Depot-specific adipocyte cell lines reveal differential drug-induced responses of white adipocytes—relevance for partial lipodystrophy

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Kovsan J, Osnis A, Maisel A, Mazor L, Tarnovskci T, Hollander L, Ovadia S, Meier B, Klein J, Bashan N, Rudich A. Depot-specific adipocyte cell lines reveal differential drug-induced responses of white adipocytes—relevance for partial lipodystrophy. Am J Physiol Endocrinol Metab 296: E315–E322, 2009. First published November 25, 2008; doi:10.1152/ajpendo.90486.2008—Intra-abdominal (IA) fat functionally differs from subcutaneous (SC) adipose tissue, likely contributing to its stronger association with obesity-induced morbidity and to differential response to medications. Drug-induced partial lipodystrophy, like in response to antiretroviral agents, is an extreme manifestation of the different response of different fat depots, with loss of SC but not IA. Investigating depot-specific adipocyte differences is limited by the low accessibility to IA fat and by the heterogeneous cell population comprising adipose tissue. Here, we aimed at utilizing immortalized preadipocyte cell lines from IA (epididymal) or SC (inguinal) fat to investigate whether they differentially respond to the HIV protease inhibitor nelfinavir. Pre-adipocytes were readily amenable to adipogenesis, as evidenced by lipid accumulation, expression of adipose-specific genes, measurable lipolysis, and insulin responsiveness. Leptin secretion was higher by the SC line, consistent with known differences between IA and SC fat. As previously reported, nelfinavir inhibited adipogenesis downstream of C/EBPβ, but similarly in both cell lines. In contrast, nelfinavir’s capacity to diminish insulin signaling, decrease leptin secretion, enhance basal lipolysis, and decrease expression of the lipid droplet-associated protein perilipin occurred more robustly and/or at lower nelfinavir concentrations in the SC line. This was despite similar intracellular concentrations of nelfinavir (23.8 ± 5.6 and 33.6 ± 12.2 μg/mg protein for inguinal and epididymal adipocytes, respectively, P = 0.46). The cell lines recapitulated depot-differential effects of nelfinavir observed in differentiated primary preadipocytes and with whole tissue explants. Thus, we report the use of fat depot-specific adipocyte cell lines for unraveling depot-differential responses to a drug causing partial lipodystrophy.

nelfinavir; lipolysis; perilipin; intra-abdominal adipocytes; subcutaneous adipocytes

A different contribution of adipose tissue from different anatomic locations to endocrine-metabolic regulation is a well-accepted phenomenon supported by a large body of experimental evidence (39, 48). Although different venous drainage of intra-abdominal (IA) fat compared with that of subcutaneous (SC) fat (to the portal vs. systemic venous systems) may partially underlie such differences (21), these two depots are functionally distinct. In particular, key functions of adipose tissue now known to regulate whole body metabolism, including adipokine secretion and lipolysis, are distinctly regulated. Moreover, the responses of these depots to clinically used medications are also distinct. For example, in response to thiazolidinedione (TZD) insulin sensitizers, SC adipose mass increases, whereas IA fat mass may actually diminish (22, 23, 34, 47). As a potential mechanism, it has been suggested that SC adipocytes express higher levels of PPARγ, the nuclear receptor target of TZDs, and respond to the drug with a greater hyperplastic response than IA fat (31). In other cases, the mechanisms underlying differential response to medications are unclear. Highly active antiretroviral therapy (HAART), known to greatly improve the prognosis of HIV-positive persons, subjects many to partial lipodystrophy, a condition in which SC fat is differentially lost whereas IA fat is either unaffected or even expands in mass (7, 19, 52). The associated health consequences are severe, as affected persons develop metabolic dysregulation subjecting them to morbidity, including type 2 diabetes and cardiovascular disease (16). The mechanisms for this differential response of adipose tissues to HAART are unclear and may range from different accumulation of the drug to different biological response to it. Yet, since IA fat in humans is rather inaccessible and rodents do not seem to develop the full features of human HAART-induced partial lipodystrophy, research in this field has been limited by the lack of depot-specific cell-based systems.

