Differential regulation of the stearoyl-CoA desaturase genes by thiazolidinediones in 3T3-L1 adipocytes

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Abstract **Two stearoyl-CoA desaturase (SCD) isoforms can be expressed during the differentiation of 3T3-L1 preadipocytes into adipocytes. Here we report on the effects of the peroxisome proliferator-activated receptor γ ligand troglitazone (TRO) on *scd1* and *scd2* mRNA levels as determined by Northern blotting, on SCD protein expression as determined by Western blotting, and on total lipid composition as determined by GC during differentiation. In preadipocytes, *scd1* mRNA and SCD protein were not detected, whereas *scd2* mRNA was detected. These cells have high levels of palmitate (16:0), stearate (18:0), and monounsaturated oleate (Δ^9-18:1) and low levels of monounsaturated palmitoleate (Δ^9-16:1). In MDI (methylisobutylxanthine, dexamethasone, and insulin)-treated cells, *scd1* mRNA and SCD protein were increased ~100-fold relative to preadipocyte levels, the *scd2* mRNA level was increased 2-fold, Δ^9-16:1 was increased ~20-fold, and 18:0 was decreased ~3-fold. In TRO-treated cells, the *scd1* mRNA level was lower than that observed in preadipocytes, while the *scd2* mRNA level was similar. TRO also decreased *scd1* mRNA in primary adipocytes. The TRO-treated cells contained a Δ^9-18:1 level typical of MDI-treated cells whereas, conversely, these cells also contained a low Δ^9-16:1 level typical of preadipocytes. The implications of these correlations for the regulatory and enzymatic mechanism(s) used to establish and maintain lipid composition are discussed.—** Kim, Y-C., F. E. Gomez, B. G. Fox, and J. M. Ntambi. **Differential regulation of the stearoyl-CoA desaturase genes by thiazolidinediones in 3T3-L1 adipocytes. *J. Lipid Res.* 2000.** 41: **1310–1316.**

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The mouse embryo-derived 3T3-L1 preadipocyte cell line represents an often-used model system for studying cellular differentiation and development (1). Under the appropriate hormonal stimuli, these cells differentiate in culture to possess morphological and biochemical characteristics typical of mature adipocytes. On differentiation, cellular levels of glycolytic, lipogenic, and lipolytic enzymes as well as other adipocyte-specific proteins rise dramatically due to an increase in gene transcription (2). Among these enzymes, the activity of stearoyl-CoA desaturase (SCD) increases up to 100-fold during differentiation. Two isoforms of SCD, encoded by the genes *scd1* and *scd2*, are known (3, 4). These isoforms have ~85% primary sequence identity (4) and can catalyze the formation of monounsaturated palmitoleate (Δ^9-16:1) and oleate (Δ^9-18:1) from palmitate (16:0) and stearate (18:0), respectively. Along with 16:0, Δ^9-16:1 and Δ^9-18:1 are the major constituents of membrane phospholipids and the triacylglycerol stores found in differentiated 3T3-L1 adipocytes and in mouse adipose tissue (5).

Because SCD is directly responsible for the synthesis of monounsaturated fatty acids, regulated expression of the *scd* isoforms can influence a variety of physiologic responses dependent on membrane composition, including insulin sensitivity (6, 7), metabolic rate (7), and adipocity (8). Indeed, *scd* gene expression and SCD activity have been shown to be elevated in the liver and adipose tissue, respectively, in various animal models of obesity (8, 9). Furthermore, a significant positive correlation between SCD activity in skeletal muscle and the percentage of body fat has been reported in humans (9). However, despite the overall similarity of the two SCD isoforms (3, 4), their tissue distribution in mouse is markedly different. Both SCD1 and SCD2 are expressed in adipose tissue, whereas only SCD1 is present in liver (1, 5, 10, 11). In contrast, only SCD2 is found in spleen (12), heart, brain, kidney (1, 11), and B cells (13), whereas T cells possess neither isoform (12, 13). The physiologic significance of this tissue distribution has not been clarified.

