The effects of protease inhibitors on basal and insulin-stimulated lipid metabolism, insulin binding, and signaling

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Abstract The objective of our research was to investigate the effects of the protease inhibitors ritonavir, saquinavir, and indinavir on triglyceride synthesis, lipolysis, insulin binding, and signaling in differentiating 3T3 L1 pre-adipocytes. Saquinavir, ritonavir, and indinavir all stimulated triglyceride (TG) synthesis. Additionally, all concentrations of protease inhibitors employed (i.e., 0.1 μM to 10 μM) significantly decreased insulin-stimulated TG synthesis. No effects of any of the protease inhibitors were observed either on basal lipolysis or after stimulation of lipolysis with 100 nM noradrenaline. Specific ^{125}I-insulin binding was observed to be decreased by exposure to all the protease inhibitors throughout the period of adipocyte phenotype development. This was mediated by indinavir through a receptor decrease and had no effect on receptor affinity. During differentiation with ritonavir (i.e., 1–11 days post addition of differentiating cocktail), insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation was ascertained (day 11) and found to be decreased in the ritonavir exposed cells when compared with control cells. The results reported herein demonstrate protease inhibitor effects on basal TG synthesis while exhibiting decreased insulin-stimulated TG synthesis at physiological concentrations of protease inhibitors. These effects may be subsequent to decreased insulin binding and/or IRS-1 tyrosine phosphorylation.—Cammalleri, C. and R. J. Germinario. **The effects of protease inhibitors on basal and insulin-stimulated lipid metabolism, insulin binding and signaling. *J. Lipid Res.* 2003.** 44: **103–108.**

Supplementary key words triglyceride synthesis • lipolysis • insulin lipogenic response • binding and signaling

Dramatic changes in the morbidity and mortality of HIV-1 have been observed in the aftermath of the use of combination therapy to treat these patients. Additionally, many patients on this highly active antiretroviral therapy (HAART) exhibited multiple metabolic perturbations such as altered body fat distribution (1–4) that included peripheral fat atrophy (1, 2) and central adiposity (3, 4). In concert with this, increased serum glycerol and triglycerides were observed. Additionally, the patients demonstrated higher than normal insulin levels suggestive of insulin resistance (5, 6). The degree of lipodystrophy in patients varies but has been reported to be greater than 80% in one study (5). The cause of these HAART associated perturbations is unknown and has been suggested to involve multiple sites (7–10). Unlike the reverse transcriptase inhibitors that suppress replication of the viral genome, the protease inhibitors inhibit virus maturation, limiting the infectivity of the virus particles (11). All this notwithstanding, the "syndrome" is reminiscent of the insulin-resistant state known as Syndrome X (12). Clearly, these metabolic perturbations may be related to the etiology of cardiovascular disease and/or diabetes.

The study reported herein investigated the effects of several protease inhibitors (PI) (e.g., saquinavir, indinavir, and ritonavir) on basal and insulin-stimulated triglyceride (TG) synthesis in differentiating 3T3 L1 pre-adipocytes in vitro. Additionally, the PI effects on lipolysis, insulin binding, and early insulin signaling have been studied. To this end, we have found that the PI induced decreased insulin-stimulated TG synthesis, insulin binding, and early insulin signaling. No effects on PI stimulated lipolysis were observed.

MATERIALS AND METHODS

Cell culture

3T3 L1 pre-adipocytes were cultured in 10% DMEM (v/v) fetal calf serum, penicillin (100 U/ml), and streptomycin (100 U/ml)

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Abbreviations: HAART, highly active antiretroviral therapy; IRS-1, insulin receptor substrate-1; PI, protease inhibitors; TG, triglyceride.

