FAT/CD36-mediated Long-Chain Fatty Acid Uptake in Adipocytes Requires Plasma Membrane Rafts

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We previously reported that lipid rafts are involved in long-chain fatty acid (LCFA) uptake in 3T3-L1 adipocytes. The present data show that LCFA uptake does not depend on caveolae endocytosis because expression of a dominant negative mutant of dynamin had no effect on uptake of [3H]oleic acid, whereas it effectively prevented endocytosis of cholera toxin. Isolation of detergent-resistant membranes (DRMs) from 3T3-L1 cell homogenates revealed that FAT/CD36 was expressed in both DRMs and detergent-soluble membranes (DSMs), whereas FATP1 and FATP4 were present only in DSMs but not DRMs. Disruption of lipid rafts by cyclodextrin and specific inhibition of FAT/CD36 by sulfo-succinimidyl oleate (SSO) significantly decreased uptake of [3H]oleic acid, but simultaneous treatment had no additional or synergistic effects, suggesting that both treatments target the same mechanism. Indeed, subcellular fractionation demonstrated that plasma membrane fatty acid translocase (FAT/CD36) is exclusively located in lipid rafts, whereas intracellular FAT/CD36 cofractionated with DSMs. Binding assays confirmed that [3H]SSO predominantly binds to FAT/CD36 within plasma membrane DRMs. In conclusion, our data strongly suggest that FAT/CD36 mediates raft-dependent LCFA uptake. Plasma membrane lipid rafts might control LCFA uptake by regulating surface availability of FAT/CD36.

INTRODUCTION

Adipocytes are the primary site for lipid storage and mobilization and, as such, one of their major roles is the uptake and release of long-chain fatty acids (LCFAs). The permeation of LCFAs across the adipocyte plasma membrane relies on a high-affinity, low-capacity carrier-facilitated transport system (Abumrad et al., 1981). Several proteins in the adipocyte plasma membrane have been implicated in fatty acid transport or binding such as plasma membrane fatty acid binding protein (FABPpm) (Stremmel et al., 1986), fatty acid transport protein (FATP) (Schaffer and Lodish, 1994), caveolin-1 (Trigatti et al., 1999), and fatty acid translocase (FAT/CD36) (Abumrad et al., 1993). The important role of FAT/CD36 for LCFA uptake in adipocytes has been extensively studied. When overexpressed in cultured fibroblasts FAT/CD36 increases saturable, high-affinity LCFA uptake (Ibrahimi et al., 1996). Moreover, FAT/CD36 knockout mice have increased serum fasting levels of nonesterified free fatty acids and show reduced uptake of oleate in isolated adipocytes (Febbraio et al., 1999).

Very recent findings indicate that specialized microdomains of the plasma membrane, termed lipid rafts, are essential for regulating LCFA uptake (Kolleck et al., 2002; Pohl et al., 2002, 2004; Razani et al., 2002; Ring et al., 2002; Vistisen et al., 2004). Rafts are membrane domains that are enriched in sphingolipids and cholesterol and form a liquid-ordered subdomain that contains a select set of membrane proteins (reviewed in Parton and Simons, 1995). On the basis of their particular properties as detergent-resistant membranes (DRMs), lipid rafts can be isolated from cell lysates (Parton and Simons, 1995). Caveolae are a specialized subset of rafts forming characteristic flask-shaped invaginations. Caveolin-1 is the major structural protein of caveolae and can bind fatty acids saturably and with high affinity (Trigatti et al., 1999). Whereas rafts are found in all cell types, caveoleae are only found in some cell types among which adipocytes express a particularly high number of caveoleae. Estimates have been made that from 15 to 30% of the adipocyte plasma membrane surface are caveoleae (Fan et al., 1983). Caveoleae participate in a large number of important cellular functions. These include the organization of numerous transmembrane signaling complexes in many cell types, the regulation of cellular cholesterol homeostasis, and formation of endocytic vesicles (Tran et al., 1987; reviewed in Parton 2003). It is known that albumin, which functions as a carrier for fatty acids, can be endocyotised by caveoleae (Schubert et al., 2001). The endocytic internalization of caveoleae requires the GTPase dynamin II (Henley et al., 1998; Orth et al., 2002). Expression of a dominant negative dynamin mutant results in loss of endocytosis by caveoleae and clathrin-coated pits (Orth et al., 2002).

