

The HIV protease inhibitor saquinavir impairs lipid metabolism and glucose transport in cultured adipocytes

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Abstract

Treatment of HIV infection using protease inhibitors is frequently associated with lipodystrophy and impaired lipid and glucose metabolism. We examined the effect of saquinavir, one of the protease inhibitors, on lipid metabolism and glucose transport in cultured adipocytes. Saquinavir inhibited lipoprotein lipase (LPL) activity in 3T3-F442A and 3T3-L1 adipocytes. The inhibition of LPL was 81% at a concentration of 20 µg/ml. Another closely related drug, indinavir, had a small inhibitory effect. Saquinavir also inhibited the biosynthesis of lipids from [¹⁴C]-acetate. Saquinavir increased the lipolysis.

Saquinavir had no significant effect on the cellular protein synthesis or protein content. Saquinavir increased the basal glucose transport threefold and decreased insulin-stimulated glucose transport by 35%. These studies suggest that some HIV protease inhibitors have direct effects on lipid and glucose metabolism. This inhibition of lipogenesis and glucose transport may explain some of the lipodystrophy, dyslipidemia and disturbed glucose metabolism with the clinical use of these drugs.

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Introduction

Protease inhibitors are a new class of drugs used for the treatment of human immunodeficiency virus (HIV) infection. These drugs block the replication cycle of HIV by inhibiting the aspartic protease, which is critical in the processing of the products of the gag-pol gene into functional protein of HIV. Gag-pol protein releases the aspartic protease that cleaves the gag-pol protein allowing the virus particle to mature (Kohl *et al.* 1988). Inhibition of the protease enzyme prevents the maturation of the viral particle and retards the infection. The protease inhibitors are peptide-like substrate analogues that bind to the active site resulting in the inhibition of the enzyme activity (Navia *et al.* 1988). Clinical studies have shown that these drugs reduce the viral load significantly and improve the CD4+ counts (Schapiro *et al.* 1996).

Although the drugs are generally well tolerated, several studies have reported that treatment of patients with protease inhibitors causes peripheral lipodystrophy, resulting in loss of fat in all regions except the abdomen, hyperlipidemia and insulin resistance (Carr *et al.* 1998, 1999). The treatment of HIV with protease inhibitors resulted in an atherogenic hyperlipidemia, with elevated total cholesterol, low density lipoprotein cholesterol and

triglycerides (Echevarria & Hardin 1999, Periard *et al.* 1999). Purnell *et al.* (2000) have reported that administration of zidovudine to healthy subjects resulted in hypertriglyceridemia but post-heparin plasma lipoprotein lipase (LPL) remained unchanged. Insulin resistance develops in many patients treated with HIV proteases, and extreme hyperglycemia has been reported with the protease inhibitor nelfinavir (Visnegarwala *et al.* 1997). These studies suggest that the protease inhibitors may have direct effects on the lipid and glucose metabolism in the fat and muscle tissue.

Previous *in vitro* studies demonstrated an inhibition of preadipocyte to adipocyte differentiation by protease inhibitors using both human and 3T3-L1 preadipocytes (Wentworth *et al.* 1999, Zhang *et al.* 1999). To further examine the effect of HIV protease inhibitors on lipid metabolism, we studied the effects of two such drugs, saquinavir and indinavir, on LPL *in vitro* in 3T3-F442A adipocyte cultures. LPL-mediated triglyceride hydrolysis is one of the important factors in adipocyte lipid accumulation in humans and rats (Hollenberg 1966, Wilson *et al.* 1973). The effects of these drugs on lipid accumulation and glucose transport were also investigated. The results showed that saquinavir inhibited LPL activity, lipid synthesis and insulin-stimulated glucose transport.

Materials and Methods

Materials

3T3-F442A and 3T3-L1 cells were obtained from Dr Howard Green (Massachusetts Institute of Technology, Cambridge, MA, USA). Media and other tissue culture supplies were from Gibco-BRL (Grand Island, NY, USA). Human recombinant insulin (Novolin) was obtained from Nordisk Pharmaceuticals (Princeton, NJ, USA). [U-¹⁴C]-Glucose, 2-deoxy-[³H]-glucose, [³⁵S]-methionine and [³H]-triolein were from New England Nuclear (Boston, MA, USA). Bovine serum albumin, triolein, lecithin, 2-deoxyglucose and peroxidase-conjugated anti-rabbit chicken IgG were purchased from Sigma Chemical Co. (St Louis, MO, USA). Saquinavir and indinavir were dissolved in 25% ethanol, filter-sterilized and used for the experiments described. Polyclonal antibodies against bovine LPL raised in rabbit were a generous gift from Dr Ira Goldberg (Columbia University, New York, NY, USA).

