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Differential regulation of fatty acid elongation enzymes in brown adipocytes implies a unique role for *Elovl3* during increased fatty acid oxidation

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Jakobsson, Andreas, Johanna A. Jörgensen, and Anders Jacobsson. Differential regulation of fatty acid elongation enzymes in brown adipocytes implies a unique role for *Elovl3* during increased fatty acid oxidation. *Am J Physiol Endocrinol Metab* 289: E517–E526, 2005. First published April 26, 2005; doi:10.1152/ajpendo.00045.2005.—The expression of the *Elovl3* gene, which belongs to the *Elovl* gene family coding for microsomal enzymes involved in very long-chain fatty acid (VLCFA) elongation, is dramatically increased in mouse brown adipose tissue upon cold stimulation. In the present study, we show that the cold-induced *Elovl3* expression is under the control of peroxisome proliferator-activated receptor-α (PPARα) and that this regulation is part of a fundamental divergence in the regulation of expression for the different members of the *Elovl* gene family. In cultured brown adipocytes, a mixture of norepinephrine, dexamethasone, and the PPARα ligand Wy-14643, which rendered the adipocytes a high oxidative state, was required for substantial induction of *Elovl3* expression, whereas the same treatment suppressed *Elovl1* mRNA levels. The nuclear liver X receptor (LXR) has been implicated in the control of fatty acid synthesis and subsequent lipogenic processes in several tissues. This regulation is also exerted in part by sterol regulatory element-binding protein (SREBP-1), which is a target gene of LXR. We found that stimulation of *Elovl3* expression was independent of LXR and SREBP-1 activation. In addition, exposure to the LXR agonist TO-901317 increased nuclear abundance of LXR and mature SREBP-1 as well as expression of the elongases *Lce* and *Elovl1* in a lipogenic fashion but repressed *Elovl3* expression. A functional consequence of this was seen on the level of esterified saturated fatty acids, such as C22:0, which was coupled to *Elovl3* expression. These data demonstrate differential transcriptional regulation and concomitantly different functional roles for fatty acid elongases in lipid metabolism of brown adipocytes, which reflects the metabolic status of the cells.

elongase; brown adipose tissue; gene expression; very long-chain fatty acid

FATTY ACIDS (FA) function not only as bulk molecules in membrane and storage lipids but have also been shown to exert signaling functions by modulation of nuclear receptor activation (4, 41). Such signaling processes implicate FA and related metabolites in cellular feedback functions to reflect the metabolic status of the cell.

The bulk of the FA synthesis in cells is performed by the cytosolic complex fatty acid synthase (FAS) (54). FAS produces medium- to long-chain FA (up to C16), which can be further elongated by membrane-bound enzymes mostly residing in the endoplasmatic reticulum (ER). Members of the *Elovl* (elongation of very long-chain fatty acids) gene family encode for enzymes, which are believed to perform the first step (condensation) in the elongation cycle to produce long-chain or very long-chain fatty acids (LCFA/VLCFA) from shorter FA. The family of enzymes consists of at least six members in mouse and human, believed to exert substrate-specific elongation with respect to FA length and unsaturation (30, 31, 36, 53, 61).

One important factor regulating FA metabolism in adipocytes is the transcription factor liver X receptor-α (LXRα) (26), which is activated by endogenous oxysterols. Along with the functionally overlapping LXRβ isoform, LXRα is involved in the control of cellular lipid homeostasis (24, 29) by activation of genes that are associated with FA and triglyceride synthesis, for example, FAS and sterol regulatory element-binding protein-1 (SREBP-1) (25, 44).

Two isoforms of SREBP-1 have been identified, SREBP-1a and SREBP-1c, originating from the same gene by the use of two different transcription start sites (60). Cleavage of the inactive SREBP precursor proteins residing in the ER, by a two-step proteolytic cascade, induces translocation of the NH_2-terminal part into the nucleus, where it exerts its effects on transcription (21, 55, 59). One recently characterized member of the *Elovl* family, long-chain elongase (*Lce*), which has an apparent substrate specificity for saturated FA in the C12-C16 range, displays a lipogenic expression profile manifested in transgenic mice overexpressing constitutively active isoforms of SREBP-1 (36).

The *Elovl1* and *Elovl3* genes have been suggested to encode for proteins involved in the elongation of saturated and monounsaturated FA in the C20-C24 range. In similarity to *Lce* and *Fas*, *Elovl1* is ubiquitously expressed in mice, whereas *Elovl3* is expressed mainly in liver, skin, and brown adipose tissue (BAT) (53). In BAT, *Elovl3* expression is very low when mice are housed at thermoneutrality but displays a dramatic upregulation during BAT activation and recruitment in response to cold stress (4°C for 3 days) (52). Increased VLCFA elongation activity in BAT in response to cold stress has previously been shown by Tvrdik et al. (53). BAT activation and recruitment processes, which include induction of *Elovl3* expression, are primarily controlled by sympathetic nerve activity and norepinephrine (NE) release. The resulting nonshivering thermogenesis primarily utilizes FA as substrate for mitochondrial respiration and subsequent heat generation through the uncoupling protein UCP1 (reviewed in Ref. 10).

Differentiation of isolated primary brown adipocytes in culture, indicated by multilocular fat accumulation and UCP1 expression, is spontaneously induced around day 5 after cell isolation, depending mainly on cell confluence and supply of nutrients from the culture medium. In cells that have already

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acquired adipocyte characteristics, NE advances final differentiation, including a dramatic increase in the expression of UCP1 and other genes associated with increased thermogenic capacity (reviewed in Refs. 10 and 39). However, to substantially induce *Elovl3* expression in cultured cells, the addition of glucocorticoids together with chronic exposure to NE is required (52).

In the present study, we show that the induced *Elovl3* expression in brown fat of cold-stimulated mice requires the transcription factor peroxisome proliferator-activated receptor-α (PPARα). By using primary brown adipocyte cultures, we further demonstrate differential regulation between *Elovl3* and the two other FA elongases, *Lce* and *Elovl1,* involved in the synthesis of saturated FA, which was correlated with FA oxidation processes.

MATERIALS AND METHODS

Reagents. L-Norepinephrine bitartrate (NE; Arterenol), dexamethasone, TO-901317, 9-*cis*-retinoic acid, WY-14643, 22(S)-hydroxycholesterol, and all other chemicals when otherwise not stated, were obtained from Sigma. NE was dissolved in water, whereas all other compounds used for cell stimulation were dissolved in ethanol. The concentrations used for NE, dexamethasone, TO-901317, 9-*cis*-retinoic acid, and WY-14643 were chosen on the basis of titration experiments and shown to give the optimal estimated effects in primary cultured brown adipocytes.

Animals. PPARα-ablated mice were a kind gift from Dr. F. Gonzales (28), backcrossed on a DBA background. As control mice, age-matched mice from this back-cross, which were bred under the same conditions as the PPARα-ablated mice, was used. Animals were housed in groups of three to four mice per cage and were fed ad libitum (Rat and Mouse Standard Diet No. 1; BeeKay Feeds, B&K Universal, Stockholm, Sweden), had free access to water, and were kept on a 12:12-h light-dark cycle at room temperature. Before the cold exposure experiments, the mice were kept at 30°C (thermoneutrality) for 6 days. After this period, animals were exposed to 22°C for the indicated times or remained at 30°C.

Cell culture. Brown adipocyte precursors were isolated from the interscapular, axillary, and cervical BAT depots of 3- to 4-wk-old male mice of the NMRI strain (B&K) and the DBA strain containing a PPARα-ablation (28) and grown in culture principally as described by Néchad et al. (37). Briefly, tissues were minced in HEPES-buffered solution containing 0.2% (wt/vol) collagenase type II (Sigma), and digestion was allowed for 30 min at 37°C. The digest was filtered through 250- and 25-μm nylon filters to remove undigested parts and mature cells. Precursor cells were pelleted by centrifugation (700 *g*), washed in Dulbecco's modified Eagle's medium, pelleted, and resuspended in 0.5 ml of culture medium/mouse. The cells were seeded in six-well plates (Corning) at a density of 1–2 × 10^4 cells/cm^2. The cells were cultured in Dulbecco's modified Eagle's medium (1 g glucose/liter) supplemented with 10% newborn calf serum, 4 nM insulin, 25 μg/ml sodium ascorbate, 10 mM HEPES, 4 mM glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin at 37°C in an atmosphere of 8% CO_2 in air. The cells were washed with Dulbecco's modified Eagle's medium on *day 1*, and the medium was changed on *days 1, 3,* and *6* of culture. Administration of agents was performed as described in the figure legends, and the respective solvents (ethanol and water) were added to control wells to exclude unspecific effects.

