Transdifferentiation of porcine satellite cells to adipoblasts with ciglitizone


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ABSTRACT: Ciglitizone, a class of thiazolidinediones, acts as a potent activator of the adipose differentiation program in established preadipose cell lines. Thiazolidinediones have also been investigated in diabetic patients and have been reported to act as peroxisome proliferator-activated receptor-γ ligands. Intramuscular adipogenesis or marbling through transdifferentiation of satellite cells in cattle was successfully conducted earlier. In this report, the effects of ciglitizone on the differentiation pathway of porcine myogenic satellite cells was investigated. Semitendinosus muscle was aseptically taken from 10-d-old piglets under general anesthesia, and porcine satellite cells were obtained and grown to near confluence. Postconfluent cells (d 0) were further cultured in differentiation medium containing an adipogenic mixture plus ciglitizone (10 μM) for 48 h. From d 2 onward, the cells were cultured only in the presence of ciglitizone until d 10. Controls were cultured in differentiation medium only. Exposure of porcine satellite cells to the adipogenic mixture plus ciglitizone generated lipid droplets on d 2, and subsequently, exposure of cells to ciglitizone alone helped in cytoplasmic lipid filling, providing them with the acquisition of adipocyte morphology. An increase (P < 0.05) in the fusion (structures containing 2 to 3 nuclei) of satellite cells was observed, and myosin heavy chain appeared with greater intensity (immunohistochemistry) in the control group from d 2 onward. Adipocyte-specific transcriptional factors (i.e., CCAAT/enhancer binding protein-α and peroxisome proliferator-activated receptor-γ) were predominant during transdifferentiation and were observed with immunohistochemistry, Western blot (∼47.2 and ∼60.4 kDa, respectively), and real-time PCR. Ciglitizone appeared to convert the differentiation pathway of satellite cells into that of adipoblasts.

Key words: adipogenesis, ciglitizone, CCAAT/enhancer binding protein-α, myogenesis, peroxisome proliferator-activated receptor-γ

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doi:10.2527/jas.2006-524

INTRODUCTION

Interest in adipocytes located within muscle has developed because of the demand for increased marbling fat and meat quality, particularly in pigs (Gondret and Lebret, 2002; Van Barneveld, 2003). However, little is known about the growth and development of intramuscular adipocytes or the regulation of intramuscular adipogenesis. This limits the development of methods to regulate the growth of these cells and to improve the marbling fat content of meat animals (Poulos and Hausman, 2005). Myoblasts and adipocytes arise from the same germ layer of the embryo, the mesoderm. Therefore, Li et al. (2005) hypothesized that it is possible to induce a direct conversion of myoblasts to adipocytes. Using a tissue model of myogenesis (G8 myoblasts), they demonstrated that these cells can differentiate spontaneously into myotubes when cultured in medium containing fetal calf serum. Subsequently, studies revealed that the expression of transcription factors such as CCAAT/enhancer binding protein-α (C/EBP-α) and peroxisome proliferator-activated receptor-γ (PPAR-γ) during the differentiation of G8 myoblasts can suppress the muscle-specific transcription factors (Hu et al., 1995). In addition, upregulation of PPAR-γ and subsequent activation of C/EBP-α, -β, and -γ can stimulate adipogenesis in fibroblasts (NIH3T3) in the presence of PPAR-γ li-
gand (Wu et al., 1996). Thiazolidinediones (TZD), synthetic activators of PPAR-γ, are considered potent stimulators of adipogenesis and are presently being used clinically to reduce hyperglycemia in type 2 diabetes (Rosen et al., 2002; Hammarstedt and Smith, 2003).

Based on the above hypothesis and because of a paucity of literature concerning transdifferentiation of porcine satellite cells, this study was conducted to determine the role of cigitizone, a class of TZD, in the transdifferentiation of porcine satellite cells to adipoblasts in vitro and to determine the possibility for increasing intramuscular marbling in pigs in vivo.