Recently we showed that in 3T3-L1 adipocytes, lipid rafts are involved in binding and uptake of LCFAs. However, the mechanism of lipid raft-mediated LCFA uptake remained elusive. Theoretically, lipid raft-dependent LCFA uptake could rely either on receptor-mediated endocytosis or on facilitated transport by a fatty acid binding protein located in lipid rafts. In conclusion, our data strongly suggest that FAT/CD36 mediates raft-dependent LCFA uptake. Plasma membrane lipid rafts might control LCFA uptake by regulating surface availability of FAT/CD36.
in plasma membrane lipid rafts. In the present study, we investigated both of these hypotheses. First we prevented caveolea endocytosis by transient expression of the dynamin II mutant K44A (Orth et al., 2002) to investigate a potential involvement of lipid raft endocytosis in LCFA uptake. Subsequently, we characterized the distribution of FATP1 and FATP4, the major representatives of the FATP family in adipose tissue (Stahl, 2004), and FAT/CD36 in raft and nonraft membrane domains of whole cell lysates and plasma membrane fractions. FAT/CD36 has previously been shown to be localized in caveolae (Souto et al., 2003; Pohl et al., 2004); however, it is unclear whether these are plasma membrane caveolea involved in LCFA uptake. The functional role of FAT/CD36 associated with lipid rafts was studied using sulfo-N-succinimidyl oleate (SSO), an oleic acid derivative that specifically binds to and cross-links FAT/CD36 on the plasma membrane, resulting in an arrest of the transport function of this protein (reviewed by Coort et al., 2002). FATP1 and FABPpm function are not affected by SSO (Van Nieuwenhoven et al., 1995). Furthermore, SSO does not permeate the plasma membrane and thus does not directly affect intracellular transport processes (reviewed by Coort et al., 2002).

MATERIALS AND METHODS

[3H]Oleic acid was purchased from Biotrend (Cologne, Germany). Fatty acid-free bovine serum albumin (BSA) (fraction V), cholesterin, dexamethasone, phloretin, EGTA, leupeptin, pepstatin, sucrase, EDTA, chymostatin, HOSu(SO4)Na, dicyclohexylcarbodiimide, N,N-dimethylformamide (DMF), and nonradiolabeled oleic acid were purchased from Sigma-Aldrich (St. Louis, MO). Ultima-Gold scintillation fluid was purchased from Packard (Groningen, The Netherlands). Dimethyl sulfoxide (DMSO) was from Merck (Darmstadt, Germany).

Cell Culture

3T3-L1 fibroblasts were obtained from the American Type Culture Collection (Rockville, MD) and cultured in DMEM supplemented with 10% (vol/vol) fetal calf serum (FCS; Invitrogen, Carlsbad, CA), 2 mM l-glutamin, 100 U/l penicillin, and 100 µg/l streptomycin at 37°C in 10% CO2 and passed at ~70% confluency. Confluent fibroblasts were induced to differentiate 1 d after reaching confluency by addition of DMEM containing 10% (vol/vol) FCS, 0.25 µg/ml insulin, 0.25 mM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 100 ng/ml b-2-mercaptoethanol. After 2 h, the medium was replaced with fresh FCS and DMEM containing insulin. Adipocytes were differentiated for 8–10 d postdifferentiation.

Synthesis of Sulfo-succinimidyl-Oleate

The synthet of unlabeled and radiolabeled sulfo-succinimidyl derivatives of oleate (SSOs) was performed as described by Harmon et al. (1991). Oleate (0.25 mmol), HOSu(SO4)Na (0.25 mmol), and dicyclohexylcarbodiimide (DCC) (0.275 mmol) were dissolved in 0.5 ml of dry DMF and stirred at room temperature overnight. For synthesis of radiolabeled SSO, trace amounts of [3H]oleic acid were initially added to the nonradioactive oleic acid. The precipitated dicyclohexylurea was removed by filtration. The filtrate was taken to dryness. Eight volumes of ethyl acetate was added and the precipitated product was collected by filtration under nitrogen in a glove Bag and then stored in a vacuum desiccator over phosphorus pentoxide. Stock solutions of SSO were made dissolving the compounds in DMSO.