The thiazolidinedione (TZD) drugs, ligands for the adipocyte-specific nuclear peroxisome proliferator-activated receptor γ (PPARγ2), enhance the conversion of preadipocytes to mature adipocytes both in vivo and in vitro (14,

Abbreviations: aP2, adipocyte lipid-binding protein; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MDI, differentiation cocktail consisting of methylisobutylxanthine, dexamethasone, and insulin; PPARγ2, peroxisome proliferator-activated receptor γ; SCD, stearoyl-CoA Δ^9-desaturase; *scd1*, gene encoding SCD isoform 1; *scd2*, gene encoding SCD isoform 2; TRO, troglitazone; TZD, thiazolidinedione.

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15). The PPARγ2 is expressed predominantly in adipose tissue early in differentiation (16–18) and enhances adipocyte-specific gene transcription (16, 19). Furthermore, ectopic expression of PPARγ2 in the presence of a ligand such as TZD enhances the conversion of NIH 3T3 fibroblasts into adipocytes (17). TZDs exert potent antidiabetic effects by enhancing the sensitivity of target tissues to insulin (20); however, the precise mechanism by which these ligands enhance insulin sensitivity via the PPARγ2 is not completely known.

Kurebayashi et al. (21) reported that *scd1* transcription was repressed in mature adipocytes in the presence of a model TZD, troglitazone (TRO), after differentiation with a cocktail containing methylisobutylxanthine, dexamethasone, and insulin (MDI). This repression was correlated with an increase in saturated fatty acids and a decrease in monounsaturated fatty acids. However, comparative information on *scd2* transcription was not obtained, and deconvolution of the individual effects of MDI and TRO was not possible because of the use of mature MDI-differentiated adipocytes as the starting cell type. In the present study we have examined the effects of MDI and TRO on the expression of both *scd* genes during the time-course differentiation of 3T3-L1 preadipocytes into mature fat cells. These studies show that the TRO-treated cells have a low level expression of the *scd1* gene and a level of expression of the *scd2* gene similar to that of both preadipocytes and MDI-treated cells. In the TRO-treated cells, there is a unique, substantial decrease in the percentage conversion of 16:0 to Δ^9-16:1 but no change in the percentage conversion of 18:0 to Δ^9-18:1. One interpretation of these detected changes in lipid composition and mRNA levels is that the two SCD isoforms present in 3T3-LI preadipocytes may have different catalytic selectivities for desaturation of 16:0- and 18:0-CoA in addition to their different patterns of transcriptional regulation.

MATERIALS AND METHODS

Materials

Troglitazone (TRO) was a gift from Parke-Davis (Ann Arbor, MI). Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, and trypsin were from GIBCO (Gaithersburg, MD). Methylisobutylxanthine was from Aldrich (Milwaukee, WI), dexamethasone, fatty acid methyl ester standards, bovine serum albumin (BSA), and collagenase type IV were from Sigma (St. Louis, MO), and insulin was from Eli Lilly (Indianapolis, IN). Calf serum was from BioWhittaker (Walkersville, MD). [α-^{32}P]dCTP (3,000 Ci/mmol) was from NEN Life Sciences (Boston, MA), nitrocellulose filters were from Schleicher & Schuell (Keene, NH), and Nylon mesh was from Spectrum (Microgon, Laguna Hills, CA).

Tissue culture of 3T3-L1 cells

3T3-L1 cells were cultured in DMEM, 10% calf serum with penicillin (100 U/mL) and streptomycin (100 μg/mL) (22). For MDI differentiation, confluent preadipocyte monolayers were incubated for 48 h in DMEM containing 10% fetal bovine serum and a differentiation cocktail consisting of methylisobutylxanthine (115 μg/mL), dexamethasone (390 ng/mL), and insulin (10 μM). After 48 h, the cells were maintained in DMEM containing 10% fetal bovine serum, antibiotics, and insulin. The medium was changed every 2 days. The cell morphology was monitored daily for the appearance of cytoplasmic lipid droplets, using a phase-contrast microscope and oil red O staining. For TRO differentiation, TRO was dissolved in dimethyl sulfoxide and added to the medium to give a concentration of 10 μM. TRO was replenished with every medium change unless otherwise indicated.