An additional complication in investigating fat depot-differential responses of adipose tissue has been the recent realization that adipose tissue in different locations may be composed of different cell populations, particularly in morbid states. For example, obesity has been demonstrated to be associated with infiltration of immune cells into adipose tissue (18, 54, 55). This process appears to occur more pronouncedly in IA fat (5, 17), hence, it is difficult to dissect out whether functional differences between SC and IA fat can be attributed to different alterations of the adipocytes per se or rather to differential alterations in cellular composition of adipose tissue and/or non-adipocyte cells composing the adipose tissue.

Here, we report the use of adipocyte cell lines derived from immortalized preadipocytes from inguinal (Ing) and peri-epididymal (Peri) mouse fat pads, representing SC and IA fat, respectively. We demonstrate that, in response to nelfinavir, an HIV protease inhibitor, both cell lines equally accumulate the drug and exhibit similar inhibition of adipogenesis. Yet, Ing adipocytes are more sensitive than Peri adipocytes to the...
induction of insulin resistance and to the increase in lipolysis, consistent with the preferential diminution of SC fat mass in HAART-induced lipodystrophy.

MATERIALS AND METHODS

Adipocytes from immortalized Ing (SC) and Peri (IA) preadipocyte cell lines. Two permanent cell lines were created as follows. Ing and Peri white adipose tissues preadipocytes were isolated from 6-wk-old mice as previously described (25). Briefly, tissues were minced and digested with collagenase and filtered through a 100-μm nylon mesh. Cells in the pellet were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 25 mM glucose, 20% fetal bovine serum (FBS), 20 mM HEPES, and 100 U/ml penicillin-streptomycin, and, after reaching 80% confluence, incubated for 24 h with the retroviral pBabe vector encoding the SV40T antigen and puromycin resistance. Following infection, the cells recovered in culture medium for 72 h, and positively infected cells were selected with puromycin (1 μg/ml) for 3 wk. Differentiation into adipocytes was performed as follows. Cells were grown until 12–24 h postconfluence in DMEM supplemented with 20% FBS, 100 U/ml penicillin-streptomycin, 20 nM insulin, 1 nM triiodothyronine (T3). Differentiation was induced by adding 0.125 mM indomethacin, 2 μg/ml dexamethasone, and 0.5 mM 3-isobutylmethylxanthine to the medium for 24 h, after which, cells were grown for an additional 6 days before being used.

Primary rat differentiated preadipocytes and whole tissue explants. All procedures were approved in advance by the Animal Care Ethics Committee at Ben-Gurion University. Sprague-Dawley (Harlan, Jerusalem, Israel) rats (weight 200–300 g) were killed by CO2 asphyxiation, and perigonadal (IA) or inguinal (SC) fat was immediately collected. Primary rat preadipocytes were obtained from the pellet (stroma-vascular fraction) of collagenase-digested adipose tissue, obtained as previously described (43). Following the last wash, cells were resuspended and cultured in DMEM supplemented with 10% FCS, 2 mM l-glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μg/ml amphotericin B. On the next day after seeding, cells were thoroughly washed with PBS to discard nondhering cells. Forty-eight hours postconfluence, differentiation was induced exactly as we described for 3T3-L1 adipocytes (44), and cells were used 7–10 days postdifferentiation induction, when exhibiting a high (typically >80%) differentiation, as assessed by light microscopy and Oil red O staining. Whole tissue explants were prepared by mincing the tissue into small pieces of 2–3 mm, as we described for human adipose tissue (2).

Nelfinavir treatment. Nelfinavir (Shanghai 21CCE Pharma, London, UK) stock solution (100 mM) was prepared in 100% ethanol. Indicated medium concentrations of nelfinavir were achieved by appropriate dilution from a 1:100 diluted stock in water. To assess nelfinavir-induced inhibition of adipogenesis (Figs. 2, 3), indicated concentrations of nelfinavir were added starting at the beginning of differentiation induction and throughout until cells were used. To assess nelfinavir-induced effects on fully differentiated adipocytes, indicated concentrations of nelfinavir were added on day 6 for cell lines or days 7–10 for primary preadipocytes, for 18 h in medium without serum, supplemented with 0.5% w/v bovine serum albumin (see Figs. 4 and 5). Protein recovery and MTT assays were used to confirm normal viability (85–110% of control) following nelfinavir treatment.

