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Evidence for protein-mediated fatty acid efflux by adipocytes

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Abstract

Aim: The hormonally controlled mobilization and release of fatty acids from adipocytes into the circulation is an important physiological process required for energy homeostasis. While uptake of fatty acids by adipocytes has been suggested to be predominantly protein-mediated, it is unclear whether the efflux of fatty acids also requires membrane proteins.

Methods: We used fluorescent fatty acid efflux assays and colorimetric assays for free fatty acids and glycerol to identify inhibitors with effects on fatty acid efflux, but not lipolysis, in 3T3-L1 adipocytes. We assessed the effect of these inhibitors on a fibroblast-based cell line expressing fatty acid transport protein 1, hormone-sensitive lipase and perilipin, which presumably lacks adipocyte-specific proteins for fatty acid efflux.

Results: We identified 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) as an inhibitor of fatty acid efflux that did not impair lipolysis or the cellular exit of glycerol but lead to an accumulation of intracellular fatty acids. In contrast, fatty acid efflux by the reconstituted cellular model for fatty acid efflux was responsive to lipolytic stimuli, but insensitive to DIDS inhibition.

Conclusion: We propose that adipocytes specifically express an as yet unidentified DIDS-sensitive protein that enhances the efflux of fatty acids and therefore may lead to novel treatment approaches for obesity-related disorders characterized by abnormal lipid fluxes and ectopic triglyceride accumulation.

Keywords adipocyte, fatty acid, lipid metabolism, lipolysis.

The controlled release of free fatty acids (FFAs) from intracellular triglyceride (TAG) stores into the circulation is a predominant feature of white adipose tissue (WAT) and comprises a key component of energy homeostasis. Primarily, WAT lipolysis can be stimulated by factors that activate the adenylyl cyclase-cyclic AMP-protein kinase A (PKA) pathway (Collins *et al.* 2001), such as ß-adrenergic receptor agonists, glucagon or GLP-1. In contrast, antilipolytic factors, including insulin, generally lower cyclic AMP levels and PKA activity. PKA activity is linked to lipolysis through its ability to phosphorylate hormone-sensitive lipase (HSL) (Stralfors & Belfrage 1983) and the lipid droplet-associated protein perilipin (Egan *et al.* 1990, Greenberg *et al.* 1991, Souza *et al.* 2002). Unphosphorylated perilipin blocks the access of HSL and other lipases, such as adipocyte triglyceride lipase, to their substrates, while PKA-dependent phosphorylation of perilipin recruits HSL from the cytosol to the lipid droplet resulting in stimulated lipolysis (Souza *et al.* 1998, Brasaemle *et al.* 2000, Martinez-Botas *et al.* 2000, Tansey *et al.* 2001). Once FFAs and glycerol are hydrolytically generated within the adipose cell, they are rapidly and efficiently released from the cell. Cellular exit of glycerol has been shown to be mediated by aquaporins (Maeda *et al.* 2008); however, it is currently unknown if any proteins are involved in FFA efflux.

While several proteins that enhance the uptake of FFAs have been identified, evidence in support of a hypothetical FFA efflux system in WAT is more circumstantial, although specific transporters mediate cellular export of other hydrophobic and amphipathic biomolecules. Organic anion transporting polypeptides [organic anion transport protein (OATP)/Slc21] are sodium-independent facilitative transporters that mediate the uptake and efflux of a plethora of hydrophilic compounds, including drugs, hormones, bile acids, pravastatin and eicosanoids (Mikkaichi *et al.* 2004). The ability to transport various prostaglandins, which are oxygenated products of the fatty acid arachidonate, highlights the potential of OATPs to transport fatty acids. Additionally, ATP-binding cassette (ABC) proteins are important for the efflux of several lipids, including phospholipids, bile and cholesterol (Pohl *et al.* 2005), and mutations in ABC transporters have been linked to several hereditary lipid disorders, including Tangiers disease, Stargardt syndrome, progressive familial intrahepatic cholestasis and adrenoleukodystrophy (ALD) (Dean 2005). Of particular relevance in this context are mutations in ATP-binding cassette transporter D1 (ABCD1) that cause ALD. ABCD1 deficiency results in the inability to transport very-long-chain fatty acid into peroxisomes where they are normally oxidized. This is important for two reasons: first, the transport step from cytoplasm into peroxisomes is topologically comparable to export from the inside to the outside of cells and second, the transported substrate is a fatty acid. Further observations that support the notion that fatty acid efflux is protein-mediated include the finding by Abumrad *et al.* (1984) that epinephrine could increase the export of FFAs from adipocytes independent of changes in lipolysis.