Cell culture

3T3-F442A cells were maintained in 75 cm² flasks in low glucose Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% calf serum and a mixture of penicillin and streptomycin. For experiments, they were subcultured in 12-well dishes. Confluent cultures were allowed to differentiate by adding DMEM containing 10% fetal bovine serum and 100 nM insulin. The cells differentiated well in 3–5 days after switching to the differentiation medium. 3T3-L1 cells were differentiated using isobutylmethylxanthine (IBMX), dexamethasone and insulin as described previously (Clancy & Czech 1990). In the experiments involving the treatment of the cells with saquinavir during differentiation, the drug was added along with insulin in the case of 3T3-F442A cells or after the 48 h treatment with differentiation cocktail in the case of 3T3-L1 cells.

3T3-F442A cells were used for studying the effect of these drugs on LPL and lipid metabolism. Since high concentration of insulin was used to differentiate and maintain 3T3-F442A adipocytes, the basal glucose transport was high and not stimulated further by insulin. Hence, for studies on insulin action and glucose transport we used 3T3-L1 adipocytes.

Glucose transport

After differentiation, 3T3-L1 adipocytes were cultured without insulin for 6–8 days to study insulin-stimulated glucose transport. Before the glucose transport assay, the cells were incubated with 1 ml serum-free DMEM for 4 h. The cells were then washed twice with Krebs-Ringer phosphate buffer and incubated with or without 10 nM

insulin in the same buffer for 30 min. 2-Deoxy-[³H]-glucose was added to give a final concentration of 0.1 mM (1 µCi/100 nmol) and incubated for 10 min. The medium was aspirated and the cells were washed three times, and dissolved in 0.5 ml 0.2 M NaOH. Radioactivity in this lysate was determined by scintillation counting. Protein content in the lysate was determined by BioRad reagent (Richmond, CA, USA). Non-carrier-mediated glucose transport was determined in the presence of 10 µM cytochalasin (Clancy & Czech 1990).

LPL assay

LPL was measured in the medium after release with heparin, as described previously. The culture medium was removed from the dishes and 1 ml fresh medium containing heparin (10 U/ml) was added and incubated for 60 min at room temperature. It was found that more than 90% of the total extractable LPL activity in these cells was released by heparin under these conditions. LPL catalytic activity was measured using an emulsified [³H]-triolein substrate as described previously (Nilsson-Ehle & Schotz 1976). After incubation of 100 µl sample with 100 µl substrate for 60 min, liberated [³H]-fatty acids were separated from the reaction mixture using the method of Belfrage & Vaughn (1969). The LPL activity is expressed as nmol fatty acids released in 60 min/mg cell protein.

Incorporation of [¹⁴C]-acetate into lipids

To study the biosynthesis of total lipids from acetate, the cells were incubated with [¹⁴C]-acetate for 1 h at 37 °C in phosphate-buffered saline and washed five times with the same buffer. Total lipids from the monolayers were extracted using 2 ml hexane:isopropanol (3:2) mixture evaporated to dryness and then the radioactivity was determined (Ranganathan & Kern 1998).

Measurement of adipocyte lipolysis

Lipolysis was determined by measuring the amount of glycerol released by the cells into the medium. The cells were treated with or without saquinavir for 18 h in 1 ml medium and the glycerol content in the medium was measured using the kit from Sigma Chemical Co.

