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Increase in membrane uptake of long-chain fatty acids early during preadipocyte differentiation

(preadipocyte differentiation/fatty acid transport/fatty acid-binding protein)

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ABSTRACT **An increase in early rates of oleate uptake, which reflected fatty acid (FA) entry into the cells, was apparent 2–3 days after confluence of differentiating BFC-1 preadipocytes. The increase was measured in cells kept without glucose and with arsenate, where >95% of intracellular radioactivity was recovered as free unesterified oleate. Uptake of retinoic acid, a molecule structurally similar to long-chain FA, remained unaltered during cell differentiation. Increase in oleate transport was related to increase in transport V_{max} (determined under arsenate treatment) from 0.2 to 2 nmol/min per 10^6 cells, whereas K_m remained unchanged (2×10^{-7} M). Oleate transport was maximal at about day 6 after cell confluence (day 0), as FA metabolism (incorporation into lipids) began to gradually increase. The increase in transport preceded induction of mRNAs for both cytosolic FA-binding protein, which appeared at day 6, and for the FA synthase, which appeared at day 10. Data indicated that increases in activities of FA transport and of lipoprotein lipase, early during cell differentiation, favored increased availability of exogenous FA at a stage when endogenous FA synthesis is limited. This result would promote FA esterification and lipid deposition by supplying a rate-limiting substrate. Furthermore, oleate addition to BFC-1 preadipocytes at confluence potentiated the effect of dexamethasone in inducing mRNA for cytosolic FA-binding protein. In adipocytes, FA from exogenous or endogenous sources was necessary to maintain levels of cytosolic FA-binding protein mRNA. Thus, the increase in FA availability might contribute to, or modulate, induction of proteins necessary for preadipocyte differentiation.**

Fatty acid (FA) permeation across the plasma membrane of adipocytes has been shown to be carrier mediated with characteristics of facilitated diffusion (1–3). Multiple lines of evidence suggested that FA transport might be important in regulating FA metabolism (4, 5). To gain more insight into the physiological significance of the transport system we studied oleate uptake and metabolism in cultured adipocytes. Adipose cells in culture offer an ideal system where transport and metabolism of FA can be related as the cell differentiates and acquires the adipose phenotype.

BFC-1 cells, established from mouse brown fat tissue, express many of the characteristics of other adipose cell lines like 3T3 L1 and 3T3 F442A. They show a high rate of differentiation with temporally well-defined stages (6). We have previously shown that BFC-1 adipocytes when compared with preadipocytes exhibited an increased rate of fatty acid uptake necessary to accommodate their higher rate of FA metabolism (7). Whether the increase in FA uptake reflected membrane or metabolic events and whether it preceded or followed acquisition of other markers of differentiation remained uncertain. Such information will help determine whether increased substrate availability is involved in the differentiation process. In this study we have characterized uptake of [^{3}H]oleate in BFC-1 cells at various stages of differentiation into adipocytes and related changes in transport to those in other parameters of FA metabolism.

MATERIALS AND METHODS

Cell Culture. Establishment and characteristics of the BFC-1 preadipocyte line from mouse brown fat has been described by Forest *et al.* (6). Our experiments were conducted with a subclone BFC-1b that exhibited >90% conversion into adipocytes. Cells were plated at 1–2 × 10^5 cells per 35-mm dish in Dulbecco's modified Eagle medium (DMEM, GIBCO)/10% fetal calf serum (Flow Laboratories)/penicillin at 200 units/ml/streptomycin at 50 μg/ml/33 μM biotin/17 μM pantothenate. At confluence (0.7–1 × 10^6 cells per dish) 1 nM triiodothyronine and 20 nM insulin were added to enhance differentiation. Cell rounding and formation of lipid droplets were apparent at about day 6 (after confluence), and differentiation was complete by about day 15.