MATERIAL AND METHODS

Materials

Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, horse serum, penicillin (100 IU/mL), and streptomycin (100 mg/mL) were obtained from American Type Culture Collection (ATCC, Manassas, VA). Pig skin gelatin, dexamethasone, insulin, isobutylmethylxanthine, biotin, acetic acid, pantothenic acid, ascorbic acid, cigitizone, and protease (Pronase (P5147)) were obtained from Sigma Aldrich (Saint Louis, MO). Porcine satellite cells were isolated from piglets.

Porcine Satellite Cell Isolation

Animal experimentation was approved by the Animal Care and Concern Committee of the National Livestock Research Institute, Suwon, Korea. Harvesting of muscle from 9 piglets (Landrace × Duroc; 10-d-old) was done aseptically under general anesthesia (xylazine, 0.05 mg/kg, and ketamine, 2 mg/kg i.m.; Yuhan Co., Seoul, Korea). A portion of the right and left semitendinosus muscles (weighing approximately 2 g each) was excised, and the surgical site was closed with suture. Postoperative care was routinely followed, and sutures were removed on postoperative d 8.

Satellite cells were isolated from semitendinosus muscle as described by Doumit and Merkel (1992). Briefly, the muscles were excised, trimmed of visible connective tissue, and minced with fine sharp scissors into dishes. Minced muscle was incubated for 50 min at 37°C in a solution of 0.8 mg of protease/mL in PBS (2 parts muscle:3 parts protease solution, vol/vol). After enzymatic digestion, the cells were separated from tissue fragments by repeated centrifugation at 1,200 and 300 × g for 15 and 5 min, respectively, followed by filtration using 50-μm-mesh nylon cloth (Mijin Co., Gy-eonggi-do, Korea). Thereafter, the filtrate was further centrifuged at 1,200 × g for 15 min and the pellet was collected and reconstituted with DMEM at a ratio of 1:1 (g/mL). Primary muscle cell isolates were stored in liquid nitrogen until used.

Cell Culture

Porcine muscle cell isolates (satellite cells) were plated on pig skin gelatin-coated dishes at a density of 2 × 10³ cells/cm² and grown in proliferation medium consisting of DMEM supplemented with 10% fetal bovine serum and 1.1% penicillin/streptomycin. Cells were kept at 37°C in a humidified incubator with 5% CO₂. The medium was changed every day, and near (i.e., almost 100%) confluence was reached in 5 d. Control experiments were performed to exclude any effects of dimethylsulfoxide.

Subsequently, confluent cells were selected for the transdifferentiation study. Postconfluent cells (d 0) were further cultured in differentiation medium containing DMEM + 2% horse serum + 1.1% penicillin/streptomycin with an adipogenic mixture consisting of ascorbic acid (20 μL), biotin, (10 μL), acetic acid (50 μL), pantothenic acid (5 μL), dexamethazone (10 μL), isobutylmethylxanthine (100 μL), and insulin (10 μL) in 10 mL of differentiation medium, plus cigitizone (10 μM) in dimethylsulfoxide for 48 h. From d 2 onward, the cells were cultured in differentiation medium containing cigitizone (10 μM) alone, and the medium was changed every 48 h until d 10. Controls were cultured only in differentiation medium, and the entire experiment was performed in triplicate.

Fusion Index

Samples were collected at different 48-h intervals as outlined above. Cells were fixed with methanol and stained with hematoxlin (Sigma Aldrich). The total number of nuclei and the number of nuclei incorporated into myotubes were scored in 10 microscopic fields (400×)/dish chosen at random. The fusion index was calculated as the percentage of nuclei incorporated into the myotubes relative to the total number of nuclei. The structures containing at least 3 nuclei were scored as representing myotubes (Dodson and Mathison, 1988).