Tissue culture of primary adipocytes

Male B10.PL(73NS)/Sn mice, 6–8 weeks old, from the Jackson Laboratory (Bar Harbor, ME) were killed and the epididymal fat pads were excised aseptically. The fat pads were minced with sharp scissors and placed in a plastic scintillation vial containing collagenase (0.5 mg/mL), DMEM, 1 M HEPES, and 4% BSA and incubated in a shaking water bath (150 cycles/min) at 37°C for 1 h. After collagenase digestion, the cell suspension was filtered through a 526-μm Nylon mesh and cells were allowed to float to the top. The adipocytes were then washed five times to remove all traces of collagenase. The resuspended cells were transferred to a polypropylene tube containing DMEM, 10% FBS and penicillin–streptomycin and incubated in either the presence or absence of 10 μM TRO in suspension with gentle shaking at 37°C for 16 h.

RNA analysis

Northern analyses were performed with total RNA isolated from 3T3-L1 cells (22). After agarose–formaldehyde gel electrophoresis, the blot was hybridized with ^{32}P-labeled cDNA probes synthesized using the divergent 5′ or 3′ untranslated regions specific for either the *scd1* or the *scd2* cDNAs (4). The membrane was then stripped and sequentially reprobed with cDNAs for the PPARγ2 and adipocyte lipid-binding protein (aP2). A pAL15 (22) cDNA probe was used as the internal loading control. The intensities of the bands were quantitated by laser densitometric scanning of autoradiograms.

Western analysis

Total cellular protein was prepared from cells grown in 100-mm dishes. The cells were washed twice with phosphate-buffered saline and lysed in 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40 (NP-40), 0.25% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM Na_3VO_4, 10 mM Na_2MoO_4, 40 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF), aprotinin (2 μg/mL), and leupeptin (1 μg/mL). The cells were lysed by passage through a 23-gauge needle, incubated at 4°C for 10 min, and then clarified by centrifugation at 10,000 *g* for 10 min at 4°C. Protein concentrations were determined by dye-binding assay, using bovine serum albumin as the standard (23). Equal amounts of protein were separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE), transferred, and immobilized on Immobilon-P transfer membranes (Millipore, Danvers, MA) at 4°C. After blocking with 10% nonfat dry milk in phosphate-buffered saline at 4°C overnight, the membranes were washed and incubated with rabbit anti-rat SCD as the primary antibody and goat anti-rabbit IgG–horseradish peroxidase (HRP) conjugate as the secondary antibody. Visualization of the SCD protein was performed with the ECL Western blot detection system (Amersham-Pharmacia Biotech, Piscataway, NJ), using procedures provided by the manufacturer.

Lipid analysis

Cells were washed twice with cold phosphate-buffered saline and total cellular lipids were extracted three times with $CHCl_3$–methanol 2:1 (v/v). The three lipid extractions were combined in a screw-capped glass tube, dried under N_2 gas at 40°C in a heating block, and resuspended in toluene. Fatty acid methyl esters were

produced from BCl_3–methanol (Alltech, Deerfield, IL), extracted with hexane, dried, and resuspended in hexane. Fatty acid methyl esters were identified with a Hewlett-Packard (Palo Alto, CA) 6890 gas chromatograph equipped with a 7683 autoinjector and an HP-5 column (30 m × 0.25 mm, 0.25-μm film thickness) connected to a flame ionization detector set at 275°C. The injector was maintained at 250°C. The column temperature was held at 180°C for 2 min after injection, increased to 200°C at 8°C/min, held at 200°C for 15 min, and then increased to 250°C at 8°C/min. Under these conditions, the Δ^9-16:1-, 16:0-, Δ^9-18:1-, and 18:0-methyl esters eluted at 9.2, 9.7, 15.3, and 16.4 min, respectively.

RESULTS

Selective action of TRO in 3T3-L1 adipocytes

Figure 1 shows a Northern blot analysis of the changes in mRNA expression of the known adipose-specific genes *scd1*, *scd2*, PPARγ2, aP2, and the control gene pAL15. Because the cDNAs of *scd1* and *scd2* cross-hybridize to the 4.9-kb mRNA corresponding to the coding regions of these genes, specific cDNA probes generated from divergent regions in the 5′ and 3′ untranslated regions of these genes were used for this study (4). In preadipocytes maintained in culture medium for 9 days (Fig. 1, lane 1), the level of *scd2* mRNA was considerably higher than the level of *scd1* mRNA. The level of mRNA for the other adipose-specific genes, PPARγ and aP2, was low in the confluent preadipocytes.