Cell Treatments. Cells were always studied the day after medium change. Before the transport assay, cells were kept in serum-free DMEM for 1 hr at 37°C. The medium was then switched to a Krebs-Ringer solution buffered with Hepes (KRH), pH 7.5 and containing 0.5% bovine serum albumin (BSA; fraction V, FA free, Sigma). The buffer contained 2 mM glucose or lacked glucose and was without or with 25 mM sodium arsenate; phosphate was omitted from buffers containing sodium arsenate. Under all conditions pH was adjusted to 7.5 before and after BSA addition and after sodium arsenate addition. Preincubations with KRH were for 0.5–2 hr, as indicated. Cells were then washed three times with 1 ml of appropriate buffer at 23°C and then assayed for oleate transport.

Transport Assay. After aspiration of the last wash, the dish was placed on a rotating shaker platform. Transport of [^{3}H]oleate (NEN) was started by adding 1 ml of isotopic medium containing oleate (80 nmol, 3000 cpm/μl) complexed (1) to the indicated amount of serum albumin (20–160 nmol). The isotopic solution also contained [^{14}C]mannitol (42 μCi/μmol, ICN; 1 Ci = 37 GBq) as extracellular marker. The cells were maintained during the transport assay on the rotating platform to ensure good mixing of the transport medium. At the desired time, 2 ml of ice-cold buffer without albumin (stop solution) was added, mixed well with the isotopic solution, and aspirated. The cells were then washed twice with 2 ml each of cold buffer and lysed in 1 ml of 0.1 M NaOH. Under these conditions no efflux or uptake of radioactivity occurred during the stop and wash procedures as determined in

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Abbreviations: FA, fatty acid(s); KRH, Krebs/Ringer/Hepes; aP2, FA binding protein; FAS, FA synthase; EF1, enhancing factor 1; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium.

separate experiments. Cell-associated radioactivity was measured by adding an aliquot (700 μl) of the cell lysate to 10 ml of scintillant (ACS, Amersham) and counting in a three-channel Beckman LS 3300 counter set up for double-isotope discrimination. Extracellularly trapped radioactivity was determined from the ^{14}C in the lysate (extracellular space equaled ≈1.5 μl per 10^6 cells) and from 0-time incubations where isotope and stop solutions were added simultaneously. Extracellularly trapped [^{3}H]oleate was subtracted from total cell-associated [^{3}H]oleate to calculate intracellular [^{3}H]-oleate.

Rates of oleate uptake were derived from the least-squares fit to time courses by using either linear or nonlinear regression. In most cases with exponentially growing cells the best fit was obtained by linear regression, whereas for postconfluent cells, nonlinear regression was required. The model used for fitting the two-phase time courses was based on the following empirical equation: $S_t = S_\infty(1 - e^{-\lambda t}) + kt$. S_t is FA taken up at time t, S_∞ is the amount taken up exponentially at steady state, λ is the fractional rate of exponential approach to steady state, and k is the rate of the slow phase of FA uptake. Initial rate of FA uptake is given by $S_{\infty\lambda} + k$. The first and second uptake phases reflect, respectively, accumulation of free label and of FA metabolites. For incubations without glucose and with arsenate, as most FA remained in the free form inside the cell, a one-compartment model was adequate, and kt was dropped from the equation. In all cases correlation coefficients for the data fit exceeded 0.95, and the residuals (difference between observed and predicted values) were randomly distributed.

For determination of uptake kinetics, rates of oleate uptake were related to the concentration of unbound FA in the medium, calculated (1) on the basis of published association constants for FA–BSA binding (8).

Determination of Oleate Incorporation into Cell Lipids. After the transport assay and the washes with cold stop solution, the cells were directly lysed in methanol and Folch-extracted (chloroform/methanol, 2:1). Oleate distribution into cellular lipid was determined by described methods (4, 7).