Adipocyte Stain Uptake and Oil Red O Staining

Measurements of stain retained by the adipoblast were done to determine the increase in the number or size of the adipoblasts. Formalin-fixed cells were washed with 60% isopropanol and air-dried. Cells were stained with freshly diluted oil red O (6 parts oil red O stock solution (Sigma Aldrich) and 4 parts distilled H₂O; the solution was 0.5% oil red O in isopropanol) for 10 min, and excess stain was removed using a small transfer pipette and 4 to 5 washings with distilled water. Thereafter, the cells were dried, and the remaining oil red O was eluted by adding 100% isopropanol and incubating for 10 min. Optical density of the eluted stain was measured at 500 nm (eluted stain index) over the 100% isopropanol as a blank in 1.5-mL Eppendorf vials (McNeil, 2005).
Oil red O staining was performed following the procedure described by Green and Kehinde (1974) with minor modifications. In brief, formalin-fixed cells were washed twice with PBS for 15 min and stained with freshly diluted oil red O for 1 h. The stain was then removed and the cells were washed twice with distilled water, with or without counterstain (Gill’s hematoxylin for 5 min) and then examined under a light microscope (20× magnification).

Adipocyte Index

The total number of adipocytes (distinguished morphologically by rounding and staining characteristics) and the number of myonuclei were scored in 3 randomly chosen microscopic fields at a magnification of 400×. The adipocyte index was calculated as a percentage of the total number of myonuclei that were identifiable as adipocytes (oil red O-positive upon microscopic inspection) in 3 randomly chosen microscopic fields.

Immunohistochemistry

The expression of myogenic and adipogenic proteins of differentiating porcine satellite cells was determined by indirect immunostaining (Michal et al., 2002). Samples were collected in triplicate at each of the 48-h incubation intervals. The cells were initially washed with PBS and then fixed with 100% methanol. To eliminate nonspecific binding of primary antibodies, 50 μL of 2% normal horse serum (ATCC) in PBS was added as a blocker and the plates were incubated overnight at 4°C. The cells were washed with PBS, and the primary antibodies to paired box transcription factor-7 [Pax-7; 1:100; antimouse monoclonal immunoglobulin (Ig) G1, R&D Systems, Minneapolis, MN], C/EBP-α (1:200; rabbit monoclonal IgG, Affinity BioReagents, Golden, CO), antomyosin heavy chain (1:400; antimouse monoclonal IgG1, Sigma Aldrich), or PPAR-γ2 (1:200; rabbit monoclonal IgG1, Sigma Aldrich) were added. All primary antibodies were diluted in PBS that contained 5% nonfat milk (Bio-Rad, Hercules, CA). After incubation at room temperature for 1 h, the plates were washed with PBS + 0.1% Tween 20 (PBST; Bio-Rad). The secondary antibody, biotinylated goat antimouse IgG (1:500 dilution; Biomedia, Foster City, CA), was added to all of the samples and incubated for 30 min at 37°C. Unbound secondary antibodies were washed from the plates with PBST and PBS. Avidin biotinylated horseradish peroxidase complex (Pierce, Rockford; IL) was added to each plate and incubated for 30 min at 37°C. After washing excess reagents from the plates with PBS, the peroxidase activity was developed using 3,3′-diaminobenzidine (Sigma Aldrich), and the cells were then examined by light microscopy (20×).

Western Blot

Postconfluent porcine satellite cell samples on d 0, 2, 4, 6, 8, and 10 were dislodged from the plates with a cell scraper and collected. The protein concentration was analyzed using Proprep (iNtRON Biotechnology, Seongnam-Si, Gyeonggi-do, Korea). The sample was lysed by boiling for 5 min at 100°C using Proprep. The lysate mixed with loading buffer was loaded onto a 15% SDS gel. Proteins were separated by electrophoresis at 80 V for 20 min and 120 V for 100 min using Tris-glycine running buffer (0.025 M Tris base, 0.192 M glycine, and 0.1% SDS, pH 8.3). Prestained molecular weight markers (Kaleidoscope, Invitrogen, Carlsbad, CA) were used to determine the molecular weight of proteins.

