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Conversion of C2C12 Myoblast into Adipoblast with Thiazolidinediones - A Possible Basis for Intramuscular Fat Generation in Meat Animals

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ABSTRACT : Thiazolidinediones (TZDs) act as potent activators of the adipose differentiation program in established preadipose cell lines. TZD`s have also been investigated in diabetic patients and reported to act as PPAR-γ ligands. In this report, the effects of TZDs on the differentiation pathway of myoblasts have been investigated. C2C12 mouse myoblasts were grown in Dulbecco`s Modified Eagles medium for 4-5 days until they reached almost 100% confluency. Post-confluent cells (day 0) were further exposed to adipogenic induction medium along with TZDs for 48 hours. Thereafter, cells were exposed only to TZDs every 48 h until day 10. The control was provided with differentiation medium without any treatment. Alterations in the cells during the differentiation programme were analyzed on the basis of fusion index, oil-red-o staining, adipocyte index, adipocyte stain uptake measurement, immuno-histochemistry and western blotting. Exposure of C2C12 mouse myoblasts to TZDs prevented the expression of myosin heavy chain with parallel increase in the expression of C/EBP-α and PPAR-γ and acquisition of adipocyte morphology, thus abolishing the formation of multinucleated myotubes. TZDs exert their adipogenic effects only in non-terminally differentiated myoblasts; myotubes were insensitive to the compound. Continuous exposure (at least 4-5 doses) to inducers after the growth arrest was essential to provide a sustained environment to the cells converting to fully matured adipoctyes. The results indicate that TZDs specifically converted the differentiation pathway of myoblasts into that of adipoblasts. (**Key Words :** TZD, Myogenesis, C/EBP-α, PPAR-γ, Adipogenesis)

INTRODUCTION

Intramuscular adipose tissue is a desirable characteristic of meat associated with meat quality and thus makes the animal more valuable. Therefore, increasing the intramuscular fat to increase the quality and value of meat has long been a major goal of animal scientists (Novakofski, 2004). Myoblasts and adipocytes arise from the same germ layer of the embryo, the mesoderm, and recent observations suggest it is possible to directly induce the conversion of myoblasts to adipocytes (Li et al., 2005). G 8 myoblasts are a tissue-culture model for myogenesis and can differentiate spontaneously into myotubes when cultured in medium containing fetal calf serum. The transcription factors C/EBP-alpha and PPAR-gamma, when expressed in G8 myoblasts, can suppress the muscle specific transcription factors i.e. Myf5, MyoD, myogenin and MRF4 (Hu et al., 1995). C/EBP-alpha, beta and gamma stimulates adipogenesis in fibroblast cell lines such as NIH3T3 (Freytag, et al., 1994; Yeh et al., 1995) which probably occurs through the up-regulation of PPAR-gamma expression (Wu et al., 1995). It has been reported that activation of PPAR-gamma requires ligand binding (Wu et al., 1996). Various classes of thiazolidinediones which are PPAR-gamma ligands are under trial in diabetic type 2 patients. This class of drug has been associated with insulin sensitizing effects. TZDs have been reported to alter the expression of many metabolically important genes in adipose tissue, liver and muscle in insulin-resistant animal models (Way et al., 2001; Albrektsen et al., 2002) and in some cell culture systems (Cha et al., 2001; Malerod et al., 2003). Genes whose expressions were altered in muscle and liver were found to have a role in the regulation of glucose metabolism and fatty acid handling (Way et al., 2001). In adipose tissue, TZDs have been shown to alter the expression of leptin, inflammatory molecules and

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circulating proteins such as tumor necrosis factor-alpha (TNF-α), which may be implicated in insulin resistance. It remains to be determined whether altered expression of these key metabolic genes accompanies the insulin-sensitizing effects of TZDs (Tonelli et al., 2004). Of particular note, TZDs increase adipocyte gene expression and plasma levels of adiponectin (Albrektsen et al., 2002; Yu et al., 2002; Iwaki et al., 2003), a circulating adipose-derived protein claimed to have favourable effects on insulin action (Tonelli et al., 2004). TZDs are the synthetic activators of PPAR-gamma and considered as potent stimulators of adipogenesis for use clinically to reduce hyperglycaemia in type 2 diabetes (Rosen et al., 2006). Keeping the above hypotheses in consideration, the present study was designed to find out the role of TZDs in transdifferentiation of C2C12 myoblasts to adipoblasts and to examine the possibility to increase intramuscular adipose tissue in meat animals.