Isolation of Cellular RNA. Cells were harvested by scraping. Total RNA was prepared using the guanidium–cesium chloride procedure (9) and was quantitated by its absorbance at 260 nm. Ethidium bromide (0.01 μg/μl) was added to RNA samples (25 μg) before subjecting them to 1% agarose-formaldehyde electrophoresis to monitor RNA integrity and the uniformity of RNA transfer onto a nitrocellulose membrane (Optibind, Schleicher & Schuell). This concentration of ethidium bromide did not interfere with RNA transfers that were generally complete. Prehybridization and hybridization with the various cDNA probes [for fatty acid-binding protein (aP2), FA synthase (FAS), β-actin, enhancing factor 1 (EF1)] were at 43°C. The conditions were the same [5× standard saline citrate (SSC)/50 mM sodium phosphate/1 mM sodium pyrophosphate/25% (vol/vol) formamide/10% (wt/vol) dextran sulfate, 5× Denhardt's solution/0.1% SDS/0.05% Nonidet P-40/torula RNA at 2 mg/ml/salmon sperm DNA at 0.1 mg/ml.] After hybridization, the membranes were washed for 20 min twice in 3× SSC/0.1% SDS at 43°C and then once or twice in 0.1× SSC/0.1% SDS at 65°C. For FAS the last wash was done in 0.2× SSC at 43°C. Transport assays and RNA isolation were done by using cells from the same transfer plated at the same cell density.

Immunodetection of aP2. Cells were washed with cold KRH buffer and then extracted and processed for immunoblotting, as described by Bernlohr *et al.* (10). Blots were incubated for 15–18 hr with anti-mouse adipose lipid-binding protein, ALBP (11) antibody (1:500), washed, and immunostained (Vectastain, ABC kit, Vector Laboratories).

RESULTS

Cell Characteristics During Differentiation Process. BFC-1 cells showed visible lipid accumulation beginning at about day 6 after confluence. At 15–18 days after confluence >90% of the cells had multiple lipid droplets. To relate changes in transport activity to those in cell-surface area and cell water, cell diameter and equilibrium space of the nonmetabolizable 3-*O*-methylglucose were estimated during various stages of cell differentiation (Fig. 1). Cell diameter and cell water increased significantly during cell differentiation. Cell diameter (Fig. 1*A*) increased 1.6-fold by day 12 after confluence. This change meant that cell-surface area, which is a function of r^2, increased 2.5-fold, and cell volume, which is a function of r^3, increased by a factor of 4.0. Cell water (Fig. 1*B*) also increased (by 2.9-fold) from 1.7 to 5 μl per 10^6 cells.

The difference between the respective increases in cell volume and cell water could be related to the increase in volume occupied by the accumulating lipid and protein. The data would indicate that ≈28% of the increase in cell volume could be related to the increased lipid content, whereas 72% was from an increase in intracellular water as a result of expansion of cell membranes.

Analysis of Time Course of Oleate Uptake During Cell Differentiation. To obtain early measurements of oleate uptake that were independent of oleate metabolism, conditions were established where most FA could be recovered intracellularly in free unesterified form. Table 1 shows that when BFC-1 adipocytes are kept in KRH buffer without glucose and in the presence of arsenate for 2 hr at 37°C and then assayed for oleate uptake at 23°C, a major fraction of the oleate taken up at the end of 2 min could be recovered in free unesterified form. This result was true for cells kept with glucose for uptake times shorter than 0.4 min. Consistent with this is the observation that the early phase of oleate uptake was similar in cells kept with glucose or without glucose and without or with arsenate (Fig. 2*B*). Slope of the late phase of uptake (time >0.4 min) was markedly decreased