The gel was subsequently transferred onto a nitrocellulose membrane (Nitopure, MSI, Westboro, MA) by electrophoresis (overnight at 100 V, 4°C) using a transfer buffer that contained 25 mM Tris base, 192 mM glycine, and 10% methanol, pH 8.1 to 8.3. The membrane was washed with PBST and blocked with 3% blocker for 1 h at room temperature. Antibodies to C/EBP-α and PPAR-γ, diluted at 1:1,000 in PBST containing 1% BSA, were incubated with the membrane for 1 h at room temperature. Thereafter, the samples were incubated for 1 h with biotinylated goat antimouse antibodies diluted at 1:3,000 in PBST containing 1% BSA. Finally, horseradish peroxidase activity was detected by enhanced substrate chemiluminescence (Bio-Rad), as described by the manufacturer, and subsequently examined.

Quantitative Real-Time PCR for the Adipocyte-Specific Cell Type Marker Genes

Total RNA samples were prepared from porcine satellite cells for the real-time PCR analysis using Trizol reagent (Life Technologies Inc., Grand Island, NY) according to the manufacturer’s instructions and quantified by absorbance at 260 nm. First-strand cDNA synthesis was performed for 1.5 h at 42°C in a final reaction volume of 20 μL containing purified total RNA, 3 μg; 5× reaction buffer, 2.5 mM; deoxy nucleotide 5′-triphosphate, 2.5 mM; 10 μM cDNA synthesis primer dT-ACP1; RNAase inhibitor (40 U/μL, Promega, Madison, WI) and superscript II reverse transcription (200 U/μL, Invitrogen). The synthesized first-strand cDNA samples were diluted by the addition of 100 μL of ultrapureified water.

To characterize the degree of transdifferentiation of porcine satellite cells to adipocytes, 2 adipocyte-specific marker genes were selected, C/EBPα and PPARγ. The mRNA expression levels for these markers and selected marker genes were analyzed by quantitative real-time PCR with specific primers. The sequences of the primers were as follows: C/EBP-α (accession #AF103944): forward 5′-TGGACAAGAACA-3′; reverse 5′-TTGTCACTGGTCAGCTCCAGC-3′; PPAR-γ (accession no. NM214379): forward 5′-ACTGTCGTTTCCAGAGTGCG-3′; reverse 5′-CACAGACTCTGGGTTGTC-3′; and 18S rRNA gene (accession no. NR002170): forward 5′-AGTCGCCATCGTTATGGTC-3′; reverse 5′-ACCJC
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GGTTCTATTTTGTTG-3′. The 18S rRNA gene was used as an internal control. Real-time reverse-transcription PCR amplification mixtures (20 μL) contained 3 μL of cDNA, 2× SYBR Green I Master Mix (Qiagen, GmbH, Hilden, Germany), and 10 μM each of the forward and reverse primers. The real-time PCR reactions began at 95°C for 15 min for predenaturation, and the condition was set at 95°C for 10 s, 56°C for 20 s, and 72°C for 30 s for 40 cycles. The PCR was conducted in a 7500 system (Applied Biosystems, Foster City, CA). The relative quantification of the target gene expression was evaluated using the ∆∆CT method [average ∆CT (sample of interest) – average ∆CT (reference sample)]. The ∆CT (CT (target gene) – CT (endogenous reference gene)) value was determined by subtracting the 18S ribosomal CT value for each sample from the target CT value. The ∆∆CT was calculated by subtracting the early stage of adipogenesis (d 0) ∆CT value from the later stages of adipogenesis (d 2 to 10) ∆CT value. The fold change in relative gene expression of the target was determined by calculating the 2−∆∆CT (Pfaffl, 2001).

Statistical Analysis

The treatment at different time intervals was analyzed by ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Significant differences were detected (P < 0.05) by Duncan’s multiple range test using SAS (SAS Inst. Inc.).

RESULTS

Porcine satellite cells displayed an almost complete morphology of multinucleated myotubes (Figure 1) when postconfluent cells were maintained in differentiation medium containing Dulbecco’s modified Eagle’s medium (DMEM) + 2% horse serum (HS) + 1.1% penicillin and streptomycin. Myogenesis was apparently present from d 2 onward. The fusion (3 or more nuclei within the membrane) rate increased (P < 0.05) from d 2 through the observation period compared with d 0 (Figure 2).