Figure 1, lane 2 shows that differentiation with MDI was associated with dramatic increases in the levels of the *scd1*, PPARγ, and aP2 mRNAs. As revealed by changes in cellular morphology detected by microscopy with oil red O staining (data not shown), preadipocytes treated with MDI were ~70% differentiated into adipocytes with the maximal levels of the adipogenic transcripts achieved after 6 to 9 days. Figure 1, lane 2 also shows only a slight increase in the level of *scd2* mRNA and no change in the level of pAL15 mRNA.

Figure 1, lane 3 shows that the addition of 10 μM TRO alone to the culture medium during the 9-day growth period caused a decrease in the level of *scd1* mRNA expression relative to preadipocytes and MDI-treated cells. However, when compared with cells differentiated in the presence of MDI alone, only a slight decrease in the levels of *scd2* and PPARγ expression was observed and the level of aP2 expression was stimulated by approximately 4-fold. The presence of some adipogenic mRNA transcripts suggests that differentiation may have been partially initiated by TRO in the absence of MDI.

Figure 1, lane 4 shows that when both MDI and TRO were added to the culture medium during the 9-day growth period, the PPARγ2 and aP2 mRNAs were present at levels comparable to those observed with MDI alone. The expression of *scd2* was slightly decreased by differentiation in the presence of both MDI and TRO. Furthermore, while the level of *scd1* mRNA was similar to that in preadipocytes and 3.2-fold higher than that present in TRO-treated cells, this level was ~95% reduced as compared with MDI-treated cells. In the MDI- and TRO-treated cells, microscopy revealed that ~100% differentiation was obtained, but the fat cells obtained were smaller than those differentiated in the presence of MDI alone.

Correlation of *scd* mRNA expression and translation

Figure 2 shows a Western analysis using a polyclonal rabbit anti-rat SCD antibody. Previous studies (24, 25) have shown that this antibody reacts with a 37-kDa SCD polypeptide (the 97-kDa band represents cross-reactivity of the polyclonal antibody with a protein not related to SCD). Figure 2, lane 1 shows that the level of 37-kDa SCD polypeptide was low in preadipocytes. On the addition of MDI (Fig. 2, lane 2), there was a substantial increase in the 37-kDa polypeptide as detected by Western blotting (presumably corresponding to increased expression of SCD1), while differentiation in the presence of 10 μM TRO (Fig. 2, lane 3) did not elicit the appearance of the SCD polypeptide (a minor band, whose identity is not known, was detected at ~45 kDa). Similar results were obtained when another TZD derivative, BRL49653 was substituted for TRO at 1 μM in growth medium (data not shown). In the presence of both MDI and TRO (Fig. 2, lane 4), the 37-kDa SCD protein was detectable at low levels, while minor cross-reactive bands of

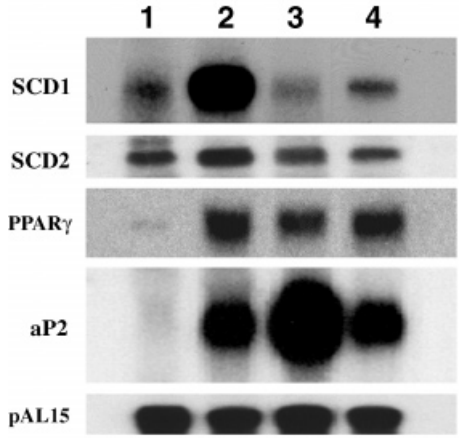

Fig. 1. Differential effects of TRO on *scd1* and *scd2* mRNA expression. Lane 1, confluent preadipocytes. Lane 2, differentiation with MDI. Lane 3, differentiation with TRO. Lane 4, differentiation with MDI plus TRO.