Treatment with [3H]SSO

Treatment of 3T3-L1 cells was performed exactly as described by Abumrad et al. (1991). Briefly, on day 10 after differentiation 3T3-L1 cells were released from dishes and washed three times with cold Krebs-Ringer-HEPES (KRH) buffer and checked for viability. Preparations were used only if >90% of cells excluded trypan blue. Cells were suspended [30% (vol/vol) in KRH buffer containing 0.2% fatty acid-free bovine serum albumin and glucose (2 mM)]. Afterward, aliquots of SSO stock solution were added to the adipocyte culture suspension to a final concentration of 400 µmol of SSO. To avoid toxicity of DMSO the concentration of DMSO was always kept <0.05%. The cells were incubated at 37°C for 30 min. At the end of the incubation, the cells were washed three times with KRH buffer containing 0.2% fatty acid-free BSA to remove any unbound sulfo-succinimidyl-oleate. The cells were either resuspended for assay of oleate transport or used for plasma membrane isolation experiments or isolation of detergent resistant membranes.

Assay for [3H]Oleic Acid Transport

The [3H]oleic acid uptake assays were performed as described previously (Stremmel et al., 1986) using confluent 3T3-L1 cell monolayers. Briefly, trace amounts of [3H]oleic acid mixed with 373 µmol/l nonradioactive oleic acid were dissolved in a defatted BSA solution (175 µmol/l) at a ratio of 1:1. 2 ml of the oleate/BSA solution was incubated with each 3T3-L1 cell monolayer in a 5-cm culture dish at 37°C. The uptake was stopped by removal of the solution and addition of 2 ml of ice-cold PBS. The stop solution was discharged after 2 min, and the culture dishes were washed by dipping them six times in ice-cold incubation buffer. NaOH (2 mol/l) was added to lyse the cells, and aliquots of the lysate were used for protein and radioactivity determination. Radioactivity was determined after the addition of 10 ml of Ultima-Gold in a 1217 Rackbeta liquid scintillation counter (Amersham Biosciences AB, Uppsala, Sweden).

Transfection of the Dynamin II Mutant K44A

Dynamin II K44A in pCMV5 was provided by S. Schmid (Scripps Research Institute, La Jolla, CA). Fifty days after differentiation of 3T3-L1 cells, transient transfection using the FuGene 6 Transfection reagent (Roche Diagnostics, Indianapolis, IN) was performed with 2 µg of the expression plasmid according to the instructions of the manufacturer (Roche Diagnostics, Basel, Switzerland). Transfections were performed 2 d before functional assays.

Functional Assays

Two days after transient transfection, caveolea-mediated endocytosis was assayed by monitoring the internalization of rhodamine-labeled cholera toxin (List Biological Laboratories, Campbell, CA). Cells were rinsed with phosphate-buffered saline (PBS) and then chilled to 4°C and incubated 15 min in DMEM containing 4 µg/ml rhodamine-cholera toxin B. Afterward, cells were rinsed four times with 4°C PBS to remove unbound toxin. These cells then were incubated ~2.5 h in DMEM at 37°C in an air incubator. Containing 0.5% (vol/vol) albumin and 200 µM phloretin. The stop solution was discharged after 2 min, and the culture dishes were washed by dipping them six times in ice-cold incubation buffer. NaOH (2 mol/l) was added to lyse the cells, and aliquots of the lysate were used for protein and radioactivity determination. Radioactivity was determined after the addition of the expression plasmid according to the instructions of the manufacturer. Briefly, cells were washed once in PBS to remove the culture medium and then once in the homogenization buffer (25 mM sucrose, 0.5 mM EDTA, 10 mM Tris). Afterward, cells were scraped in 3 ml of homogenization buffer and centrifuged at 1000 × g for 5 min. The pellet was resuspended in 2 ml of homogenization buffer and cells were lysed by 10 passages through a fine syringe needle followed by treatment with a tight-fitting Dounce homogenizer. The homogenate was centrifuged at 10000 × g for 10 min to obtain a postsynaptic supernatant. The supernatant was centrifuged at 100,000 × g for 40 min and resuspended in 1 ml of homogenization buffer containing 25% (vol/vol) isodixol. The cell suspension was loaded on a nine-step OptiPrep gradient, which consisted of 25, 22, 19, 16, 13, 10, 7, 4, and 1% (vol/vol) isodixol. Centrifugation was done in Beckman SW41Ti rotor at 200,000 × g for 3 h at 4°C. Eighteen fractions were collected from the bottom of each centrifuge tube. A quarter of each fraction was analyzed with SDS-PAGE and Western blotting.