Exposure of postconfluent cells to the differentiation medium with added adipogenic mixture plus ciglitzone on d 2 and ciglitzone alone thereafter led to an absolute change of the myotube formation vanishing, with a drastic increase in the number of small cells containing lipid droplets and formation of adipoblasts. Completely round and oil red O-positive cells were considered adipoblasts and were counted in 3 randomly chosen fields at 400×. The total number of adipoblasts was counted over the satellite cell nuclei and expressed as a percentage. The adipoblast count increased (P < 0.05) from d 2 onward until d 6 during differentiation (Figure 2). Microscopically, the size and number of the transformed cells appeared to increase (P < 0.05) with a longer incubation time. The increase in the number and size of the adipoblasts was confirmed by measuring the adipocyte stain uptake, which was determined spectrophotometrically at 500 nm. During differentiation, stain uptake by the adipocytes showed an increase (P < 0.05) soon after exposure of the cells to the adipogenic mixture plus ciglitzone until 48 h of incubation and subsequently with ciglitzone alone until the end of the experiment (Figure 2).

Oil red O staining was performed to confirm the effect of ciglitzone on the conversion of satellite cells to adipoblasts (Figure 3). Porcine satellite cells, during differentiation in the presence of the adipogenic mixture plus ciglitzone for 48 h and subsequently with ciglitzone alone until the end of the experiment, started to swell and acquired a morphology similar to adipocytes. The cells, which assumed a large, round shape from d 2 of treatment, stained intensely red with oil red O, confirming these cells as differentiated adipocytes.

Incubation of porcine satellite cells with only secondary antibody as a negative control yielded minimal background. Intense staining was observed when the cells were incubated with anti-Pax-7, the monoclonal
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Figure 2. Porcine satellite cells during differentiation. Postconfluent cells cultured in differentiation medium fused to form myotubes (fusion index), whereas when the cells were cultured in differentiation medium with the adipogenic mixture plus ciglitizone for 48 h and later with ciglitizone alone, they showed differentiation to adipoblasts (adipoblast index). An increase in the optical density of eluted oil red O from stained adipoblasts (eluted stain index) represents an increase in the number or the size of adipoblasts during differentiation. Bars are means ± SE and represent a total of 9 piglets. *Indicates a significant difference (P < 0.05) within an item compared with the undifferentiated (d 0) cells.

Antibodies to cellular proteins used for immunohistochemistry were also tested on electrophoresed lysates of porcine satellite cells during differentiation. The C/EBP-α was detected as a band of ∼47.2 kDa after incubation with anti-C/EBP-α. Expression of PPAR-γ was quite apparent in the lysates in the ciglitizone-treated groups and resulted in a band size of ∼60.4 kDa (Figure 5).

The fold change in the relative gene expression pattern of C/EBP-α during the differentiation program was found to have an increasing trend (P < 0.05) from d 6 onward, consistent with the effects of ciglitizone. The relative gene expression pattern of PPAR-γ (P < 0.05) increased following exposure of the cells to the adipogenic mixture plus ciglitizone on d 2 and subsequently to ciglitizone alone. However, expression of PPAR-γ decreased (P < 0.05) from d 6 onward (Figure 6).

DISCUSSION

Restriction of cell lineages during embryonic development in lesser developed animals explains the existence of exclusive pathways and generates the possibility of “transdifferentiation” from one cell type to another (Eguchi and Kodama, 1993). Transdifferentiation has also been observed when cells are transplanted to ectopic sites where the environmental cues impose new constraints (Baron, 1993; Le Douarin and Ziller, 1993). Increased amounts of intramuscular adipose tissue are desirable in meat animals and are of interest to meat scientists. Raising lipid synthesis through feed is not economical (Novakofski, 2004). Therefore, assuming that myoblasts and adipoblasts have similar germinal origins (Li et al., 2005), the possibility of transdifferentiation was hypothesized and tested to generate marbling or intramuscular adipogenesis.