MATERIALS AND METHODS

Materials

C2C12 myoblast cell line, Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), horse serum and penicillin- streptomycin (PS) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Dexamethasone, insulin, isobutylmethylxanthine (IBMX), biotin, ascorbic acid, pantothenic acid, ascorbic acid and TZDs were obtained from Sigma (Sigma Aldrich, Saint Louis, Missouri, USA).

Cell culture

C2C12 cells were plated at a density of $2\times10^3/cm^2$ and grown in DMEM supplemented with 10% FBS and 1.1% of 100 IU/ml penicillin, and 100-mg/ml streptomycin (Proliferation media). Cells were kept at 37°C in a humified incubator under 5% CO_2. Media was changed every day and the confluence (almost 100%) was reached within 5 days. Contents of the adipogenic mixture were ascorbic acid (0.1 M) in distilled water (DW) , biotin, (33 mM) in DW, acetic acid (10 mM) in DW, pantothenic acid (34 mM) in DW, dexamethazone (5 mM) in ethanol, isobutylmethylxanthine (0.5 mM) in ethanol, insulin (10 μg/ml) in DW and Thiazolidinediones (50 μM) in dimethylsulfoxide. Control experiments were performed to exclude any effects of dimethylsulfoxide. Post-confluent cells (day 0) were further exposed to adipogenic induction medium (DMEM supplemented with 5% horse serum and 1.1% PS) along with TZDs for 48 h. Thereafter, cells were exposed only to TZDs every 48 h until day 10. The control was provided with differentiation medium without any treatment and the entire experiment was performed in triplicate.

Fusion index

Samples were collected at different intervals as committed in the methods. Cells were fixed with methanol and stained with hematoxylin stain (Sigma Aldrich, Saint Louis, Missouri, USA). The total number of nuclei and the number of nuclei incorporated in myotubes were scored in 10 microscopic fields/dish chosen at random at 400×. The fusion index was calculated as the percentage of nuclei incorporated in the myotubes relative to the total number of nuclei. The structures containing at least three nuclei were scored as representing myotubes (Dodson and Mathison, 1988).

Oil-red-O staining/Adipocyte index

Oil-red-O (Sigma Aldrich, Saint Louis, Missouri, USA) staining was performed following the procedure described by Green and Kehinde (1974) with minor modifications. In brief, cells were washed twice with phosphate buffer saline (PBS) and fixed with 10% formalin in PBS for 15 min. After two washings in PBS, cells were stained for at least 1 h in freshly diluted Oil-red-O solution (six parts Oil-red-O stock solution and four parts H_2O; Oil-red-O stock solution is 0.5% Oil-red-O in isopropanol). The stain was then removed and the cells were washed twice with water, with or without counter stain (0.25% giemsa for 15 min) and then photographed using light microscopy. The total number of adipocytes (round and filled up bodies with Oil-red-O) and number of nuclei were scored in 3 microscopic fields/dish chosen at random at 400×. The adipocyte index was calculated as a percentage from the total number of adipocytes over the number of nuclei in 3 microscopic fields at random.

Adipocyte stain uptake measurement

Cells were washed initially with PBS and fixed with 10% formalin. All the formalin was removed and dishes dried thereafter; cells were washed with 60% isopropanol and then dishes were allowed to air dry. Oil red-O stain was then added for 10 min without touching the walls of the dishes. All the stain was removed using a small transfer pipette and dishes were washed with distilled water 4-5 times. Cells were then dried and remaining Oil-red-O was eluted by adding 100% isopropanol and incubating for about 10 min. Eluted stains were transferred to 1.5 ml eppendorf vial and optical density was measured at 500 nm over the 100% isopropanol as blank (McNeil, 2005).

