The plasma membrane lipid order of 3T3F442A cells was examined during the course of adipocyte differentiation by measuring the fluorescence polarization of 1-[4-(trimethylamino)phenyl]-6-phenylhexatriene. This cationic fluorophore labels the plasma membrane but does not rapidly redistribute to intracellular organelar membranes and can, therefore, be used to specifically probe the plasma membrane of intact cells. Studies with whole cells demonstrated that the plasma membrane of 3T3F442A cells becomes less ordered during the course of adipocyte conversion and that this alteration begins relatively early during the differentiation process. In addition, the lipid order of plasma membranes isolated from adipocyte-stage cells was found to be lower than the lipid order of the early, fibroblast-stage cells. Analysis of membrane lipid composition suggests that the molecular bases for the decrease in adipocyte plasma membrane lipid order are a large increase in the level of monounsaturated phospholipid acyl chains and a decrease in the molar ratio of cholesterol to phospholipid. The alteration in plasma membrane lipid composition may be specifically required for integral membrane protein function, since the differentiation-dependent fatty acid desaturase activity is known to be maintained even in the absence of triacylglycerol accumulation.

3T3F442A preadipose cells, a clonal cell line derived from Swiss mouse fibroblasts by Green and Kehinde (1), have been used as a model system for studying the growth, development, and biochemical properties of adipocytes (2). The cells exhibit fibroblast-like characteristics during exponential growth, but confluent cells differentiate into adipocytes spontaneously (1) or upon treatment with a combination of drugs, hormones, and/or nutrients (3-5). Adipocyte conversion is accompanied by dramatic increases in the activities of enzymes involved in de novo fatty acid and triacylglycerol synthesis (3, 6-8), which lead to the accumulation of cytoplasmic triacylglycerol droplets (9, 10). In addition, changes in the activities of several integral membrane functions occur during the course of differentiation. These include alterations in insulin binding (11), potassium transport (12), insulin-responsive glucose transport (13), and plasma membrane (Na⁺,K⁺)ATPase activity (12). All of these integral membrane functions are reported to be sensitive to their membrane lipid environment (14, 15).

Limited evidence exists as to the development of membrane lipid physical properties during mammalian cellular differentiation (16). DeLaat et al. (17) reported that the lateral diffusion rate of lipids in neuroblastoma neurite membranes increased during differentiation, suggesting an increase in membrane lipid fluidity. Conversely, an increase in membrane lipid order during myoblast differentiation, as measured by electron paramagnetic resonance techniques, has been recently described (18). Transmembrane lipid asymmetry has also been observed to change during myoblast differentiation. Sessions and Horwitz (19) found an increase in external leaflet phosphatidylyserine and phosphatidylethanolamine content as cells developed from chick embryo fibroblasts to myoblasts. Similar increases in outer leaflet aminophospholipids have been reported to occur during erythrophoiesis (20).

The present studies were conducted in order to determine whether plasma membrane lipid order changes during the adipose conversion of 3T3F442A cells. Since this adipocyte cell line exhibits membrane functional changes during the course of differentiation, and since important lipid synthetic changes are known to occur as well, it is conceivable that membrane lipid structural changes play a role in functional expression and in the regulation of membrane properties. We have used the fluorescence polarization of 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMADPH), a cationic derivative of 1,6-diphenylhexa-1,3,5-triene (DPH), to monitor the order of the plasma membrane lipids in intact cells. In situ measurements of plasma membrane properties are desirable, since during subcellular fractionation procedures, some degree of contamination with endomembranes is unavoidable, and unrepresentative membrane regions may be selected. In addition, fewer cells are generally required for in situ determinations.