The current study was based on the observations that myogenic satellite cells differentiate into myoblasts (Hu et al., 1995; Lee et al., 2000), but the process was applied to adipogenesis by the use of ciglitizone. The morphological changes in porcine satellite cells were investigated after culturing postconfluent cells in differentiation medium with the adipogenic mixture plus ciglitizone for 48 h and subsequently in ciglitizone alone from d 2 onward. Postconfluent porcine satellite cells grown only in differentiation medium appeared to form myotubes, and no restriction to their committed myogenic lineage was observed. Similar observations were reported when C2C12 myoblasts and primary myoblasts (satellite cells) from mice were grown in DMEM with 5% horse serum, which strictly formed myocytes (Asakura et al., 2001).

Postconfluent porcine satellite cells acquired histological similarity to adipocytes (lipid-filled cytoplasms and eccentric nuclei) when grown in the presence of...
the adipogenic mixture plus ciglitizone for 48 h and subsequently with ciglitizone alone. The adipoblasts formed were positive for oil red O staining, in agreement with the findings of Tamori et al. (2002), who demonstrated the overexpression of PPAR-γ in the presence of ciglitizone (TZD-PPAR-γ ligand) in mature 3T3L1 adipocytes, leading to their increased affinity to oil red O staining. We demonstrated an increased number and size of adipoblasts when the adipogenic mixture and ciglitizone combination was used (for 48 h only) and when ciglitizone alone was used thereafter. The intensity of the retained oil red O stain was observed spectrophotometrically, reflecting the increase in the number or size of the adipoblasts.

To obtain better adipogenic induction, we formulated the adipogenic mixture containing dexamethasone, insulin, isobutylmethylxanthine, acetic acid, ascorbic acid, biotin, and pantothenic acid as a source of adipogenic conditioning based on the literature (Pollard and Walker, 1989; Tahilian and Beinlich, 1991; Wu et al., 1996; Mie et al., 2000; Reusch et al., 2000; Novakofski, 2004; Hong et al., 2006). However, the contents of the adipogenic mixture were not tested independently during this study. Conditioning of the postconfluent cells for 48 h was done with the adipogenic mixture to provide a suitable environment for ciglitizone to perform its expected role in transdifferentiation, because in our pilot studies, ciglitizone did not perform as expected without the inclusion of inducers.

Recent advances in the understanding of the transcriptional basis of adipogenesis has allowed meat scientists to directly examine the ability of myogenic cells to enter the adipogenic pathway. Expression of Pax-7 in the cells harvested from porcine confirmed that Pax-7 is an essential requirement of myogenic satellite cells (Seale et al., 2000; Zammit and Beauchamp, 2001; Chen and Goldhamer, 2003). We agree that satellite cells isolated from piglets in the current study behaved as “currency” to myoblasts, as hypothesized by Anderson (2006). We further assumed that these cells are like stem cells or are stem cells themselves and have the ability to become plasticized to a different cell type, provided appropriate conditioners are supplemented. Early expression of PPAR-γ followed by C/EBP-α has been reported in various cell culture models (Tontonoz et al., 1994a,b). The expression of PPAR-γ and C/EBP-α was noticed in porcine satellite cells during transdifferentiation on d 2 and 4, respectively, as shown by the immunohistochemical and Western blot results, which were consistent with the above reports. When C/EBP-α mRNA is expressed at or near fat cell levels, it synergizes with PPAR-γ to stimulate adipogenesis (Tontonoz et al., 1994b). Both PPAR-γ and the C/EBP are known to be direct transcriptional activators of several fat cell
Figure 4. Expression of myogenic and adipogenic transcription factors during proliferation and differentiation of porcine satellite cells. The negative control was incubated with only secondary antibodies. Immunostaining for myosin heavy chain (arrows indicating staining in the cytoplasm), paired-box transcription factor (Pax-7; arrows indicating staining in the nucleus), CCAAT/enhancer binding protein-α (C/EBP-α; arrows indicating light staining in the adipoblasts), and peroxisome proliferator-activated receptor-γ (PPAR-γ; arrows indicating staining in the adipoblasts). Gill’s hematoxylin was used to counterstain the nucleus. The original magnification was 20×; the top 2 micrographs were taken using phase contrast, whereas the bottom 6 micrographs were taken using standard brightfield without phase contrast. The results are representative of 3 separate experiments. DMEM = Dulbecco’s modified Eagle’s medium; HS = horse serum; FBS = fetal bovine serum.
Figure 5. Western blot analysis of porcine satellite cells for CCAAT/enhancer binding protein-α (C/EBP-α) and peroxisome proliferator-activated receptor-γ (PPAR-γ) during differentiation. Electrophoresed protein was transferred to a nitrocellulose membrane by electrophoretic blotting. The membrane was incubated with primary antibodies for (A) PPAR-γ or (B) C/EBP-α and biotinylated goat antirabbit immunoglobulin G secondary antibody. The signal was amplified with streptavidin-horseradish peroxidase (Bio-Rad, Hercules, CA) and detected colorimetrically using Opti-4CN substrate (Bio-Rad). The results are representative of 3 separate experiments.

