Adipose Differentiation Related Protein (ADRP) Expressed in Transfected COS-7 Cells Selectively Stimulates Long Chain Fatty Acid Uptake*

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Adipose differentiation related protein (ADRP) is a 50-kDa novel protein cloned from a mouse 1246 adipocyte cDNA library, rapidly induced during adipocyte differentiation. We have examined ADRP function, and we show here that ADRP facilitates fatty acid uptake in COS cells transfected with ADRP cDNA. We demonstrate that uptake of long chain fatty acids was significantly stimulated in a time-dependent fashion in ADRP-expressing COS-7 cells compared with empty vector-transfected control cells. Oleic acid uptake velocity increased significantly in a dose-dependent manner in ADRP-expressing COS-7 cells compared with control cells. The transport $K_m$ was 0.051 μM, and $V_{max}$ was 57.97 pmol/10^5 cells/min in ADRP-expressing cells, and $K_m$ was 0.093 μM and $V_{max}$ was 20.13 pmol/10^5 cells/min in control cells. The oleate uptake measured at 4 °C was only 10% that at 37 °C. ADRP also stimulated uptake of palmitate and arachidonate but had no effect on uptake of medium chain fatty acid such as octanoic acid and glucose. These data suggest that ADRP specifically enhances uptake of long chain fatty acids by increasing the initial rate of uptake and provide novel information about ADRP function as a saturable transport component for long chain fatty acids.

Long chain non-esterified free fatty acids (FA) and their derivatives have multiple functions as either essential components of membrane, efficient sources of energy, or important effective molecules that regulate metabolism and mediate gene expression (1, 2). Adipose tissue is the main source of lipids and fatty acids in the body where they play key roles in the regulation of energy balance in mammals. Proteins involved in FA and triglyceride synthesis and accumulation as well as utilization of exogenous lipid are induced during adipocyte differentiation (3, 4). Increase of enzymatic activities regulating lipogenesis and lipolysis is also ongoing at this stage. These multiple roles of FA suggest that careful regulation of cellular aspects of FA metabolism including cellular uptake in liver, fat, cardiac and skeletal muscles, and other organs is essential.

The mechanism by which long chain free FA enter the cells is not completely understood. It has long been postulated that the movement of long chain FA across the cell membrane is invariably passive (5, 6). Studies (5, 6) have suggested that FA penetrate cardiac myocytes by a passive unregulated mechanism rather than by a specific facilitated process and that saturation of an intracellular metabolism step is the cause for apparent saturation of uptake in other studies. Others (7) have also shown that the entry of FA into hepatocytes reflected their passive partitioning into the lipid component of the cell membrane.

However, recent observations indicated that at least a portion of FA uptake might occur by a carrier-mediated transport system (8, 9). Studies with liver, fat cells, cardiac tissue, and skeletal myocytes have shown that free FA uptake exhibited many of the kinetic properties of a facilitated process, namely saturation, trans-stimulation, cis-inhibition, stereospecificity, and counter transport (8–12). Several studies have indicated that FA uptake by adipocytes was phloretin-inhibitable and blocked by antibody raised against specific binding proteins (10, 13). These features could not be explained by diffusion. It is then possible to assume that facilitated and passive uptake processes both occur simultaneously in cells (12), with the facilitated transport being the predominant process. In support of these findings, five putative mammalian free FA transporters have been identified so far in the plasma membrane of several tissues (14–18). cDNA clones have been isolated for three of them, specifically plasma membrane fatty acid-binding protein (FABPpm) (14), fatty acid translocase (FAT) (15), and fatty acid transport protein (FATP) (16). In addition, small molecular weight cytosolic fatty acid-binding proteins have been characterized, cloned, and extensively studied (for review see Ref. 19).

Adipose differentiation related protein (ADRP) is a novel 50-kDa protein originally cloned by differential hybridization from a cDNA library of differentiated mouse 1246 adipocytes (20). The 1.7-kilobase pair ADRP mRNA was induced 50–100-fold a few hours after the onset of adipocyte differentiation in 1246 cells, thus making ADRP an early marker of the adipose differentiation program (20). It has been shown that ADRP mRNA was expressed at high levels in adipose tissue (20) and also in many different types of cells and tissues where lipids were accumulated or synthesized, although at lower levels than

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1 The abbreviations used are: FA, fatty acids; ADRP, adipose differentiation related protein; BSA, fatty acid-free bovine serum albumin; DME-F12, 1 to 1 mixture of Dulbecco’s modified Eagle’s and Ham’s F12 media; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; KRP, Krebs-Ringer phosphate buffer; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; FAT, fatty acid translocase; FATP, fatty acid transport protein; FABPpm, plasma membrane fatty acid-binding protein.