We attempt to address the topic of FFA export from adipocytes in this study using a pharmacological approach and several commercially available compounds: phloretin, verapamil, glyburide and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS). Phloretin is known to alter the protein-mediated transport processes of many charged and uncharged substrates including glucose (Fuhrmann *et al.* 1992), urea (Toon & Solomon 1987), glycerol and chloride (Owen *et al.* 1974). Verapamil, glyburide and DIDS have all been shown to inhibit various ABC transporters (Prehm & Schumacher 2004). DIDS is also an inhibitor of anion exchangers and organic anion transport polypeptides (OATP/Slc21) and was shown to potently inhibit Cl^- exchangers (IC_{50} 2 μM) (Cabantchik & Greger 1992) and OATP3 (IC_{50} 115 μM) (Walters *et al.* 2000). Here, we present evidence that 3T3-L1 adipocytes express an as yet unidentified protein involved in FFA efflux that is sensitive to inhibition by antagonists of various transporters of hydrophobic and amphipathic biomolecules leading to an accumulation of intracellular FFAs without affecting glycerol efflux.

Material and methods

Antibodies and reagents

Polyclonal antisera against the C termini of FATP1 and FATP4 were raised as described previously (Stahl *et al.* 1999, Doege *et al.* 2006). 14-C oleic acid was obtained from American Radiolabeled Chemicals (St. Lois, MO, USA). Essentially fatty acid-free bovine serum albumin (BSA), forskolin, 3-isobutyl-1-methylxanthine (IBMX), phloretin, DIDS, glyburide and verapamil were purchased from Sigma (St. Louis, MO, USA).

Cell culture and treatment

3T3-L1 fibroblasts (ATCC) were grown in DMEM containing 10% foetal bovine serum with 2 mM L-glutamine and 1% penicillin/streptomycin (DMEM/FBS). A cell differentiation protocol was followed as previously described (Baldini *et al.* 1992, Stahl *et al.* 2002). Mature 3T3-L1 adipocytes were used in experiments on days 8–12 of differentiation.

Quencher-based FFA efflux assay

We modified a quencher-based fluorescent FFA uptake assay (Liao *et al.* 2005) that utilizes a cell-impermeable quencher of extracellular fluorescent fatty acid analogues. 3T3-L1 adipocytes were seeded on black-wall/clear-bottom 96-well plates (Costar, Corning Life Sciences, Lowell, MA, USA) and loaded with 2 μM of the fluorescent lipid 4,4-difluoro-5-methyl-4-bora-3a, 4a-diaza-S-indacene-3-dodecanoic acid (C1-BODIPY508/512-C12; Molecular Probes, Invitrogen, Carlsbad, CA, USA) bound to 0.1% BSA in Hank's buffered salt solution (HBSS) for 30 min, followed by a 30 min preincubation with or without the lipolytic stimuli 20 μM forskolin and 1 mM IBMX, which jointly work to raise cyclic AMP levels and activate PKA. This was replaced with a solution of 200 μM quenching agent Q-Red.1 (Molecular Devices, Sunnyvale, CA, USA), fresh lipolytic stimuli and 0.1% BSA in HBSS with the indicated inhibitors. Assay plates were immediately read with a fluorescent plate reader (Molecular Devices) utilizing a bottom-read setting.

Colorimetric FFA and glycerol efflux assays

3T3-L1 adipocytes were seeded on 12-well plates and treated with lipolytic stimulation buffer consisting of 20 μM forskolin, 200 μM IBMX and 0.1% BSA in HBSS with the indicated inhibitors. Aliquots of the buffer

were removed at different time points, centrifuged to pellet cell debris, and the resultant supernatant was tested for the presence of FFAs using the FFA Detection Kit (WAKO, Richmond, VA, USA) or glycerol using Free Glycerol Reagent (Sigma) as per manufacturer's instructions. Intracellular FFA concentrations were measured using cell lysates with the FFA Detection Kit.

Radiolabeled FFA assay

It was necessary to substitute colorimetric FFA and glycerol efflux assays with radiolabeled assays for all experiments using DIDS as we discovered that DIDS inhibits the bacterial long-chain acyl-coA synthetase (LACS) utilized by the colorimetric assays. 3T3-L1 adipocytes were seeded on 12-well plates and incubated for 1 h in a buffer of 2 μM C14-oleate and 200 μM oleate bound to 0.1%BSA in HBSS. Next, cells were washed three times with 0.1% BSA in HBSS and treated with lipolytic stimulation consisting of 20 μM forskolin, 200 μM IBMX and 0.1% BSA in HBSS, with or without the addition of DIDS. Aliquots of the buffer were removed at different time points and centrifuged to pellet cell debris. Radioactivity in the supernatant was quantified in a liquid scintillation counter (Packard, Meriden, CT, USA).