Immuno-histochemistry

The expression of myogenic and adipogenic proteins of differentiating C2C12 myoblast cells was determined by indirect immuno-staining (Michal et al., 2002). Samples were collected in triplicate at each of the different intervals on incubation at 37°C and 5% CO_2. Cells were initially

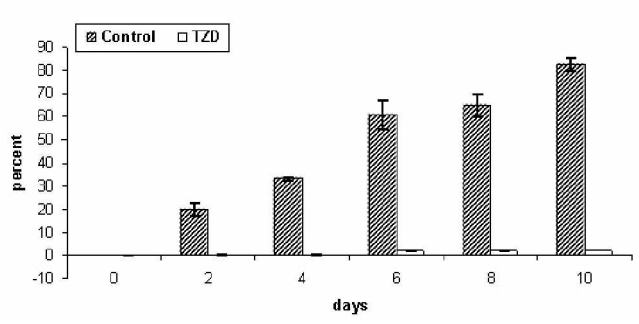

Figure 1. Fusion index of C2C12 myoblasts during differentiation. Post-confluent C2C12 myoblasts (day 0) were exposed to DMEM+5% HS+1.1% PS with adipogenic mixture in conjunction with TZDs for 48 h. Subsequently only TZDs were supplemented every 48 h till day 10. Controls were kept devoid of adipogenic mixture and TZDs.

washed with PBS and then fixed with 100% methanol. To eliminate nonspecific binding of primary antibodies, 50 μl of 2% normal horse serum (ATCC, Manassas, VA, USA) in PBS was added as a blocker and the plates were incubated overnight at 4°C. Cells were washed with PBS, and the primary antibodies to Pax-7 (1:100) (Anti-mouse Monoclonal IgG1; R&D System; Minneapolis; MN; USA), C/EBP-α (1:200) (Rabbit Polyclonal IgG; Affinity BioReagents; CO; USA), anti-myosin heavy chain (1:400) (Sigma Aldrich; Saint Louis; Missouri; USA) and PPAR-γ2 (1:200) (Sigma Aldrich, Saint Louis, Missouri, USA) were added. All primary antibodies were diluted in PBS that contained 5% non-fat milk (Biorad; Alfred Nobel Dr., Hercules, CA, USA). After incubation at room temperature for 1hour, plates were washed with PBS+0.1% Tween20 (TPBS). The secondary antibody, biotinylated Goat Anti-mouse IgG antibody (1:500 dilution; Biomeda, Foster City, CA, USA) was added to all the samples and incubated for 30 min at 37°C. Unbound secondary antibodies were washed from the plates with TPBS and PBS. ABC peroxidase (Pierce; Rockford, IL, USA) 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 stain (Sigma Aldrich; Saint Louis; Missouri; USA), and cells were then examined by light microscopy.

Western blot

Post-confluent C2C12 myoblast samples on day 0, 2, 4, 6, 8 and 10 were collected and protein concentration was analysed using pro-prep (iNtron Biotechnology). The samples were lysed by boiling for 5min at 100°C, using pro-prep. The lysate was mixed with loading buffer and loaded on 15% SDS gel. Proteins were separated by electrophoresis at 80 volts for 20 min and 120 volts for 100 min using Tris-glycine running buffer (0.025 M Tris base, 0.192 M glycine, and 0.1% Sodium dodecyl sulfate (SDS), pH 8.3). Prestained molecular marker was used to determine molecular weight of proteins (Kaleidoscope; Invitrogen). The gel was subsequently transferred onto a nitrocellulose membrane (Nitropure; MSI; Westboro; MA) by electrophoresis overnight at 100 volts, 4 degree using a transfer buffer that contained 25 mM Tris base, 192 mM glycine, 10% methanol, pH 8.1-8.3. The membrane was washed with PBST and blocked with 3% blocker for one hour at room temperature. Antibodies to C/EBP-alpha and PPAR-gamma diluted at 1:1000 in PBST containing 1% bovine serum albumin (BSA) were incubated with the membrane for 1 hour at room temperature. Thereafter, the membrane was incubated with biotinylated goat antimouse antibody for 1 h diluted at 1:3000 in PBST containing 1% BSA. After two washes in PBST for 5min, the membrane was incubated with 1:1,000 Streptavidin-HRP for 30 min. Then, it was washed twice with PBST and incubated with BAR (biorad amplification reagent) for 10 min. Further, it was washed with 20% DMSO/PBST four times for 5 min each. Then, it was incubated again with 1:1000 Stretavidin-HRP for 30 min and thereafter two more washes were given with PBST. Horse-radish peroxidase activity was detected by enhanced chemiluminescence's substrate (biorad) as directed by the manufacturer's instructions and subsequently photographed.