Measurements of intracellular nelfinavir concentrations. Adipocytes grown on 10-cm dishes were treated for 18 h with 30 μM nelfinavir. Following three freeze-thaw cycles, cells were scraped in 1 ml of PBS, homogenized using a glass homogenizer, and frozen and stored at −70°C. For analysis, samples were thawed, vortexed, and extracted with 5 ml of tert-butylmethyl ether for 10 min. The lower organic layer was evaporated at 30–40°C under nitrogen, and the residue was reconstituted with 300 μl of mobile phase and vortexed again for 1 min prior to injection. HPLC separation (Jasco PU-2089) was on C18, 5 μm 4.6 × 150 mm columns (Phenomenex, Torrance, CA) using a series of gradients of acetonitrile and potassium phosphate, pH 5.6, at a flow rate of 1.6 ml/min. Detection was by a UV-Vis detector (Jasco-1570) set at 210 nm, based on nelfinavir standards, with a retention time of 12.2 min.

Other assays. Oil red O staining was performed as described (51) and quantified by solubilizing cells in DMSO followed by spectrophotometric detection as suggested by the manufacturer (Sigma). Western blot analyses were performed exactly as previously reported (45, 50), using the following antibodies: anti-Akt/PKB, p-Ser473-Akt/ PKB (Cell Signaling), peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT-enhancer-binding protein-α (C/EBPα) and C/EBPβ (Santa Cruz Biotechnology), GLUT1 and GLUT4 (Chemicon), and β-actin (Sigma). Anti-HSL (hormone-sensitive lipase) and perilipin were kindly provided by Dr. Greenberg, Tufts University. Peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG were from Amersham Biosciences. Glycerol release was assayed spectrophotometrically exactly as we previously reported (27). Leptin and adiponectin secretion to the medium were determined in media collected at the end of the 18-h incubation with either vehicle or nelfinavir, using commercial kits according to the manufacturer’s instructions. A leptin mouse ELISA kit was from Ray Biotech, and a mouse/rat adiponectin ELISA kit was from B-Bridge International. 2-Deoxylucose uptake and glycerol release to the medium (lipolysis and insulin-mediated anti-lipolysis) were performed exactly as described before (40, 46).

Statistical analysis. The data are expressed as means ± SE. Each treatment was compared with control, and statistical significance of differences between two groups was evaluated using Student’s t-test. The criterion for significance was set at P < 0.05.

RESULTS

Fat depot-specific preadipocyte cell lines. Adipocyte cell lines derived from preadipocytes from Ing (SC) or Epi (IA) fat depots were generated as described in MATERIALS AND METHODS and for brown adipocytes in Refs. 13 and 24–26. Both cell lines had fibroblast-like morphology and did not exhibit significant lipid accumulation prior to differentiation. Upon adipocyte differentiation, both lines retained a similar capacity to accumulate triglycerides, as detected by Oil red O staining (Fig. 1A). To further confirm terminal differentiation of the two cell lines, we measured protein levels of the lipid droplet-associated protein perilipin, HSL, GLUT4, and the adipogenic transcription factor PPARγ (Fig. 1B). Adipogenesis induced the expression of these typical adipocyte markers. Importantly, PPARγ expression was higher in Ing than in Epi, consistent with a higher expression of this transcription factor in the SC fat depot (56). In addition, both cell lines exhibited hormone-regulated lipolysis (Fig. 1C): β-adrenergic stimulation with isoproterenol increased glycerol release, with a fold stimulation in Epi higher than in Ing, also consistent with depot differences between IA and SC adipocytes (47a, 48a). Importantly, insulin responsiveness could be demonstrated by insulin-mediated anti-lipolysis (Fig. 1C), insulin-stimulated phosphorylation of Akt/PKB on Ser473 (Fig. 1D), and glucose uptake (Fig. 1E). The twofold higher expression of total Akt/ PKB in Epi compared with Ing adipocytes (P = 0.005) was consistent with observations in human omental vs. subcutaneous abdominal adipose tissue (2). Finally, both cell lines secreted leptin and adiponectin to the media, with higher leptin secretion by Ing adipocytes than from Epi (P < 0.05; Table 1), consistent with the known higher expression of leptin in SC compared with IA fat (37, 53). Medium adiponectin concen-
Table 1. Leptin and adiponectin secretion to the medium by inguinal and epididymal adipocyte cell line in response to nelfinavir