The present studies demonstrate that TMADPH polarization can be used to investigate plasma membrane lipid order in intact 3T3F442A cells. It is found that adipocyte conversion is accompanied by a decrease in the lipid order of the plasma membrane and that this alteration occurs relatively early during the differentiation process. Specific changes in plasma membrane lipid composition appear to be responsible for the observed decrease in lipid order. In particular, a dramatic increase in the level of monounsaturated phospholipid acyl
chains is observed. This is likely due to the differentiation-dependent expression of stearyl-coenzyme A desaturase (21, 22). Since this activity is maintained in the bombin-deficient state (21), when fatty acid synthesis and triacylglycerol accumulation are absent, it suggests that the conversion of plasma membrane acyl chains and the consequent decrease in plasma membrane lipid order may be among the primary events (23) in the differentiation of 3T3F442A adipocytes.

MATERIALS AND METHODS

Cell Culture—Surface cultures of 3T3F442A preadipocyte fibroblasts were grown to confluence in Dulbecco-Vogt modified Eagle's medium supplemented with 10% calf serum. Cells were fed three times per week and maintained at 37 °C in a humidified atmosphere of 95% CO₂. Adipose conversion of confluent monolayers was then induced by substitution of the bovine serum with 10% fetal calf serum (FCS) and, where indicated, 5 mg/ml of insulin. Greater than 85% of the cells converted to adipocyte morphology following 7–10 days of FCS feeding, as evidenced by their rounded morphology, accumulation of large cytoplasmic lipid droplets, and Oil Red O staining. Parallel cultures of 3T3C2 cells, which undergo adipose conversion with very low frequency (1), were maintained and fed under identical conditions. Cultures were harvested with treatment with 0.1% trypsin and 1 mM EDTA in isotonic buffer for 5 min at 37 °C. Cells were sampled at different times during the course of adipocyte differentiation, as follows: day 0 designates confluent fibroblasts, day 1 are cells which have been fed with FCS for 1 day, day 2 have been fed with FCS for 2 days, etc.

Fluorophores and Fluorescence Measurements—DPH was purchased from Kodak, and TMADPH and 12-([anthroyloxy]stearatate (12AS) were from Molecular Probes (Eugene, OR).

Steady state fluorescence polarization measurements were performed immediately following cell harvesting. Cells were washed twice with isotonic phosphate-buffered saline (137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.4, room temperature) (PBS) and resuspended at a concentration of 2 × 10⁶ cells/ml. Further dilutions were made with PBS as indicated in the text. The plasma membrane lipid concentration was estimated by assuming a 1:1 ratio of protein:lipid (w/w) in plasma membrane and a concentration of 40 μg plasma membrane protein/10⁶ cells (12). TMADPH was added from ethanolic stock solutions such that the final ethanol concentration was <1% volume %, and the plasma membrane lipid probe molar ratio was approximately 80:1. For example, 3.2 μl of a 0.1 mM stock of TMADPH was added with gentle swirling to 0.4 ml of a cell suspension of 10⁶ cells/ml for a final concentration of 0.8 μM probe and a final ethmol volume of 0.8%. A 2-fold increase or decrease in the lipid:probe ratio did not influence the polarization values obtained (data not shown). Measurements were taken at 24 °C in 3 × 3-mm cuvettes containing approximately 0.25 ml of sample with an SLM 4800 (27). Modulation lifetimes are used since they are relatively insensitive to sample turbidity in comparison with phase values. Lifetimes were measured at 18 mHz with the excitation polarizer set at 35 ° from the vertical (24, 27). Ten measurements were made for each sample and data points were ±0.2 ns.