In vitro *TAG hydrolase activity assay*

We assessed the ability of 400 μM glyburide and 400 μM phloretin to inhibit lipase activity as described by (Duncan *et al.* 2008). Reactions proceeded for 10 min in the presence of 100 μM 3H-triolein before fatty acids were extracted and their radioactivity quantified.

Fluorescent-activated cell sorter (FACS)-based FFA efflux assay

Hormone-sensitive lipase-FATP1-perilipin (HFP) cells were lipid loaded overnight with 120 μM each palmitate and oleate and 2 μM C1-BODIPY508/512-C12 bound to 1.1% BSA. Cells were washed, trypsinized and diluted in DMEM with 0.1% BSA and centrifuged for 4 min at 400 ***g*** at 37 °C. The supernatant was aspirated, and pelleted cells were resuspended in either a control solution of 0.1% BSA in HBSS or 0.1% BSA in HBSS with the addition of 20 μM forskolin and 1 mM IBMX or 1 μg mL^{-1} insulin. Cells were sorted via FACS after a 30-min incubation period.

HFP cell generation

Hormone-sensitive lipase-FATP1-perilipin cells were generated by first stably transfecting NIH 3T3 fibroblasts with an expression vector for HSL and a construct for concurrent expression of murine FATP1 and perilipin. The dual expression construct was based on pBudCE 4.1 (Invitrogen, Carlsbad, CA, USA). HFP cells were loaded overnight with 120 μM each palmitate and oleate and 2 μM C1-BODIPY 508/512-C12 bound to 1.1% BSA. Cells with the highest lipid content were isolated by FACS and expanded in tissue culture.

HFP cell lipid loading media

5.46 μM solutions of palmitate and oleate were prepared in water and 2.5% 1 N NaOH, respectively, and heated at 70 °C. Following fatty acid solubilization, the solutions were combined and 750 μL of this mixture was rapidly pipetted into 2.5 mL of PBS and 5% BSA. The solution was filter sterilized, and 800 μL was added to 25 mL of DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 1% BSA to create a 120 μM solution of each fatty acid for overnight lipid loading.

Statistics

All data are reported as mean ± SD. All results are representative of a series of experiments, and *n* refers to the number of wells, lysates or suspensions of cells used per experiment unless otherwise noted. Statistical significance was assessed by one-way ANOVA, with P values <0.05 considered significant. Linear regression, IC50 and all other statistics were analysed using PRISM version 5.0 (Graphpad Software, La Jolla, CA, USA).

Results

Identification of FFA efflux inhibitors

To examine the possibility that adipocytes express an as yet unidentified fatty acid exporter, we performed pharmacological tests using several commercially available compounds with known inhibitory effects on transporters of hydrophobic or amphipathic biomolecules: (phloretin, verapamil, glyburide and DIDS). We first investigated the effects of phloretin, a broad inhibitor of many cellular transport processes, on FFA efflux from 3T3-L1 adipocytes with a novel quencher-based fluorescent FFA efflux assay. We determined the real-time loss in fluorescence from C1-BODIPY508/512-C12-loaded adipocytes following treatment with a lipolytic stimulus (Fig. 1a). The addition of 500 μM phloretin reduces the FFA efflux rate by approx. 50% from 29 relative fluorescent units (RFU) min^{-1} to 14 RFU min^{-1} during the linear phase, supporting our notion that FFA efflux occurs via a protein-mediated process rather than by passive diffusion.