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<tr>
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<th>Inguinal</th>
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<td>Leptin</td>
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<tr>
<td>pg/ml</td>
<td>%Decrease</td>
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<tr>
<td>Cont</td>
<td>302.7±20.0</td>
<td>46.1±17.2†</td>
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<tr>
<td>Nelf 10 μM</td>
<td>198.9±38.7</td>
<td>34.6±8.55</td>
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<td>Nelf 40 μM</td>
<td>43.3±0.2*</td>
<td>85.7±1.0</td>
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<td>Adiponectin</td>
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<td>μg/ml</td>
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<tr>
<td>Cont</td>
<td>10.9±5.7</td>
<td>15.5±4.3</td>
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<tr>
<td>Nelf 10 μM</td>
<td>8.0±4.2</td>
<td>26.6±0.8</td>
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<tr>
<td>Nelf 40 μM</td>
<td>6.4±4.1</td>
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Values are means ± SE; n = 2–3 independent experiments. Nelf, nelfinavir; Cont, control. Inguinal and epididymal adipocytes were treated with vehicle (ethanol) or nelfinavir at indicated concentrations for 18 h. Before nelfinavir treatment, the medium was changed and collected at the end of the 18-h incubation period, and the amounts of leptin and adiponectin secreted to the medium were assessed by specific ELISA assays, as detailed in materials and methods. *P < 0.05 vs. control cells; †P < 0.05 vs. inguinal adipocytes.

trations were within the wide concentration range reported in 3T3-L1 adipocytes (1a, 4a), and similar to serum concentrations did not significantly affect total cell viability as determined by measuring glycerol release into the medium, as detailed in MATERIALS AND METHODS. Insulin treatment was performed by including insulin (100 nM) during incubation with isoproteranol (10 nM). Each experiment was conducted ≥3 times. *P < 0.05 vs. basal and vs. isoproteranol + insulin. D: lysates were prepared from differentiated (day 6) adipocytes stimulated or not with insulin (100 nM for 7 min) and blotted for pS473-Akt/PKB or total Akt/PKB. Shown are representative results of 3 independent experiments. E: 2-Deoxyglucose uptake was measured after a 20-min incubation with or without 100 nM insulin. *P < 0.05 between insulin-stimulated and unstimulated cells of the same line.

**Figure 1.** Characteristics of mouse inguinal and epididymal adipocyte cell lines. Preadipocytes isolated and immortalized from inguinal (subcutaneous) or epididymal (intra-abdominal) fat pads were differentiated into adipocytes as detailed in MATERIALS AND METHODS. A: Oil red O staining reveals equivalent levels of triglyceride storage in both cell lines at days 0, 3, and 6 of the differentiation protocol. Shown are representative results of 5 independent experiments. B: total cell lysates were prepared from preadipocytes (day 0) or differentiated cells (day 6) and blotted for perilipins, hormone-sensitive lipase (HSL), GLUT4, and PPARγ. Shown are representative blots of 2–6 independent experiments. C: basal and isoproteranol-stimulated lipolysis of differentiated adipocytes (day 6) of both cell lines was determined by measuring glycerol release into the medium, as detailed in MATERIALS AND METHODS. Insulin treatment was performed by including insulin (100 nM) during incubation with isoproteranol (10 nM). Each experiment was conducted ≥3 times. *P < 0.05 vs. basal and vs. isoproteranol + insulin. D: lysates were prepared from differentiated (day 6) adipocytes stimulated or not with insulin (100 nM for 7 min) and blotted for pS473-Akt/PKB or total Akt/PKB. Shown are representative results of 3 independent experiments. E: 2-Deoxyglucose uptake was measured after a 20-min incubation with or without 100 nM insulin. *P < 0.05 between insulin-stimulated and unstimulated cells of the same line.