Plasma Membrane Isolation—Suspensions of plasma membranes from day 0 cells (confluent fibroblast morphology) and from fully differentiated adipocytes (generally day 5–11) were prepared as described by Thom et al. (28). Briefly, 10–12 100-mm dishes of confluent cell monolayers were harvested, washed with PBS, then with isotonic borate (0.05 M boric acid, 0.05 M NaCl, 1 mM CaCl₂, 1 mM MgCl₂, pH 7.2), and resuspended in 1 pellet volume of isotonic borate. The concentrated suspension pellet was added slowly with stirring to 100 volumes of cold hypotonic borate (0.02 M boric acid, 0.2 mM EDTA, pH 10.2). After stirring for 10 min, 0.08 volume of 0.5 M boric acid, pH 10.2, was added and stirring continued for 5 min. The lysed cell suspension was filtered through two layers of 110-μm mesh Nytex nylon gauze and nuclei were then removed by centrifugation at 1,500 × g for 10 min. The postnuclear supernatant was spun at 16,000 rpm in a Sorvall SS34 rotor for 40 min at 4 °C. The pellet was resuspended in PBS plus 1 mM of CaCl₂ and MgCl₂ (PBS+), homogenized with a loose fitting Dounce homogenizer, and 13 ml were layered over a 25-ml cushion of 35% sucrose (w/w) in PBS+ in an SW27 centrifuge tube (40 ml capacity). Samples were spun for 45 min at 18,000 rpm and membranes collected from the interface were washed in PBS and pelleted in a type 65 rotor at 40,000 rpm for 30 min. Plasma membranes were determined by the method of Mitchell et al. (29) using bovine serum albumin as standard. The purity of the plasma membrane suspensions was evaluated using marker enzymes. The mean specific activity ratios (purified plasma membranes/crude cell homogenates) for the fibroblast and adipocyte stages were: alkaline phosphatase (plasma membrane marker, Ref. 30), 8.0 ± 1.3 and 7.2 ± 0.1 (mean ± S.E. for six fibroblast and five adipocyte membrane preparations): glucose-6-phosphatase (microsomal marker, Ref. 31), 3.02 ± 0.01 and 0.02 ± 0.01: succinic acid dehydrogenase (mitochondrial marker, Ref. 32), 0.10 and 0.06.

Lipid Analyses—Total lipids were extracted from membranes by the method of Bligh and Dyer (35) or as described by Folch et al. (34). The total phospholipid content was quantified by complete ashing of the lipid extract (35) followed by estimation of inorganic phosphate (36). Cholesterol content was determined by the method of Chomarat and Henry (37). Lipid extracts were separated into polar and nonpolar fractions by silicic acid chromatography as described previously (38). The fatty acid composition of each fraction was determined by gas chromatography. Fatty acid methyl esters were quantitatively prepared using boron trifluoride (14%) w/v as catalyst (39) and analyzed using a Varian Aerograph 2400 gas chromatograph and a flame ionization detector interfaced with a Hewlett-Packard 3380A integrator. The 6-foot glass column was packed with 10% SP-2330 on 100/120 mesh Chromosorb (Supelco, Bellefonte, PA), and column temperature was 150 °C for 10 min followed by an increase of 4 °C/min to 250 °C. Injection temperature was 220 °C and carrier gas flow rate was 10 ml/min. Retention times of authentic standards (Supelco) were used to identify sample peaks.

Fluorescence Microscopy—Fluorescence microscopy was carried out with a Zeiss UVM microscope equipped with epifluorescence and interfaces with a Venum image intensification camera and Dage video recorder.

RESULTS

Localization of TMADPH in Whole Cells—Studies in model membrane systems have shown that the charged amino group of TMADPH anchors the probe at the membrane-water interface, with the fluorescent portion of the molecule intercalated between the upper region of the fatty acyl chains (40). As such, TMADPH is useful for labeling the plasma membrane of intact cells, since unlike its neutral parent molecule DPH, it should not distribute to endomembranes (41, 42).

Addition of TMADPH at room temperature to cells in suspension or to cells grown on coverslips

Excited state lifetimes (τ = fluorescence lifetimes) were determined at 24 °C by the phase modulation technique using the SLM 4900 (27). Modulation lifetimes are used since they are relatively insensitive to sample turbidity in comparison with phase values. Lifetimes were measured at 18 mHz with the excitation polarizer set at 35 ° from the vertical (24, 27). Ten measurements were made for each sample and data points were ±0.2 ns.
Membrane Lipid Order in 3T3F442A Differentiation

FIG. 1. Localization of DPH and TMADPH in 3T3F442A adipose cells. Adherent cells on coverslips or cell suspensions were incubated at room temperature in the presence of 1 μM fluorophore, and fluorescence images were obtained within 10 min of probe addition. A and B, DPH and TMADPH, respectively, in suspended cells. C, Nomarski image of adherent cells and D, DPH fluorescence image of these cells. E, TMADPH fluorescence image of an identical group of adherent adipocytes. Arrows point to intracellular lipid droplets.