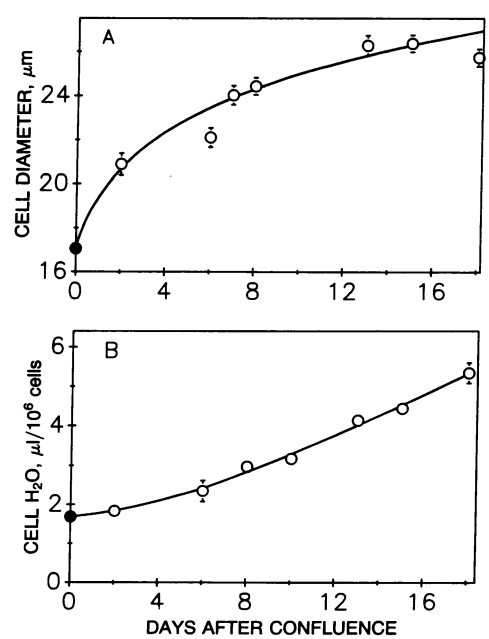

FIG. 1. Changes in cell diameter (*A*) and intracellular water (*B*) in BFC-1 cells during adipose conversion process. BFC-1 cells were grown in DMEM/10% fetal calf serum, which was supplemented at confluence (day 0, filled symbol) with 1 nM triiodothyronine and 20 nM insulin. Cell diameter (n = 100 for each point) was determined microscopically with dilute (2%) cell suspensions. Cell water (n = 10 for each point) was estimated from the equilibrium space of 3-*O*-methylglucose. Data are from two separate cell series.

Table 1. Intracellular free [^{3}H]oleate recovered (at 0.4 and 2 min) in cells kept in the presence or absence of glucose (the latter with and without arsenate treatment)

	Free oleate, % of total			
	Preadipocytes		Adipocytes	
Incubation condition	0.4 min	2 min	0.4 min	2 min
With glucose	72	15	65	12
Without glucose	79	65	69	76
with arsenate	93	88	95	85

Preadipocytes (1 day before confluence) and adipocytes (16 days after confluence) were processed and assayed for oleate uptake at 23°C, as described in legend for Fig. 2*A*. Separation of major lipid fractions to determine percent of free oleate was done as described (1, 7).

in adipocytes kept without glucose and/or treated with arsenate. In preadipocytes, because the early-uptake phase was very low, arsenate treatment reduced FA entry to almost undetectable levels (data not shown). That glucose deprivation and arsenate treatment greatly reduced FA esterification without affecting initial uptake rate indicates that esterification is not involved in FA entry and does not influence entry rate. This fact also suggests that metabolic energy is not required for the entry step (1).

Fig. 3*A* shows time courses of oleate uptake determined at various stages during adipose differentiation for cells kept and assayed in the presence of glucose. Time courses were generally biphasic with a fast early phase that was complete in ≈0.4 min. The early phase was barely detectable in just confluent cells (filled symbols day). Fig. 2 showed that this early phase of oleate uptake was independent of FA metabolism and reflected FA entry into the cells and approach of intracellular FA to isotopic equilibrium. The slower phase reflected FA incorporation into glycerides. As shown, uptake rates increased significantly as cells differentiated. The increase involved both phases of the time course. Increase in the early phase of uptake was significant within 2 days after confluence and addition of insulin and triiodothyronine to the culture medium. On the other hand, increase in the late phase of the time course was more gradual in onset.

Early rates of oleate uptake were determined from curve fitting of complete time courses, like the ones shown in Fig. 3*A*, for cells at various stages during culture. Cells were assayed in the presence of glucose or in the absence of glucose without or with arsenate (Fig. 3*B*). The data obtained with all three treatments of the cell preparations were similar