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-**Inguinal**
  - Leptin: 302.7±20.0 pg/ml (Cont), 198.9±38.7 pg/ml (Nelf 10 μM), 43.3±0.2* pg/ml (Nelf 40 μM).
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-**Epididymal**
  - Leptin: 46.1±17.2† pg/ml (Cont), 39.3±21.4† pg/ml (Nelf 10 μM), 24.4±5.3† pg/ml (Nelf 40 μM).
  - Adiponectin: 15.5±4.3 μg/ml (Cont), 11.4±2.8 μg/ml (Nelf 10 μM), 9.6±2.5 μg/ml (Nelf 40 μM).

Values are means ± SE; n = 2–3 independent experiments. Nelf, nelfinavir; Cont, control. Inguinal and epididymal adipocytes were treated with vehicle (ethanol) or nelfinavir at indicated concentrations for 18 h. Before nelfinavir treatment, the medium was changed and collected at the end of the 18-h incubation period, and the amounts of leptin and adiponectin secreted to the medium were assessed by specific ELISA assays, as detailed in materials and methods. *P < 0.05 vs. control cells; †P < 0.05 vs. inguinal adipocytes.

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tions of 20 μM or more (Fig. 2). Yet, both cell lines exhibited similar sensitivity to the inhibitory effect of nelfinavir on lipid accumulation. Nelfinavir at 40 μM present during and after induction of adipocyte differentiation almost completely prevented triglyceride accumulation in cells derived from both fat depots. To verify this response in molecular terms and to determine at what stage of adipocyte differentiation nelfinavir exerted its inhibitory effects, we measured the protein levels of several major transcription factors and adipocyte differentiation markers. Although the presence of nelfinavir during and following differentiation did not affect the expression of C/EBPβ, decreased expression of C/EBPα, and more evidently of PPARγ, could be observed with nelfinavir concentrations 20 μM or more (Fig. 3A). HSL, the lipid droplet-associated protein perilipin (Fig. 3B), and GLUT4 (not shown) were used as terminal adipocyte differentiation markers. In response to increasing concentrations of nelfinavir, decreased expression was observed, with a dose dependency that corresponded to the decrease in PPARγ expression (Fig. 3A) and to the diminished lipid accumulation (Fig. 2B). However, these findings, largely confirming reports in 3T3-L1 adipocytes (11), did not display a differential response between the Ing and Epi cell lines.

To corroborate these findings in a nonimmortalized adipose cell system, we isolated preadipocytes from rat SC and perigonadal (PG) fat pads, as detailed in MATERIALS AND METHODS. After confluence, preadipocytes (exhibiting fibroblast-like morphology) were subjected to a differentiation protocol in the presence or absence of increasing concentrations of nelfinavir. Six days after induction of differentiation, lysates were prepared and the protein level of transcription factors regulating adipogenesis (A) or markers of terminal adipocyte differentiation (B) were determined by Western blot analysis. C: primary preadipocytes were isolated from collagenase-digested rat inguinal (subcutaneous, SC) and epididymal (perigonadal, PG) fat pads. Cells were grown to confluence and differentiated into adipocytes in the absence or presence of different nelfinavir concentrations. Cell lysates were prepared at day 7 and blotted for peroxisome proliferator-activated receptor-γ (PPARγ), with β-actin serving as a loading control. All experiments were performed ≥5 times, with similar results.

Fig. 2. Nelfinavir similarly inhibits triglyceride accumulation during adipogenesis of inguinal and epididymal preadipocytes. Inguinal and epididymal preadipocytes were differentiated into adipocytes in the absence or presence of different concentrations of nelfinavir. A: 6 days after induction of differentiation, cells were stained with Oil red O, and representative images were obtained at ×400 magnification by light microscopy. B: for quantification, cells were solubilized in DMSO, absorbance was determined, and results were expressed as percent of absorbance in control cells. Results are means ± SE of 3–6 independent experiments. *P < 0.05 vs. control values (no significant differences were observed between the 2 cell lines in any of the nelfinavir concentrations).