resulted in rapid (=seconds) insertion of the probe into the plasma membrane. When added to suspended cells, DPH distributed throughout the interior of the cell with the exception of the nucleus (Fig. 1A). TMADPH, on the other hand, shows the characteristic ring stain of a surface label (Fig. 1B). The Nomarski image shown in Fig. 1C demonstrates the presence of large intracellular lipid droplets in differentiated adipocytes on a coverslip. Although DPH labels these droplets (Fig. 1D), TMADPH staining is again apparently only on the cell surface (Fig. 1E).

Although TMADPH is seen to initially localize to the plasma membrane, it can be expected that after sufficient time it will partition into intracellular compartments, either by transmembrane flip-flop or by endocytosis. For example, Gibbs et al. (43) have shown that at 37 °C, 3T3-L1 adipocytes in the presence of insulin have an endocytic rate of <1.5% of cell volume/5 min. In order to determine the temporal stability of the plasma membrane labeling by TMADPH, the effect of incubation time on fluorescence polarization was determined. Cell suspensions from different stages of cell differentiation were loaded with TMADPH and polarization measured at various times between 3 and 35 min following probe addition. The results showed that P values remained constant for at least 10 min following TMADPH addition (data not shown). Where changes were observed, P values decreased, as expected, since intracellular membranes have been found to have lower lipid order than the plasma membrane (44, 45).

All data in the following experiments were obtained within 5 min of probe addition and at 24 °C. Thus, the TMADPH signal is assumed to arise from a plasma membrane-bound probe.

Plasma Membrane Lipid Order in Whole Cells during Adipocyte Differentiation—The fluorescence polarization of TMADPH was measured in cell suspensions harvested during the course of adipocyte conversion. The accumulation of cytoplasmic triacylglycerol droplets during differentiation results in an increased turbidity of cell suspensions. Since...
Membrane Lipid Order in 3T3F442A Differentiation

FIG. 3. TMADPH polarization in intact cells during adipocyte differentiation. A, the intrinsic polarization ($P_i$) values for TMADPH were determined as a function of cell density, as detailed under “Materials and Methods” and demonstrated in Fig. 4. Confluent 3T3F442A ($\Delta$, ○) or 3T3C2 (□) cells were fed under identical conditions with 10% FCS plus 5 µg/ml insulin, and $P_i$ of TMADPH was determined. B, observed values of TMADPH polarization ($P_{obs}$) in $10^6$ cells/ml labeled with 0.8 µM TMADPH as described under “Materials and Methods.” Confluent cells were fed with 10% FCS (△) or 10% FCS plus 5 µg/ml insulin (□).

turbidity can alter fluorescence polarization measurements through scatter depolarization of both excitation and emission light (25, 26), it was important to determine the effect of turbidity-dependent depolarization as a function of the state of cell differentiation. Fig. 2 shows the effect of increasing cell concentration on the observed value of TMADPH polarization. When cells are phenotypically fibroblast-like, little effect of cell concentration is seen. Scattering depolarization becomes significant, however, as adipocyte differentiation proceeds.

The results in Fig. 3 show that the polarization of TMADPH decreases with time of differentiation, and this decrease reflects a decrease in adipocyte plasma membrane lipid order. Polarization values corrected for turbidity ($P_t$) were determined as described above, and the results shown in Fig. 3A demonstrate that a decrease in plasma membrane lipid order is associated with cell differentiation. Since the 3T3C2 cell line undergoes adipocyte differentiation at an extremely low frequency (1, 46), these cells were used to determine whether switching serum had any effect on TMADPH polarization, since the 3T3F442A cells are grown to confluence in calf serum and then switched to FCS to induce differentiation. As Fig. 3A demonstrates, the $P_t$ value for TMADPH in 3T3C2 cells is essentially identical to that of fibroblast-stage 3T3F442A cells. Moreover, although $P_t$ decreases during differentiation of the 3T3F442A cells, no
change was observed in the 3T3C2 controls despite equivalent exposure to medium with FCS. In addition, 3T3F442A cells maintained in calf serum after reaching confluence exhibited no change in TMADPH polarization (data not shown). Thus, the observed decrease in lipid order related to adipocyte differentiation and is not simply due to time spent in culture.