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at 37°C in a 5% CO_2 humidified incubator. On reaching confluence (~4 days), the cells were exposed to differentiation medium (containing 167 nM insulin, 0.5 mM isobutylmethylxanthine (IBMX), and 0.25 μM dexamethasone in 10% DMEM) for 3 days (13). The cells were then incubated in 10% DMEM with 167 nM insulin for another 3 days, after which they were maintained in 10% DMEM for 4 days (media not containing insulin). All HIV PI were dissolved in DMSO and diluted with 10% DMEM. The highest concentration of DMSO employed in experiments was 0.001%. PI was added throughout differentiation of the 3T3 L1 cells. In some cases, acute exposure was employed and this is noted in the experiments so performed.

Triacylglycerol synthesis

3T3 L1 pre-adipocytes were grown and differentiated in 24 well plates at 1 × 104 cells/ml/well. At precisely 3 or 11 days post-induction, 3T3 L1 cells were serum starved overnight in MEM, 5 mM D-glucose, and 0.1% BSA. The cells were then incubated with insulin in MEM for 6 h. D-[^{14}C(U)]glucose (2.2 dpm/pmol) was then added for 18 h at 37°C in a humidified incubator with 5% $C0_2$. The cells were then placed on ice and the medium removed after which the monolayers were washed 3× with PBS at 4°C. Triglycerides were then extracted for 30 min 2× with 1 ml isopropanol-heptane (2:3; v/v) and collected after extraction with alum (14, 15). The incorporation of D-[^{14}C]glucose into triglycerides was determined by scintillation counting. Cell monolayers were dissolved in 1 ml 1 N NaOH and protein measured by the Lowry method (16).

Lipolysis

Lipolysis experiments were conducted using a glycerol assay from Sigma Diagnostics (procedure #337). The glycerol produced from the hydrolyzed triglycerides was measured after 6 h or 24 h with or without 100 nM noradrenaline. At this point, 10 μl of media from each sample was added to 1 ml of GPO-Trinder reagent in a cuvet and the OD was read at 540 nm. All samples were corrected for absorbance blanks and the concentration in each sample was determined per unit of protein using the Lowry method for determination of cell protein.

Insulin binding

3T3 L1 adipocytes throughout differentiation were washed twice with 20°C Hank's HEPES balanced salt solution (HHBS) containing 0.1% BSA, pH 7.4.

The cells were then exposed to ^{125}I-insulin (1 ng/ml) alone or with a large excess of unlabeled insulin (40 μg/ml) (non specific binding) for 2 h on a rotary shaker at room temperature (17). At this time, the cell monolayers were washed 4× with 4°C HHBS. Samples were solubilized in 1 ml of 1 N NaOH and counted in a gamma counter. Protein analysis was performed on separate samples using the Lowry method (16). For studies on competitive displacement of labeled ^{125}I-insulin by cold insulin, slight modifications of the protocol were employed. Cold insulin was employed from 0.6 ng/ml to 40,000 ng/ml.

Preparation of whole cell extracts

3T3 L1 adipocytes were washed twice with 4°C PBS and solubilized in RIPA buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM sodium vanadate, and 1 mM sodium fluoride). The samples were gently rocked for 15 min at 4°C to aid in solubilization of the cells. The lysates were centrifuged at 11,000 *g* for 15 min at 4°C. Protein content was determined using a modified Lowry assay for the supernatant fluid which represented the whole cell lysate.

Immunoprecipitation

Two hundred fifty microliters of cell lysate protein was placed in a microcentrifuge tube to which we added 2 μg of anti IRS-1. After gentle shaking overnight at 4°C, the immunocomplex was captured by adding 50 μl of Protein A agarose bead slurry (25%). The reaction mixture was shaken at 4°C for 2 h. The agarose beads were collected by centrifugation at 1,500 *g* in the cold for 5 min. The beads were washed three times with PBS and then resuspended in 50 μl of 2× Laemmli sample buffer with β-mercaptoethanol and boiled for 5 min. The beads were pelleted and Western analysis performed on the supernate.