Fig. 3. Nelfinavir similarly affects the expression of transcription factors regulating adipogenesis and terminal adipocyte differentiation markers in inguinal and epididymal adipocytes. A and B: inguinal and epididymal preadipocytes were differentiated into adipocytes in the absence or presence of different concentrations of nelfinavir. Six days after induction of differentiation, lysates were prepared and the protein level of transcription factors regulating adipogenesis (A) or markers of terminal adipocyte differentiation (B) were determined by Western blot analysis. C: primary preadipocytes were isolated from collagenase-digested rat inguinal (subcutaneous, SC) and epididymal (perigonadal, PG) fat pads. Cells were grown to confluence and differentiated into adipocytes in the absence or presence of different nelfinavir concentrations. Cell lysates were prepared at day 7 and blotted for peroxisome proliferator-activated receptor-γ (PPARγ), with β-actin serving as a loading control. All experiments were performed ≥5 times, with similar results.
navir induced insulin resistance by interfering with insulin-stimulated activation of Akt/PKB, but with normal signal transmission to PI 3-kinase (3, 46). Fully differentiated Ing and Epi adipocytes were exposed to different concentrations of nelfinavir for 18 h, after which cells were rinsed and stimulated for 7 min with insulin. Although no significant effect of nelfinavir was observed on basal Akt/PKB phosphorylation, insulin-stimulated Ser473 phosphorylation of Akt was diminished in a dose-dependent manner by nelfinavir (Fig. 4A). Yet, Ing adipocytes were more sensitive to this effect, demonstrating an already considerable decrease in p-Ser-Akt with 10 μM nelfinavir (Fig. 4B), and significantly different from Epi adipocytes at 20 and 30 μM (Fig. 4, A and B). Similar results were observed with differentiated primary rat preadipocytes: whereas in SC-derived adipocytes 30 μM nelfinavir nearly fully inhibited insulin-stimulated Akt/PKB phosphorylation, adipocytes derived from the PG fat depot still retained a measurable capacity, albeit diminished, to mount a phospho-Akt/PKB above non-insulin-stimulated cells (Fig. 4C). Thus, as with the observations on adipogenesis, the two cell lines recapitulated the results in primary cells, demonstrating that SC-derived adipocytes are more sensitive than IA adipocytes to nelfinavir-induced insulin resistance.

Fig. 4. Inguinal adipocytes are more sensitive to nelfinavir-induced inhibition of insulin-stimulated Akt/PKB phosphorylation. Differentiated adipocytes were treated with indicated concentrations of nelfinavir for 18 h. A: lysates were prepared, and the protein level of pS473-Akt/PKB and total Akt/PKB were determined by Western blot analysis. B: results of densitometry analysis of 5–8 independent experiments is shown. Results are expressed as means ± SE. *P < 0.05 inguinal vs. epididymal cell line. C: similar experiment as in A, performed in primary differentiated preadipocytes.

SC-derived adipocytes are more sensitive to the lipolytic effect of nelfinavir and to its tendency to diminish leptin secretion despite similar intracellular concentrations of the drug. Differentiated 3T3-L1 adipocytes exposed for 18 h to nelfinavir exhibit an increased lipolytic rate in basal (non-β-adrenergic-stimulated) state, as assessed by glycerol release to the medium (46). Ing adipocytes already exhibited this response when exposed to 10 μM nelfinavir, whereas concentrations exceeding 30 μM were required to observe this response in Epi (Fig. 5A). The lipolytic effect of nelfinavir in 3T3-L1 adipocytes was shown to be mediated by decreased expression of perilipin (27). Consistently, perilipin expression in both adipocyte cell lines was decreased by nelfinavir, but with a differential dose effect (Fig. 5B): Ing cells already exhibited decreased perilipin with 10 μM nelfinavir, corresponding to the increase in glycerol release at this concentration, whereas Epi cells required higher concentrations. To verify that this finding truly represents the effect of nelfinavir in adipose tissue, we incubated mouse PG and SC fat pad explants with the drug for 18 h. Both tissues exhibited decreased perilipin expression in response to incubation with nelfinavir; but in SC fat, perilipin decreased by 56.8 ± 5.6%, whereas PG fat by 20.5 ± 4.5% (n = 5 independent experiments, P < 0.05). Collectively, SC fat tissue and adipocytes are more sensitive to the lipolytic action of nelfinavir and to a decrease in perilipin expression. Moreover, the lipolytic response of SC-derived adipocytes to nelfinavir occurs with concentrations of the drug that are reported in serum of treated patients (12, 30, 38).