Statistical analysis

Statistical analyses of fusion, adipocyte counting and adipocyte staining data were performed by one-way analysis of variance (ANOVA). Significant differences were detected ($p<0.05$) by Duncan's multiple range tests using a PC statistical package (SAS, release 8.01, SAS Institute, Inc., Cary, NC).

RESULTS

Fusion index

C2C12 cells displayed an almost complete morphology of multinucleated myotubes when postconfluent (day 0) cells were maintained in Dulbecco's medium with 5% HS and 1.1% PS without any treatment either with adipogenic mixture or TZDs. Myogenesis was apparently present from day 2 onwards. Samples were collected on day 0, 2, 4, 6, 8 and 10 and cells were stained with Gill's haematoxylin and those with three or more nuclei within a membrane were considered fused. Fusion rate was significantly ($p<0.05$) increased from day 2 of the observation period compared to day 0 (Figure 1). Exposure of post-confluent cells to adipogenic mixture and TZDs on day 2 and only TZDs thereafter led to a net decrease in the number of multinucleated myotubes with a parallel increase in the number of small cells containing lipid droplets and

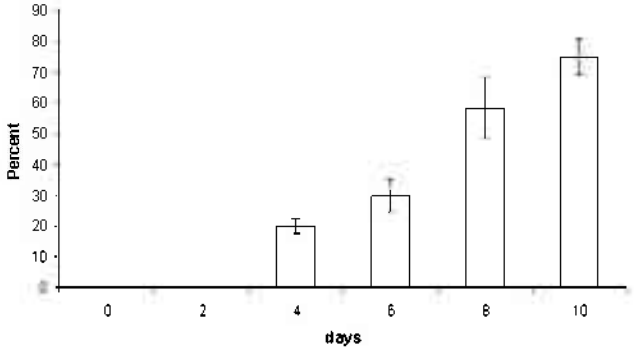

Figure 2. Adipogenic index (adipoblast formation) of C2C12 myoblast during differentiation. Post-confluent C2C12 myoblasts (day 0) were exposed to DMEM+5% HS+1.1% PS with adipogenic mixture in conjunction with TZDs for 48 h. Subsequently only TZDs were supplemented every 48 h till day 10. Controls were kept devoid of adipogenic mixture and TZDs.

adipoblast formation.

Oil-red-O staining/adipocyte index

Oil-red-O staining was performed to confirm the effect of adipogenic induction and TZDs on conversion of myoblast to adipoblast as shown in Figure 4. Adipogenic induction and TZD treatment of post-confluent cells showed abundant production of lipid droplets from day 2 itself which acquired Oil-red-O stain. Myoblast cells started to swell from their middle portion and acquired morphology similar to preadipocytes with many oil droplets in their cytoplasm. Such structure stained feebly with Oil-red-O.

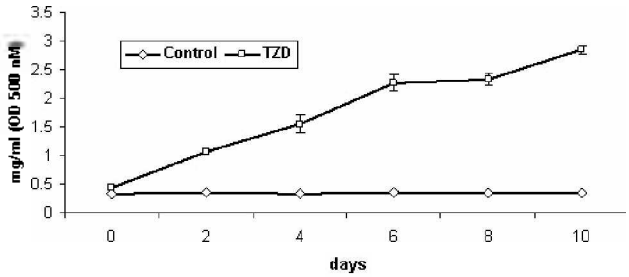

Figure 3. Adipoblast stain uptake measurement of C2C12 myoblasts during differentiation. Post-confluent C2C12 myoblasts (day 0) were exposed to DMEM+5% HS+1.1% PS with adipogenic mixture in conjunction with TZDs for 48 h. Subsequently only TZDs were supplemented every 48 h till day 10. Controls were kept devoid of adipogenic mixture and TZDs.