Western analysis

Cell lysate proteins (i.e., 40 μg per lane) were mixed with 4× Laemmli buffer. The blots were washed with PBST (0.1% Tween) and probed with rabbit anti mouse polyclonal antibodies in appropriate blocking reagents for 1 h at room temperature. The membranes were washed again with PBST and incubated with horseradish peroxidase (HRP) secondary antibody. The blots were then incubated with the chemiluminescence detection reagents (ECL Amersham) prior to exposure to Kodak X-Omat Blue film. The intensity of the bands was quantitated using UNSCAN-IT computer software (Silk Scientific).

Statistics

All analyses represent triplicate experiments with triplicate plates in each experiment. The statistical analyses employed herein include the Student's *t*-test and one-way ANOVA. The level of significance was set at $P < 0.05$. All data ± SEM.

RESULTS

It can be seen from the data in **Fig. 1** that insulin-stimulated triglyceride synthesis at both 3 and 11 days post-induction. These time intervals were chosen since they represent early and fully mature 3T3-L1 adipocytes. In these groups, the peak times of TG synthesis were similar. The basal and insulin-stimulated levels of triglyceride synthesis were ~6-fold higher in the day-11 cells at 3.35 nM insulin while at the same concentration of insulin in the day-3 cells a 2-fold increase was seen. Additionally, our interests were on the effects of several PI on triglyceride synthesis in the absence of 3.35 nM insulin. The highest concentration of PI chosen was 10 μM (i.e., a physiologically relevant concentration) and three PI were chosen for our investigations (i.e., saquinavir, ritonavir, indinavir). Subsequently, our first question was to determine if triglyceride synthesis (i.e., no insulin used) was affected by ritonavir, saquinavir, or indinavir. In **Table 1**, cells that were induced to differentiate for 3 days showed a concentration dependent increase in TG synthesis. Both saquinavir and ritonavir at 1.0 μM and 10.0 μM concentrations exhibited significant increases in TG synthesis ($P < 0.05$; one-way ANOVA; n = 3). Similarly, a significant increase in TG synthesis was seen for the indinavir group throughout the range of concentrations employed ($P < 0.05$; one-way ANOVA; n = 3). At 10 μM, no effects on total cell protein were observed on 3T3 cells after 11 days exposure to ritonavir or indinavir (i.e., in control cells 0.6 ± .06 mg protein/plate was determined while in 10 μM ritonavir, 0.67 ± 0. 08 mg p/plate and in 10 μM indinavir-exposed cells 0.72 ± 0.01 mg p/plate was determined; n =

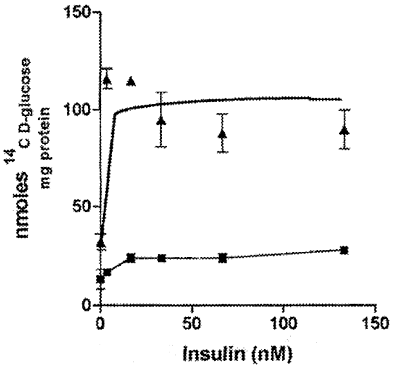

Fig. 1. The effect on insulin on 3T3 L1 adipocytes 3 days and 11 days postinduction of the adipocyte phenotype. Three-day (squares) and 11-day cells (triangles) are shown. The data represent the average of three experiments (triplicate plate in each experiment) ± SEM.

11–14). Similar results on total cell protein were seen at 1.0 μM PI throughout the differentiation period (days 0, 4, 7, and 11; data not shown). Additionally, at 10 μM PI, less than 10% toxicity was observed for the three PI employed in this study (CellTiter, Promega cell proliferation assay).