RNA analysis. Cells were harvested in 1 ml of Ultraspec solution (Biotec, Houston, TX), and total RNA was isolated according to the procedure described by the manufacturer. Ten micrograms of total RNA were separated by electrophoresis in an ethidium bromide-containing agarose-formaldehyde gel and blotted onto Hybond N membranes (Amersham). Prehybridization and hybridization were carried out in 50% formamide, 5× SSC, 5× Denhardt's solution, 50 mM sodium phosphate, pH 6.5, 0.5% SDS, and 100 μg/ml degraded herring sperm DNA at 42°C overnight. Membranes were washed once in 2× SSC and 0.1% SDS at room temperature for 15 min and once in 0.1× SSC and 0.1% SDS at 42°C for 15–30 min. cDNA fragments, identified by BLAST (1) and verified by sequencing for *Elovl1* and *Elovl3* (53), SREBP-1 (51), 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1; IMAGE EST clone gi:14605089), and LCE (gi:15715540), were [^{32}P]dCTP-labeled using a random-primed DNA labeling kit (Amersham Biosciences) and used as probes. Phosphor-imaging plates were exposed to the hybridized membranes for 24–72 h and subsequently analyzed in a FLA-3000 reader (Fuji). 18S rRNA was detected using a 473-nm laser in the FLA-3000 reader and used for normalization. Blots were quantified using Fuji Film Science Lab software and statistically analyzed by repeated-measures ANOVA and Tukey's multiple comparison test in GraphPad PRISM.

Isolation of subcellular protein fractions. Crude nuclear extracts were isolated according to Andrews and Faller (2). Medium was removed, and cells were scraped into 1 ml of cold phosphate-buffered saline, pelleted for 10 s, and resuspended in 400 μl of cold *buffer A* [10 mM HEPES-KOH, pH 7.9, at 4°C, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, complete miniprotease inhibitor cocktail (Roche)] by flicking the tube. Cells were allowed to swell on ice for 10 min and were then vortexed for 10 s. Nuclei were pelleted by centrifugation at 2000 *g* for 1 min, and the supernatant (S1 fraction) was transferred to a new tube. Pellets were resuspended in 50 μl of cold *buffer B* [20 mM HEPES-KOH, pH 7.9, at 4°C, 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, complete miniprotease inhibitor cocktail (Roche)] and incubated on ice for 20 min for high salt extraction. Cellular debris was removed by centrifugation (20.000 *g*) for 2 min at 4°C, and the supernatant (containing DNA-binding proteins) was stored at −80°C. The S1 fraction was centrifuged at 20,000 *g* for 15 min, and the supernatant was discarded, whereas the pellet (containing mitochondria and peroxisomes) was resuspended in 30 μl of cold *buffer B* and stored at −80°C. Protein concentration was determined by protein assay kit (Bio-Rad) for the isolated fractions before gel electrophoresis. Recovery of the nuclear and mitochondrial/peroxisomal proteins in the subcellular fractions was assessed by detection of marker proteins [cAMP response element-binding protein (CREB; not shown), UCP1, and acyl-CoA oxidase (AOx), respectively].

Protein analysis. Nuclear proteins (7.5 μg) and 3 μg of proteins from the 20,000-*g* fraction were separated on NuPAGE 10% Bis-Tris gels (Invitrogen) using NuPAGE MOPS-SDS running buffer according to the manufacturer's protocol and blotted onto nitrocellulose membranes. Membranes were blocked in 5% milk, washed in Tris-buffered saline containing 0.1% Tween-20 (TBST), incubated with primary antibody diluted in TBST and 5% BSA overnight, washed three times in TBST, and incubated with horseradish peroxidase-linked secondary antibody (Cell Signaling) for 1 h. Proteins were visualized using the ECLplus kit (Amersham Biosciences) and detected in a LAS-1000 charge-coupled device camera (Fuji). Proteins were quantified and statistically analyzed as described for RNA. SREBP-1 antibodies were purchased from Santa Cruz Biotechnologies and diluted 1:500, UCP1 antibodies were generated by Neosystem (COOH-terminal part, 1:2,000 dilution), and AOx (58) and multifunctional enzyme type 1 (MFE-1) (42) antibodies (1:2,000 dilution) were kindly provided as a gift from Dr. S. H. Alexsson, Karolinska Hospital and Dr. J. K. Hiltunen, University of Oulu.

Lipid analysis. Exogenous [^{14}C]palmitic acid (0.1 μCi/ml, 16 μM; GE Healthcare) bound to BSA (0.5%) was added to the culture medium 4 h before harvest of the cells. Cells were scraped in cold phosphate-buffered saline, pelleted, vigorously vortexed in the lipid extraction solvent mixture chloroform(C)-methanol(M)-water (4:8:3) for 1 min, left at room temperature for 20 min, and reextracted in C-M (2:1). Extracts were pooled, evaporated in speed-vac and redissolved in C-M (2:1). Lipid aliquots were applied to a silica gel 60 TLC plate (VWR International), and the plate was developed as previously

described by Downing (13). Briefly, the plate was prewashed with chloroform and placed in 105°C for 30 min before sample application. Development was as follows: first phase using hexane (to the end of the plate), air dried for 15 min; second phase using toluene (end of the plate), air dried for 15 min; third phase containing hexane-ether-acetic acid (70:30:1) run on half the plate. The thin-layer chromatography (TLC) plate was sprayed with sulfuric acid-ethanol (1:1) and charred at 150°C. Lipids were detected in the LAS-1000 and quantified as described earlier. Lipid identities were determined by standards run in parallel.

FA analysis. Cellular lipids were extracted in the presence of authentic internal standards by the method of Folch et al. (14) using chloroform-methanol (2:1, vol/vol). Approximately 25 million cells per treatment from three independent experiments were pooled and used for FA analysis. Individual lipid classes within each extract were separated by preparative TLC, as described previously (56). Authentic lipid class standard compounds were spotted onto the two outside lanes of the TLC plate to enable localization of the sample lipid classes. Each lipid fraction was scraped from the plate and *trans*-esterified in 3 N methanolic-HCl in a sealed vial under a nitrogen atmosphere at 100°C for 45 min. The resulting FA methyl esters were extracted from the mixture with hexane containing 0.05% butylated hydroxytoluene and prepared for gas chromatography by sealing the hexane extracts under nitrogen.

FA methyl esters were separated and quantified by capillary gas chromatography using a gas chromatograph (Hewlett-Packard model 6890, Wilmington, DE) equipped with a 30-m DB-225MS capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector as described previously (56).

FA oxidation. Fifty microliters of NCSII tissue solubilizer (GE Healthcare) was applied on pieces of filter paper left to dry overnight. [^{14}C]palmitic acid (0.1 μCi/ml, 16 μM; GE Healthcare) was added to cell culture wells as described for lipid analysis, and the culture plates were sealed using super sealing film (Life systems) with NCSII-treated filter papers attached over each well to collect released CO_2 from cells. After 4 h of incubation at 37°C, filters were collected and the amount absorbed CO_2 was quantified using liquid scintillation counting.

RESULTS

Reduced Elovl3 expression in BAT of cold-exposed PPARα-ablated mice. In liver, PPARα controls the expression of several genes involved in FA oxidation (3). Because the adrenergic pathway stimulates UCP1-mediated heat production, via increased FA β-oxidation and uncoupled cellular respiration (reviewed in Ref. 10), along with increased *Elovl3* synthesis (52), we investigated whether *Elovl3* expression was effected in PPARα-ablated mice upon cold stimulation. As seen in Fig. 1*A*, the expression of *Elovl3* was severely reduced in the PPARα-ablated mice after both 12 h and 3 days of mild cold exposure. This effect was specific for *Elovl3,* since the mRNA levels for the elongase *Elovl1* was not affected by the PPARα ablation.