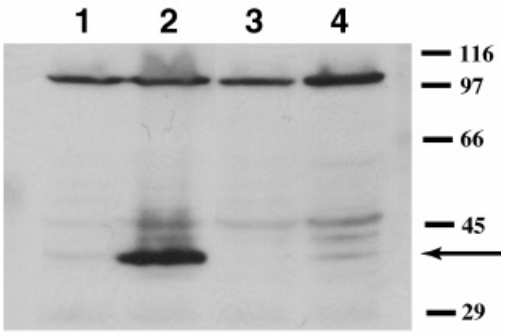

Fig. 2. Western analysis of SCD protein expression. Lane 1, confluent preadipocytes. Lane 2, differentiation with MDI. Lane 3, differentiation with TRO. Lane 4, differentiation with MDI plus TRO. The arrow indicates the 37-kDa SCD protein.

~40 and ~45 kDa were again observed. Correlation of the mRNA expression results of Fig. 1 and the Western blotting results of Fig. 2 suggests that both *scd1* mRNA expression and the appearance of the 37-kDa polypeptide (likely SCD1) were stimulated by MDI and inhibited by TRO. In contrast, *scd2* mRNA expression was only slightly decreased by either TRO or MDI plus TRO.

Changes in lipid composition associated with TRO differentiation

Table 1 shows the percentage distribution of 16:0, Δ^9-16:1, 18:0, and Δ^9-18:1 in the total lipid fraction obtained from the various differentiation protocols. In confluent preadipocytes, the most abundant saturated fatty acids were 18:0 (29.9%) and 16:0 (16.5%), while the predominant monounsaturated fatty acid was Δ^9-18:1 (12.8%); Δ^9-16:1 was present at a considerably lower level (1.4%).

After differentiation with MDI alone (Table 1), the percentage of 16:0 increased, consistent with the commitment to de novo fatty acid biosynthesis caused by differentiation, while the percentage of 18:0 decreased by ~3-fold. There was also an increase in the percentage of total monounsaturated fatty acids, and this was mainly due to an ~20-fold increase in Δ^9-16:1 as opposed to only an ~1.3-fold increase in Δ^9-18:1.

On differentiation with TRO alone (Table 1), the percentages of both 16:0 and 18:0 were high, with 16:0 markedly elevated relative to confluent preadipocytes. For both saturated fatty acids, the percentages were comparable to those observed in cells differentiated by the addition of MDI alone, implying that some aspects of de novo fatty acid biosynthesis were stimulated by the presence of TRO. However, despite the substantial increase in the percentage of the requisite substrate 16:0, the percentage of Δ^9-16:1 did not increase in the TRO-treated cells whereas the percentage of Δ^9-18:1 increased to a level similar to that observed in both preadipocytes and cells treated with MDI alone.

Effect of TRO on *scd* mRNA levels in mature adipocytes

Kurebayashi et al. (21) reported that TRO repressed *scd1* mRNA expression when added to fully differentiated 3T3-L1 adipocytes produced by treatment with MDI for 9 days. However, no information was provided for *scd2* mRNA expression. **Figure 3A** shows the time-course effect of TRO on the expression of both *scd* genes in mature adipocytes as obtained by the use of specific cDNA probes. Addition of 10 μM TRO to fully differentiated 3T3-L1 adipocytes reduced the level of *scd1* mRNA by approximately 50% within 6 h, suggesting blockage of new *scd1* gene expression and a half-life for *scd1* mRNA in adipose tissue that corresponds well with that previously determined in liver tissues (10). The *scd1* mRNA level continued to drop for the 24 h, and eventually reached a steady state level ~20% of that observed before addition of TRO. In contrast, no significant change in the levels of *scd2*, aP2, and pAL15 mRNAs was observed.

TABLE 1. Fatty acid composition in adipocyte cells

	Treatment[a]				
Fatty Acid	Control[b]	MDI	TRO	MDI + TRO	Human[c]
16:0	16.5 (1.0)	23.3 (0.1)	26.3 (1.2)	25.1 (1.1)	20.4 (2.2)
Δ^9-16:1	1.4 (0.1)	25.0 (1.8)	3.0 (1.0)	18.1 (0.6)[d]	6.1 (1.5)
18:0	29.9 (2.5)	9.3 (0.9)	31.3 (2.0)	12.1 (0.2)	4.9 (1.1)
Δ^9-18:1	12.8 (1.1)	16.9 (1.1)	15.0 (0.50)	18.8 (0.7)	42.5 (2.4)

[a] Values are the percentage of total fatty acids in 3T3-L1 adipocytes produced by the indicated differentiation protocol (mean of n ≥ 4 differentiation experiments at day 9; standard deviation shown in parentheses).
[b] Confluent preadipocytes.
[c] Average value obtained from ~10^5 determinations of lipid composition in human adipose tissue.
[d] Significantly different from the MDI result, $P < 0.05$.