We found that insulin-stimulated TG synthesis was quantitatively different between 3T3 L1 cells that are at 3 days differentiation versus 11 days differentiation (Fig. 1). Although we observed increased TG synthesis with all the concentrations of PI tested, we saw that insulin-stimulated TG synthesis in differentiating day 3 cells was significantly decreased at all concentrations of PI tested (i.e., 0.1, 1.0 and 10.0 μM) ($P < 0.05$; one-way ANOVA; n = 3) (**Table 2**). A maximal concentration of insulin (i.e., 3.35 nM) (Fig. 1.) was employed and TG synthesis was reduced by PI (i.e., nmoles D-[^{14}C]glucose incorporated into TG /mg protein/18 h). On average, insulin-stimulated TG synthesis was decreased 34–35% by saquinavir and indinavir, and 54% by ritonavir at 10.0 μM concentrations of these PI (Table 2.). In mature adipocytes (day 11 cells), decreased insulin-stimulated TG synthesis was seen in the ritonavir-exposed group at a concentration of 10.0 μM ritonavir (~40%; data not shown).

TABLE 1. The effect of different protease inhibitors on basal triglyceride synthesis in 3T3 L1 adipocytes 3 days postinduction

	Triglyceride Synthesis			
Treatment	0 μM	0.1 μM	1.0 μM	10 μM
	nmoles D-[^{14}C]glucose/mg protein/18 h			
Protease inhibitor				
Saquinavir	8 ± 0.05	10.1 ± 1.0	11 ± 0.02[a]	17 ± 1.0[a]
Indinavir	8 ± 0.05	10.5 ± 0.01[b]	13.5 ± 0.02[b]	17.5 ± 0.01[b]
Ritonavir	8 ± 0.05	10.4 ± 0.13	12 ± 0.02[c]	17 ± 1.5[c]

Cells were exposed to drugs continually (4 days pre-differentiation and 3 days postinduction of differentiation). Basal means that no PI (and no insulin) was present in the media. Only PI were added to the treatment media (i.e., 0.1 μM to 10 μM). Triglyceride synthesis was determined as described in Materials and Methods. All data ± SEM.

[a] Significantly elevated, one-way ANOVA, $P < 0.05$, n = 3.
[b] Significantly elevated, one-way ANOVA, $P < 0.05$, n = 3.
[c] Significantly elevated, one-way ANOVA, $P < 0.05$, n = 3.

TABLE 2. The effect of different protease inhibitors on insulin-stimulated triglyceride synthesis in 3T3 L1 adipocytes three days postinduction of differentiation

	Triglyceride Synthesis			
Treatment	0 μM	0.1 μM	1.0 μM	10.0 μM
	D-[^{14}C]glucose/mg protein/18 h			
Protease inhibitor				
Saquinavir	43 ± 2	30 ± 0.1[a]	30 ± 0.5[a]	28 ± 2
Indinavir	43 ± 2	29 ± 0.3[b]	28 ± 1[b]	28.5 ± 0.8[b]
Ritonavir	43 ± 2	28 ± 2[c]	30 ± 0.2[c]	20 ± 4[c]

Cells were treated as described in Table 1. The control data represented maximal stimulation of TG synthesis with no PI present. For insulin stimulation, the cells were exposed to 3.35 nM insulin for 6 h, followed by an 18 h pulse with D-[^{14}C]glucose. Triglyceride synthesis was determined as described in Materials and Methods. All data ± SEM.

[a] Significantly decreased, one-way ANOVA, $P < 0.05$, n = 3.
[b] Significantly decreased, one-way ANOVA, $P < 0.05$, n = 3.
[c] Significantly decreased, one-way ANOVA, $P < 0.05$, n = 3.

Our next series of experiments was directed at determining the effects of ritonavir, saquinavir, and indinavir on lipolysis. We found that 1.0 μM and 10.0 μM of saquinavir, ritonavir, or indinavir exhibited no increased lipolysis (i.e., as measured by glycerol release) in day 11 adipocytes. All values ranged between 3.25 mM ± 0.13 mM glycerol release to 4.8 mM ± 0.8 mM glycerol release for controls and PI at all concentrations tested, ($P > 0.05$; one-way ANOVA; n = 3). Increasing lipolysis with noradrenaline (100 nM) lead to increased lipolysis in the control and PI treated groups. Again, no differences in lipolysis were observed in the noradrenaline plus PI treated groups, the range of values being 12.2 mM ± 0.5 mM glycerol released for controls to 16.8 mM ± 4 mM glycerol release for all PI tested, ($P > 0.05$; one-way ANOVA; n = 3).