Elevated Elovl3 expression and FA oxidation by PPARα ligand Wy-14643 in brown fat primary cultures. Cultured primary brown adipocytes are the only cell culture system described that expresses significant amounts of endogenous *Elovl3*. As shown earlier, 3 days of chronic NE stimulation in combination with glucocorticoids (dexamethasone) induces the expression of *Elovl3* in cultured brown adipocytes (52). Because NE, in conjunction with PPARα, stimulates the expression of *Elovl3* in vivo as shown above, we asked whether *Elovl3* expression could be augmented along with improved thermogenic capacity of the cultured brown adipocytes by stimulating the cells with a synthetic PPARα agonist (Wy-14643). Activation of PPARα has been shown to increase the rate of β-oxidation through upregulation of both mitochondrial and peroxisomal enzymes (6, 17, 27). As seen in Fig. 1*B*, Wy-14643, both alone (W) and in combination with NE, dexamethasone, and RXR ligand 9-*cis*-retinoic acid (heterodimerizing partner of PPARs) (NDRW), increased the level of the peroxisomal enzymes *AOx* and *MFE-1*, which are known PPARα target genes. Wy-14643 also induced an increase in the mitochondrial protein content of UCP1 when added in combination with adrenergic, retinoid, and glucocorticoid stimuli (NDRW), which implies an increased capacity for uncoupled respiration. In agreement with this, the oxidative response to adrenergic stimuli and PPARα activation (NDRW), measured by released CO_2 generated from β-oxidation of exogenously added labeled palmitic acid, was also increased compared with vehicle-treated cells (Fig. 1*C*). The level of *Elovl3* expression correlated with the oxidative capacity of the brown adipocytes; i.e., it was further elevated by the addition of PPARα ligand Wy-14643 (Fig. 1*D*). In accord with the in vivo data, the *Elovl3* expression was markedly reduced in NDRW-stimulated brown adipocytes obtained from PPARα-ablated mice (Fig. 1*E*). As expected, no expression was detected by Northern blot in unstimulated cells. *Elovl3* expression could not be detected in wild-type cells stimulated with dexamethasone, WY-14643, or 9-*cis*-retinoic acid alone (not shown), implying that adrenergic stimuli are essential for the activation processes involving elevated *Elovl3* expression in brown adipocytes.

Induction of Elovl3 expression is not mediated via induction of lipogenic factors LXR and SREBP-1. Activation of LXRs by natural and synthetic agonists induces *SREBP-1* mRNA and protein expression, and subsequently also elevated levels of mature SREBP-1 in the nucleus, which in turn can induce transcription of downstream lipogenic genes as shown for *Fas* and *Lce* (7, 36). To elucidate the signals inducing *Elovl3* expression, we investigated whether the NDRW mixture activated the transcription factors LXR and SREBP-1 in brown adipocytes. Interestingly, no increase in the nuclear abundance of SREBP-1 was detected when maximal induction of *Elovl3* expression was obtained (Fig. 2, *A* and *B*).

To further investigate whether LXR- and SREBP-1-mediated signaling pathways can, in fact, be activated in cultured primary brown adipocytes and, as a consequence, stimulate *Elovl3* expression, cells were stimulated with the synthetic LXR agonist TO-901317. Ligand activation increased the amount of *SREBP-1* mRNA and nuclear protein levels (Fig. 2, *A* and *B*). In contrast, NDRW-induced *Elovl3* mRNA levels were markedly suppressed by the LXR agonist (Fig. 3*A*). In addition, *Elovl3* expression was not restored upon simultaneous addition of 22(S)-hydroxycholesterol, which inhibits SREBP-1 cleavage and nuclear translocation without interfering with activation of LXR (11, 23, 55) (Figs. 2*B* and 3*A*). This suggests that changes in nuclear levels of SREBP-1 did not contribute to the LXR-mediated suppression of *Elovl3* mRNA levels in NDRW-stimulated brown adipocytes. In contrast, and as expected, the expression profile of the two other elongases, *Elovl1* and *Lce,* followed the level of nuclear SREBP-1 (Figs. 2*B* and 3, *B* and *C*).