Fig. 3B demonstrates the effect of turbidity on $P_{\infty}$. As seen by comparison with Fig. 3A, the magnitude of the decrease in $P_{\infty}$ is greater than in $P_{t}$ (−15% and −10%, respectively). Both measurements, however, exhibit a similar time course, and thus, even the uncorrected $P_{\infty}$ values reflect the correct trend. As also seen in Fig. 3B, addition of insulin results in a further decrease in lipid order, presumably reflecting greater conversion efficiency, but does not change the time course of the alteration.

Excited state lifetimes ($\tau$) of TMADPH in whole cell suspensions were measured during the course of adipocyte differentiation. The results indicate that lifetimes remain roughly constant at $\approx 7$ ns until day 5 of differentiation, after which a small decrease to about 6 ns is observed. Since for constant motional constraint, a decrease in lifetime would result in an increase in $P$ value, the observed decrease in $P$ value together with the constant or slightly decreased value of $\tau$ indicates that there is reduced motional constraint ($i.e.$ reduced lipid order) on the probe in plasma membranes of differentiated cells.

**Isolated Plasma Membranes from Fibroblast and Adipocyte Stages**—Plasma membranes were purified and characterized from confluent fibroblasts and mature adipocytes as described under “Materials and Methods.” Steady state fluorescence polarization of TMADPH, DPH, and 12AS was determined in these isolated membrane subfractions, and the results are shown in Table I. Polarization values for all three membrane probes were lower in adipocyte plasma membranes than in fibroblast-stage membranes. These results provide further evidence that a decrease in plasma membrane lipid order occurs during adipocyte differentiation of 3T3F442A cells.

To determine how the observed change in lipid order is related to the membrane lipid composition, total lipid extracts of three preparations each of fibroblast and adipocyte plasma membranes were prepared, and the fatty acyl chain composition was analyzed. In addition, the total lipid extracts of two preparations each of fibroblast and adipocyte plasma membranes were separated into nonpolar and polar fractions and their respective fatty acid compositions determined. As shown in Table II, there was an approximately 6-fold increase in the total membrane level of the monounsaturated palmitoleic acid (16:1, $p < 0.001$) in adipocyte as compared to fibroblast plasma membranes and a 45% increase ($p < 0.05$) in oleic acid (18:1) content. On the other hand, decreases of between 2- and 3-fold were observed in the saturated acyl chains myristate (14:0), palmitate (16:0), and stearate (18:0). Thus, the ratio of saturated to monounsaturated acyl chains in the adipocyte plasma membranes decreased 5-fold compared to fibroblast membranes ($p < 0.01$). The acyl chain composition of the membrane polar lipid fraction showed similar dramatic increases in the level of the monounsaturated chains, as well as a 2-fold increase in the percentage of total unsaturated fatty acids (Table II). TLC separation of the neutral lipid fraction indicated that virtually no triacylglycerol was found in either the fibroblast or adipocyte membrane preparations (data not shown).

Cholesterol and phospholipid levels were determined as described under “Materials and Methods.” The molar ratio of cholesterol/phospholipid in fibroblast membranes was 0.70 ± 0.06 ($n = 3$), significantly higher than the ratio of 0.51 ± 0.06 ($n = 4$, $p < 0.05$) found in adipocyte membranes.