Cells assumed a large round shape from the 4th day of treatment and stained intensely red. Completely round and intensely red cells were considered adipoblasts and were counted in three randomly chosen fields at 400×. Total number of adipoblasts counted were divided by the number of myoblast nuclei and expressed as a percentage. Adipoblast count significantly increased ($p<0.05$) from day 4 onwards until the final day of observation compared to day 0 (Figure 2).

Adipocyte stain uptake measurement

Consequent to adipogenic induction and TZD treatment myoblast morphology began to change with plenty of lipid droplets in the cytoplasm. Later with the passage of time many droplets coalesced together to form a bigger droplet

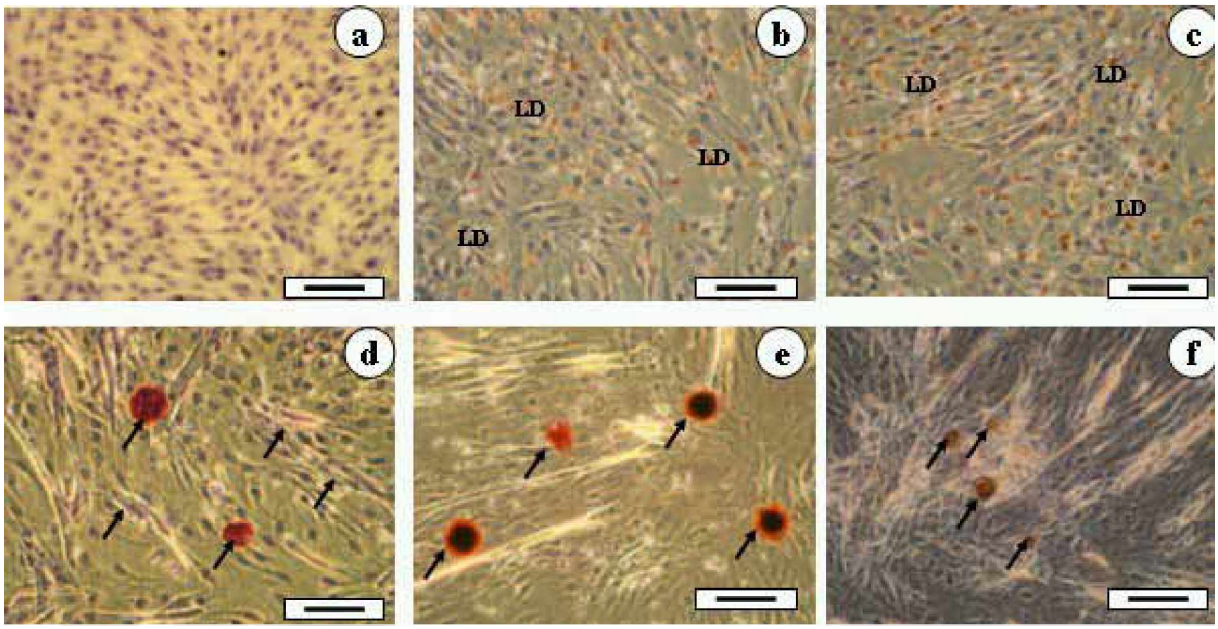

Figure 4. Lipophilic staining (Oil-Red-O) of C2C12 myoblasts during differentiation showing formation of lipid droplet (LD) and adipocytes (arrows) formation at different phases of cell growth and development. Pictures a, b, c, d, e and f represents day 0, 2, 4, 6, 8 and 10 respectively. Slight percentage of myotube formation were also evident from day 6 onwards. The original magnification was 20×. Results are representative of three separate experiments.