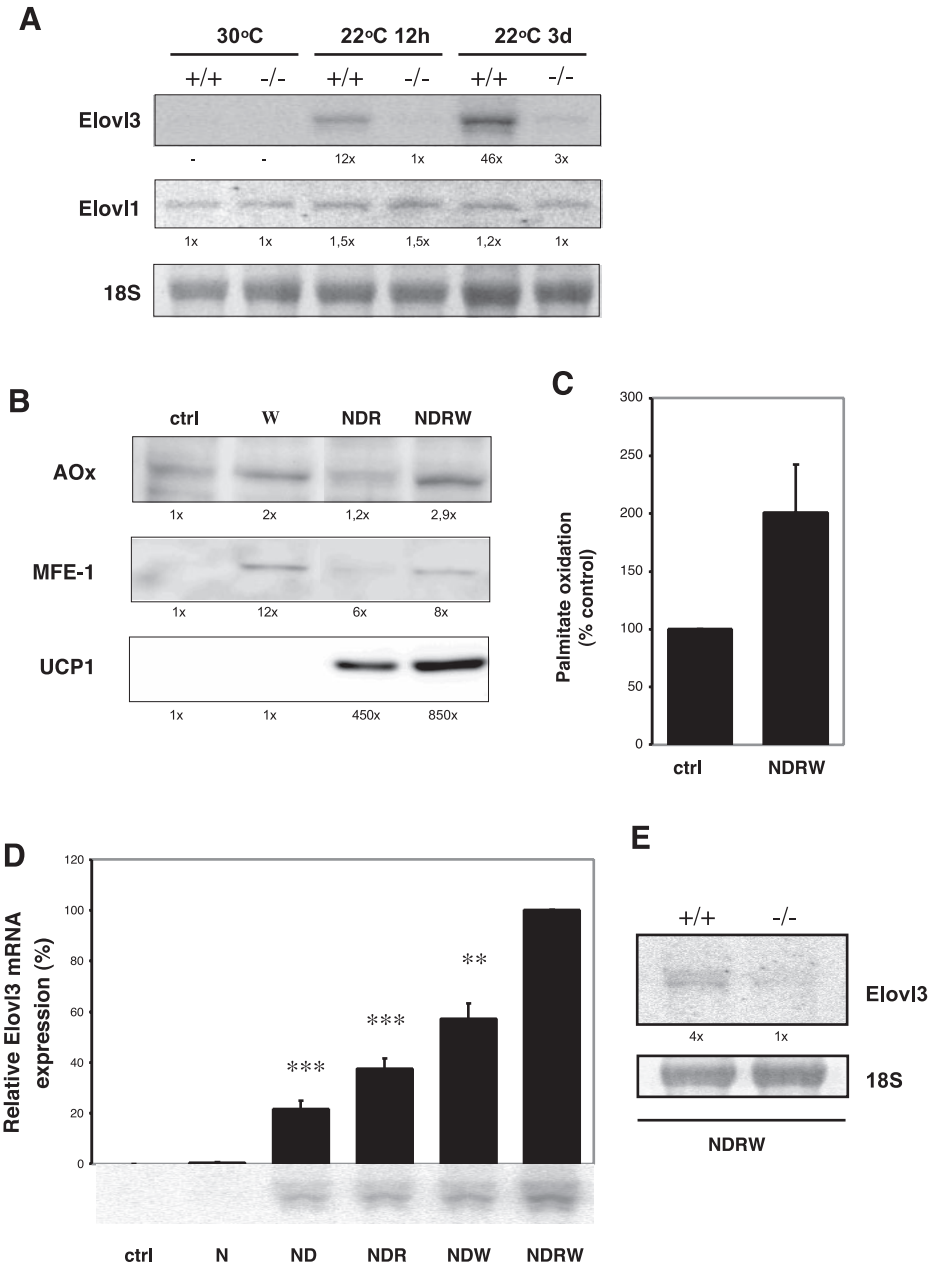

Fig. 1. Peroxisome proliferator-activated receptor-α (PPARα)-regulated *Elovl3* mRNA expression in conjunction with increased fatty acid (FA) oxidation in brown adipocytes. *A*: representative Northern blots showing RNA levels of *Elovl3*, *Elovl1*, and ribosomal RNA, respectively, in control and PPARα-ablated mice exposed to 30 or 22°C for indicated times. RNA levels were quantified, indicated as fold induction, by densitometric analysis. Primary brown adipocytes were cultured for 6 days up to confluence. From *day 6*, cells were treated (when indicated) with 0.1 μM norepinephrine (N) (every 12 h) and/or 1 μM dexamethsone (D) for 3 days. Wy-14643 (20 μM, W) and/or 500 nM 9-*cis*-retinoic acid (R) was added (when indicated) on *day 7.5* (for 36 h). *B*: proteins in subcellular fraction containing mitochondria and peroxisomes were separated on SDS-PAGE, and typical marker proteins of FA β-oxidation, acyl-CoA oxidase 1 (AOx), multifunctional enzyme type 1 (MFE-1), and uncoupling protein-1 (UCP1), were detected by immunoblotting and quantified. *C*: oxidation of palmitic acid was measured on *day 9* as released CO_2. Bars represent means ± SE of 4 experiments performed in triplicate. The difference between the 2 groups was not statistically significant. *D*: total RNA was isolated on *day 9* and analyzed by Northern blot. Membranes were probed with *Elovl3* cDNA and results normalized to 18S rRNA (see EXPERIMENTAL PROCEDURES). Bars represent means ± SE of 3 experiments performed in duplicate (autoradiogram shows representative blot). Significance at $^{**}P < 0.01$ and $^{***}P < 0.001$ by comparing *Elovl3* mRNA levels in respective treatments with (NDRW; set to 100%). *E*: total RNA isolated from NDRW-stimulated cells, as in *D*, obtained from control and PPARα-ablated mice.

Elovl3 expression correlated with expression of the main regulatory enzyme in glucocorticoid metabolism. Because the regulation of *Elovl3* expression did not follow an ordinary lipogenic pattern controlled by LXR and SREBP-1, we asked whether other factors related to lipid metabolism would show a similar expression profile to *Elovl3*. We (52) have previously shown glucocorticoid stimulation of *Elovl3* gene expression in cultured brown adipocytes, which is also shown here in Fig. 1. The glucocorticoid-metabolizing enzyme *11β-HSD1* primarily activates cellular glucocorticoids to sustain proper glucocorticoid signaling, although the enzyme has also been suggested to catalyze the reverse, inactivating reaction at a slower rate. To investigate whether *Elovl3* expression was paralleled by changes in the regulation of glucocorticoid turnover, *11β-HSD1* expression was measured. As shown for *Elovl3*, a combination of NE and dexamethasone (NDRW mixture) increased the *11β-HSD1* expression, whereas LXR activation decreased *11β-HSD1* mRNA levels (Fig. 4) in parallel with *Elovl3* expression. Again, similar to *Elovl3* expression, the TO-901317-induced suppression was not blocked by addition of 22(S)-hydroxycholesterol, why a SREBP-1 dependent mechanism was excluded. This suggests that the regulation of local glucocorticoid activation and *Elovl3*-expression in BAT are closely associated and in a similar way restrained by LXR.

Elovl3 expression and lipid composition. To assess a functional consequence of altered *Elovl3* expression in the brown adipocytes, we analyzed the lipid composition by TLC and gas chromatography as described in MATERIALS AND METHODS. In NDRW-treated cells where *Elovl3* expression was high, incorporation of ^{14}C label (originating from exogenous FA) into sterol esters and fatty alcohols was selectively increased (Fig. 5*A*). This effect was abolished by the addition of LXR agonist,

both in the presence and in the absence of 22(S)-hydroxycholesterol, as shown earlier to inhibit SREBP maturation. In addition, in NDRW-treated cells, 22(S)-hydroxycholesterol did not have any effect on the incorporation of ^{14}C label into sterol esters and fatty alcohols, which was in accord with sustained high *Elovl3* expression in these cells.

To look for changes in specific FA incorporation, which could be linked to the level of *Elovl3* expression, we analyzed the sterol ester fractions further. To obtain a sufficient amount of material for measurements of the FA composition, ~25 million cells per treatment were pooled from three independent experiments. Noticeably, the FA composition in the sterol ester fractions displayed alterations in behenic acid (22:0) content between the different treatments (Fig. 5*B*), which was in accordance with the amount of incorporated FA label into sterol esters (Fig. 5*A*) and the level of *Elovl3* expression (Fig. 3*A*); high levels in NDRW-treated cells and low levels in cells stimulated by the LXR agonist. The alterations in sterol ester

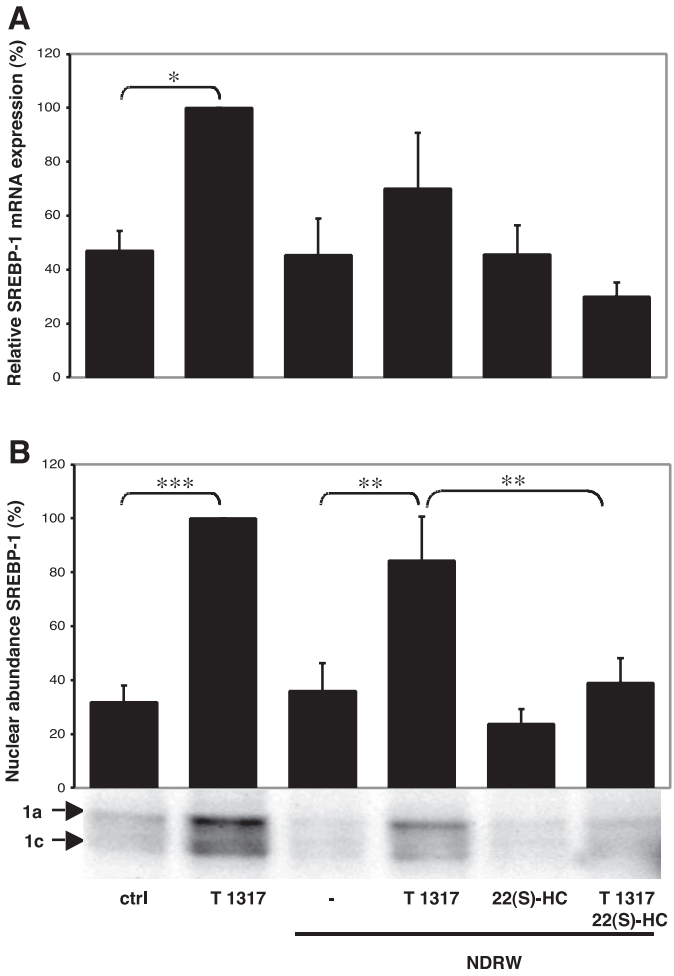

Fig. 2. Effects of nuclear receptor agonists and adrenergic brown adipocyte activation on abundance of sterol regulatory element-binding protein (SREBP)-1. Brown adipocytes were cultured and treated as described in Fig. 1. *SREBP-1* mRNA levels (*A*) and SREBP-1 nuclear protein levels (*B*) were analyzed following indicated treatments. Visible bands are 1a and 1c isoforms, as indicated by arrows. TO-901317 (T1317) and 22(S)-hydroxycholesterol (22(S)-HC) (20 μM) were added to cells on *day 7.5* (for 36 h). Bars represent 3 individual experiments in duplicate ± SE. Significance at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