Incorporation of [³⁵S]-methionine into protein

The cells were incubated with [³⁵S]-methionine for 2 h. The monolayers were washed five times with phosphate-buffered saline and dissolved in 1 ml 0.2 M NaOH in a microfuge tube. These cell extracts were treated with trichloroacetic acid to give a final concentration of 15% for 2 h on ice. The tubes were centrifuged and the pellets

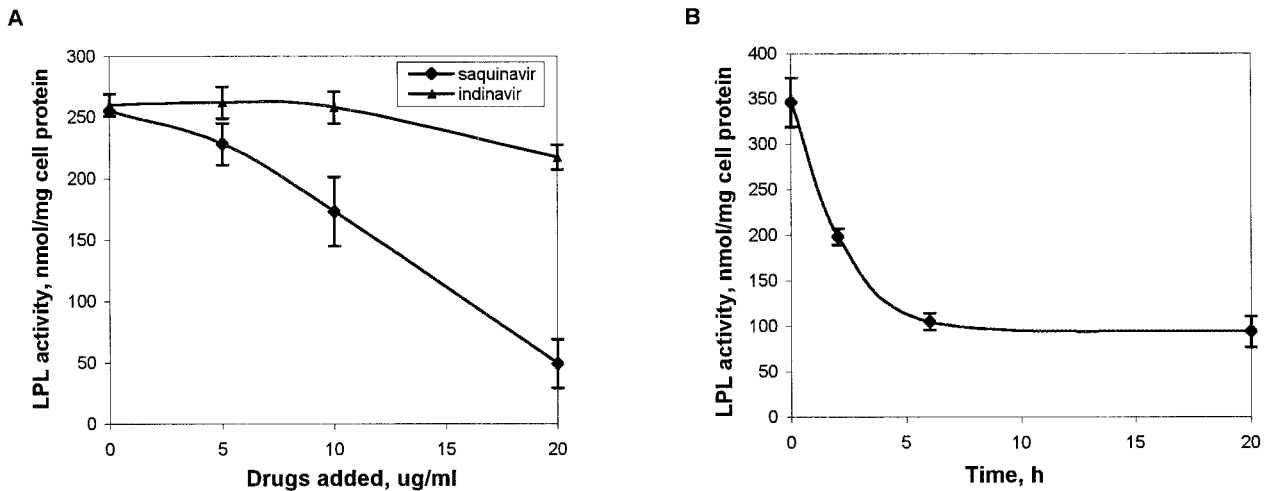


Figure 1 Effect of indinavir and saquinavir on LPL activity in 3T3-F442A adipocytes. (A) Differentiated 3T3-F442A adipocytes were treated with the indicated concentrations of saquinavir and indinavir for 24 h and the heparin-releasable LPL activity was determined. (B) Differentiated 3T3-F442A adipocytes were treated with saquinavir (20 μ g/ml) for the indicated time-periods and the heparin-releasable LPL activity was determined. Each value is the mean of independent analysis from triplicate wells and the error bars represent s.d.

were washed three times with 10% trichloroacetic acid. The pellets were finally dissolved in 0.2 M NaOH and the radioactivity was determined.

RNA extraction and Northern blot analysis

RNA was isolated from the cells using guanidine thiocyanate as described by Chomczynski & Sacchi (1987). RNA (10 μ g) was electrophoresed on a 1% agarose-formaldehyde gel, transferred to nylon membrane and hybridized with 32 P-labeled cDNA probe for mouse LPL.

Western blot analysis

The cells from each well were lysed in 1.0 ml of a buffer containing Triton X-100 and phenylmethylsulfonyl fluoride. The lysates were centrifuged and 10 μ l of the clear sample was subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. LPL was detected using polyclonal antibodies raised against bovine LPL in rabbit and peroxidase-conjugated second antibody. The bands were visualized using chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Statistics

All data are expressed as the mean \pm s.d. of triplicate cell cultures, and all statistical comparisons were made using the Student's *t*-test.

Results

To examine the effect of protease inhibitors on LPL activity, differentiated 3T3-F442A adipocytes were

treated with saquinavir and indinavir for 24 h. The data presented in Fig. 1 show that saquinavir inhibited LPL activity in a dose-dependent manner. The inhibition was 10% with 5 μ g/ml saquinavir ($P=0.1$), and 33 and 81% ($P<0.01$) with 10 and 20 μ g/ml respectively. Plasma concentrations of saquinavir have been reported to be as high as 7 μ g/ml during treatment (van Heeswijk *et al.* 2000). Indinavir caused no inhibition at 5 and 10 μ g/ml, but inhibited LPL activity by 17% ($P=0.1$) at 20 μ g/ml. The time-course of LPL inhibition by saquinavir is also shown in Fig. 1. At a concentration of 20 μ g/ml, the drug inhibited 43% ($P<0.01$) of the LPL activity within 2 h of treatment. At 6 h, the inhibition was 72% ($P<0.01$) and there was no further decrease in activity at 20 h. Saquinavir did not inhibit LPL activity when added directly to the assay (data not shown).