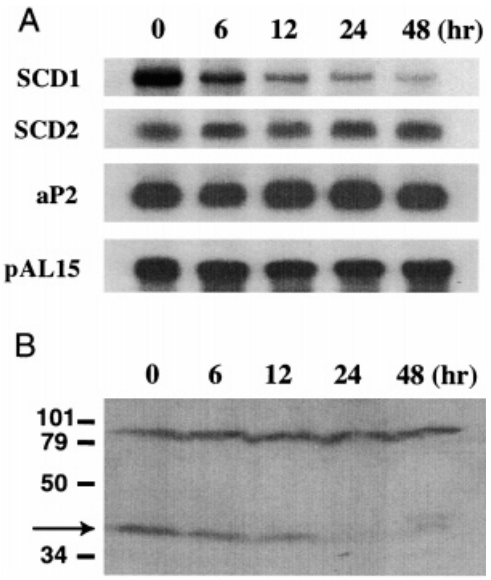

Fig. 3. Effect of TRO on *scd* mRNA and SCD protein expression in mature adipocytes. MDI-differentiated adipocytes (day 9) were treated with TRO (10 μM). (A) Total cellular RNA was extracted at the hour indicated and Northern blot analysis was performed. (B) Total cellular protein was extracted at the hour indicated and Western blot analysis was performed. The arrow indicates the 37-kDa SCD protein.

Figure 3B shows the time-course effect of TRO on the presence of SCD as obtained by Western blotting. During the time course, the 37-kDa polypeptide showed the same time-dependent decrease as observed for the *scd1* mRNA. These results establish a correspondence between *scd1* mRNA levels and SCD protein levels, and are also consistent with the half-life for proteolytic degradation of SCD determined elsewhere (24, 25).

Figure 4 shows the effect of TRO on *scd1* mRNA expression in primary adipocytes. Treatment of primary adipocytes in suspension with 10 μM TRO resulted in ~60% reduction in expression of *scd1* mRNA consistent with our previous demonstration that TRO decreases *scd1* mRNA expression in mouse adipose tissue in vivo (26).

DISCUSSION

Effect of TRO on *scd* mRNA expression

PPARγ ligands such as TRO are known to promote adipocyte differentiation but also to repress *scd1* gene expression (21). These opposing effects give rise to differences

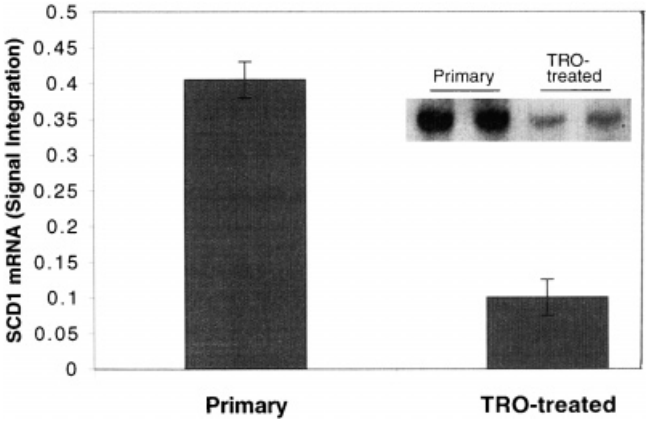

Fig. 4. The effect of 10 μM TRO on expression of mouse *scd1* mRNA in primary adipocytes isolated from B10.PL(73NS)/Sn mice. The signal was quantitated by densitometric scanning of Northern blots, and numbers on the *y* axis represent the ratio of the intensity of *scd1* mRNA to aP2 mRNA used as a positive expression control. The inset shows two separate experiments, in which 4.9-kb *scd1* mRNA levels from primary adipocytes and primary adipocytes treated with TRO for 16 h in suspension, are compared.

in both the lipid composition (Table 1) and the phenotypic characteristics of 3T3-L1 adipocytes produced by differentiation with MDI alone as compared with those produced by differentiation with TRO. Both Northern (Fig. 1) and Western (Fig. 2) blotting show that *scd1* gene expression is inhibited by TRO, whereas Northern blotting shows that *scd2* gene expression is essentially independent of either MDI or TRO. Figure 4 shows that TRO decreased *scd1* mRNA in primary mouse adipocytes as well.