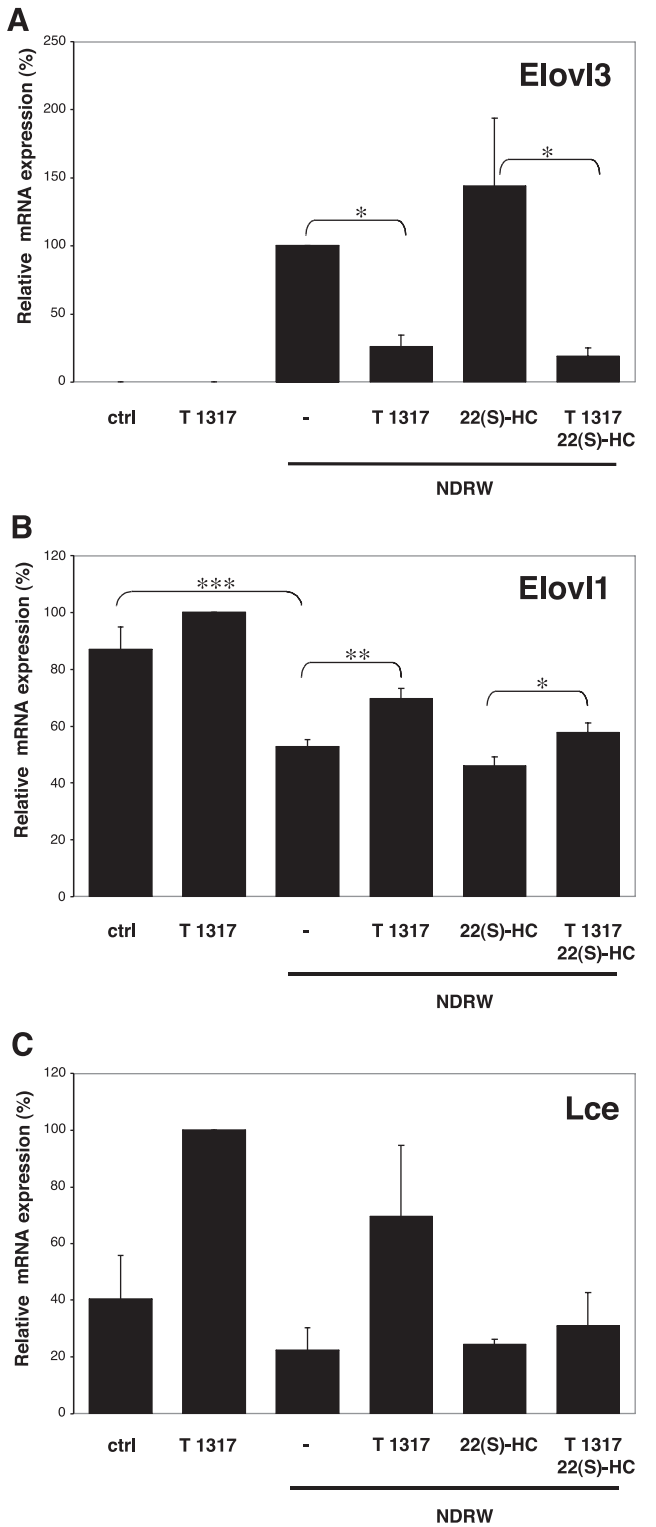

Fig. 3. Contrasted *Elovl3* mRNA expression pattern from *Elovl1* and *Lce*. Cells were cultured and treated as described in Fig. 1. Shown are relative mRNA levels following indicated treatments, as in Fig. 2, for *Elovl3* (*A*), *Elovl1* (*B*), and *Lce* (*C*), analyzed by Northern blot. TO-901317 treatment is set to 100%, except for *Elovl3* where NDRW represents 100%. Bars represent 3 individual experiments in duplicate ± SE. Significance at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

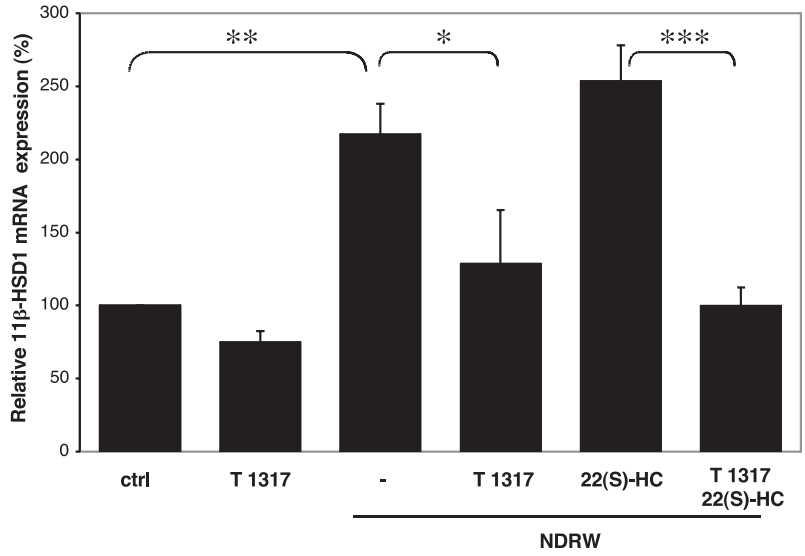

Fig. 4. Liver X receptor (LXR) agonist suppresses 11β-hydroxysteroid dehydrogenase-1 (11β-HSD1) mRNA levels. *11β-HSD1* mRNA levels in brown adipocytes analyzed by Northern blot, following indicated treatments as in Fig. 2. Bars represent 3 individual experiments in duplicate ± SE. Significance at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

behenic acid content was specific, as no similar changes was seen for LCFA, such as palmitic acid (16:0; Fig. 5*B*), in response to the respective treatments. This is of particular interest because *Elovl3* is suggested to be involved in the synthesis of saturated VLCFA in that range of C22–24 acyl-chain lengths (Ref. 53 and A. Jacobsson, unpublished observations).

These data suggest a function for *Elovl3* in the formation of saturated VLCFA, such as behenic acid, in a situation when the rate of cellular FA oxidation is stimulated. Ligand activation of LXR appears to inhibit similar lipid synthesis, in conjunction with a suppressive effect on *Elovl3* expression.

DISCUSSION

The stimulatory effect of LXR agonists on genes involved in FA synthesis has been suggested to be mediated both directly through LXR and via SREBP-1c (25, 45). From our experiments, we conclude that, although LXR and SREBP-1 could be activated in primary brown adipocytes in response to a synthetic LXR agonist, the induction of *Elovl3* expression by NDRW stimuli was not mediated via activation of LXR- or SREBP-mediated signaling pathways. In contrast to *Lce* and *Elovl1*, the LXR agonist TO-901317 exerted a negative effect on *Elovl3* expression, although all of these three *Elovl* enzymes have been suggested to elongate saturated FA. Stimulation of *Elovl3* expression was instead accomplished by the addition of a stimulatory mix including a PPARα agonist, which proved to be a unique regulatory feature among the *Elovl* genes in the present study. The PPARα regulation of *Elovl3* expression in brown adipocytes was confirmed both in cultured cells obtained from PPARα-ablated mice and in whole animals when exposed to cold.

PPARα and LXRα have been proposed to form atypical heterodimers, working as negative regulators of the respective target genes (15, 35). The suppressive effect of LXR agonist on PPARα agonist-stimulated *Elovl3* expression putatively suggests similar regulation in BAT, but the same relative suppression of *Elovl3* expression in response to LXR agonist was seen when 9-*cis*-retinoic acid and Wy-14643 was excluded from the stimulatory mix. This indicates that the suppressive effect on *Elovl3* levels is not dependent on downregulation of PPARα- and RXR-mediated signals.

The assembly of nuclear receptor coactivator/corepressor complexes is an important determinant of the impact on transcriptional activity. Recent data on transcriptional control of lipid synthesis by the PPARγ coactivators PGC-1α and PGC-1β, which have been shown to coactivate, e.g., PPARα and LXRα, suggest a gene-specific displacement of corepressors by PGC-1α upon nuclear receptor ligand activation (16) and PGC-1β mediated activation during energy storage (33). A specific recruitment of PGC-1α and PGC-1β on different promoters, such as for *Lce*, *Elovl1,* and *Elovl3*, may explain, in part, the discrepancy in regulation by LXR on the expression of these genes.