**DISCUSSION**

The results of this investigation demonstrate that the plasma membrane lipid order of 3T3F442A cells decreases during adipocyte conversion, as evidenced by the decrease in fluorescence polarization of TMADPH (Fig. 3). The change occurs relatively early during the course of differentiation and is apparently related specifically to the differentiation process rather than to exposure to FCS or time in culture. The lower degree of lipid order in the mature adipocyte plasma membranes is further suggested by the small decrease in TMADPH excited state lifetime in adipocyte as compared to fibroblast stage cells. The fluorescence lifetime of TMADPH has been shown to be shorter in fluid phase as compared to gel phase phosphatidylcholine bilayers (40, 47).

TMADPH was synthesized and characterized by Prendergast et al. (40) as a cationic derivative of the frequently used lipophilic membrane probe, DPH. Its polar trimethylamino function tethers it at the aqueous interface of the membrane, and it has, therefore, proven useful for examining the lipid order of the upper region of the bilayer acyl chains (38). Kuhry and co-workers (41, 42) introduced the study of intact cell plasma membrane lipid order with TMADPH, and the present study demonstrates that TMADPH can be utilized in situations where changes in cell turbidity are occurring. The probe is taken up rapidly and localizes exclusively to the plasma membrane of the 3T3F442A cells, in distinct contrast.

**Table I**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Fibroblast</th>
<th>Adipocyte</th>
</tr>
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<tbody>
<tr>
<td>TMADPH</td>
<td>0.364 ± 0.005 (7)</td>
<td>0.346 ± 0.003 (4)*</td>
</tr>
<tr>
<td>DPH</td>
<td>0.292 ± 0.010 (7)</td>
<td>0.262 ± 0.002 (4)*</td>
</tr>
<tr>
<td>12AS</td>
<td>0.130 ± 0.004 (3)</td>
<td>0.117 ± 0.005 (3)</td>
</tr>
</tbody>
</table>

*Differs from fibroblast membrane ($p < 0.05$) by $t$ test of independent means.

**Table II**

<table>
<thead>
<tr>
<th>Fatty acid composition of total and polar lipid fractions of fibroblast and adipocyte plasma membranes</th>
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<tbody>
<tr>
<td>Component</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Total lipid extract</td>
</tr>
<tr>
<td>Fatty acids (wt. %)</td>
</tr>
<tr>
<td>14:0</td>
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<tr>
<td>14:1</td>
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<tr>
<td>16:0</td>
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<td>16:1</td>
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<tr>
<td>20:3</td>
</tr>
<tr>
<td>20:4</td>
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<tr>
<td>22:6</td>
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| Unsaturated fatty acids (%) | 35 ± 3 | 66 ± 1* |
| Ratio saturated/mono-unsaturated fatty acids | 2.8 ± 0.5 | 0.6 ± 0.1* |

*Values are mean ± S.E. for three separate preparations.

†Values are the average of two separate preparations.

‡Diffs from fibroblast total lipid extract ($p < 0.06$ or lower) by $t$ test of independent means.
to the ubiquitous cellular localization of its neutral parent fluorophore, DPH (Fig. 1). A plasma membrane localization of TMADPH is also suggested by measurement of the fluorescence polarization values of the probe in intact cells versus disrupted cells. TMADPH polarization (P_{obs}) in samples of differentiated adipocytes (day 10 cells) was 0.346 ± 0.006. Cells were then sonicated on ice for 3 s, and the polarization value obtained for these disrupted cells was 0.315 ± 0.002. In addition, cells were similarly sonicated prior to TMADPH addition, and the polarization value obtained was 0.312 ± 0.005. It should be noted that this marked decrease in P_{obs} occurred despite the decrease in turbidity which occurs in cell suspensions upon sonication. Thus, the observed decrease in TMADPH polarization, i.e. in lipid order, following cell disruption is likely due to probe redistribution to less ordered intracellular membranes and lipid droplets (44, 45). The rapid uptake and rapid redistribution suggest that the plasma membrane localization of TMADPH in an intact cell is due to its inability to “flip” across the plasma membrane bilayer. This in turn implies that the observed TMADPH polarization reflects the lipid order of the outer hemileaflet of the plasma membrane.