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in adipocytes (21). Sequencing of ADRP did not provide any information about its possible function in adipocytes. Immunolocalization studies done at different times during the adipose differentiation program in 1246 and 3T3-L1 adipocytes indicated that ADRP was localized in the vicinity of the plasma membrane in cells that started to differentiate and was found on the surface of lipid droplets in mature adipocytes (20–21). Moreover, ADRP expression was found to be induced in the liver of mice treated with the carnitine palmitoyltransferase I inhibitor, etomoxir, which caused neutral lipid accumulation in the organ (22). These various studies suggested that ADRP might be involved in the formation or stabilization of lipid droplets in adipocytes. As a first step to investigate this hypothesis, we have examined here whether ADRP was involved in FA uptake using ADRP-transfected COS-7 cells as an experimental model system. The results presented in this paper show that ADRP plays a role in facilitated FA transport in COS-7 cells expressing the protein.

EXPERIMENTAL PROCEDURES

Construction of ADRP-pcDNA3 Expression Vector and Transient Transfection of COS-7 Cells—COS-7 cells (American Type Culture Collection, Manassas, VA) were cultivated in DME-F12 medium (1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s nutrient F12 medium) supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Inc.) in T-75-cm² plates. Cell stocks were cultivated in these conditions until nearly confluent and subcultured at 1:20 dilution or plated for an experiment. For expression into COS-7 cells, ADRP cDNA was cloned into the mammalian expression vector pcDNA3 (Invitrogen, Carlsbad, CA). Mouse ADRP cDNA fragment containing the 1.3-kilobase pair ADRP open reading frame was cloned in the sense orientation in XbaI and BamHI sites of pcDNA3. Expression of green fluorescent protein (GFP, CLONTECH, Palo Alto, CA) was used to monitor transfection efficiency. GFP plasmid DNA was co-transfected into COS-7 cells with pcDNA3 empty vector (control cells) or pcDNA3-ADR3 vector plasmid DNA.

Transient Transfection of Plasmid DNA into COS-7 Cells—Transient transfection of plasmid DNA into COS-7 cells was carried out by the DEAE-dextran method (23) established for COS cells. Briefly, 2 ml of a solution containing plasmid DNA and DEAE-dextran was prepared by adding twice with culture medium and then cultivated in DME/F12 medium supplemented with 10% fetal bovine serum (1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s nutrient F12 medium) supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Inc.) in T-75-cm² plates. Cell stocks were cultivated in these conditions until nearly confluent and subcultured at 1:20 dilution or plated for an experiment. For expression into COS-7 cells, ADRP cDNA was cloned into the mammalian expression vector pcDNA3 (Invitrogen, Carlsbad, CA). Mouse ADRP cDNA fragment containing the 1.3-kilobase pair ADRP open reading frame was cloned in the sense orientation in XbaI and BamHI sites of pcDNA3. Expression of green fluorescent protein (GFP, CLONTECH, Palo Alto, CA) was used to monitor transfection efficiency. GFP plasmid DNA was co-transfected into COS-7 cells with pcDNA3 empty vector (control cells) or pcDNA3-ADR3 vector plasmid DNA.

Transfection efficiency was determined by counting the number of cells expressing GFP (co-transfected with either ADRP-pcDNA3 or with pcDNA3 empty vector) when compared with the total number of cells counted with a hemocytometer.

