seem to be relevant to only mature phase of adipocytes. The dual mode regulation of PPARγ mRNA expression by a thiazolidinedione, which we observed in the time course study for the first time, should be taken into consideration in the use of this drug as an insulin-sensitizing agent for type 2 diabetic patients. The molecular switch from up-regulation to down-regulation of PPARγ mRNA expression by a thiazolidinedione should be elucidated.

In summary, a PPARγ agonist, pioglitazone, affected the early stage of adipocyte differentiation and enhanced growth arrest, protein synthesis, commitment and hypertrophy of 3T3-L1 cells. Pioglitazone, only when added from the induction stage, enhanced basal glucose transporting activity without changing insulin action, whereas it failed to stimulate glucose transporting activity in mature adipocytes. Finally, pioglitazone regulated PPARγ mRNA expression in a unique dual mode manner. These new findings about the stage-specific effects of a thiazolidinedione may help us to understand better the effects of a thiazolidinedione on diabetic patients with obesity and insulin resistance, and should be of help for further investigations into how PPARγ signaling coordinates proliferation, differentiation and insulin actions in adipocytes.

## References

Ailhaud, G., 1992. Some new aspects on adipose tissue development. Diabetes Metab. Rev. 8, 3–7.

Beck-Nielsen, H., Hother-Nielsen, O., 1996. Obesity in non-insulin-dependent diabetes mellitus. In: LeRoith, D., Taylor, S.I., Olefsky, J.M. (Eds.), Diabetes Mellitus. A Fundamental and Clinical Text. Lippincott-Raven, Philadelphia, pp. 475–484.

Berger, J., Bailey, P., Biswas, C., Cullinan, C.A., Doebber, T.W., Hayes, N.S., Saperstein, R., Smith, R.G., Leibowits, M.D., 1996. Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-γ, binding and activation correlate with antidiabetic actions in db/db mice. Endocrinology 137, 4189–4195.

Betz, A.L., Drewes, L.R., Gilboe, D.D., 1975. Inhibition of glucose transport into brain by phlorizin, phloretin and glucose analogues. Biochim. Biophys. Acta 406, 505–515.

Betz, A.L., Csejtey, J., Goldstein, G.W., 1979. Hexose transport and phosphorylation by capillaries isolated from rat brain. Am. J. Physiol. 236, C96–C102.

Camp, H.S., Whitton, A.L., Tafuri, S.R., 1999. PPARγ activators down-regulate the expression of PPARγ in 3T3-L1 adipocytes. FEBS Lett. 186, 186–190.

Caro, J., Dohm, L.G., Pories, W.J., Sinha, M.K., 1989. Cellular alterations in liver, skeletal muscle, and adipose tissue responsible for insulin resistance in obesity and type II diabetes. Diabetes Metab. Rev. 5, 665–689.

Chomczynski, P., Sacchi, N., 1987. Single-step isolation by acid guanidium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Forman, B.M., Tontonoz, P., Chen, J., Brun, R.P., Spiegelman, B.M., Evans, R.M., 1995. 15-Deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ is a ligand for adipocyte determination factor PPARγ. Cell 83, 803–812.

Green, H., Kehinde, O., 1973. Subline of mouse 3T3 cells that accumulate lipid. Cell 1, 113–116.

Green, H., Kehinde, O., 1975. An established preadipose cell line and its differentiation in culture: II. Factors affecting the adipose conversion. Cell 5, 19–27.

Green, H., Meuth, M., 1974. An established pre-adipose cell line and its differentiation in culture. Cell 3, 127–133.

Hauser, S., Adelmant, G., Sarraf, P., Wright, H.M., Mueller, E., Spiegelman, B.M., 2000. Degradation of the peroxisome proliferator-activated receptor γ is linked to ligand-dependent activation. J. Biol. Chem. 275, 18527–18533.

Hofmann, C.A., Colca, J.R., 1992. New oral thiazolidinedione antidiabetic agents act as insulin sensitizers. Diabetes Care 15, 1075–1078.

Hu, E., Tontonoz, P., Spiegelman, B.M., 1995. Transdifferentiation of myoblasts by the adipogenic transcription factors PPARγ and C/EBPα. Proc. Natl. Acad. Sci. U. S. A. 92, 9856–9860.

Iwamoto, Y., Kuzuya, T., Matsuda, A., Awata, T., Kumakura, S., Inooka, G., Shiraishi, I., 1991. Effect of new antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. Diabetes Care 14, 1083–1086.

Kahn, S.E., Porte, J.D., 1997. The pathophysiology of type II (noninsulin-dependent diabetes mellitus, implication for treatment. In: Porte, J.D., Sherwin, R.S. (Eds.), Ellenberg and Rifkin's Diabetes Mellitus. Appleton & Lange, Stamford, pp. 487–512.

Kletzien, R.F., Clarke, S.D., Urlich, R.G., 1992. Enhancement of adipocyte differentiation by an insulin-sensitizing agent. Mol. Pharmacol. 41, 393–398.

Kliewer, S.A., Lenhard, J.M., Willson, T.M., Patel, I., Morris, D.C., Lehmann, J.M., 1995. A prostaglandin $J_2$ metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell 83, 813–819.

Kobayashi, M., Iwasaki, M., Ohgaku, S., Maegawa, H., Watanabe, N., Shigeta, Y., 1983. A new potentiator of insulin action post-receptor activation in vitro. FEBS Lett. 163, 50–53.

Sandouk, T., Reda, D., Hofmann, C., 1993. Antidiabetic agent pioglitazone enhances adipocyte differentiation of 3T3-F442A cells. Am. J. Physiol. 264, C1600–C1608.

Sparks, R.L., Strauss, E.E., Zygmunt, A.I., Phelan, T.E., 1991. Antidiabetic AD4743 enhances adipocyte differentiation of 3T3 T mesenchymal stem cells. J. Cell. Physiol. 146, 101–109.

Tafuri, S.R., 1996. Troglitazone enhances differentiation, basal glucose uptake, and Glut1 protein levels in 3T3-L1 adipocytes. Endocrinology 137, 4706–4712.

Tokunaga, K., Taniguchi, H., Yoda, K., Shimizu, M., Sakiyama, S., 1986. Nucleotide sequence of a full-length cDNA for mouse cytoskeletal beta-actin mRNA. Nucleic Acids Res. 14, 2829.

Tontonoz, P., Hu, E., Graves, R.A., Budavari, A.I., Spiegelman, B.M., 1994a. mPPARγ2, tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8, 1224–1234.

Tontonoz, P., Hu, E., Spiegelman, B.M., 1994b. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell 79, 1147–1156.

Traxinger, R.R., Marshall, S., 1990. Suitability of 2-deoxyglucose for measuring initial rates of glucose uptake in isolated adipocytes. Biochem. Int. 22, 607–615.

Vidal-Puig, A., Jimenez-Linan, M., Lowell, B.B., Hamann, A., Hu, E., Spiegelman, B., Flier, J.S., Moller, D.E., 1996. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. J. Clin. Invest. 97, 2553–2561.

Willson, T.M., Cobb, J.E., Cowan, D.J., Wiethe, R.W., Correa, I.D., Prakash, S.R., Beck, K.D., Moore, L.B., Kliewer, S.A., Lehmann, J.M., 1996. The structure–activity relationship between peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. J. Med. Chem. 39, 665–668.