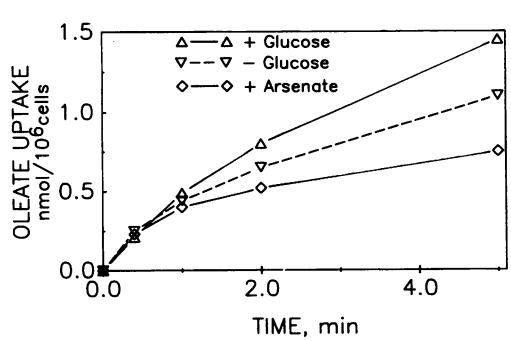

FIG. 2. Effect of glucose deprivation without or with arsenate treatment on oleate uptake by differentiated BFC-1 cells. After the 1-hr incubation in serum-free DMEM, cells were maintained for 2 hr at 37°C in KRH buffer/0.5% BSA with either 2 mM glucose, no glucose, or no glucose with 25 mM sodium arsenate. At end of incubation cells were washed with 2 ml of the appropriate buffer and assayed for [^{3}H]oleate uptake at 23°C, as described. Distribution of intracellular [^{3}H]oleate radioactivity for the three treatments is shown in Table 1.

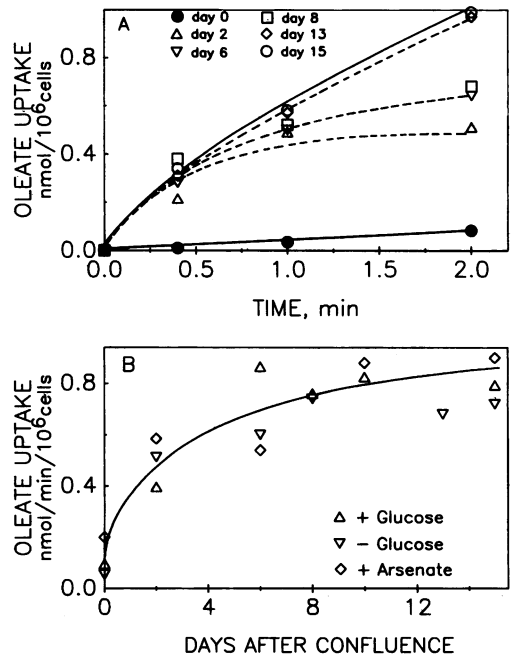

FIG. 3. (*A*) Time course of oleate uptake in differentiating BFC-1 cells on various days after confluence (day 0, filled symbols) and addition of insulin and triiodothyronine. Before assay cells were kept at 37°C for 1 hr in serum-free DMEM and then switched to KRH buffer/0.5% albumin/2 mM glucose for 30-min stabilization period. Cells were washed with fresh KRH buffer at 23°C to remove released FA from the extracellular space. Uptake of [^{3}H]oleate was assayed at 23°C. The isotopic solution contained 2 mM glucose, 80 nmol of [^{3}H]oleate (0.3 μCi/nmol) complexed to 40 nmol of BSA, and [^{14}C]mannitol (25 μCi/nmol) as a marker for the extracellular space. Dashed lines illustrate progressive changes in shapes of time courses, which occurred as cells differentiated. (*B*) Early rates of oleate uptake at various days after cell confluence (day 0) measured in cells treated as described under legend to *A*. Early rates were obtained from curve fitting of time courses (four time points in duplicates), as described in text. Assay times chosen were for preconfluent cells at 0.4, 0.8, 2.0, and 5.0 min and for confluent cells, at various stages of differentiation at 0.1, 0.4, 1.0 and 2 min. Data for each treatment are averages of experiments conducted with two different cell series.

and demonstrated that the rate of oleate entry into the cells increased 4- to 7-fold during the first 2 days after confluence to ≈65% of its final value. On the other hand, oleate metabolism (determined from measurements >2 min in cells kept in glucose) progressively increased starting at ≈6 days after confluence and continued to increase beyond the eighth day of the differentiation period in parallel with capacity of the cells to incorporate oleate into lipids (data not shown).

The increase in FA transport was not a simple result of increases in cell-surface area or in cytosolic volume. At day 2 postconfluence, cell-surface area had undergone only 33% of its eventual 2.5-fold increase. Similarly cell water had undergone <10% of its eventual 2.9-fold increase. In contrast, oleate transport had achieved 65% of its final 5- to 9-fold increase. The increase in transport was more than three times the increase in cell-surface area and more than twice the increase in cell volume.