Determination of ADRP Expression in Transfected Cells—ADR3 protein expression in transfected COS-7 cells was examined in cell lysates prepared from transfected COS-7 cells by Western blot analysis using rabbit anti-ADR3 antibody. Briefly, cells were washed once with PBS and lysed in 1 ml of SDS sample buffer (6.25 % Tris-HCl, pH 6.8, 2% SDS, 10% glycerol) without β-mercaptoethanol and bromphenol blue (all chemicals were from Bio-Rad). The cell lysate was then sonicated at 40%, 20-watt output for 10 s using a Vibra Cell sonicator (Sonics & Materials Inc., Danbury, CT), and centrifuged at 10,000 x g for 10 min, and the supernatant was collected. The protein concentration of cell lysate was measured by using micro-BCA protein assay reagent kit (Pierce). After adding 1/10 volume of 10% loading dye (50% 2-Me, 1% bromphenol blue), equal amounts of protein (20 μg) from ADRP- and empty vector-transfected COS-7 cells were analyzed by polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate (10%) gel followed by Western blot analysis to measure ADRP expression in both cell types. Conditions for SDS-PAGE and Western blot analysis were similar to the ones described previously to measure ADRP expression in adipocytes (24).

Immunolocalization of ADRP in Transfected COS-7 Cells—COS-7 cells were plated in chamber slides (Becton Dickinson) in DME/F12 medium supplemented with 10% FBS. Transfection of ADRP or empty vector plasmid DNA was carried out as described above. After 48 h, cells were washed with PBS, fixed with 2% paraformaldehyde, and permeabilized with 0.2% Triton X-100 using a previously described method (19) and stained with a suspension of sodium dodecyl sulfate antibody for 1 h at 37 °C, followed by incubation with FITC-conjugated goat anti-rabbit secondary antibody (Bio-Rad). After extensive washing with PBS, slides were mounted in buffer glycerol solution and examined with an Olympus BX40 fluorescence microscope equipped with an Olympus camera.

Preparation of Albumin-bound Fatty Acids—[9,10-3H]Oleic acid (1.2 μCi/ml) (New England Nuclear Science Products) and unlabeled oleic acid sodium salts were dissolved in 10 ml of water at 40 °C to give a concentration of about 320 μM (48 μCi/ml). When the solution was completely clear after ~10 min, fatty acid-free BSA (Sigma) from a concentrated stock solution (20%) was added with gentle mixing to obtain a final concentration of 80 μM BSA and an oleic acid/BSA molar ratio of 4.0. For the assays, an aliquot of the stock solution of FA/BSA (320 μM) was diluted 1:8 with PBS containing or not containing additional fatty acid-free BSA to obtain the final desired concentrations of oleic acid (40 μM) and of fatty acid-free BSA (10, 13, 20, 40, and 80 μM) and oleic acid/BSA molar ratios of 4.0, 3.0, 2.0, 1.0, and 0.5, respectively. The final concentration of fatty acids in the assay was 20 μM [3H]oleic acid (3 μCi/ml) with various concentrations of fatty acid-free BSA. The unbound oleic acid in the tubes was separated by centrifugation at 4°C, 16,826 g for 10 min, and the supernatant was routinely measured and subtracted from experimental values. Background radioactivity representing isotope trapped extracellularly and bound nonspecifically by the cells was measured from zero time incubation determined by adding stop solution to the cells before adding radiolabeled fatty acid-BSA complex.

Fatty acid uptake data were normalized with the transfection efficiency that had been determined by counting the number of fluorescent cells expressing GFP compared with the total cell number. Since non-transfected cells could also uptake fatty acid, it was necessary to remove their contribution to the total FA uptake in order to determine the contribution due to the expression of ADRP in the cells. To achieve this, the uptake of FA by non-transfected cells was determined by multiplying the value of total FA uptake in non-transfected control cells by the percentage of non-transfected cells (100% – transfection efficiency). Non-transfected control cells were treated similarly to transfected cells but without adding plasmid DNA of vectors used for transfection. The value for the remaining FA uptake contributed by the transfected cells was obtained by subtracting the nontransfected cells uptake from the total uptake measured experimentally. Then the remaining FA uptake of transfected cells was normalized to the transfection efficiency. The final amount of uptake was expressed as pmol per 10⁶ transfected cells.