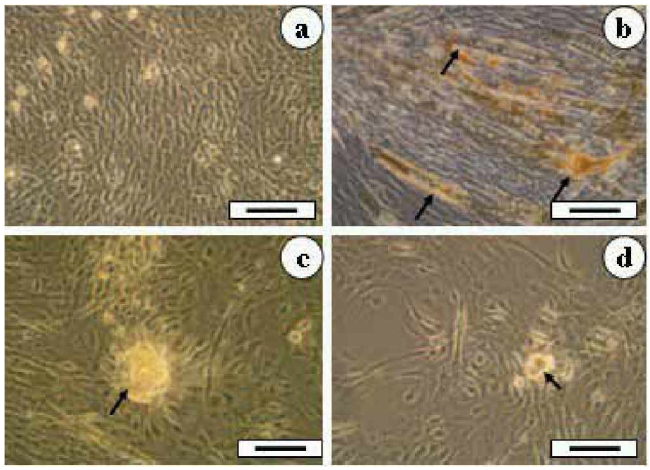

Figure 5. Expression of myogenic and adipogenic transcription factors during differentiation of C2C12 myoblast. The cells were then subjected to immuno-staining for myosin heavy chain ((b) on day 4; arrows indicating brown staining of the cytoplasm), PPAR-γ ((c) on day 2; arrows indicating brown staining in the adipoblasts), C/EBP-α ((d) on day 6; arrows indicating brown staining in the adipoblasts). The original magnification was 20×. Control samples were incubated with only secondary antibodies (a).

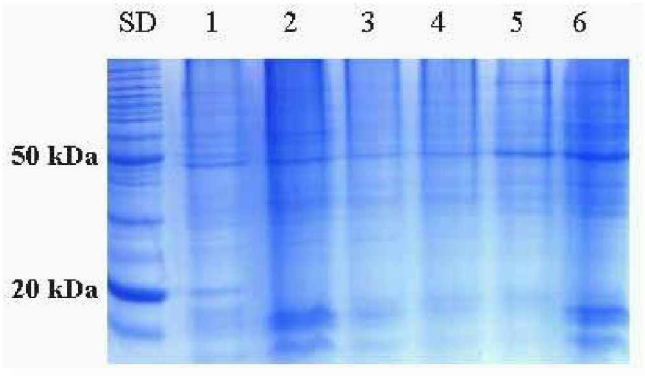

Figure 6. Commasie blue staining of SDS-PAGE run for 20 min at 80 V and for 90 min at 120 V. Lane 1-6 represents day 0, 2, 4, 6, 8 and 10 respectively. Loading volume was 30 µl for each lane. Standard marker was with 15 bands from 220 to 10 kDa with two bands of 50 kDa and 20 kDa with greater intensity.

Figure 7. Western blot analysis of C2C12 myoblasts for C/EBP-α and PPAR-γ during differentiation. Expression of myogenic and adipogenic transcription factors during differentiation of C2C12 myoblasts. Electrophoresed protein was transferred to nitrocellulose membrane by electrophoretic blotting. Membrane was incubated with primary antibodies for C/EBP-α (A) and PPAR-γ (B) and biotinylated goat anti-rabbbit IgG secondary antibody. The membrane was further amplified with streptavidin-HRP and was colorimetrically detected using Opti-4CN substrate. SD: Standard marker with 10 bands from 170.8 to 5.7 kDa. A. lane 1 to 5 represents day 4, 6, 8, 10 and 0. B: lanes 1-6 represent day 0, 8, 6, 4, 2 and 10 respectively.

and filled most of the cytoplasm. Consequently, the cytoplasm appeared thin with an eccentric nucleus. Microscopically, the size of the transformed cells appeared to increase significantly with more incubation time. Increase in the size of the adipoblast was further confirmed by measuring the adipocyte stain uptake which was determined spectrophometrically at 500 nM. During the differentiation the stain uptake by the adipocyte significantly increased ($p<0.05$) soon after the adipogenic induction and TZD treatment (Figure 3).