SREBPs are important transcription factors in the regulation of lipogenic processes, as many of the target genes are involved in the synthesis of lipids. SREBP-2 is suggested to primarily regulate cholesterol homeostasis, whereas the two characterized isoforms of SREBP-1 (a and c) are implicated in FA and triglyceride synthesis, as suggested from studies in transgenic mice (19, 47, 48). Although several studies indicate distinct functions for the two SREBP-1 isoforms (20, 32, 49), we could not detect any difference in the induction or translocation of the mature SREBP-1a and -1c proteins in response to LXR agonist (Fig. 3*C*).

A possible candidate to be involved in the regulation of *Elovl3* expression is the main regulatory enzyme in glucocorticoid metabolism, 11β-HSD1, which proved to be regulated in a similar way to *Elovl3* at the mRNA level in primary brown adipocytes. In white adipose tissue, glucocorticoids enhance adipogenesis by inducing 11β-HSD1 activity in preadipocytes, which creates a feed-forward loop contributing to adipocyte differentiation (9, 18). In addition to fluctuating glucocorticoid levels in the circulation, this enzyme constitutes a local determinant of the active glucocorticoid levels within peripheral tissues, as 11β-HSD1 catalyzes the interconvertion of inactive keto- and active hydroxycorticosteroids in most tissues favoring the dehydrogenase (activating) reaction. In this context, it is noteworthy that the synthetic glucocorticoid dexamethasone is also metabolized by the normal cellular mechanisms, but at

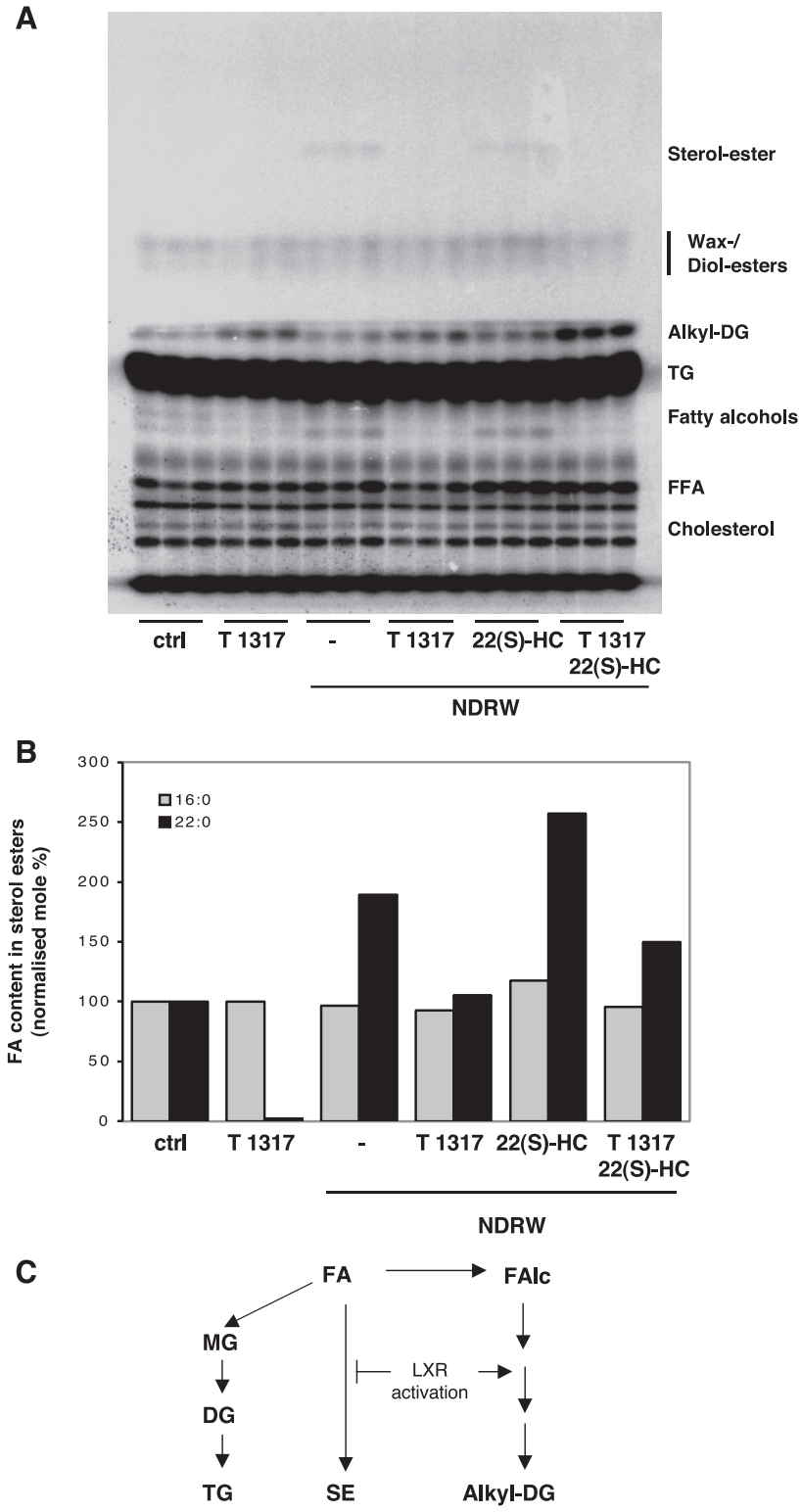

Fig. 5. *Elovl3* expression and FA incorporation. *A*: thin-layer chromatography (TLC) separation of labeled cellular lipids from total lipid extract. Incorporation of ^{14}C label originating from [U-^{14}C]palmitic acid into cellular lipids was detected by phosphorimaging after TLC separation and identified by lipid standards run in parallel. Cells were cultured and treated as described in Fig. 2. [U-^{14}C]palmitic acid was added 4 h before harvest. Samples are shown in triplicates. *B*: content of palmitic (16:0) and behenic acid (C22:0) expressed as normalized mole percent of total FA content within the sterol ester fraction, analyzed by gas chromatography. *C*: scheme FA esterification pathways. MG, monoglycerides; DG, diglycerides; TG, triglycerides; SE, sterol esters; Falc, fatty alcohols.

a slower rate compared with endogenous glucocorticoids due to its structural properties (12). A decrease in the cellular expression of *11β-HSD1* in response to LXR activation, which has previously also been shown in 3T3-L1 cells by Stulnig et al. (50), may result in lower levels of endogenous as well as synthetic active glucocorticoids. Although the enzyme may play a more-or-less different role in BAT than in white adipose tissue, the inhibitory effect of TO-901317 on *Elovl3* expression may in part be explained by a decrease in glucocorticoid receptor signaling (Fig. 6).

In contrast to our findings, Hummasti et al. (22) recently reported a positive effect of LXR agonist GW-3965 on *Elovl3* expression in differentiated 3T3-L1 white adipocytes. However, gene expression in a highly oxidative tissue such as BAT

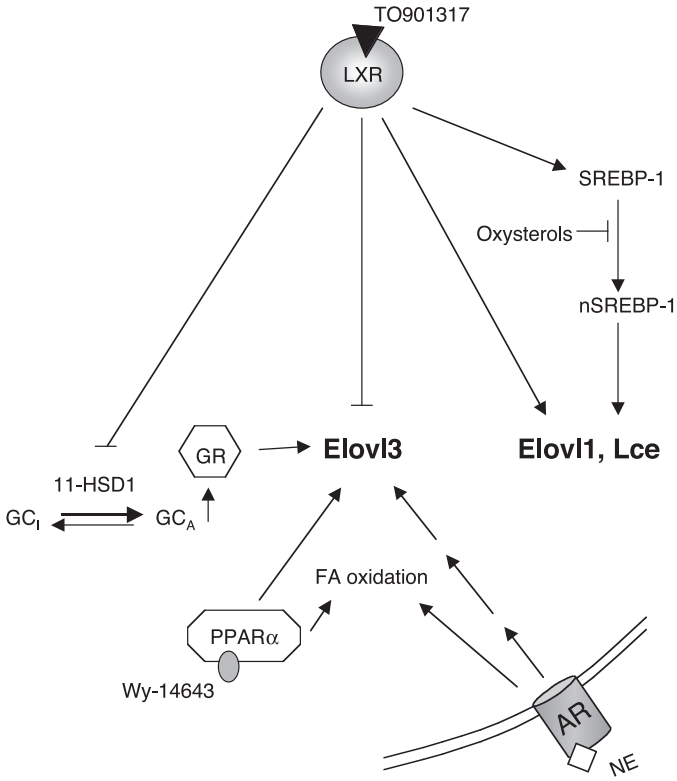

Fig. 6. Regulation of *Elovl* genes in brown adipocytes. Schema of hormonal and metabolic regulation of *Elovl* genes with respect to regulatory factors correlated with oxidative or lipogenic events in brown adipocytes. nSREBP-1, SREBP-1; GR, glucocorticoid receptor; GC_i, inactive glucocorticoid; GC_a, active glucocorticoid; AR, adrenoreceptors; NE, norepinephrine.