Bligh-Dyer Extraction and Thin Layer Chromatography of Lipids—The distribution of labeled oleate in intracellular lipids in ADRP-transfected or empty vector-transfected COS-7 cells was examined after uptake was performed. Uptake was carried out as described above. At various time points after uptake was stopped, cells from triplicate samples (6x10⁵ cells) were washed by centrifugation. Lipids were immediately extracted by the method of Bligh and Dyer (27) from cells
RESULTS

ADRP Expression in COS-7 Cells Transiently Transfected with ADRP-pcDNA—To test whether ADRP promoted FA transport, the pcDNA3 expression vector containing ADRP cDNA insert was transiently transfected into COS-7 cells. Plasmid DNA from empty pcDNA3 vector was transfected into COS-7 cells. In contrast, cells transfected with the ADRP-pcDNA3 expression vector expressed high levels of ADRP protein expression was very low in empty vector-transfected COS-7 cells. In contrast, cells transfected with the ADRP-pcDNA3 expression vector expressed high levels of ADRP protein equivalent to the one in 1246 adipocytes known to express high level of ADRP (19, 24) and used as positive controls. These results demonstrated that ADRP protein was effectively expressed in COS-7 cells and that the transfected COS-7 cells could be used as a model of ADRP protein overexpression to examine the function of ADRP in mammalian cells.

Localization of ADRP in transfected COS-7 cells was examined by immunofluorescence staining of fixed cells with anti-ADRP antibody followed by FITC-conjugated goat anti-rabbit secondary antibody. Fluorescence microscopy revealed a specific pattern of staining at the cell periphery in ADRP-transfected COS-7 cells (Fig. 2 top panel) which was not observed in empty vector-transfected COS-7 cells (Fig. 2 bottom panel) or in cells incubated with preimmune IgG used as negative control (data not shown). Additional immunostaining was also found associated with the nucleus in ADRP-transfected cells as well as in control cells although with a lesser degree. These data show that ADRP is preferentially found associated with the plasma membrane in the transfected COS-7 cells.

Time Course of [3H]Oleic Acid Uptake in ADRP-transfected and in Control COS-7 Cells—Assays of oleic acid transport were performed in control and in ADRP-expressing COS-7 cells. [3H]Oleate at a final concentration of 20 μM corresponding to an oleate/BSA molar ratio of 4:1 was incubated with 10^5 cells at 37 °C from 15 s to 30 min. Uptake was stopped by adding 5 ml of an ice-cold stop solution containing 200 μM phloretin and 0.1% BSA in PBS. Since the stop solution removed surface-bound [3H]oleate while blocking efflux from the cells of oleate already internalized (8), accurate quantitation of cumulative cellular oleate uptake could be obtained after stop solution treatment. The [3H]oleate uptake measured as the total [3H]oleate accumulated in ADRP or empty vector-transfected cells was normalized to the transfection efficiency as described under “Experimental Procedures.” Fig. 3 showed the time course of oleate uptake in transfected COS-7 cells determined at 2.30 μM unbound oleic acid with an oleate/BSA ratio of 4:1 (mol/mol). The time course was biphasic with a fast early phase linear for up to 0.5 min (Fig. 3A) followed by a slower phase (Fig. 3B) both for control and ADRP-transfected cells. In the later slower phase, the oleate uptake rate decreased although the cells continued to accumulate oleate. ADRP increased [3H]oleate uptake by about 3-fold over the uptake in control cells at all time points measured. Each of the two phases were significantly faster in ADRP-transfected cells than in control cells (p < 0.01 in each case). By 30 min, ADRP-transfected COS-7 cells had incorporated 401.0 ± 57.6 pmol/10^5 cells and control cells 194.6 ± 42.6 pmol/10^5 cells, respec-
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The kinetics of [3H]oleic acid uptake by ADRP was assessed by examining oleate uptake over a 15-s period within a range of unbound oleic acid concentrations from 0.043 to 2.30 μM corresponding to a molar ratio of oleate/BSA of 0.5 to 4, respectively. As shown in Fig. 4, the uptake in control and ADRP-transfected cells was saturable. [3H]Oleate uptake velocity in control cells and ADRP-transfected cells reached a plateau at concentrations above 0.11 μM unbound oleic acid. After a 15-s incubation, the [3H]oleic acid uptake velocity in ADRP-transfected COS-7 cells was significantly higher than that of control cells at this range of oleic acid concentrations (p < 0.01). From a double-reciprocal plot of these data, the transport Km of ADRP was determined to be 0.051 μM and Vmax was 57.97 pmol/10⁵ cells/min, whereas Km was 0.983 μM and Vmax was 20.13 pmol/10⁵ cells/min in control cells. These results suggested that ADRP had the function of facilitating the uptake of fatty acid by increasing fatty acid uptake velocity.