Saturation of oleate uptake, in adipocytes, was seen at low levels of unbound FA ($K_m = 2 \times 10^{-7}$ M). These values were similar to those observed in isolated rat adipocyte and indicated the presence of a high-affinity system for long-chain FA (2). Preadipocytes exhibited a K_m similar in range to that in adipocytes. The increase in uptake rates seen with differentiation reflected an increase in V_{max} from ≈0.2 to 2 nmol/min per 10^6 cells (determined in arsenate-treated cells, data not shown).

Uptake of Retinoic Acid During Differentiation of BFC-1 Preadipocytes. To determine specificity of the increase in FA transport we measured early rates of retinoic acid uptake at various stages during conversion of preadipocytes. Retinoic acid was chosen because it shares structural similarities with oleate, which include a hydrocarbon chain and a carboxyl group; in addition, similarly to long-chain FA, retinoic acid binds to both albumin and adipose aP2 (11). Early-uptake rates for retinoic acid (30 μM, complexed to 30 μM BSA) are shown in Fig. 4. Rates, determined at two concentrations of retinoic acid (30 and 60 μM), remained constant throughout the differentiation process.

Temporal Relationship Between the Increase in FA Transport and Induction of mRNA for aP2 and for FA Synthetase. The increase in oleate uptake was demonstrated in the absence of oleate metabolism, which eliminated the possibility that it reflected increased activity of a metabolic step. But the increase could still be consequent to an increase in intracellular binding sites for FA. Adipocyte differentiation is associated with a large induction of the cytosolic aP2. That this induction might account for the increase in oleate uptake rates was therefore investigated. Cultures from the cell series assayed for FA uptake were used for RNA preparation to determine the time at which induction of aP2 mRNA occurred. Fig. 5 shows that aP2 mRNA could not be detected on day 2 after confluence, and a significant increase was observed only at days 8–10. This result indicated that aP2 induction could not explain the increase in oleate uptake because this induction followed the oleate increase by at least 4 days. The data do not rule out the possibility that another FA-binding protein was induced at day 2 after confluence; however, no evidence exists for such a protein.

The increase in uptake of exogenous FA also preceded the increase in cellular lipogenesis. Fig. 5, upper row, shows that mRNA for FAS enzyme complex was not seen before day 10 postconfluence, so it followed the increase in FA transport by ≈6–8 days.

Possible Role of FA in Modulating Expression of Proteins Involved in Lipid Metabolism. Because the increase in FA transport occurred early during cell differentiation, determining whether increased availability of cellular FA might modulate the levels of differentiation markers was of interest. For this, the effects of FA addition (60 μM oleate complexed to 40 μM BSA; initial concentration of unbound FA, ≈0.15 μM) on mRNA levels for aP2 in preadipocytes and adipocytes was tested (Fig. 6). In preadipocytes (Fig. 6*A*) oleate, at 60 μM, slightly induced mRNA for aP2 and potentiated the effect of dexamethasone, a known inducer of the aP2 gene (15). Marked induction of aP2 mRNA by various FA was observed by Amri *et al.* (16) in a detailed study with ob1771

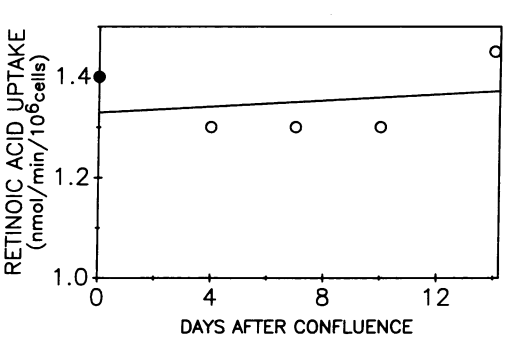

FIG. 4. Early rates of retinoic acid uptake during differentiation of BFC-1 cells. Cells, maintained with glucose, were processed as described for oleate uptake (legends for Figs. 2 and 3). Retinoic acid was used at two concentrations (30 and 60 μM with 30 μM BSA). Rates shown are from time courses (0.1–1 min) conducted at 30 μM with two cell series.