Immuno-histochemistry

Incubation of C2C12 myoblasts with only secondary antibody as a negative control yielded minimal background. Significant staining was not observed when the cells were incubated with anti-Pax-7 during proliferation, the monoclonal antibody that has been associated with satellite cells. Cells grown in media without any treatment were incubated with anti-myosin heavy chain, the monoclonal antibody that binds to myosin heavy chain, which resulted in dark brown staining in the cytoplasm of myotubes. Treatment groups incubated with anti-C/EBP-alpha and anti-PPAR-gamma yielded light brown staining of the round adipoblast structure. PPAR-gamma appeared as early as day 2 of the treatment whereas C/EBP-alpha was significantly observed from day 6 of the treatment and by day 10 staining had become comparatively less intense (Figure 5).

Western blot

Antibodies to cellular proteins used for immunohistochemistry were also tested on electrophoresed lysates of C2C12 myoblasts during differentiation and the results are shown in Figure 7. C/EBP-alpha was detected as bands between approximately 47.1 to 35.1 kDa after incubation with anti-C/EBP-α, ranging from approximately 45 kDa. PPAR-γ expression was identified in the lysates quite clearly in the treated groups compared to the control (day 0), and resulted in a band between 60.4 to 47.2 kDa, nearing 50 kDa.

DISCUSSION

Mutual conversion of fat cells to muscle cells or muscle cells to fat cells with expression and suppression of certain related transcription factors formed the basis for meat scientists to examine the possibility of intramuscular fat generation with this particular approach. Further, this

concept, propagated as the origin for fat and muscle cell during embryonic development, was found to have a common germinal layer i.e. mesoderm (Li et al., 2005). Embryonic development is accompanied by the restriction of cell lineages to distinct pathways leading to the formation of functionally differentiated cell types. There are examples, particularly in avian and amphibian tissues, in which regeneration is important that explain the evidence of exclusive pathways which allow 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). Morphological changes were evident (Oil-Red-O) in C2C12 myoblasts soon after exposure to adipogenic mixture and TZD treatment. C2C12 myoblasts grown in the absence of adipogenic mixture and TZD treatment appeared to form myotubes and no restriction to their committed myogenic lineage was observed. Asakura et al. (2001) also reported C2C12 myoblasts and primary myoblasts from mice strictly formed myocytes when grown in DMEM with 5% HS. We formulated the adipogenic cocktail with dexamethasone, insulin and isobutylmethylxanthine as a source for adipogenic conditioning from the literature to obtain better adipogenic induction. IBMX in preadipocytic cell cultures (3T3L1) has been shown as a source of cAMP and cAMP initiates adipogenesis via the transcription factor cAMP response binding factor (CREB) (Reusch et al., 2000). Supplementation of growth medium with dexamethazone (Wu et al., 1996), acting as an adipogenic transcriptional factor inducer, and insulin acting through an IGF-1 receptor enhanced lipid filling (Novaksaki, 2004), eventually improved the adipogenic induction of C2C12 myoblast. Additional supplementation of acetic acid (Hong et al., 2006), ascorbic acid (Mie et al., 2000) and biotin (Pollard and Walker, 1989) proved to be beneficial inducers of adipogenesis in C2C12 myoblasts.

Presence of biotin along with other supplementation improved lipid accumulation and adipogenesis in C2C12 myoblasts as determined with Oil red-O staining. However, Levert et al. (2002) reported that presence of biotin treatment in 3T3L1 blocked the induction of PPAR-gamma and inhibited the lipid accumulation. Contradictory notions about biotin require further determination.