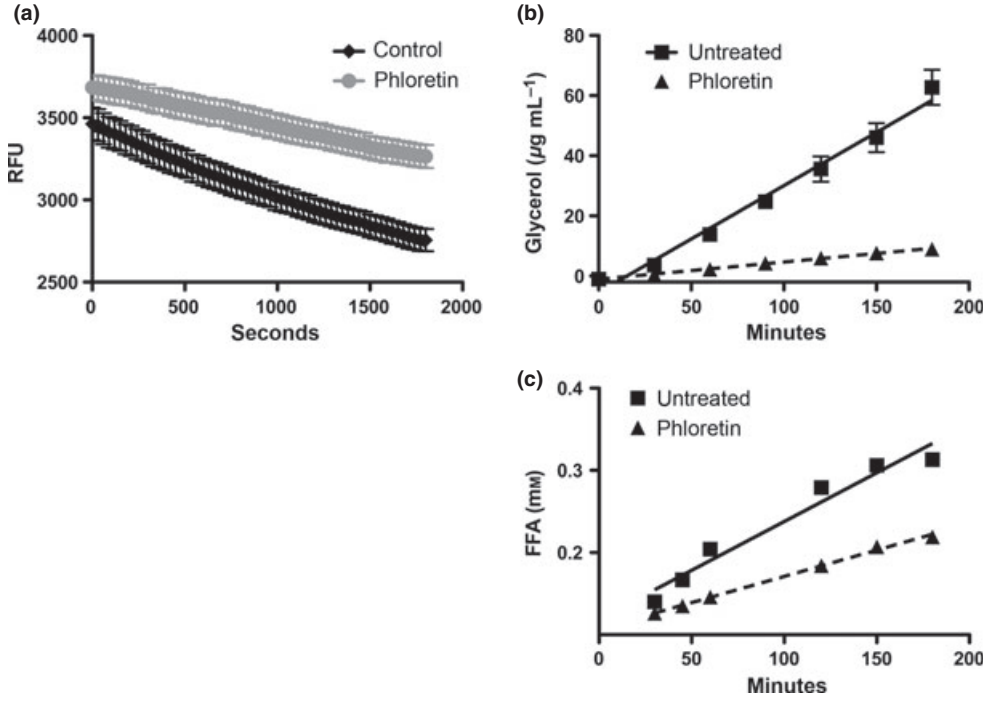

Figure 1 Effect of phloretin on glycerol and free fatty acid (FFA) efflux. (a) Intracellular fluorescence of 3T3-L1 adipocytes treated with 0 μM (black) or 500 μM phloretin (grey) detected after 30-min incubation with forskolin/IBMX. Phloretin was added at the beginning of assay; $n = 6$. Colorimetric assays to determine glycerol (b) and FFA (c) efflux over time from forskolin/IMBX stimulated 3T3-L1 adipocytes in the presence of 0 μM (squares) or 500 μM phloretin (triangles). Lines indicate linear regression through data points to calculate efflux rates; $n = 3$.

We sought to identify inhibitors with distinct effects on FFA efflux vs. lipolysis using colorimetric assays for glycerol and FFAs. A time course with 3T3-L1 adipocytes treated with phloretin demonstrates that both FFA and glycerol efflux are linear over the recorded time frame (Fig. 1b,c). Treatment with 0.5 or 1 mM phloretin significantly reduces both glycerol and FFA release from adipocytes (Fig. 2a,b). At 50 and 100 μM concentrations, verapamil reduces glycerol release by 55.3 and 63.2%, respectively, but neither concentration has an effect on the release of FFAs (Fig. 2a,b). Hundred micromolar glyburide treatment results in a reduction in both glycerol and FFA release at 31.6 and 28%, respectively (Fig. 2a,b). Inhibition of glycerol release concomitant with inhibition of FFA release, as seen with phloretin and glyburide treatment, could be due either to inhibition of lipolysis or simultaneous inhibition of both transport processes. To distinguish between these two possibilities, we performed an *in vitro* TAG hydrolase activity assay and found that while phloretin treatment results in a 17.6% reduction in lipolysis, glyburide has no inhibitory effect and in fact increases lipolysis by 11.5% (Fig. 2c). Thus, glyburide most likely has separate inhibitory effects on FFA and glycerol release. DIDS inhibits enzyme-based colorimetric FFA assays that rely on bacterial LACS, so all FFA and glycerol efflux experiments with DIDS utilized radiolabeled tracers. At 50 and 100 μM concentrations, DIDS inhibits FFA release by 48 and 60%, respectively (Fig. 2b), without affecting glycerol release (Fig. 2a). Titration of DIDS shows that an apparent IC_{50} of 102 μM for inhibition of FFA efflux from forskolin/IBMX stimulated 3T3-L1 adipocytes (Fig. 2e). If DIDS inhibits a FFA export protein, one would expect to see an increase in intracellular FFAs in treated cells. Indeed, following lipolytic stimulation, we detect a 2.2-fold and twofold increase in intracellular FFA levels in DIDS or phloretin treated cells, respectively (Fig. 2d). Glyburide and DIDS have dose-dependent inhibitory effects on FFA efflux, but not lipolysis, which suggests that these inhibitors act specifically on a FFA exporter. Furthermore, propidium iodide toxicity assays following each experiment showed no toxic effects by glyburide and DIDS on experimental cells over the recorded time frame.