Isolation of Detergent-resistant Membranes

Detergent extraction with 3-[3-cholamidopropyl]dimethylammonio]-propane-sulfonate (CHAPS) was performed as described previously (Fiedler et al., 1993). Cells were rinsed with ice-cold PBS and scraped on ice into 300 µmol/l 3 mM Tris-HCl (pH 7.4), 150 mM NaCl, 3 mM EDTA (TNE) buffer containing leupeptin, pepstatin, chymostatin, and antipain (each at 25 µg/ml). Cells were homogenized 15 times through a 22-gauge needle followed by 10 strokes with a pestle. Dounce homogenizer. The lysate was centrifuged for 5 min at 3000 rpm to obtain a postsynaptic supernatant, which was subjected to extraction with 20 mM CHAPS in TNE buffer on ice. The extracts were adjusted to 40% sucrose
and overlaid with a discontinuous sucrose gradient (6 ml of 30% sucrose in TNE or 2 ml of TNE without sucrose). The gradients were centrifuged at 200,000 × g in a Beckman SW41 rotor for 16–22 h at 4°C. Fractions (1 ml each) were obtained and used for liquid scintillation counting and Western blotting.

**Western Blot Analysis**

For determination of the respective proteins, aliquots of membrane fractions were separated with SDS-PAGE and Western blotting, as we have described previously (Pohl et al., 2004). The origin of the antibodies is specified in Materials and Methods. Antibody binding was visualized using the enhanced chemiluminescence reagents (Amersham Biosciences). Immunoreactive bands on autoradiography films were scanned (Epson GT 9600; Epson, Tokyo, Japan) and quantified using Raytest image software (Raytest, Straubenhardt, Germany).

**RESULTS**

**Inhibition of Endocytosis by Transient Expression of the Dynamin II Mutant K44A**

To investigate whether lipid raft-mediated LCFA uptake in 3T3-L1 adipocytes is mediated by an endocytotic transport process, we transiently expressed the dominant negative dynamin II mutant K44A linked to green fluorescent protein (GFP). Twenty-four hours after transfection, ~60% of cells expressed GFP. To confirm the functional inhibition of caveolae-mediated endocytosis by K44A in 3T3-L1 adipocytes, we showed that the uptake of rhodamine-labeled cholera toxin B was inhibited by expression of K44A (Figure 1). Cholera toxin B is a glycosphingolipid-binding ligand known to be internalized by caveolae-mediated endocytosis (Parton 1994; Lencer et al., 1999). Instead of accumulating within intracellular compartments, the toxin remained concentrated at the surface of dynamin II-GFP–positive cells. However, when cells were transiently transfected and incubated with [3H]oleic acid for 5 min, there was no effect on [3H]oleic acid uptake compared with controls (10.4 ± 1.2 mmol oleate/mg protein in controls compared with 11.3 ± 1.2 mmol oleate/mg protein in transfected cells). Thus, under our experimental conditions endocytosis was not essential for LCFA uptake to occur.

**Expression of FATP1, FATP4, and FAT/CD36 in DRMs**

To investigate the presence of known LCFA binding proteins in lipid rafts, detergent lysates were subjected to centrifugation on sucrose density gradients. Due to their insolubility, lipid rafts localize to the upper (low-density) fractions. As shown in Figure 2, caveolin-1 was exclusively located in fraction 11 (DRM fraction), whereas the transferrin receptor (a marker protein of detergent-soluble membranes) was present in only the lower fractions (DSM fractions). FAT/CD36 was expressed in caveolin-1-enriched DRMs as well as in nonlipid raft fractions. In contrast, FATP1 and FATP4 were exclusively expressed in DSMs (Figure 2). To further elucidate the functional significance of FAT/CD36 expression in lipid rafts, we inhibited FAT/CD36 function and lipid raft function by treatment with SSO and by cyclodextrin, respectively. Both treatments resulted in a significant decrease of oleate uptake (Figure 3). However, combining both treatments did not show any additional effect on oleate incorporation. These results suggest that both treatments inhibit the same pathway of LCFA uptake.