Figures 1 and 3, and Table 1, show that the combination of MDI and TRO in experiments with mature 3T3-L1 adipocytes (this work and others; see ref. 21) does not produce the same pattern of effects (mRNA levels, lipid composition) given by the addition of either MDI alone or TRO alone. In the concentration regimen used here (and elsewhere), adipocytes differentiated with both MDI and TRO have an *scd1* mRNA level, SCD protein level, and a lipid composition intermediate between those of TRO-treated and MDI-treated cells. However, even with the decreased levels of *scd1* mRNA and SCD protein present in the MDI plus TRO-treated cells, there appears to be sufficient catalytic activity to produce a conversion of 16:0 to Δ^9-16:1 nearly equivalent to that observed in MDI-treated cells.

Effect of TRO on lipid composition and cell morphology

As observed in preadipocytes (Fig. 1, lane 1), cells differentiated by TRO (Fig. 1, lane 3) primarily exhibit *scd2* mRNA, implying that SCD2 may be the major isoform present to catalyze desaturation of either 16:0 or 18:0. However, in both preadipocytes and TRO-treated cells, this putative SCD2 was apparently able to provide the observed conversion of 18:0 to Δ^9-18:1 while at the same time was apparently unable to convert the substantial quantities of 16:0 present into elevated levels of Δ^9-16:1 (Table 1).

The inability of the TRO-treated cells to produce substantial quantities of Δ^9-16:1 may also be associated with the inability of these cells to assume the characteristic phenotype produced by differentiation with MDI. This correlation suggests that an important physiologic role of SCD1 catalysis may be to maintain fluidity of the accumulating lipid droplets via conversion of 16:0 to Δ^9-16:1. In the absence of this bulk transformation of the lipid composition, the higher melting temperature of excess saturated fatty acid may place severe constraints on cell size, thus preventing the change to the characteristic phenotype. These observations are consistent with previous studies (27), where adipocytes isolated from TZD-treated animals were smaller in size and contained smaller fat droplets.

Catalytic selectivity of SCD isoforms

SCD1 and SCD2 are members of a family of integral membrane enzymes that contain eight conserved histidine residues (28, 29). This diiron-center binding motif has been identified in many other membrane enzymes that require NADPH, O_2, and nonheme iron for catalysis (30, 31), including desaturases, hydroxylases (32, 33), sterol methyl oxidase (34), aldehyde decarbonylase (35), plasmologen (36) and ceramide (37) desaturases, epoxidases, and acetylenase (38). Site-directed mutagenesis studies have suggested that a relatively small number of amino acids will be responsible for this catalytic diversity (39). Within this enzyme family, the Δ^6-, Δ^9-, Δ^{12}-, Δ^{15}-, and other desaturases have different selectivities for the length of acyl chain, the position of double bond insertion, and the type of polar head group on the preferred substrate (30). Furthermore, interactions of acyl chains with desaturases and other enzyme have been shown to give free energy changes used for either catalytic enhancement (40) or binding enhancement (41–44). One intriguing possibility is that SCD1 and SCD2 may share these properties with other members of this family, and thus have different selectivities for acyl chain length in addition to their different regulatory origins.