FFA efflux and glycerol release by adipocytes are not dependent on chloride

As many transport processes of organic anions are coupled to chloride uptake, we examined the dependency of FFA efflux on this substrate using colorimetric

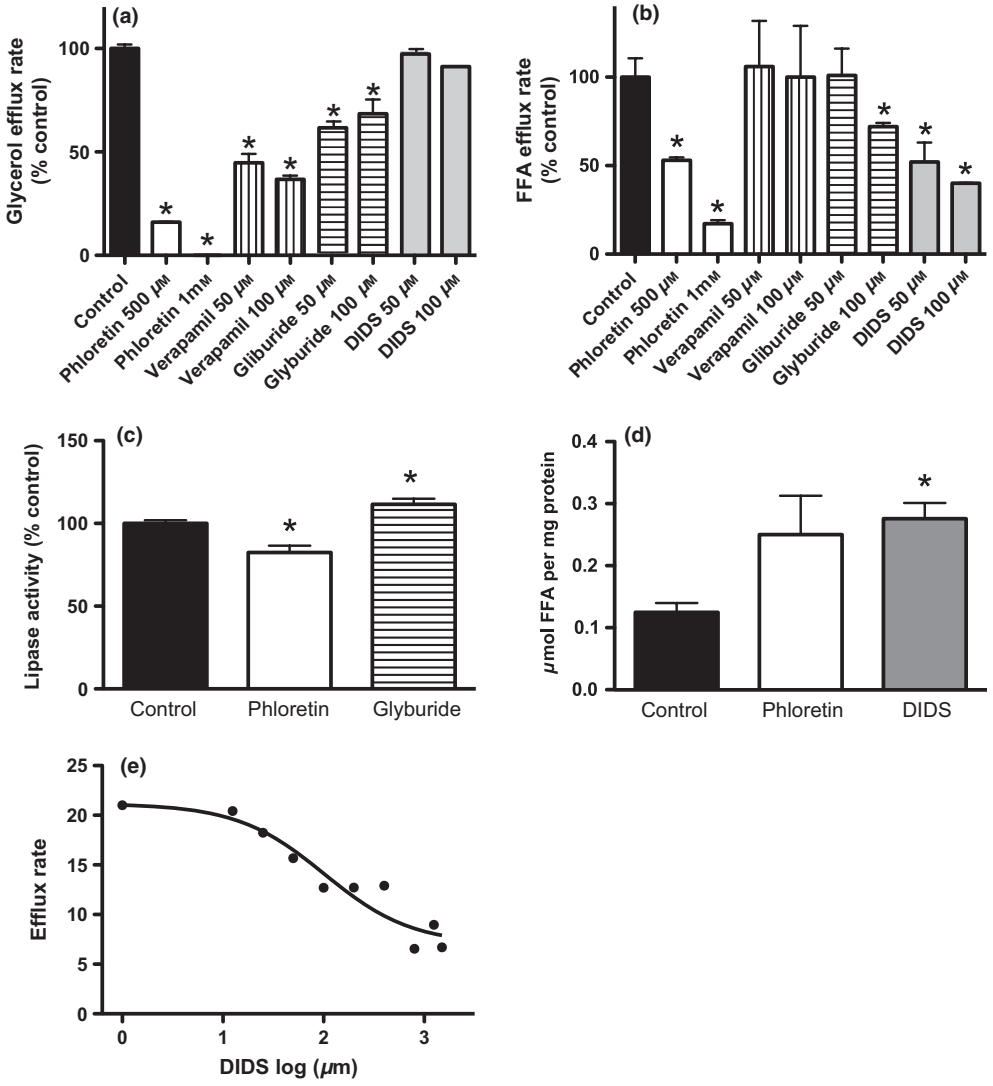

Figure 2 Modification of cellular efflux. Glycerol (a) and free fatty acid (FFA) (b) efflux rates from forskolin/IMBX stimulated 3T3-L1 adipocytes in the presence of phloretin, verapamil, glyburide (n = 8) or 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) (n = 3) at the indicated concentrations. (c) ^{3}H triolein hydrolysis assay in the presence of 400 μM phloretin or glyburide. n = 3 cell lysates. (d) Intracellular FFA concentration in control, phloretin (500 μM) or DIDS (250 μM) treated and forsklolin/IBMX stimulated 3T3-L1 adipocytes; n = 6. (e) Inhibition of ^{14}C-oleate efflux from 3T3-L1 adipocytes by DIDS 30 min after initial lipolytic stimulation. Non-linear fit of data indicates an IC_{50} of 102 μM.

assays and found that a lack of chloride in the extracellular medium has no significant effect on FFA and glycerol efflux (Fig. 3). While not completely precluding the possibility, these results argue strongly against the involvement of an anion exchanger in FFA export.