Experimental manipulations could bring about the activation of certain genetic expression in cell culture systems. It has been shown that the nuclei from muscle cells can cause the activation of muscle genes when the nuclei share cytoplasm in heterokaryons (Blau, 1992). Recently, ectopic expression of the muscle regulatory gene MyoD has been shown to stimulate myogenesis in a number of cell lines including fibroblasts, liver cells, neuroendocrine epithelial cells, and preadipocytes (Weintraub et al., 1989). Recent advances in the understanding of the transcriptional basis of adipogenesis allowed meat scientists to directly examine the ability of myogenic cells to enter the adipogenic pathway. Pax-7 was not observed immunohistochemically in C2C12 myoblasts in the present study which provided a clue that myoblasts are certainly different from myogenic satellite cells. Recently, it has come to notice that Pax-7 is an essential requirement of myogenic satellite cells (Seale et al., 2000). Authors opine that the myoblast being a son of satellite cells (stem like) definitely inherited properties of satellite cells to be plasticised to a different cell type provided particular conditioners are supplemented. PPAR- is an orphan member of the nuclear hormone receptor superfamily that is specifically expressed in fat (Tontonoz et al., 1994a). In addition, its expression is induced very early in several cell culture models of adipogenesis. Ectopic expression of PPAR-γ induces several fibroblastic cell lines to differentiate into adipocytes in a PPAR-γ activator dependent manner i.e. TZDs (Tontonoz et al., 1994b). PPAR-γ expression was noticed in C2C12 myoblasts during transdifferentiation on day 2 itself as evidenced by immunohistochemistry and western blot results. C/EBP-α, while expressed in many tissues *in vivo*, is also induced during adipogenesis later in the time course than PPAR-γ and was noticed on the 6th day of observation after the onset of adipogenesis confirmed with immunohistochemistry and western blotting. Findings of the present study coroborated those of Freytag et al. (1994) that ectopic expression of C/EBP-α can trigger differentiation in preadipocytes and a cause of adipogenesis in several fibroblastic cell types. When C/EBP-α mRNA is expressed at or near fat cell levels, it powerfully synergizes with PPAR-γ to stimulate adipogenesis (Tontonoz et al., 1994b). Both PPAR-γ and the C/EBPs are known to be direct transcriptional activators of several fat cell genes, and the best characterized adipocyte-specific regulatory sequences have been shown to contain binding sites for both factors (Tontonoz et al., 1994a; Tontonoz et al., 1995).

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. Importantly, the myogenic inhibitory activity of these factors is temporarily and functionally separable from their ability to stimulate overt adipogenesis. However, in C2C12 myoblasts the expression of both the factors during the differentiation under the influence of TZDs could not bring about the 100% adipogenesis and ability to form myotubes was not completely lost. The present authors opine that the presence of a higher percentage (5%) of horse serum might have restricted the complete conversion of myoblast to adipoblast (Fedoroff, 1979). Contrary to findings of the

present study. Erding et al. (1995) explained that when both the factors are expressed in G8 cells in the absence of PPAR-γ activators and other adipogenic hormones, there was no morphological or biochemical evidence of fat development. However, expression of these factors clearly interfered with the expression of myogenic transcriptional regulators and myofibrillar proteins. When a PPAR activator and adipogenic hormones are applied, these cells differentiate into adipocytes as efficiently as established fat cell lines such as 3T3L1. Complete inhibition of myogenesis or suppression of myogenic regulatory proteins was not accomplished in the present study. Slight formation of myotubes was noticed in conjunction with massive adipogenesis in C2C12 myoblast during transdifferentiation. However, it has been reported that inhibition of myogenesis can be accomplished by expression of C/EBP-α alone, to a certain extent, and expression of PPAR-γ alone is not effective in blocking the expression of muscle end-product genes in response to the PPAR-γ activators in G8 myoblasts (Erding et al., 1995). One of the important features noticed in the present study was that the chronic dosage of TZDs was not required for adipose differentiation. Secondly, TZDs were absolutely indifferent to myotubes and exerted their effects only on non-terminally differentiated C2C12 myoblasts. Findings of the present study demonstrate that thiazolidinediones and the adipogenic mixture used promoted the transition from myogenic lineage to that of adipogenic lineage and inferred that this conversion event occurs at the end of the growing phase of committed myoblasts. Katagiri et al. (1994) also reported similar features in describing the conversion of the differentiation pathway of C2C12 myoblasts into osteoblasts upon bone morphogenetic protein-2 treatment. The above observations together provide an insight into the plasticity of the committed myoblast and the crucial role of TZDs and adipogenic mixture on the fate of these cells towards adipogenic lineage.

IMPLICATION

The findings of the present study offer a valuable cellular model to study the cellular and molecular events of mesodermal cell commitment. In addition, even if it is premature to conclude that transition from the myoblast to the adipoblast cell lineage could occur *in vivo*, it would be of interest to meat scientists to investigate the similar possibility in primary culture of various meat animals and in the animals as such.

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