We next investigated any changes in specific ^{125}I-insulin binding that might occur in the presence of the various PI. The differences seen in specific ^{125}I-insulin binding during the course of differentiation are shown in **Fig. 2**. It can be seen that the decreased specific ^{125}I-insulin bound was apparent on all days postinduction of differentiation in the presence of 1.0 μM PI. Further, the data in **Fig. 3** indicated that at the 1.0 μM and 10.0 μM concentrations of PI employed, ^{125}I-insulin specifically bound was decreased. In short, all PI significantly decreased ^{125}I-insulin binding to the 3T3 L1 adipocytes ($P < 0.05$; one-way ANOVA; n = 3) in a dose dependent fashion. The differences seen in the binding between Fig. 2 and Fig. 3 can be attributable to the state of the cells, the degree of differentiation, or experimental variability. In **Fig. 4A** and 4B, we show the competitive displacement of labeled insulin by increasing amounts of unlabeled insulin in the form of a Scatchard plot. In Fig. 4A, the data gave us the K_d for the high affinity binding site for control cells (e.g., 0.69 nM), while in Fig. 4B the K_d for the indinavir-exposed cells was 0.57 nM. The total insulin bound was higher in the control cells versus indinavir-exposed cells (e.g., being 10.5 vs. 6 fmoles/mg p, respectively). The low affinity sites had similar slopes yielding a K_d for control cells of 21 nM and 14 nM for the indinavir-exposed cells. The indinavir-exposed cells had decreased insulin binding versus the control cells (i.e., 9.5 vs. 29.5 fmol/mg p, respectively). This suggested that the

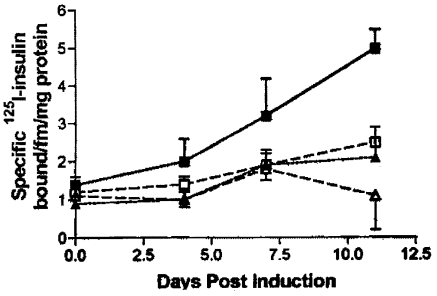

Fig. 2. The changes in specific ^{125}I-insulin bound during adipocyte induction in control (closed squares) or in the presence of 1 μM of ritonavir (open triangles), saquinavir (closed triangles), and indinavir (filled squares). The data represent three experiments (triplicate plates in each experiment) ± SEM.

affinity of the receptor for insulin was not different but that the differences in specific ^{125}I-insulin binding reside at the receptor number (18).

There has been much controversy concerning the effects or lack thereof of PI on insulin signaling (9, 19, 20). Thus, we decided to investigate insulin effects on IRS-1 in 3T3 L1 adipocytes 11 days postinduction of differentiation. The data in **Fig. 5A** indicated that up to 11-day exposure to 1.0 or 10.0 μM ritonavir did not affect the total IRS-1 protein. The ratio of insulin-control (+20 nM insulin for 15 min) of total IRS-1 protein was similar for all groups (e.g., 1.4 for control, 0.6 for 1 μM ritonavir, and 1.0 for 10.0 μM ritonavir). In Fig. 5B, the data obtained for tyrosine phosphorylated IRS-1 showed a large increase in the insulin-control group (3.75:1), while the same ratio obtained for the 1.0 μM and 10.0 μM ritonavir groups was considerably lower (1.51 and 1.53, respectively). The data indicated that tyrosine phosphorylated IRS-1 was not stimulated as effectively in 11 day exposed cells. There were trends seen toward a difference in total IRS-1 protein ± PI but no significant differences were found.