To determine whether the inhibition of LPL activity could be reversed, differentiated 3T3-L1 adipocytes were treated with 20 μ g/ml saquinavir for 18 h and the cells were washed and incubated further in the absence of the drug. The data presented in Fig. 2 show that the LPL activity was inhibited by 75% as a result of incubation with saquinavir. After removal of the drug, the LPL activity remained low for 6 h and after 24 h 70% of the original activity was restored.

Northern blot analysis was carried out to determine if the expression of LPL mRNA was affected by saquinavir. The data shown in Fig. 3 indicate that saquinavir treatment did not inhibit the expression of LPL mRNA even though there was a large decrease in LPL activity. Western blot analysis of the heparin-releasable fraction demonstrated that there was no change in LPL protein although the inhibition of enzyme activity was more than 75% (Fig. 3).

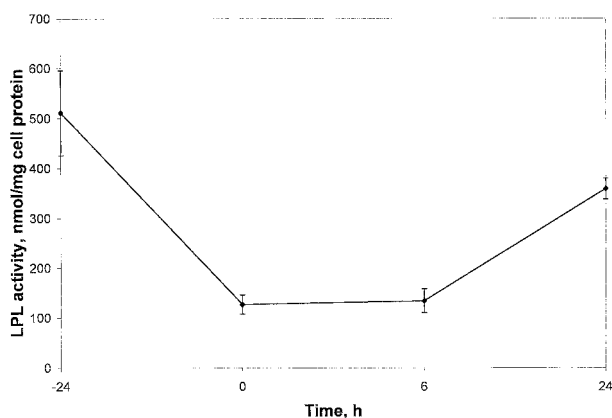


Figure 2 Reversal of the inhibition of LPL activity after removal of saquinavir in 3T3-F442A adipocytes. Differentiated 3T3-F442A adipocytes were treated with saquinavir (20 µg/ml) for 24 h. The cells were washed and incubated with fresh medium without saquinavir for the indicated periods and the heparin-releasable LPL activity was determined. All values are means from triplicate analyses and the error bars represent s.d.

In order to examine whether the protease inhibitors have any effect on lipid biosynthesis, the incorporation of [¹⁴C]-acetate into total lipids was studied. The data presented in Fig. 4A show that saquinavir inhibited lipid synthesis by 74% ($P < 0.01$) after 24 h treatment at a concentration of 20 µg/ml. No significant changes were observed at 5 and 10 µg/ml saquinavir. The time-course of the changes in lipid biosynthesis is shown in Fig. 4B. The inhibition of lipid synthesis was detected as early as 2 h after the addition of saquinavir, and the inhibition was 53% and 82% after 5 h and 20 h respectively ($P < 0.01$). Indinavir had no effect on lipid biosynthesis under similar conditions (data not shown).

To determine whether the inhibitory effect of saquinavir on LPL and lipid synthesis was due to a general cytotoxic effect, the incorporation of [³⁵S]-methionine into total proteins was determined. The results shown in Table 1 indicate that the rate of protein synthesis and the cellular protein concentration remained unchanged after saquinavir treatment. Therefore, saquinavir did not inhibit total protein synthesis in these cells.

The lipodystrophy resulting from the clinical treatment with protease inhibitors could be due to increased lipolysis of triglyceride from fat cells. Therefore, we examined the effects of saquinavir on the release of glycerol from 3T3-F442A adipocytes. The release of glycerol is a measure of triglyceride hydrolysis catalyzed by hormone-sensitive lipase. The data in Table 2 show that 20 µg/ml saquinavir increased the basal rate of glycerol release by 82% ($P < 0.01$). No significant changes were observed at lower concentrations of saquinavir.