and a lipid storage tissue such as white adipose tissue may be subject to differential regulation as a consequence of different sets of transcription factors and their cell-specific modulations. In addition, *Elovl3* expression is normally very low in white adipose tissue, and the ways of inducing adipocyte differentiation in cultured cell lines, such as 3T3-L1, may interfere with the physiological response to various stimuli. Analysis of the *Elovl3* promoter region revealed putative binding sites for, e.g., cAMP response element-binding protein (CREB; NE-cAMP stimulation), PPARα, and glucocorticoid receptor (GR). Whether any of these elements is actually involved in the *Elovl3* expression has yet to be investigated.

The physiological activator of brown adipocytes, NE, confers the basis for induction of FA oxidation, including peroxisomal β-oxidation (38), and thermogenesis in BAT.

The stimuli required for induction of *Elovl3* expression, further emphasizes the "unique" cellular condition leading to transcription of this gene. As *Elovl3* mRNA levels are correlated with increased β-oxidation, the function of *Elovl3* may be to replenish intracellular pools of specific FAs when the FA turnover rate is high. In vitro elongation experiments on microsomes from murine BAT, as well as functional complementation studies in yeast, indicated ELOVL3 activity for saturated and monounsaturated FA substrates in the C16-C24 range (53) (A. Jacobsson, unpublished observations). Our data from this study on the correlation between cellular behenic acid (22:0) content and the level of *Elovl3* expression are in accordance with this. Even though we did not see any major differences between the different treatments in total FA incorporation within the triglyceride fraction, we cannot rule out that there is a specific difference in FA content similar to the sterol ester factions. However, the analysis was not readily performed due to very high basal FA incorporation.

Although the FA composition in both phospholipids and triglycerides from BAT is altered toward increased content of saturated C22 and C24 species in response to cold acclimation (40, 46), which is in agreement with elevated VLCFA elongation activity in cold-acclimated mice (53), the rationale for a shift toward saturated VLCFA in the cold is not clear. An increased amount of saturated VLCFA within the phospholipids would result in a more ordered (rigid) membrane structure to maintain barrier integrity during thermogenesis. It has also been shown that long hydrophobic FAs are important components in the formation of fat droplets (43). Although there is a decrease in triglyceride content during cold exposure, there is at the same time an increased triacylglycerol/FA turnover in BAT, which can be detected already during the first day of cold exposure (8, 34). The decrease in triacylglycerol content seen with NE-mediated BAT activation is secondary to activation of FA oxidation (5). Our data on *Elovl* mRNA levels support a role for ELOVL3 during intense FA turnover. Recent data show an impaired sterol ester and triglyceride formation in the sebocytes of *Elovl3*-ablated mice (57), suggesting that the function for ELOVL3 in brown adipocytes relates to the need for restoration of specific intracellular lipid pools when the FA turnover rate is high.

In conclusion, functional diversity and specificity of FA-modifying enzymes enables the cell to maintain a dynamic metabolic balance in response to hormonal signals and varying nutritional status. Our data show a fundamental divergence in transcriptional regulation of the genes controlling saturated FA synthesis in the cell, which implicates certain functions of these elongases in different metabolic contexts.

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REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, and Lipman DJ.** Basic local alignment search tool. *J Mol Biol* 215: 403–10, 1990.
2. **Andrews N and Faller D.** A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res* 19: 2499, 1991.
3. **Aoyama T, Peters JM, Iritani N, Nakajima T, Furihata K, Hashimoto T, and Gonzalez FJ.** Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J Biol Chem* 273: 23–28, 1998.
4. **Auwerx J.** Regulation of gene expression by fatty acids and fibric acid derivatives: an integrative role for peroxisome proliferator activated receptors. *Horm Res* 38: 269–277, 1992.
5. **Baht HS and Saggerson ED.** Effect of noradrenaline on triacylglycerol synthesis in rat brown adipocytes. *Biochem J* 258: 369–373, 1989.
6. **Barbera MJ, Schluter A, Pedraza N, Iglesias R, Villarroya F, and Giralt M.** Peroxisome proliferator-activated receptor alpha activates transcription of the brown fat uncoupling protein-1 gene. A link between

regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. *J Biol Chem* 276: 1486–1493, 2001.
7. **Bennett MK, Lopez JM, Sanchez HB, and Osborne TF.** Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. J *Biol Chem* 270: 25578–25583, 1995.
8. **Brooks BJ, Arch JR, and Newsholme EA.** Effect of some hormones on the rate of the triacylglycerol/fatty-acid substrate cycle in adipose tissue of the mouse in vivo. *Biosci Rep* 3: 263–267, 1983.
9. **Bujalska IJ, Kumar S, Hewison M, and Stewart PM.** Differentiation of adipose stromal cells: the roles of glucocorticoids and 11beta-hydroxysteroid dehydrogenase. *Endocrinology* 140: 3188–3196, 1999.
10. **Cannon B and Nedergaard J.** Brown adipose tissue: function and physiological significance. *Physiol Rev* 84: 277–359, 2004.
11. **Cases S, Novak S, Zheng YW, Myers HM, Lear SR, Sande E, Welch CB, Lusis AJ, Spencer TA, Krause BR, Erickson SK, and Farese RVJ.** ACAT-2, a second mammalian acyl-CoA:cholesterol acyltransferase. Its cloning, expression, and characterization. *J Biol Chem* 273: 26755–26764, 1998.
12. **Diederich S, Eigendorff E, Burkhardt P, Quinkler M, Bumke-Vogt C, Rochel M, Seidelmann D, Esperling P, Oelkers W, and Bahr V.** 11Beta-hydroxysteroid dehydrogenase types 1 and 2: an important pharmacokinetic determinant for the activity of synthetic mineralo- and glucocorticoids. *J Clin Endocrinol Metab* 87: 5695–5701, 2002.
13. **Downing DT.** Photodensitometry in the thin-layer chromatographic analysis of neutral lipids. *J Chromatogr* 38: 91–99, 1968.
14. **Folch J, Lees M, and Sloane Stanley GH.** A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497–509, 1957.
15. **Gbaguidi GF and Agellon LB.** The atypical interaction of peroxisome proliferator-activated receptor alpha with liver X receptor alpha antagonizes the stimulatory effect of their respective ligands on the murine cholesterol 7alpha-hydroxylase gene promoter. *Biochim Biophys Acta* 1583: 229–236, 2002.
16. **Guan HP, Ishizuka T, Chui PC, Lehrke M, and Lazar MA.** Corepressors selectively control the transcriptional activity of PPARgamma in adipocytes. *Genes Dev* 19: 453–461, 2005.
17. **Gulick T, Cresci S, Caira T, Moore DD, and Kelly DP.** The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc Natl Acad Sci USA* 91: 11012–11016, 1994.
18. **Hauner H, Schmid P, and Pfeiffer EF.** Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells. *J Clin Endocrinol Metab* 64: 832–835, 1987.
19. **Horton JD, Shimomura I, Brown MS, Hammer RE, Goldstein JL, and Shimano H.** Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J Clin Invest* 101: 2331–2339, 1998.
20. **Horton JD, Shimomura I, Ikemoto S, Bashmakov Y, and Hammer RE.** Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J Biol Chem* 278: 36652–36660, 2003.
21. **Hua X, Nohturfft A, Goldstein JL, and Brown MS.** Sterol resistance in CHO cells traced to point mutation in SREBP cleavage-activating protein. *Cell* 87: 415–426, 1996.
22. **Hummasti S, Laffitte BA, Watson MA, Galardi C, Chao LC, Ramamurthy L, Moore JT, and Tontonoz P.** Liver X receptors are regulators of adipocyte gene expression but not differentiation: identification of apoD as a direct target. *J Lipid Res* 45: 616–625, 2004.
23. **Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, and Mangelsdorf DJ.** Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. *Proc Natl Acad Sci USA* 96: 266–271, 1999.
24. **Janowski BA, Willy PJ, Devi TR, Falck JR, and Mangelsdorf DJ.** An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* 383: 728–731, 1996.
25. **Joseph SB, Laffitte BA, Patel PH, Watson MA, Matsukuma KE, Walczak R, Collins JL, Osborne TF, and Tontonoz P.** Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J Biol Chem* 277: 11019–11025, 2002.
26. **Juvet LK, Andresen SM, Schuster GU, Dalen KT, Tobin KA, Hollung K, Haugen F, Jacinto S, Ulven SM, Bamberg K, Gustafsson JA, and Nebb HI.** On the role of liver X receptors in lipid accumulation in adipocytes. *Mol Endocrinol* 17: 172–182, 2003.
27. **Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, and Wahli W.** Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci USA* 90: 2160–2164, 1993.
28. **Lee SST, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, and Gonzalez FJ.** Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 15: 3012–3022, 1995.
29. **Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA, and Willson TM.** Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem* 272: 3137–3140, 1997.
30. **Leonard AE, Bobik EG, Dorado J, Kroeger PE, Chuang LT, Thurmond JM, Parker-Barnes JM, Das T, Huang YS, and Mukerji P.** Cloning of a human cDNA encoding a novel enzyme involved in the elongation of long-chain polyunsaturated fatty acids. *Biochem J* 350: 765–770, 2000.
31. **Leonard AE, Kelder B, Bobik EG, Chuang LT, Lewis CJ, Kopchick JJ, Mukerji P, and Huang YS.** Identification and expression of mammalian long-chain PUFA elongation enzymes. *Lipids* 37: 733–740, 2002.
32. **Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, and Brown MS.** Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem* 277: 9520–9528, 2002.
33. **Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, Yang W, Pei L, Uldry M, Tontonoz P, Newgard CB, and Spiegelman BM.** Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell* 120: 261–273, 2005.
34. **Lindberg O, Bieber LL, and Houstek J.** Brown adipose tissue metabolism: an attempt to apply results from in vitro experiments on tissue in vivo. In: *Regulation of Depressed Metabolism and Thermogenesis*, edited by Jansky L and Musacchia XJ. Xxxx, OH: Thomas Springfield, 1976, p. 117–136.
35. **Miyata KS, McCaw SE, Patel HV, Rachubinski RA, and Capone JP.** The orphan nuclear hormone receptor LXR alpha interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. *J Biol Chem* 271: 9189–9192, 1996.
36. **Moon YA, Shah NA, Mohapatra S, Warrington JA, and Horton JD.** Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. *J Biol Chem* 276: 45358–45366, 2001.
37. **Néchad M, Kuusela P, Carneheim C, Björntorp P, Nedergaard J, and Cannon B.** Development of brown fat cells in monolayer culture. I. Morphological and biochemical distinction from white fat cells in culture. *Exp Cell Res* 149: 105–118, 1983.
38. **Nedergaard J, Alexson S, and Cannon B.** Cold adaptation in the rat: increased brown fat peroxisomal β-oxidation relative to maximal mitochondrial oxidative capacity. *Am J Physiol Cell Physiol* 239: C208–C216, 1980.
39. **Nedergaard J, Herron D, Jacobsson A, Rehnmark S, and Cannon B.** Norepinephrine as a morphogen?—its unique interaction with brown adipose tissue. *Int J Develop Biol* 39: 827–837, 1995.
40. **Ohno T, Ohinata H, Ogawa K, and Kuroshima A.** Fatty acid profiles of phospholipids in brown adipose tissue from rats during cold acclimation and repetitive intermittent immobilization: with special reference to docosahexaenoic acid. *Jpn J Physiol* 46: 265–270, 1996.
41. **Ortiz-Caro J, Montiel F, Pascual A, and Aranda A.** Modulation of thyroid hormone nuclear receptors by short-chain fatty acids in glial C6 cells. Role of histone acetylation. *J Biol Chem* 261: 13997–14004, 1986.
42. **Palosaari P and Hiltunen J.** Peroxisomal bifunctional protein from rat liver is a trifunctional enzyme possessing 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and delta 3, delta 2-enoyl-CoA isomerase activities. *J Biol Chem* 265: 2446–2449, 1990.
43. **Raclot T.** Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog Lipid Res* 42: 257–288, 2003.
44. **Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, and Mangelsdorf DJ.** Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev* 14: 2819–2830, 2000.

45. **Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, and Shan B.** Role of LXRs in control of lipogenesis. *Genes Dev* 14: 2831–2838, 2000.
46. **Senault C, Hlusko MT, and Portet R.** Effects of diet and cold acclimation on lipid composition of rat interscapular brown adipose tissue. *Ann Nutr Alim* 29: 67–77, 1975.
47. **Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, and Goldstein JL.** Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* 98: 1575–1584, 1996.
48. **Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, and Goldstein JL.** Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* 99: 846–854, 1997.
49. **Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, and Brown MS.** Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev* 12: 3182–3194, 1998.
50. **Stulnig TM, Oppermann U, Steffensen KR, Schuster GU, and Gustafsson JA.** Liver X receptors downregulate 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. *Diabetes* 51: 2426–2433, 2002.
51. **Tontonoz P, Kim JB, Graves RA, and Spiegelman BM.** ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. *Mol Cell Biol* 13: 4753–4759, 1993.
52. **Tvrdik P, Asadi A, Kozak LP, Nedergaard J, Cannon B, and Jacobsson A.** *Cig30*, a mouse member of a novel membrane protein gene family, is involved in the recruitment of brown adipose tissue. *J Biol Chem* 272: 31738–31746, 1997.
53. **Tvrdik P, Westerberg R, Silve S, Asadi A, Jakobsson A, Cannon B, Loison G, and Jacobsson A.** Role of a new mammalian gene family in the biosynthesis of very long chain fatty acids and sphingolipids. *J Cell Biol* 149: 707–717, 2000.
54. **Wakil SJ, Stoops JK, and Joshi VC.** Fatty acid synthesis and its regulation. *Ann Rev Biochem* 52: 537–579, 1983.
55. **Wang X, Sato R, Brown MS, Hua X, and Goldstein JL.** SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77: 53–62, 1994.
56. **Watkins SM, Lin TY, Davis RM, Ching JR, DePeters EJ, Halpern GM, Walzem RL, and German JB.** Unique phospholipid metabolism in mouse heart in response to dietary docosahexaenoic or alpha-linolenic acids. *Lipids* 36: 247–254, 2001.
57. **Westerberg R, Tvrdik P, Undén AB, Månsson JE, Norlén L, Jakobsson A, Holleran WM, Elias PM, Asadi A, Flodby P, Toftgård R, Capecchi MR, and Jacobsson A.** Role for ELOVL3 and fatty acid chain length in development of hair and skin function. *J Biol Chem* 279: 5621–5629, 2004.
58. **Wilcke M and Alexson S.** Differential induction of peroxisomal populations in subcellular fractions of rat liver. *Biochim Biophys Acta* 1544: 358–369, 2001.
59. **Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, and Brown MS.** Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110: 489–500, 2002.
60. **Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, Goldstein JL, and Brown MS.** SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell* 75: 187–197, 1993.
61. **Zhang K, Kniazeva M, Han M, Li W, Yu Z, Yang Z, Li Y, Metzker ML, Allikmets R, Zack DJ, Kakuk LE, Lagali PS, Wong PW, MacDonald IM, Sieving PA, Figueroa DJ, Austin CP, Gould RJ, Ayyagari R, and Petrukhin K.** A 5-bp deletion in *ELOVL4* is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet* 27: 89–93, 2001.