DISCUSSION

The data reported herein demonstrate that the PI (ritonavir, saquinavir, and indinavir) increased the basal (i.e., no insulin, no PI) rate of TG synthesis. Even with this increased rate of PI-increased TG synthesis, it was observed that insulin-stimulated TG synthesis was significantly decreased at 0.1 μM concentrations (as well as 1.0 μM and 10.0 μM) of the PI tested (i.e., saquinavir, ritonavir, or indinavir). This clearly indicated that exposure to varying concentrations of PI resulted in reduced insulin-stimulated TG synthesis. Additionally, others (21) have shown that PI can inhibit insulin-stimulated TG synthesis. Further, they showed decreased TG synthesis employing PI alone. However, they employed concentrations of 66 μM to 330 μM indinavir and 15 μM to 70 μM ritonavir at considerably higher concentrations than those employed herein, and for the most part nonphysiological. As far as we know, no other reports have been published on these effects of PI studied on TG synthesis.

Next, we investigated if TG lipolysis was affected by the PI employed herein. Our data indicated that 1.0 μM and 10.0 μM of saquinavir, ritonavir, or indinavir had no effect on basal (i.e., no insulin and no PI present) lipolysis. Further, increased lipolysis induced by 100 nM nor adrenaline did not uncover any additional lipolytic effects induced by the PI tested while 100 nM noradrenaline increased lipolysis nearly 4-fold. These results on lipolysis did not agree with those reported by others (8, 19, 20). The differences, however, could have been due to the use of a different PI (i.e., Nelfinavir) in the studies cited (8, 19, 20) and the PI concentrations employed (maximum concentrations used in other studies (8, 22–24) (>20.0 μM). Also, our protocol had PI present during fat cell differentiation while others did not expose the adipocytes until differentiation was complete, upon which the PI was added for 18 h–48 h before measuring lipolysis (8). Additionally, several different adipocyte models were employed. These included the 3T3 L1 pre-adipocytes (this study), 3T3 F442A pre-adipocytes (19), and C3H10T1/2 cells (8) Further, the concentrations of PI used in this study are in the physiological range (3–5). The clinical evidence on insulin resistance and adiposity indicated that multiple sites are affected in fat tissue (23, 24). Our data indicate that the effects of PI while elevating basal TG synthesis are not manifested by increased insulin-stimulated TG synthesis. In concert with this, the lack of effect on lipolysis have further demonstrated the complexity of the effects of PI on the adipose tissue mass (25, 26). Peripheral fat and visceral fat have been shown to behave differently in vivo (25, 26). We feel that the data presented

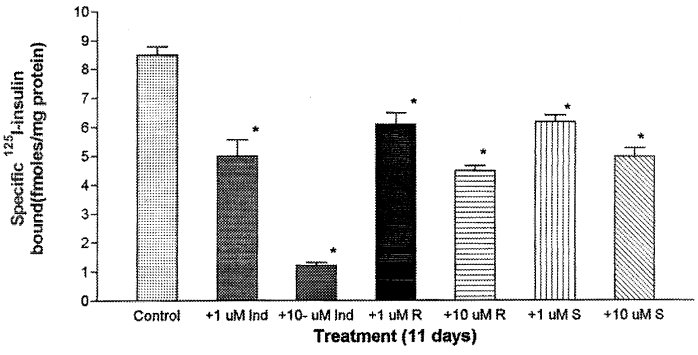

Fig. 3. The effects of different concentrations of protease inhibitor on ^{125}I-insulin specifically bound. The 11-day 3T3 L1 adipocyte control is compared with 3T3 L1 cells differentiating in the presence of 1.0 μM and 10.0 μM concentrations of indinavir (Ind), ritonavir (R), and saquinavir (S). All groups exposed to protease inhibitors express significantly decreased ^{125}I-insulin binding (n = 3, one-way ANOVA, * $P < 0.05$). Data ± SEM.