The decrease in plasma membrane lipid order in differentiated cells was confirmed by fluorescence polarization measurements in isolated plasma membrane fractions. The results in Table I show that both TMADPH and DPH polarization were significantly lower in adipocyte as compared to fibroblast plasma membranes, demonstrating further the increase in lipid fluidity that occurs during differentiation. The TMADPH polarization values were always higher in whole cells as compared to the isolated plasma membrane fraction prepared from the cells. This difference is likely due to the unavoidable presence of endomembranes in the plasma membrane preparations, which as mentioned above would be expected to result in lower polarization values (44, 45). (It is important to note that the fibroblast and adipocyte plasma membrane preparations were equally purified and thus expected to contain equivalent levels of endomembranes.)

The lipid composition of isolated plasma membranes suggests that the molecular bases for the decrease in lipid order of adipocyte plasma membranes may be the large increase in phospholipid acyl chain monounsaturation, as well as a decrease in the membrane cholesterol/phospholipid ratio. The results show a 5-fold increase in the relative levels of monounsaturated to saturated fatty acids (p < 0.01). Acyl chain unsaturation is an important determinant of bilayer lipid order, as evidenced by a wide variety of studies in both model and biological membranes (45, 48, 49). In particular, the largest decrease in membrane lipid order and increase in lipid rotational mobility arises from insertion of the first double bond, whereas further unsaturation produces changes of smaller magnitude (50–52). Thus, the dramatic increase in acyl chain monounsaturation found here would appear to account, at least in part, for the decrease in membrane lipid order during adipocyte differentiation. The metabolic basis for this change in lipid composition is most likely the differentiation-dependent induction of stearoyl-coenzyme A desaturase activity, as reported by Kasturi and Joshi (21) for the 3T3-L1 preadipocyte cell line. The gene encoding this protein has been recently shown to have elements upstream of the coding region which have homology to the putative fat-specific transcriptional element PSE-2 (22).

The present study also shows a decrease in cholesterol/phospholipid ratio in adipocyte as compared to fibroblast plasma membranes (p < 0.05). Alterations in membrane cholesterol content are also known to influence membrane physical properties. For example membrane lipid order, as determined by the fluorescence polarization of DPH, has been shown to vary directly with the cholesterol/phospholipid molar ratio (53). In addition, Straume and Leitman (54) have recently shown that cholesterol incorporation resulted in increased bilayer ordering as reported by both DPH and TMADPH. The results, therefore, demonstrate that the decrease in plasma membrane lipid order during adipocyte differentiation is likely due to both increased acyl chain unsaturation and a decreased cholesterol/phospholipid ratio.

The decrease in membrane lipid order and/or the alteration in lipid composition may be related to functional differentiation of the adipocyte. For example, Resh (12) has reported an increase in both insulin-stimulated (Na+,K+)ATPase activity and glucose transport in differentiated adipocytes (12). The (Na+,K+)ATPase is known to be stimulated by decreasing membrane lipid order (14, 55). In addition, membrane cholesterol has been shown to specifically inhibit the activity of the reconstituted erythrocyte glucose transporter (56). Thus, the present studies suggest that alterations in the composition and physical properties of the plasma membrane may play a role in adipocyte differentiation of 3T3F442A cells. Although the changes in TMADPH polarization are seen to occur early in the conversion process, it is not yet known whether these changes are required for differentiation to proceed. It is interesting to note, however, that in the biotin-deficient state, intracellular triacylglycerol accumulation is absent (23), the large induction of the fatty acid Δ9-desaturase is still maintained (21). Thus, the acyl chain unsaturation is not related simply to intracellular lipid storage. This suggests that the changes in plasma membrane acyl chain composition and hence lipid order are likely to be independent of triacylglycerol accumulation as well and thus may be important for 3T3F442A adipocyte differentiation.

Acknowledgments—We thank Dr. Howard Green and Onyiji Kehinde for providing us with cells and cell culture facilities in the early part of this work and for generously providing training and invaluable advice during the course of these investigations. We are also indebted to Drs. Morris Karnovsky and Tom Wright for generous assistance with the fluorescence microscopy.

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