With the inhibition of LPL activity, lipid synthesis, and increased basal lipolysis, one would expect to observe a decrease in total cell lipid accumulation. To investigate the

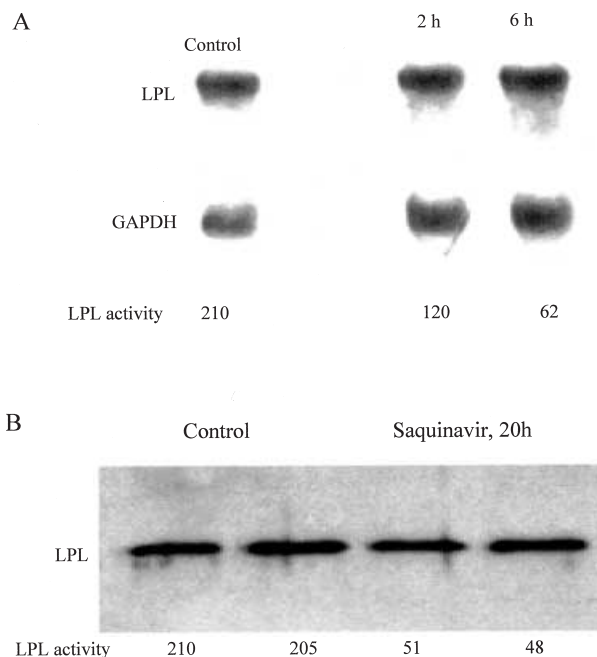


Figure 3 Expression of LPL mRNA and protein in 3T3-F442A adipocytes. (A) Differentiated 3T3-F442A adipocytes were treated with saquinavir (20 µg/ml) for 2 and 6 h. RNA was isolated and subjected to Northern blot analysis. The blots were hybridized with ³²P-labeled cDNA probes for LPL and glyceraldehyde phosphate dehydrogenase (GAPDH). (B) Differentiated 3T3-F442A adipocytes were treated with saquinavir (20 µg/ml) for 20 h. Western blot analysis was carried out in the heparin-releasable fraction, as described in Materials and Methods. The LPL activities are shown as nmol fatty acids released/mg cell protein.

effect of saquinavir on lipid accumulation, saquinavir was added to 3T3-L1 and 3T3-F442A preadipocytes at the time of differentiation. As shown in Table 3, saquinavir inhibited lipid accumulation in both the cell lines during the differentiation process.

Glucose transport and insulin action

Protease inhibitor treatment in humans causes disturbances in carbohydrate metabolism. In order to study whether protease inhibitors have any direct effect on glucose transport, 3T3-L1 cells were treated with saquinavir and indinavir and the basal and insulin-stimulated glucose transport was determined. The data shown in Fig. 5A indicate that saquinavir increased the basal glucose transport 2.7-fold ($P < 0.05$) and decreased the insulin-stimulated glucose transport by 30% ($P < 0.05$). Under the same conditions, indinavir increased basal glucose transport by 40% ($P < 0.05$) and had no effect on the insulin-stimulated glucose transport (Fig. 5B).

Discussion

The data presented in this paper show that saquinavir has significant effects on LPL activity, *de novo* lipid synthesis