A reconstituted model for cellular FFA efflux

To test whether adipocytes possess additional protein components for efficient FFA efflux, we established a reconstituted cellular model for FFA efflux. To this end, we created a NIH 3T3 fibroblast cell line that can readily load and remobilize stored fatty acids in response to lipolytic stimuli. This cell line is based on the stable overexpression of HSL, fatty acid transport protein 1 (FATP/Slc27A1) and perilipin, to facilitate lipolysis, FFA loading and lipid droplet formation, respectively (Fig. 4a). The resultant cell line (HFP cells) accumulates lipid droplets with a median diameter of 1.9 μm following overnight lipid loading of 2 μM C1-BODIPY508/512-C12 in the presence of 120 μM each oleate and palmitate (Fig. 4b,c). We performed a quencher-based FFA efflux assay to determine the real-time FFA efflux kinetics of lipid-loaded HFP cells treated with lipolytic stimuli (Fig. 4d) and saw an

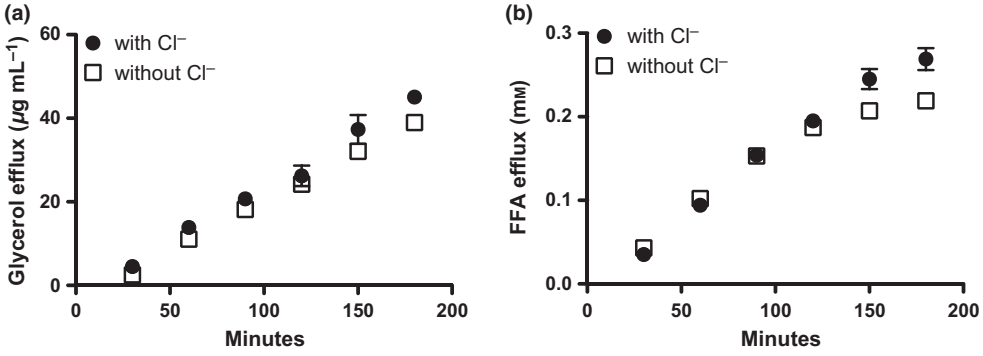

Figure 3 Effect of chloride on glycerol and free fatty acid (FFA) efflux. Glycerol (a.) and FFA (b.) efflux time course from forskolin/IBMX stimulated 3T3-L1 adipocytes with or without the presence of chloride; $n = 3$.