Nelfinavir also displayed a differential effect on adipokine secretion by the two cell lines (Table 1). Adiponectin secretion tended to decrease in a dose-dependent manner but with seemingly similar sensitivity in both cell lines. In contrast, the decrease in leptin secretion reached a >85% inhibition in Ing but only ~45% in Epi (P < 0.05), consistent with previous results using human adipose tissue fragments (9).

Given the apparent higher sensitivity of Ing adipocytes to nelfinavir actions, we determined whether this could be a result of the drug concentrating to higher intracellular levels in Ing than in Epi adipocytes. Ing or Epi adipocytes were incubated for 18-h with 30 μM nelfinavir. This concentration induced a stronger inhibition of insulin-stimulated Akt/PKB phosphorylation (Fig. 4A), increased basal lipolysis to a higher level (Fig. 5A), and decreased perilipin expression more in Ing than in Epi adipocytes (Fig. 5B). By use of HPLC analysis of cell extracts, intracellular nelfinavir concentrations were 23.8 ± 5.6 and 33.6 ± 12.2 μg/mg protein for Ing and Epi adipocytes, respectively (P = 0.46). This result does not support the possibility that differences in the transport, efflux, and/or metabolism of nelfinavir between adipocytes derived from different depots are the reason for higher sensitivity of SC fat to nelfinavir.

DISCUSSION

HAART-induced partial lipodystrophy, a manifestation of fat depot-differential response to a xenobiotic, is an interesting phenomenon but is not unprecedented. IA and SC adipose tissues differ in the expression of various developmental genes and exhibit differential functions, including in lipolytic rate, response to hormones, and secreted products (28, 32, 56). In obesity cellular composition differs, as IA fat is more infl-
Fig. 5. Nelfinavir increases basal lipolysis and decreases perilipin protein levels to a higher extent in inguinal than in epididymal adipocytes. A: differentiated inguinal and epididymal adipocytes were treated with the indicated concentrations of nelfinavir for 18 h. Then cells were incubated for an additional hour in Krebs-Ringer phosphate buffer, and glycerol release was assessed. B: cells were lysed and analyzed by Western blot using specific antibodies against perilipin. Representative blots and densitometry analysis of 4–7 independent experiments are shown and expressed as means ± SE. *P < 0.05 inguinal vs. epididymal cell line.

trated by macrophages (17), stress-sensing signaling pathways are differentially activated (2, 42), and even the response to excess nutrients may be depot specific, with IA fat favoring hypertrophy whereas SC fat favors hyperplasia (10). This depot-specific biology may also underlie the differences in the response of IA vs. SC fat to other drugs, including TZDs (56). Currently, investigating fat depot-differential characteristics of adipose tissue relies mainly on direct analyses of fat tissue explants (14) [also used in assessing differential effects of HPIs (9)] or on in vitro differentiation of preadipocytes derived from such samples, which retain some depot-specific characteristics (49). Although yielding important insights, these experimental approaches suffer from several limitations, the major one being the low accessibility of human IA fat. This is particularly true for obtaining “control samples” from lean, healthy people. In addition, adipose tissue consists of a mixed population of cells. Its composition differs between fat depots and may change dynamically in response to nutritional and genetic factors. To assess which depot-specific characteristics can be attributed to the adipocytes themselves vs. nonadipocytes (the stroma-vascular fraction), the different cell populations comprising adipose tissue are separated after the tissue is disintegrated, typically by collagenase digestion. Although suitable to many investigations, this experimental procedure has been demonstrated to induce many functional alterations (15, 41), raising the risk of introducing artifacts and overriding “real” differences that reflect the in vivo environment.