Both C/EBP-α and PPAR-γ have profound effects on the process of myogenesis. Their expression is sufficient to block muscle differentiation and cause a “transdifferentiation” of myoblasts to fat cells. In the absence of C/EBP-α and PPAR-γ expression in the control group (without treatment), myosin (heavy chain) was predominant immunohistochemically. However, in the treated group, upregulation of the C/EBP-α gene during differentiation was quite consistent with the effect of ciglitizone and was seen during the terminal phases of adipogenesis. Upregulation of PPAR-γ occurred soon after the treatment; however, the level of expression diminished during the later phases of adipogenesis, as shown with real-time PCR quantification. Further, the upregulation and downregulation of C/EBP-α and PPAR-γ during transdifferentiation of porcine satellite cells appear to be a cooperative interplay that forms a positive feedback loop to reinforce and maintain the expression of each other (Semsarian et al., 1999). This cooperative interplay activates the essential adipogenic genes required for adipocyte functions and maintains the terminally differentiated state (Morrison and Farmer, 2000; Rosen et al., 2000). Wu et al. (1996) further emphasized that signals for adipogenesis begin with the activation of PPAR-γ in the presence of PPAR-γ ligand (i.e., ciglitizone). McKnight et al. (1989) have already established that expression of PPAR-γ precedes expression of C/EBP-α, which is consistent with the results of the current study. Once C/EBP-α becomes prevalent (Yeh et al., 1995), it regulates terminal adipocyte differentiation and turns on the group of fat-specific genes re-

Figure 6. Quantitative real-time PCR for the adipocyte-specific cell type marker genes in porcine satellite cells. For the first 48 h, the confluent cells were treated with adipogenic mixture plus ciglitizone and subsequently with ciglitizone alone during differentiation. The bars are the means ± SE for 9 piglets. *Indicates a significant difference (P < 0.05) within an item compared with the undifferentiated (d 0) cells.
quired for the synthesis, uptake, and storage of long-chain fatty acids (Erding et al., 1995).

The transition of porcine satellite cells from myogenic lineage to adipogenic lineage under the influence of ciglitizone (TZD), the adipogenic mixture, or both observed during this study corroborated the findings of Teboul et al. (1995), who also reported the potential of TZD (pioglitazone) and fatty acids to transdifferentiate C2C12N myoblasts to adipoblasts. Similarly, the transition of C2C12 myoblasts to osteoblasts upon bone morphogenetic protein-2 treatment (Katagiri et al., 1994) and to adipoblasts upon rosiglitazone treatment (Yeow et al., 2001) also has been reported. Thiazolidinediones have been reported to have physiologically relevant effects on semitendinosus muscle of postnatal pigs (S-V cell culture) in potentiating marbling or intramuscular adipogenesis (Poulos and Hausman, 2005).

In summary, ciglitizone, the adipogenic mixture, or both together were able to withdraw porcine satellite cells from their myogenic lineage and promote the expression of a typical adipose conversion program. In addition, the information gathered from in vitro findings about the transition of porcine satellite cells to an adipogenic lineage could help meat scientists explore avenues toward the development of intramuscular adipogenesis or marbling and improve meat quality. Additionally, determination of certain other adipogenic markers during the transdifferentiation program through a proteomic approach would aid in absolute confirmation of this study.

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