Uptake of [3H]oleate was also examined at 4 °C and compared with the one measured at 37 °C. After 2 min incubation, oleic acid uptake at the unbound oleic acid concentration of 2.30 μM obtained from 5 replicate determinations was 90% lower in ADRP-transfected cells at 4 °C (5.17 ± 0.33 pmol per 10⁵ cells) than in ADRP-transfected cells at 37 °C (61.52 ± 11.08 pmol per 10⁵ cells). This low uptake value was similar to the one measured with control transfected cells at 4 °C.

Specificity of Fatty Acids Uptake—Experiments were carried out to examine the uptake of other fatty acids by COS cells transfected with ADRP or with empty vector. Uptake was performed at 37 °C with a 4:1 molar ratio of fatty acid/BSA and a total of 20 μM fatty acid. We measured the uptake of medium chain fatty acid such as octanoic acid and long chain fatty acids either saturated such as palmitic acid or polyunsaturated such as arachidonic acid. As shown in Table I, uptake of palmitic acid and arachidonic acid was significantly increased in ADRP-transfected cells when compared with control groups similarly to what was observed for oleic acid. In contrast, there was no significant difference between ADRP and control groups in the uptake of radiolabeled medium chain octanoic acid. These data indicate that ADRP only regulated long chain fatty acid uptake.

Uptake of 2-[14C]Deoxy-D-glucose—In contrast to FA transport, transport of 2-deoxyglucose (Fig. 5) was not significantly altered by ADRP expression. 2-[14C]Deoxy-D-glucose uptake
TABLE I

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Uptake in ADRP-COS cells</th>
<th>Uptake in EV-COS cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanoic acid (C8:0)</td>
<td>0.23 ± 0.05</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>109.65 ± 4.12</td>
<td>39.79 ± 4.12*</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>58.01 ± 9.48</td>
<td>21.12 ± 0.46*</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>36.68 ± 9.02</td>
<td>16.35 ± 6.65*</td>
</tr>
</tbody>
</table>

*p < 0.05.
bp < 0.01.

FIG. 5. Time course of glucose uptake in ADRP and empty vector-transfected COS-7 cells. ADRP- (closed squares) and control empty vector (open squares)-transfected cells were kept at 37 °C for 3 h in serum-free DMEM and then switched to KRP buffer for a 2-h stabilization period. Uptake of 2-[(14)C]deoxyglucose was measured at 37 °C by adding 200 μM 2-[(14)C]deoxyglucose into KRP buffer for various times. Data for each treatment were mean ± S.D. of six replicates.

**DISCUSSION**

In this study, we examined whether ADRP expression stimulated fatty acid uptake. These studies were carried out using COS-7 cells expressing high level of ADRP by transient transfection of ADRP cDNA as an experimental system. Immunolocalization of ADRP in transfected COS-7 cells indicated that ADRP was found preferentially associated with the plasma membrane. This was also confirmed by analyzing the distribution of ADRP immunoreactivity by SDS-PAGE and Western blot analysis of fractions obtained after subcellular fractionation of transfected COS cell lysates. ADRP was found in the 105,000 × g particulate fraction, whereas cytosol fraction was negative (data not shown).

Uptake studies presented here show that ADRP expression increased in FA uptake in transfected COS-7 cells. We observed saturation of oleate uptake velocity as a function of the unbound FA concentration in the medium. The data indicate that ADRP expression resulted in a 3-fold increase in the initial velocity of oleic acid uptake when compared with empty vector-transfected COS-7 cells. There was a 2.6-fold increase in the Vmax for the saturable component of oleate uptake in ADRP-expressing COS-7 cells. The K0.5 of the uptake component contributed by ADRP expression decreased by 2.2-fold when compared with control cells. These data suggested that the saturable component of fatty acid uptake could be facilitated by ADRP expression. It has been shown that this influx of non-esterified FA into adipocytes is phloretin-inhibitable, saturable, and independent of cellular metabolism (9, 13).