**[3H]SSO Binds to FAT/CD36 Located in DRMs**

To investigate whether lipid raft FAT/CD36 binds LCFA, 3T3-L1 adipocytes were treated with [3H]SSO for 30 min followed by extraction of DRMs by sucrose density gradient centrifugation. Scintillation counting of the fractions showed the major peak of [3H]SSO cofractionated with DRMs and some [3H]SSO was found in the DSM fractions as well (Figure 4A). SDS-PAGE demonstrated that within the DRM fraction, [3H]SSO was specifically bound to FAT/CD36. The 88-kDa band representing FAT/CD36 (as confirmed by Western Blotting) was excised from the gel and assessed for the presence of [3H]SSO. Figure 4B shows that ~80% of the total [3H]SSO content in the DRM fraction were associated with the FAT/CD36 band, whereas only 20% were found in the rest of the gel. In contrast, [3H]SSO in the DSM fractions

**Figure 1.** Inhibition of cholera toxin uptake by the dominant negative dynamin II mutant K44A. (A) Rhodamine-labeled cholera toxin. (B) GFP-labeled K44A. (C) Merged image showing that cells transiently expressing K44A do not efficiently take up cholera toxin, which remains at the cell surface (40× magnification).
was not predominantly associated with FAT/CD36. Because specific binding of SSO in live cells is limited to FAT/CD36 on the plasma membrane, the presence of [3H]SSO in the DSM fractions probably reflects nonspecific interactions with the plasma membrane. As opposed to live cells, [3H]SSO incubated with total cell homogenates mainly associated with FAT/CD36 in the DSM fractions (Figure 4C). This pattern is due to binding of [3H]SSO to previously intracellular FAT/CD36 in DSM and parallels the quantitative distribution of FAT/CD36 in DSMs and DRMs.

Together, these results show that at the plasma membrane level [3H]SSO is predominantly bound to FAT/CD36 located in DRMs, whereas there is only very little binding to FAT/CD36 in DSMs. However, in total cell lysates the majority of FAT/CD36 is located in DSMs, suggesting that this might represent an intracellular FAT/CD36 pool that normally does not get into contact with extracellular SSO. Alternatively, FAT/CD36 located in DSMs on the plasma membrane level might not bind SSO efficiently. To address these open questions, we studied the subcellular distribution of FAT/CD36 by membrane fractionation.

**Differential Subcellular Distribution of FAT/CD36, FATP1, FATP4, and Caveolin-1**

To investigate the subcellular distribution of FAT/CD36, FATP1, FATP4, and caveolin-1 in 3T3-L1 adipocytes, we fractionated cell homogenates by a discontinuous iodixanol OptiPrep step gradient. We used antibodies against Na-K-ATPase, gp26/27, and calreticulin as markers for the plasma membrane, Golgi-network, and endoplasmic reticulum (ER), respectively. Figure 5A shows that fractions containing

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*Figure 2.* Isolation of a lipid raft-enriched membrane fraction. CHAPS-insoluble lipid rafts (DRMs) were separated from soluble membranes (DSMs) on sucrose gradients and immunoblotted with antibodies to the transferrin receptor (TfR), caveolin-1, FAT/CD36, FATP1, and FATP4. Representative blots are shown. For quantification of the respective protein a given fraction (indicated on the y-axis) has been set to 100, and the other fractions were expressed relative to this fraction. Values are means ± SD of three independent experiments.