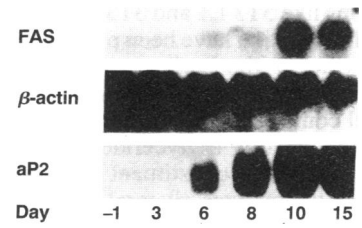

FIG. 5. Appearance of mRNA for the cytosolic aP2 and for FAS in differentiating BFC-1 cells. mRNA for aP2 and FAS were detected, respectively, with cDNA for mouse aP2 (12) and for rat liver FAS (13). Total RNA (25 μg) was probed as described. β-actin was used as control. Decrease in mRNA for β-actin seen after cell confluence was reproducible and did not interfere with data interpretation, as it occurred in opposite direction to the changes in aP2 and FAS. Data shown are for cell series assayed for oleate uptake (Fig. 3) and are typical of two other experiments. Numbers refer to various days during cell culture. Cell confluence is designated as day 0.

preadipocytes. In differentiated BFC-1 cells (Fig. 6*B*) FA from endogenous or exogenous sources appeared important for maintaining basal mRNA levels for aP2. BFC-1 adipocytes kept for 15–18 hr in serum-free DMEM lacking glucose and biotin to impede lipogenesis had very low mRNA levels for aP2, as compared with cells kept in regular serum-free DMEM. Under the same conditions, mRNA levels for the brain/erythrocyte glucose transporter GLUT 1 and for lipoprotein lipase remained unchanged. On the other hand, mRNA levels for FAS were decreased (data not shown). Addition of oleate (60 μM) significantly increased levels of mRNA for aP2 (Fig. 6*B*) and had no effect on mRNA levels for GLUT 1, lipoprotein lipase, or FAS. Levels of the aP2 protein, detected by reaction with antibody against mouse adipose lipid-binding protein ALBP P2, showed changes that paralleled those in mRNA. In preadipocytes induction of the aP2 protein (one band at ≈15 kDa) by dexamethasone was potentiated by FA. In adipocytes changes in protein levels paralleled those in mRNA but were of a lesser magnitude. Removal of glucose and biotin decreased aP2 protein levels by ≈40%. Addition of FA kept aP2 protein levels at ≈120% of those in control cells.

DISCUSSION

BFC-1 cells, established from mouse brown fat tissue, do not express the uncoupling protein typical of brown fat. However, they exhibit many of the general characteristics of other

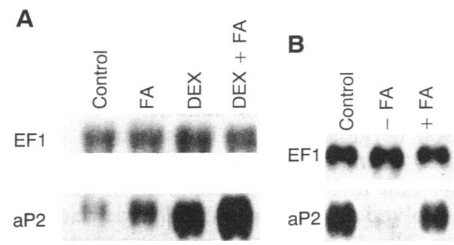

FIG. 6. Effect of oleate addition on level of mRNA for aP2 in preadipocytes and adipocytes. (*A*) Preadipocytes (2 days postconfluence) were transferred to serum-free DMEM for 24 hr with or without added 60 μM oleate complexed to 40 μM FA-free BSA; controls received 40 μM BSA. Dexamethasone (DEX) was added from a concentrated stock to final concentration of 250 nM. (*B*) Adipocytes (16 days postconfluence) were transferred to serum-free DMEM without glucose and biotin to impede endogenous FA synthesis and kept without (FA-free) or with 60 μM oleate (+FA) for 18 hr. Total RNA was prepared as described. Ethidium bromide (0.01 μg/μl), added to RNA samples (25 μg) before agarose electrophoresis, demonstrated RNA integrity and completeness of RNA transfer to the nitrocellulose filter. EF1 (14) was used as control.