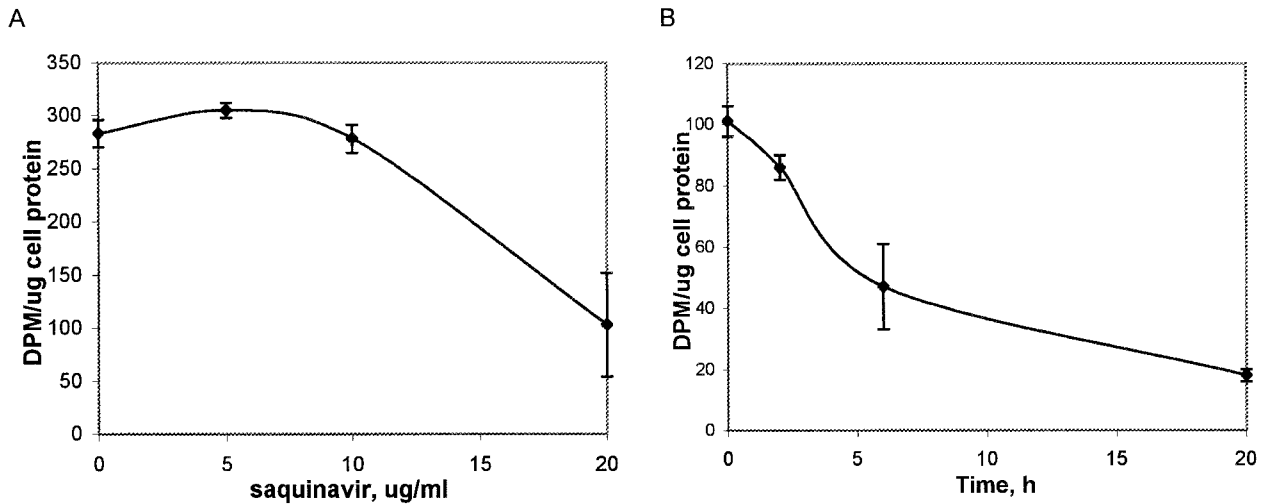


Figure 4 Effect of saquinavir on lipid synthesis from [^{14}C]-acetate in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were incubated with the indicated concentrations of saquinavir for 24 h (A) or 20 $\mu\text{g/ml}$ saquinavir (B) for the indicated periods of time and the incorporation of [^{14}C]-acetate into total lipids was determined as described in Materials and Methods. The values in the figures represent means and s.d. from triplicate analyses.

Table 1 Effect of saquinavir on protein synthesis and protein content in 3T3-F442A adipocytes. Each value is the average \pm s.d. from triplicate analyses

Saquinavir added	[^{35}S]-Met incorporation (DPM/mg protein $\times 10^{-3}$)	Protein content (μg)
None	56.5 \pm 19.5	424 \pm 9
5 $\mu\text{g/ml}$	56.3 \pm 13.8	439 \pm 25
10 $\mu\text{g/ml}$	58.8 \pm 24.7	439 \pm 24
20 $\mu\text{g/ml}$	57.9 \pm 11.1	421 \pm 19

Differentiated 3T3-F442A adipocytes were incubated with [^{35}S]-methionine (10 $\mu\text{Ci/ml}$) for 2 h. The monolayers were washed five times with phosphate-buffered saline and dissolved in 1 ml 0.2 M NaOH and transferred to a microfuge tube. The radioactivity in the trichloroacetic acid-precipitable material and the amount of total protein were determined as described in the text.

and lipolysis in adipocytes. Lipid accumulation in adipose tissue requires both LPL-mediated triglyceride hydrolysis and *de novo* fatty acid synthesis. The inhibitory effects of saquinavir on LPL, fatty acid synthesis and stimulation of lipolysis may be partly responsible for the lipodystrophy in patients treated with this drug.

In patients treated with a combination of saquinavir and nelfinavir, the plasma levels of saquinavir and nelfinavir reached 2.4 $\mu\text{g/ml}$ and 6 $\mu\text{g/ml}$ respectively (Rommel *et al.* 1999). In another study, plasma concentration of saquinavir has been reported to be as high as 6.8 $\mu\text{g/ml}$ (van Heeswijk *et al.* 2000). We did not observe any effects of saquinavir at a concentration of 5 $\mu\text{g/ml}$. It is difficult to equate plasma levels of a drug with *in vitro* data, since tissue levels of these drugs may be higher. Although saquinavir inhibited LPL activity and lipid synthesis, another HIV protease inhibitor, indinavir, had only minimal effects on

Table 2 Effect of saquinavir on glycerol release by 3T3-F442A adipocytes. The values are means \pm s.d. from triplicate wells

Saquinavir added	Glycerol release (μmol glycerol/mg protein)
None	7.9 \pm 0.1
5 $\mu\text{g/ml}$	9.2 \pm 0.1
10 $\mu\text{g/ml}$	8.4 \pm 0.5
20 $\mu\text{g/ml}$	14.4 \pm 0.1

Differentiated 3T3-F442A adipocytes were treated with the indicated concentrations of saquinavir for 18 h in 1 ml medium. The glycerol content in the medium was measured using the kit from Sigma Chemical Co.

adipocytes. The reasons for this difference between saquinavir and indinavir are not clear. The potencies of these drugs to inhibit HIV protease and dosages used for the treatment are very close (Deeks *et al.* 1997). It is possible that the inhibitory action of saquinavir on lipid metabolism in adipocytes may be unrelated to the inhibition of HIV protease. Although head-to-head comparisons of the effects on lipid and carbohydrate metabolism have not been performed, both these drugs are associated with 'syndrome X'-like presentations. Purnell *et al.* (2000) found that normal healthy subjects developed hypertriglyceridemia when treated with ritonavir, but no changes in post-heparin plasma lipase activities were observed.