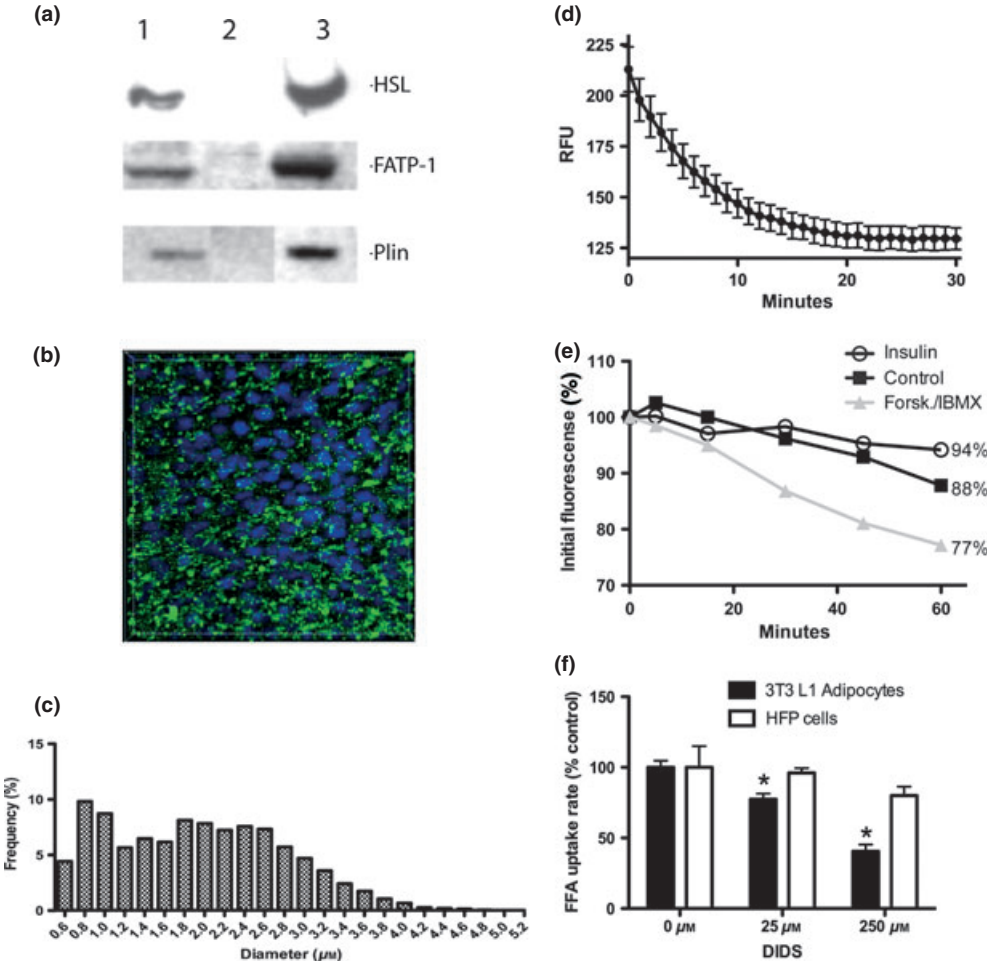

Figure 4 Hormone-sensitive lipase-FATP1-perilipin (HFP) cells are a reconstituted cellular model for free fatty acid (FFA) efflux. (a) Western blot of HFP (lane 1), 3T3-L1 fibroblast (lane 2) and 3T3-L1 adipocyte (lane 3) lysates probed for stable expression of hormone-sensitive lipase, FATP1 and perilipin (Plin). (b) 3D reconstruction of confocal images of HFP cells following overnight lipid loading in the presence of fluorescent fatty acids (green); nuclei are shown in blue. (c) Analysis of lipid droplet diameter based on 3D reconstruction shown in B. $n = 5200$ lipid droplets. (d) Intracellular fluorescence of HFP cells incubated overnight with lipids and C1-BODIPY-C12 stimulated with forskolin/IBMX at time = 0; $n = 4$. (e) Mean fluorescence of HFP cell populations loaded overnight with lipids and C1-BODIPY-C12 and stimulated for 30 min with insulin, forskolin/IBMX or nothing; $n = 4$. (f) Dose-dependent effect of 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid on FFA efflux rate from forskolin/IBMX stimulated HFP cells and 3T3-L1 adipocytes; $n = 3$.

initial robust release of FFAs that levelled off after 20 min.

A FACS-based assay was employed as a secondary method to detect FFA efflux from HFP cells, where the decrease in side scatter (granularity) correlates with loss of lipid content. We utilized the FACS-based FFA efflux assay to measure the hormonal control of FFA release by HFP cells. The resultant data reflect the known inhibitory effect of insulin and stimulatory effect of forskolin and IBMX on lipolysis in adipocytes (Fig. 4e). We found that basal FFA efflux causes a 12% reduction in initial fluorescence and insulin or forskolin/IBMX treatments result in a 6 and 23% reduction, respectively. Thus, HFP cells constitute a minimal but sufficient cellular model for FFA efflux because they load FFAs and demonstrate hormonal regulation of FFA efflux. We reasoned that FFA efflux from HFP cells should be dominated by passive diffusion, as these cells are likely to lack the adipocyte-specific protein(s) necessary for efficient FFA export. To support this notion, we compared the DIDS effect on FFA release from HFP cells and adipocytes and found that DIDS treatment causes a dose-dependent reduction in efflux in adipocytes but not HFP cells (Fig. 4f). This result strongly suggests that DIDS affects an as yet unidentified FFA transporter required for efficient FFA efflux present in adipocytes, but not fibroblasts.

Discussion

While several proteins are linked to FFA uptake in adipocytes, the mechanism of FFA export from adipocytes remains unknown. To address this issue, we developed assays and a cell line for FFA efflux quantification and generated evidence to suggest that adipocytes express an as yet unidentified fatty acid exporter. We demonstrated that DIDS and glyburide block FFA export without affecting lipolysis. DIDS is of particular interest because it has a dose-dependent inhibitory effect on FFA efflux from adipocytes, without affecting glycerol release. Further, we created a minimal reconstituted cellular model (HFP cells) to measure hormonally regulated FFA efflux. Importantly, while HFP cells faithfully replicate hormonal effects of lipolysis, FFA efflux from HFP cells is not susceptible to inhibition by DIDS. This is in contrast to the dose-dependent inhibitory effect by DIDS on adipocyte FFA efflux and argues for the existence of an adipocyte FFA efflux protein.