adipose cell lines like 3T3 L1 and 3T3 F442A. Early and late markers of differentiation have been previously described for BFC-1 cells. One of the earliest markers is the enzyme lipoprotein lipase that is optimally induced in activity at about day 4 after cell confluence (6). This enzyme functions in the hydrolysis of exogenous triglycerides (from chylomicrons and lipoproteins) to their constituent FA and glycerol. Late markers include the enzyme glycerol phosphate dehydrogenase and the fatty acyl CoA ligase, which maximally increase in activity between 6 and 8 days after cell confluence (6). Both enzymes are important for FA esterification and lipid deposition. Other late markers are the cytosolic aP2, postulated to facilitate intracellular transport of FA, and the lipogenic enzyme complex (FAS), which functions in FA synthesis. As shown in Fig. 5, mRNA for both proteins increases between 6–10 days after cell confluence.

Our study showed that an increase in the membrane transport of long-chain FAs was a very early event during differentiation of cultured preadipocytes. Under the conditions of this study (23°C and FA/BSA ratio of 2:1) oleate esterification was ≈0.2 nmol of FA per min per 10^6 cells (data not shown). This result meant that FA transport would have been significantly rate-limiting in adipocytes if it did not increase during differentiation. Uptake V_{max} in preadipocytes averaged ≈0.2 nmol/min per 10^6 cells vs. 1 nmol/min per 10^6 cells in adipocytes.

The increase in FA uptake occurred at a stage where exogenous FA was the main source of FA for the cells, as it preceded the increase in lipogenesis. Simultaneous increases in activities of both lipoprotein lipase and FA transport would cooperate to increase availability of exogenous FA, providing a limiting substrate for ester synthesis. The data suggest that an increase in intracellular FA levels might provide one of the early signals for increased lipid deposition. Furthermore, the increased levels of cellular FA might play a role toward sustaining increased triglyceride formation by contributing to the regulation of enzymes or proteins involved in FA metabolism. Addition of FA to the cell culture medium of differentiating BFC-1 cells significantly increases (30–40%) the activity of acyl CoA ligase and the triglyceride content per adipocyte (6). Our data (Fig. 6) indicate that FA can modulate mRNA for aP2 in BFC-1 adipocytes and can potentiate its regulation by dexamethasone in preadipocytes. Under the same conditions, we did not observe any effect of FA on mRNA for lipoprotein lipase or FAS (data not shown); however, these observations will have to be tested further with a range of FA concentrations and possibly different cell-culture conditions. In ob1771 preadipocytes, FA induced mRNA for the long-chain FA-acyl CoA ligase (in addition to aP2), and the effects involved increases in gene transcription (16). Thus FA, as with other small lipophilic molecules, such as retinoic acid (17), could have selective and possibly tissue-specific gene-regulating actions.

Regulation of gene activity by substrate is common in bacteria. For example, long-chain FA have been shown in *Escherichia coli* to be potent inducers of genes coding for proteins involved in their intracellular degradation (18). In mammalian cells, there are multiple documented examples of substrates that can regulate their cellular use, both acutely and by altering the expression of key proteins. Effects of glucose deprivation or addition to modulate expression of glucose transporters (19, 20) and of enzymes related to glucose metabolism, such as phospho*enol*pyruvate carboxykinase (21) and pyruvate kinase (22) have been described. Cholesterol has been shown to regulate transcription of the gene coding for hydroxymethylglutaryl-CoA reductase (23). Thus, possibly FA availability early during the differentiation process helps regulate pathways for FA metabolism, both by providing a limiting substrate and by selective modulation of the expression of proteins related to FA metabolism. Dietary manipulations, such as high fat feeding have been reported to modulate the mRNA for multiple enzymes of FA metabolism (24–26). These modulatory effects are generally believed secondary to alterations in the hormonal milieu; however, they could be, at least in part, mediated directly by FA.

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