The oleate uptake mediated by ADRP expressed into COS cells was temperature-dependent, since the uptake at 4 °C was only 10% that at 37 °C. This suggested that a specific carrier-mediated process was involved in FA transport systems in COS-7 cells. The results presented here suggest that this process is facilitated by ADRP expression. Analysis of the distribution of label in intracellular lipids showed no difference after oleate uptake in ADRP and empty vector control cells suggesting that ADRP did not influence the fate of fatty acid in intracellular pools but merely facilitated its uptake.

Comparison of uptake of various fatty acids indicated that ADRP facilitated uptake of long chain fatty acids whether they were saturated, mono-unsaturated, or polyunsaturated. In contrast, ADRP had no effect on the uptake of medium chain FA octanoic acid and on the uptake of glucose. These data suggested that ADRP mediates specifically uptake of long chain fatty acid and that uptake of medium chain fatty acid occurs by a different mechanism.

All of the results demonstrated that ADRP indeed specifically enhanced uptake of long chain fatty acids by increasing the initial uptake rate. Together with localization studies showing that ADRP localized near the membrane in transfected COS-7 cells and in cells starting to differentiate into adipocytes (20), these data strongly support the idea that ADRP acts as a fatty acid carrier protein.

Although it has long been considered that the movement of long chain FA across the cell membrane is invariably passive (7, 23, 26), increasing evidence suggests that a specific and selective facilitated transport mechanism is responsible for the transmembrane flux of free FA (10, 13, 28). FA-binding protein of plasma membrane (FABPpm) (14), fatty acid translocase (FAT) (15), fatty acid transport protein (FATP) (16), and two other putative proteins (17, 18) have been identified as FA transporters in several tissues. In Escherichia coli, a peripheral membrane FA acceptor and a distinct transmembrane transporter, which composes the facilitated FA transport system, have been well characterized (29, 30). The features of FABPpm, FAT, and FATP as FA transporters have been investigated extensively in adipocytes, hepatocytes, and myocytes (8, 10, 11, 16).

Preliminary studies carried out in our laboratory have shown that inhibition of ADRP expression in adipocyte precursors by ADRP antisense oligodeoxynucleotides resulted in inhibition of lipid droplet accumulation with a concomitant decrease in triglyceride accumulation.

The studies presented in this report provide the first time a function for ADRP as a protein involved in carrier-mediated FA influx into cells. These results are important since ADRP sequence did not provide any clue as to its functional properties and since ADRP does not have sequence similarity with the well known small molecular weight fatty acid-binding proteins (21) and with other fatty acid transporters recently cloned from adipocytes (15–16). It is presently unclear whether in the adipocytes ADRP acts solely as a mediator of FA uptake, whether it is involved in the transport systems in cooperation with other cellular proteins also involved in lipid metabolism, or whether it has some other regulatory role not directly involved in transport systems.

Hormonal stimulation of ADRP expression in adipocyte precursors shows that it is stimulated by cyclooxygenase inhibitors (24) and also by long chain FA. Regulation of gene activ-

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2 B. Condon and G. Serrero, manuscript in preparation.
3 J. Gao, H. Ye, and G. Serrero, submitted for publication.
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ities by substrate is common in mammalian cells. The expression of the glucose transporter (GLUT1) is modulated by glucose deprivation (31). Enzymes involved in glucose metabolism are also regulated by glucose (32, 33). It has been shown that the transcription of the gene coding for hydroxymethylglutaryl-CoA reductase is modulated by cholesterol (34). Expression of the fatty acid-binding protein gene apo2 is stimulated by fatty acids (35). Thus, the fact that a protein involved in FA transport and/or metabolism is modulated by FA could provide a regulatory mechanism to control FA transport and/or metabolism.

In conclusion, the present investigation provides a function for ADRP, identifies a new protein involved in fatty acid uptake, and suggests that ADRP could play a role in regulating lipid accumulation in adipocytes by increasing FA uptake. Adipocytes have the ability to uptake or synthesize fatty acids for esterification into triacylglycerol and to hydrolyze these triglycerides in response to lipolytic stimuli. Based on the results presented here and on the localization of ADRP during adipose differentiation, it is then tempting to speculate that ADRP may serve as a “shuttling” protein of lipid substrate to the lipid droplets. Additional studies are underway to investigate this hypothesis and to understand structurally the mechanism by which ADRP may act as a FA transport-associated protein.

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