Analogous transport systems in adipocytes should be examined for potential involvement in FFA efflux. Flip-flop transport of FFAs across the plasma membrane is bidirectional by nature; however, the majority of FFA uptake by adipocytes has been shown to occur via a rapid, saturable, substrate-specific and hormonally regulated mechanism indicative of protein-mediated processes (Black & DiRusso 2003, Bonen *et al.* 2003, Stahl 2004). While the major proteins involved in FFA uptake by adipocytes are CD36, acyl-CoA synthetase 1 and fatty acid transport protein 1 (FATP1/Slc27A1) (Schaffer & Lodish 1994, Must *et al.* 1999, Ibrahimi & Abumrad 2002), there is little evidence to suggest that these transporters could act bidirectionally. FATP1 is not a probable candidate for a FFA exporter because deletion of FATP1, the predominant FATP expressed by adipocytes, results in decreased uptake of FFAs and unimpaired efflux (Wu *et al.* 2006). CD36 is a scavenger receptor thought to respond to the accumulation of FFAs at the plasma membrane; however, CD36 null animals display no obvious defects in FFA release from adipocytes as their serum levels are actually elevated (Febbraio *et al.* 1999). It is also unlikely that LACSs are involved in FFA efflux because their mode of action, trapping FFAs via activation to acyl-CoA, is unidirectional (Ellis *et al.* 2010). Analogous FFA efflux transport systems in cardiomyocytes (Carley *et al.*, 2010) and hepatocytes (Sorrentino *et al.* 1992, Sorrentino *et al.* 1994) may also provide insight into the adipocyte FFA efflux mechanism. Further studies are required to determine whether the proposed FFA exporter is unique to adipose tissue or also found in heart and liver.

Given the evidence that DIDS and glyburide inhibit FFA efflux, one could speculate that the proposed FFA exporter is either part of a novel family or previously identified group of proteins inhibitable by these compounds. Thus, when anion exchangers are excluded because FFA efflux appears to occur via a chloride-independent process, the most conceivable identity for the proposed FFA efflux protein(s) is an OATP or ABC transporter. Alternatively, FFA efflux may be dependent on plasma membrane lipid rafts such as caveolae. Caveolin is the structural protein component of caveolae, which is small flask-shaped invagination of the plasma membrane. Caveolae may be involved in fatty acid transport as caveolin has been shown to bind fatty acids (Trigatti *et al.* 1999). More importantly, caveolin 1 knockout mice display defects in lipid mobilization and fail to increase serum FFA levels in response to a cold challenge (Cohen *et al.* 2005), supporting a role of caveolin in lipolysis and/or FFA efflux. However, it is currently unclear whether and how DIDS could affect caveolin, its ability to bind FFAs or the structure of caveolae. Further characterization with more specific inhibitors could be useful in narrowing this set of potential FFA exporters.

While we accumulated evidence that FFA export from adipocytes is protein-mediated and has some notion of the types of proteins involved in this process, future progress relies on a more detailed strategy for identifying the proposed plasma membrane FFA efflux

transporter. HFP cells will be useful for expression cloning gain-of-function studies, as HFP cells that demonstrate high levels of efflux after the introduction of adipocyte cDNAs can be selected for further analysis. DIDS can aid in the discovery of novel proteins when performing secondary assays with clones of interest as a tool for distinguishing inhibition of FFA efflux from inhibition of lipolysis or glycerol efflux. Additionally, a candidate gene or genome-wide loss-of-function approach based on RNA interference in 3T3-L1 adipocytes could be used to identify proteins required for efficient FFA efflux. This would be particularly useful for evaluating caveolin 1 and specific members of the OATP and ABC transport families for their involvement in FFA efflux.

During fasting, the majority of circulating FFAs in serum are derived from the breakdown of stored triacylglycerides in adipose tissue. Chronically elevated serum FFA levels have been linked to obesity-associated pathologies including insulin resistance (Bray 2004), hepatosteatosis (Marchesini *et al.* 2001) and pancreatic ß-cell lipotoxicity (Lee *et al.* 1994). Elucidating the mechanisms and proteins involved with FFA efflux from adipocytes could have important implications for the treatment of obesity-related disorders characterized by abnormal lipid fluxes. Particularly, sequestering FFAs within adipocytes could be an attractive strategy for alleviating the abnormally high levels of circulating FFAs characteristic of obesity-related conditions. Our evidence supports the notion that FFA efflux by adipocytes is a protein-mediated process, which justifies further studies into the identity of this export protein and its presumed role in physiological and pathological conditions.

Conflict of interest

The authors have no conflict of interest to disclose.

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