Our data show that LPL translation and the immunoreactive mass of LPL are unaffected by saquinavir treatment in spite of strong inhibition of enzyme activity. Therefore, saquinavir appears to inhibit post-translational processing, resulting in the synthesis of LPL protein with low activity. This mechanism appears to be similar

Table 3 Inhibition of lipid accumulation in 3T3-L1 and 3T3-F442A adipocytes by saquinavir. The values are means \pm s.d. from triplicate determinations

	3T3-F442A (μg triglyceride/ mg protein)	3T3-L1 (μg triglyceride/ mg protein)
Control	57.5 \pm 5.0	38.7 \pm 2.1
Saquinavir (20 $\mu\text{g}/\text{ml}$)	20.5 \pm 3.0	25.8 \pm 4.5

3T3-F442A and 3T3-L1 cells were cultured until they were confluent and saquinavir was added at the beginning of the differentiation period. In the case of 3T3-L1 cells, saquinavir was added after the 2-day treatment with IBMX/dexamethasone/insulin mixture. The culture was continued for 4 days and the lipid accumulation was measured by total cellular triglyceride content using the triglyceride assay kit.

to the inhibition of LPL activity by thiazolidinediones (Ranganathan & Kern 1998) and regulation of LPL by feeding and fasting (Doolittle *et al.* 1990).

These data may yield some insight into the lipodystrophy associated with treatment of patients with HIV protease inhibitors. Adipose tissue lipid content represents the result of a constant flux of new lipid accrual and ongoing lipolysis. As demonstrated previously (Hollenberg 1966), the increase in lipid content of rat adipose tissue was due to the accrual of predominantly unsaturated (plasma-derived) fatty acids, as opposed to saturated lipid from endogenous synthesis. Human adipose tissue also

derives most of its lipid for storage from circulating triglycerides (Shrago *et al.* 1969, Wilson *et al.* 1973). Thus, adipose tissue lipid accumulation is dependent on the uptake of lipids from triglyceride-rich lipoproteins, and probably to a small extent from endogenous *de novo* fatty acid synthesis. On the other hand, patients with LPL deficiency are not deficient in adipose tissue triglyceride (Peeva *et al.* 1992), suggesting that adipocytes will synthesize lipid *de novo* if the need arises.

Adipose tissue lipid accumulation is also determined by the degree of lipolysis. Hormone-sensitive lipase (HSL) is the primary mediator of lipolysis in adipocytes (Osuga *et al.* 2000). Hormones that stimulate cAMP activate protein kinase A, which then reversibly phosphorylates HSL (Stralfors *et al.* 1984, Holm *et al.* 1997) and releases non-esterified fatty acids from adipocytes. The increased release of non-esterified fatty acids plays a central role in insulin resistance (Kelley & Simoneau 1994, Unger 1995). In addition, an elevated expression of HSL in adipocytes led to reduced adipose lipid accumulation, along with the delayed expression of late markers of adipocyte differentiation (Sztalryd *et al.* 1995). Thus, the control of adipose tissue lipid accumulation may result in alterations in expression of other adipose tissue genes. These changes in adipocyte gene expression could be due to an inhibition of differentiation. Previous studies have reported that protease inhibitors inhibited the differentiation of pre-adipocytes in culture as measured by glycerophosphate dehydrogenase, lipid accumulation and LPL expression