*Figure 3.* Effects of cyclodextrin and SSO on [3H]oleate uptake. Pretreatment of cells with βcyclodextrin (10 mM for 30 min at 37°C) or SSO (400 μM for 30 min at 37°C) resulted in a significant reduction in the rate of [3H]oleate uptake over the course of 5 min. However, combining both treatments had no additional effect on [3H]oleate uptake. The asterisks indicate statistical significance (p < 0.05). Values are means ± SD of three independent experiments.
Figure 4. Binding of \([^{3}H]SSO\) to lipid raft-enriched membrane fractions and FAT/CD36. After incubating live 3T3-L1 adipocytes (A and B) or total cell homogenates (C) with 400 \(\mu M\) \([^{3}H]SSO\) for 30 min, the reaction was stopped by an ice-cold solution containing 0.5\% (wt/vol) albumin and 200 \(\mu M\) phloretin. Afterward, CHAPS-insoluble lipid rafts were separated from soluble membranes on sucrose gradients. (A) Binding of \([^{3}H]SSO\) to DRMs of live cells. When radioactivity of DRM and DSM fractions was determined the major peak of \([^{3}H]SSO\) co-fractionated with DRMs. (B) Binding of \([^{3}H]SSO\) to FAT/CD36 in DRM and DSM fractions of live cells. One hundred microliters of each CHAPS fraction was separated by 12\% SDS-PAGE, and the 88-kDa band representing FAT/CD36 (as confirmed by Western blotting) was excised from the gel and assessed for radioactivity. The figure shows \([^{3}H]SSO\) content associated with the FAT/CD36 band (closed squares) and the rest of the gel (open diamonds). (C) Binding of \([^{3}H]SSO\) to FAT/CD36 in DRM and DSM fractions of cell homogenates. As opposed to live cells, \([^{3}H]SSO\) incubated with total cell homogenates mainly associated with FAT/CD36 bands (closed squares) in DSM fractions. However, there was also modest radioactivity that was not associated with FAT/CD36 (open diamonds). Values in A, B, and C are means ± SD of five independent experiments.

Figure 5. Membrane fractionation of 3T3-L1 cell homogenates. (A) Expression of marker proteins for plasma membrane (anti-Na,K-ATPase), Golgi network (gp 26/27), and ER (calreticulin). (B) Expression of proteins involved in LCFA uptake in membrane fractions. Fractionation was performed using a discontinuous OptiPrep step gradient, and equal amounts of protein were separated by SDS-PAGE, blotted, and probed with antibodies against the indicated proteins. Signals were quantified by densitometry, and protein content was expressed relative to a given fraction (indicated on the \(y\)-axis) that has been set to 100. Values are means ± SD of three independent experiments. (C) Binding of \([^{3}H]SSO\) to membrane fractions. Membrane fractionation was performed after incubation of 3T3-L1 adipocytes for 30 min with 400 \(\mu M\) \([^{3}H]SSO\). Values are means ± SD of five independent experiments.
plasma membrane (nos. 1–7), Golgi network (nos. 7–14), and ER (nos. 14–18) could be separated. FAT/CD36 was present in the plasma membrane and Golgi fractions (Figure 5B). In contrast, FATP1 and FATP4 were expressed on the plasma membrane and in the ER. A large pool of caveolin-1 was present in the plasma membrane and Golgi fractions and a somewhat lower amount in the ER fractions as well.

Using this assay, we also confirmed that [³H]SSO did not tether significantly to any intracellular membranes when incubated with live cells (Figure 5C). The failure of [³H]SSO to recognize FAT/CD36 in DRMs of live cells but not homogenates (Figure 4, B and C) indicates that FAT/CD36 in DRMs is restricted to the intracellular compartment.

This prediction was indeed confirmed by isolation of DRMs from plasma membrane fractions and intracellular membrane fractions of 3T3-L1 adipocytes (Figure 6). Whereas FAT/CD36 expression at the plasma membrane was restricted to DRMs intracellular FAT/CD36 was expressed predominantly in DSMs.

**DISCUSSION**

A number of recent studies in cell lines and caveolin-1 knockout mice have suggested that lipid rafts/caveolae and adipocytes (Souto et al., 2003; Pohl et al., 2004). Immunogold labeling and electron microscopy of the adipocyte plasma membrane confirmed that caveolae have a limited protein composition with caveolins, semicarbazide-sensitive amine oxidase, and FAT/CD36 being their major proteinaceous constituents (Souto et al., 2003). Our finding of FAT/CD36 expression in both the plasma membrane and intracellularly contrasts with a recent report by Stahl et al. (2002) describing abundant expression of FAT/CD36 on the adipocyte plasma membrane but no intracellular pools visualized by confocal laser scanning microscopy. However, in myocytes (Bonen et al., 1999, 2000; Luiken et al., 2002) and COS-7 cells (Frank et al., 2002) FAT/CD36 is present on the plasma membrane and inside cells as found by us in 3T3-L1 adipocytes.