With these in mind, depot-specific cell lines may constitute an added tool, even if not perfect, for studying depot-specific characteristics of adipocytes. Immortalized cell lines are useful in biomedical and life science research, despite the fact that they may lose many cell-specific characteristics and in many cases poorly represent the original cells they were derived from. This may particularly raise concerns when the immortalized cell line is a model of naturally nondiending, terminally differentiated cells. Nevertheless, immortalized cell lines are widely used not only because they are accessible and relatively easy to utilize, but also because very frequently they do recapitulate and predict biological processes then found in in vivo systems. In adipocyte biology, the 3T3-L1 and 3T3-F442A murine cell lines are used extensively in studying multiple fat functions, including adipogenesis, insulin signaling, lipolysis regulation, and adipokine regulation. Very recently, several additional exciting approaches to generate differentiating preadipocyte cell lines have been described (29, 33, 36). Yet, the cell lines do not represent depot-specific adipocyte characteristics, and the novel approaches for generating differentiating preadipocyte lines have not yet been tested in parallel on cells originating from different depots. Here, we show that the Ing- and Epi-derived cell lines can be effectively differentiated into adipocytes despite the use of SV40T antigen for immortalization, a strategy proposed to interfere with differentiation (8). An adipocyte phenotype following differentiation was based on lipid accumulation, expression of adipogenic transcription factors and adipocyte genes like PPARγ, GLUT4, and perilipin, measurable lipolysis, adipokine secretion, and insulin responsiveness (Figs. 1–3).

What is the evidence that the two cell lines retain fat depot-specific characteristics? A higher expression of total Akt/PKB in IA fat compared with SC fat has been shown in human adipose tissue (2) and is seen here (Fig. 1D). Likewise, higher expression and secretion of leptin have been previously reported in SC compared with IA fat (37, 53), consistent with the higher leptin secretion by Ing vs. Epi adipocytes (Table 1). In addition, in response to the HPI nelfinavir, a constituent of HAART-induced partial lipodystrophy, Ing cells were more susceptible to become insulin resistant, diminished leptin secretion more robustly, and were more vulnerable to nelfinavir’s lipolytic action than Epi cells. This differential response seems to correspond to the clinical phenotype of partial lipodystrophy: Both impaired insulin responsiveness and enhanced basal lipolysis could constitute mechanisms for decreased fat mass, which preferentially occurs in this depot and not in IA fat. Intriguingly, both cell lines accumulated similar concentrations of the drug, suggesting that the depot differences could not be attributed to altered uptake, export, or metabolism of the drug but rather to a different biological response. Moreover, the ability of nelfinavir to inhibit adipogenesis occurred similarly in both cell lines, suggesting that it may not be a primary process differentially affecting SC vs. IA fat. This finding is consistent with a very recent study using other HPIs on primary human preadipocytes (20). Consistently in the present study, both the non-depot-differential effect on adipogenesis and the depot-differential effect on insulin signaling and on lipolysis or perilipin expression recapitulated observation in primary cells and tissue explants (Figs. 3C and 4C).

Despite the above results supportive of the usefulness of the two cell lines for investigating depot-specific differences in adipocytes, like other model systems, it is clear that there are limitations to the use of these cell lines. This was exemplified...
by the fact that the nelfinavir doses required to inhibit lipolysis were much higher than those required in primary preadipocytes [though similar to previous results in 3T3-L1 adipocytes (4, 11)]. Clearly, immortalization strategies used to generate such cell lines and the unique differentiation protocol could alter the response to various endogenous and exogenous cues. An additional example is the relative expression of perilipin. Whereas in lysates from whole adipose tissues of mice perilipin expression was higher in Epi than in Ing, the Ing adipocyte cell line exhibited a higher expression of perilipin than the Epi cells (Fig. 1B). Thus, clearly, not all depot-specific features are represented by the cell lines, and caution should be practiced when they are utilized for studying depot-specific differences. Despite these potential limitations, the two cell lines enable us to suggest that, among the various mechanisms proposed to underlie HPI-induced lipodystrophy, inhibition of differentiation is unlikely to explain depot-differential effects of these drugs. Rather, inhibition of insulin signaling, adiponectin secretion, and induction of lipolysis may represent multiple targets through which nelfinavir induces selective loss of SC fat.

In conclusion, herein we have demonstrated the use of depot-specific preadipocyte immortalized cell lines to study, upon successful adipogenesis, fat depot-differential responses to a drug that induces partial lipodystrophy. The cell lines nicely recapitulated effects also observed in primary cells and whole tissue, providing novel insights on possible cellular mechanisms for HAART-induced partial lipodystrophy.

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REFERENCES


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FAT DEPOT DIFFERENTIAL EFFECTS OF NELFINAVIR