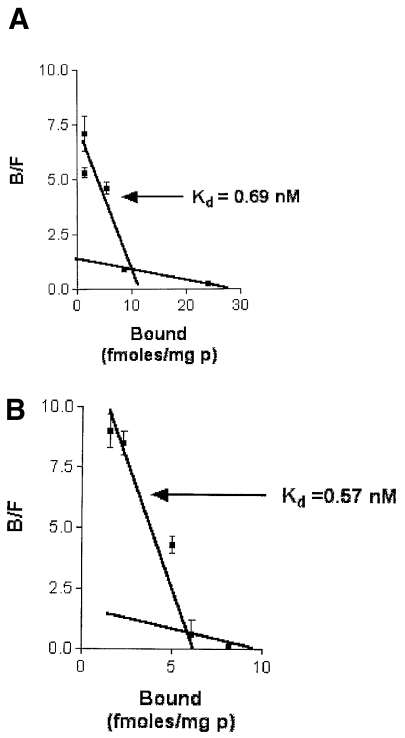

Fig. 4. Scatchard analysis of ^{125}I-insulin binding in 3T3 L1 adipocytes in control and 10 μM indinavir-exposed cells for 11 days. A: Control 3T3 L1 adipocytes. B: Indinavir-exposed 3T3 L1 adipocytes. The K_d are in nM units and the binding units are fmoles/mg protein. All data represent the mean of three experiments ± SEM.

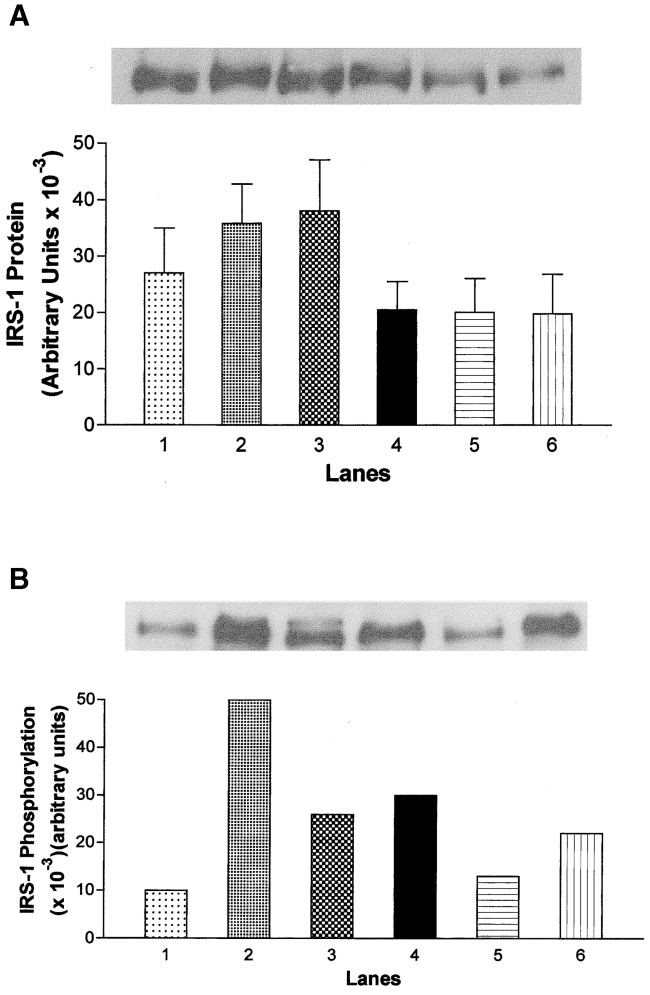

Fig. 5. A: The effect of ritonavir on IRS-1 tyrosine phosphorylation. A representative scan of total IRS-1 protein (A) in control versus ritonavir-treated cells 11 days (40 μg protein present in each lane) and tyrosine phosphoylated IRS-1. B: Insulin exposure was for 15 min to cells exposed as in A (11 days to ritonavir). Lanes 1–6 are the same for both panels: lane 1, control; lane 2, +2 nM insulin; lane 3, +1 μM ritonavir; lane 4, +1 μM ritonavir, +20 nM insulin; lane 5, +10 μM ritonavir; lane 6, 10 μM ritonavir +20 nM insulin. The data is representative of two different experiments.

herein show key events on TG synthesis and breakdown that may help explain the effects of treatment with HAART. This is especially so since different fat depots handle free fatty acids differently (26–28). Further, it has been argued succinctly that not all fat is the same (28).