The subcellular localization of FATP1 and FATP4 was addressed by only a few investigators (Schaffer and Lodish 1994; Stahl et al., 2002). Stahl et al. (2002) used differential centrifugation to demonstrate expression of FATP1 and FATP4 at the plasma membrane and in intracellularsome fractions of adipocytes. In our study, we found FATP1 and FATP4 in DSMs of the plasma membrane and the ER fraction of adipocytes, but not in DRM. Caveolin-1 was highly expressed at the plasma membrane level but also was found in abundance in Golgi fractions and to a lesser extent in the ER. This finding is consistent with previous work by Furuchi and Anderson (1998) that demonstrated caveolin-1 expression at the same locations.

Together, our results strongly suggest that LCFA uptake requires binding to FAT/CD36 located in lipid rafts. Because caveolae/lipid rafts are involved in endocytosis of different solutes (Tran et al., 1987; Schubert et al., 2001), it is conceivable that after binding of LCFA to FAT/CD36 budding of caveolae from the plasma membrane is involved in the mechanism of LCFA incorporation. However, inhibition of endocytosis by transient overexpression of a mutant dynamin II resulted in decreased incorporation of rhodamine-labeled cholera toxin, but it had no effect on uptake of radiolabeled oleate. These results indicate that endocytosis is of minor importance for direct LCFA uptake in our experimental setting.

Function of FAT/CD36 is crucial for LCFA uptake in adipocytes. This study gives evidence that lipid rafts are necessary to target FAT/CD36 to the plasma membrane and therefore provide an explanation for our previous finding that disruption of lipid rafts results in inhibition of LCFA uptake in adipocytes (Pohl et al., 2004). We suggest that lipid rafts are involved in LCFA uptake by regulating FAT/CD36 expression and function at the plasma membrane level. This putative mechanism might also involve caveolin-1. Frank et al. (2002) reported that in COS-7 cells FAT/CD36 was targeted to the plasma membrane only when cotransfected with caveolin-1. Furthermore, coexpression of FAT/CD36 with caveolin-1 shifts the cellular distribution of FAT/CD36 from the Golgi to the plasma membrane (Frank et al., 2002). These findings are in line with the results of the present study and indicate that caveolin-1 might induce targeting of FAT/CD36 to caveolae at the plasma membrane level. Therefore, malfunction of FAT/CD36 might be the underlying mechanism for severely increased free fatty acid levels and resistance to diet-induced obesity in caveolin-1 knockout mice (Razani et al., 2002). However, it is important to emphasize that isolation of DRMs does not distinguish caveolae from noncaveolar rafts. Hence, FAT/CD36 is localized in rafts, but it remains to be determined whether it is in caveolae. Contradictory results as to whether CD36 is present in caveolar rafts or not have been reported. Souto et al. (2003) immunopurified caveolin-1 from adipocytes and
found FAT/CD36 copurified with caveolin-1. Zeng et al. (2003) suggested that in Chinese hamster ovary cells FAT/CD36 is localized in lipid rafts but not in caveolae. Only little is known about the regulation of FAT/CD36 function, but several lines of evidence point toward a translocation mechanism for increasing LCFA uptake by FAT/CD36. In muscle cells, the distribution of FAT/CD36 between intracellular membranes and the plasma membrane plays a role for the regulation of this protein's function in LCEA uptake (Bonen et al., 1999, 2000; Luiken et al., 2002). A very recent report by Vistisen et al. (2004) suggested that in sarcolemmal FAT/CD36 colocalizes with the muscle-specific caveolae marker protein caveolin-3, indicating that caveolae may regulate cellular fatty acid uptake by FAT/CD36. This mechanism could hold true for adipocytes as well, because we found that FAT/CD36 in 3T3-L1 adipocytes is present in both an intracellular pool and at the plasma membrane level where it is associated with a caveolin-1–positive lipid raft fraction. In summary, we have shown that at the cell surface FAT/CD36 is exclusively located within lipid rafts, whereas intracellularly FAT/CD36 is found in nonlipid raft membranes cofractionating with the Golgi apparatus. Thus, lipid raft microdomains might control fatty acid uptake by regulating FAT/CD36 surface availability. We propose a model in which FAT/CD36 recycles from intracellular nonlipid raft domains to lipid raft regions of the plasma membrane. Caveolin-1 might target FAT/CD36 to the plasma membrane. However, the mechanism of action and regulation of FAT/CD36 as well as the other proteins involved in LCFA uptake is not well understood and is the subject of ongoing investigations by a number of groups.

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