Figure 5 diagrams how catalytic selectivity could enhance the control of lipid composition initiated by tran-

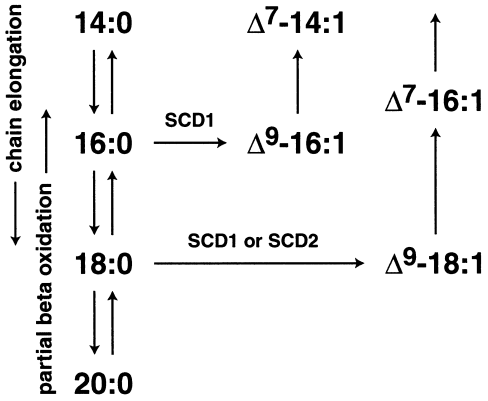

Fig. 5. Enzymatic interconversions of C16 and C18 fatty acids in mature 3T3-L1 adipocytes. SCD1 is proposed to desaturate either 16:0-CoA or 18:0-CoA, depending on the relative concentrations of these acyl-CoAs in the cell, while SCD2 is proposed to exhibit selectivity for desaturation of 18:0 relative to 16:0 (see text).

scriptional regulation. A previous steady state kinetic study of the catalytic selectivity of the purified rat liver microsomal SCD for acyl chain length revealed a slight, ~1.2-fold $V_{apparent}/K_M$ preference for 18:0-CoA desaturation relative to 16:0-CoA desaturation (45). Because this purified rat enzyme is comparable to SCD1 of 3T3-L1 adipocytes (5), SCD1 may also be relatively nonselective for either 16:0 or 18:0. In MDI-treated adipocytes, the increased formation of Δ^9-16:1 would arise from saturation of abundant SCD1 with 16:0 acyl chains derived from de novo fatty acid biosynthesis. The increased formation of Δ^9-16:1 would decrease the flux of fatty acid biosynthesis toward chain elongation to 18:0, and for the 18:0 formed, subsequent desaturation to Δ^9-18:1 could be catalyzed by either SCD2 or the elevated levels of SCD1. Thus a combination of diversion to Δ^9-16:1 and desaturation of 18:0 to Δ^9-18:1 would then be responsible for the observed decrease in 18:0.

Because the Northern and Western experiments (Figs. 1–3) suggest that SCD1 may be present at only low levels in preadipocytes and in TRO-treated adipocytes, it is feasible that SCD2 provides the major proportion of desaturase activity in these cells (presently, there is no evidence of the existence of other Δ^9-desaturase isoforms in 3T3-L1 cells). Because the 60% increase in 16:0 observed in TRO-treated cells relative to preadipocytes was associated with no significant accumulation of Δ^9-16:1, SCD2 would apparently have a low catalytic selectivity for 16:0 and may instead have a catalytic selectivity for 18:0 acyl chains (5- to 9-fold, on comparison of the percentages of Δ^9-18:1 and Δ^9-16:1 in the control and TRO columns of Table 1). This latter possibility is supported by the enhanced accumulation of Δ^9-18:1 in both preadipocytes and in TRO-treated cells, where SCD2 may predominate. In this light, it is perhaps noteworthy that mouse brain tissue, where only *scd2* mRNA expression has been detected, has 33% of Δ^9-18:1 in the total lipid fraction (46).

Figure 5 suggests other types of enzymatic processes that may also contribute to the production and maintainence of lipid composition, such as chain elongation, product inhibition when the membrane reaches an optimal lipid composition, partial β oxidation, and the conversions of acyl chains between phospholipid, triacylglycerol, and CoA forms. The impact of TRO treatment on these processes is not known at present.

Comparison with human SCD activity

By averaging the published percentages obtained from ~10^5 individual determinations of the lipid composition in the adipose tissue of normal weight humans (47–53), the average percentage lipid composition shown in Table 1 was obtained. The human lipid composition, dominated by Δ^9-18:1, is considerably different than that found in the MDI-differentiated 3T3-L1 adipocytes (Table 1), where Δ^9-16:1 is dominant. If humans have only one isoform of SCD, as is currently thought (54), this isoform could be more selective for conversion of 18:0 to Δ^9-18:1 than for conversion of 16:0 to Δ^9-16:1 in order to support the observed accumulation of Δ^9-18:1.

CONCLUSIONS

In 3T3-L1 cells, a combination of transcriptional responses (16, 17), mRNA stabilities (10), and the action of specific proteases (24, 25) has been identified to affect SCD expression and stability. However, the potential contributions of the SCD1 and SCD2 isoforms to the production and maintainence of lipid composition are less well understood. We suggest that differences in the catalytic selectivity of SCD isoforms may also contribute to establishing the lipid composition of the cell, as finer control can be provided by regulated expression of two isoforms with differing selectivities than by expression of either one or two isoforms with the same selectivity.

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