Our data has shown that 1.0 μM ritonavir, saquinavir, or indinavir lead to decreased specific ^{125}I-insulin binding throughout the period of induction of adipocytes. At this concentration (i.e., 1.0 μM), no apparent effects of PI on adipocyte differentiation have been seen (10, 19, 26). Specific ^{125}I-insulin binding was decreased with increasing concentrations of PI employed with indinavir exhibiting the maximal insulin binding inhibition. This data agreed in part with that published by others (19) using indinavir in 3T3-F442A adipocytes. In the study reported herein, Scatchard analysis of the insulin binding data indicated that receptor affinity was not affected, but total insulin binding was decreased by indinavir exposure when compared with control cells.

Our data on early induction of differentiation on the 3T3 L1 pre-adipocyte showed that total IRS-1 protein was not appreciably changed by long-term exposure to 1.0 μM or 10.0 μM ritonavir (11 days). This was in the presence or absence of 20 nM insulin for 20 min. Analysis of tyrosine phosphorylation of IRS-1 demonstrated an increased ratio in the insulin-treated group versus versus control cells (3.75:1). In the ritonavir-treated group, a decreased tyrosine phosphorylation ratio of IRS-1 ± insulin was observed at both 1.0 μM and 10.0 μM ritonavir (±insulin treatment groups, 1.5 and 1.53, respectively). Others (9, 19, 20) have reported that no early signal changes could be observed with the use of PI, while decreased PKB phosphorylation has been shown to occur with the use of Nelfinavir (20). The differences seen could relate to the protocols employed in our study when compared with others that were quite different. The differences included *1*) the type and concentration of PI employed, such as Nelfinavir (19) versus ritonavir (this study); *2*) the exposure time to the PI, e.g., 4 h (9), 18 h (20) versus 11 days (this study); *3*) the cell type used 3T3 F442A pre-adipocyte (19) versus 3T3 L1 preadipocyte (this study); and *4*) the concentration of insulin used, e.g., 100 nM (19, 20), 1,000 nM (9) versus 20 nM (this study). Regarding the latter studies (9), indinavir was employed at a concentration of 100 μM for 4 h and insulin at 1,000 nM. These studies are not directly comparable to those

described herein. Also, 100 μM indinavir is higher than what is seen physiologically (8). The changes seen in the differentiating 3T3 L1 pre-adipocyte indicated that mature adipocytes versus differentiating adipocytes respond differently to different PI.

In summary, we have shown that basal and insulin-stimulated TG synthesis is modulated by saquinavir, ritonavir, and indinavir at physiological concentrations. No effects of PI on lipolysis were observed. However studies on specific ^{125}I-insulin binding indicated that low PI (i.e., saquinavir, ritonavir, and indinavir) concentrations resulted in decreased specific^{125}I-insulin binding in the PI exposed groups. This lower specific^{125}I-insulin binding is the result of no change in insulin-receptor affinity but reflects a decreased insulin receptor number or interaction at the insulin-receptor interface. Further, we have observed that IRS-1 tyrosine phosphorylation was decreased in the ritonavir-exposed cells. The exact mechanism(s) by which the PI generate this multiple series of perturbations is not exactly known, but the results indicated that a more exacting picture of the mechanism(s) involved in PI exposed cells can be obtained. Of considerable interest are the differences seen between studies on differentiating adipocytes and mature adipocytes in vitro.

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