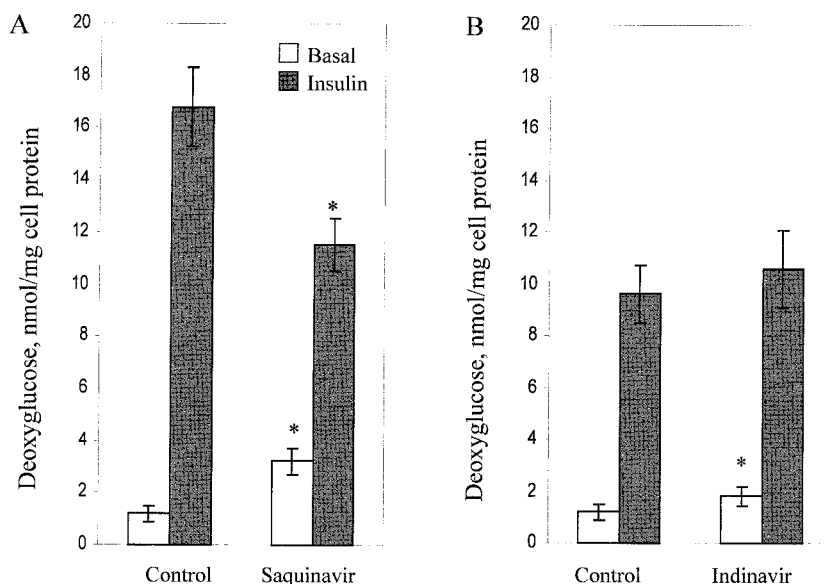


Figure 5 Effect of (A) saquinavir and (B) indinavir on basal and insulin-stimulated glucose transport in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were treated with 20 $\mu\text{g}/\text{ml}$ saquinavir or indinavir for 24 h and the basal and insulin-stimulated [^3H]-2-deoxyglucose was determined as described in Materials and Methods. The results are expressed as the mean of triplicate analyses and the error bars represent s.d. * $P < 0.05$ compared with the corresponding control values.

(Wentworth *et al.* 1999, Zhang *et al.* 1999, Lenhard *et al.* 2000a). Our studies show that saquinavir has potent effects on LPL activity and lipolysis in mature adipocytes also. Lenhard *et al.* (2000a) have also reported that some of the protease inhibitors including saquinavir increase lipolysis in cultured C3H10T1/2 adipocytes. Caron *et al.* (2001) have recently reported that indinavir impairs preadipocyte differentiation in 3T3-F442A cells.

Although decreased lipogenesis and increased lipolysis would be predicted to cause adipose wasting, patients treated with these drugs demonstrate a redistribution of adipose tissue lipids, suggesting that these drugs have depot-specific actions. There are numerous metabolic differences between different adipose tissue depots in humans. For example, gluteofemoral adipocytes have higher LPL activity, whereas the adipocytes in the central fat depot have higher lipolytic activity and rapid turnover of fatty acids (Despres *et al.* 1995, Samaras & Campbell 1999). Recently, Lenhard *et al.* (2000b) have shown that the protease inhibitors nelfinavir and ritonavir stimulated triglyceride synthesis in HepG2 cells and increased plasma triglyceride in mice (Lenhard *et al.* 2000b). The variable effects of the protease inhibitors in different tissues may be cause for the lipodystrophy in specific regions in the body.

The treatment of patients with protease inhibitors also causes insulin resistance and hyperglycemia in some individuals. The studies presented in this paper have shown direct effects of saquinavir on glucose transport and insulin action in cultured adipocytes. The data indicate that saquinavir decreased the insulin-stimulated glucose transport in 3T3-L1 adipocytes, although there was an increase in basal glucose transport. Although decreased adipocyte glucose transport is often highly correlated with decreased whole body glucose disposal rate (Kolterman *et al.* 1981), most whole body glucose disposal occurs in muscle (DeFronzo *et al.* 1981). It is not known whether changes in adipose tissue insulin action subsequently affect muscle glucose transport, or whether saquinavir has direct effects on muscle. In addition, it is not clear why indinavir did not have any effect on glucose transport or insulin action in these *in vitro* studies, since the clinical syndrome associated with indinavir is similar to that of saquinavir. Caron *et al.* (2001) have reported that treatment of 3T3-F442A preadipocytes with indinavir for 6 days during the differentiation period inhibited the insulin-stimulated mitogen-activated protein kinase activation. However, insulin receptor substrate-1 phosphorylation was not affected.

The cellular actions of the HIV protease inhibitors that would account for the effects in adipose tissue are not known. Tumor necrosis factor- α (TNF α) is secreted by the adipocytes and it is known to inhibit LPL and increase lipolysis. The inhibition of LPL activity by TNF α is transcriptionally mediated (Cornelius *et al.* 1988). Since we observed no changes in LPL mRNA, the inhibition of LPL by saquinavir is not likely to be mediated through stimulation of TNF α secretion.

In summary, these data show that the treatment of adipocytes with one of the HIV protease inhibitors resulted in a decrease in LPL activity and lipogenesis, increase in lipolysis, and a decrease in insulin-stimulated glucose transport. These data may explain some of the adverse effects observed in the patients treated with protease inhibitors, but raise many questions regarding depot specificity, and the mechanism of cellular signaling in adipocytes.

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