HIV protease inhibitors (PIs) acutely and reversibly inhibit the insulin-responsive glucose transporter Glut 4, leading to peripheral insulin resistance and impaired glucose tolerance. Minimal modeling analysis of glucose tolerance tests on PI-treated patients has revealed an impaired insulin secretory response, suggesting additional pancreatic β-cell dysfunction. To determine whether β-cell function is acutely affected by PIs, we assayed glucose-stimulated insulin secretion in rodent islets and the insulinoma cell line MIN6. Insulin release from MIN6 cells and rodent islets was significantly inhibited by the PI indinavir with IC_{50} values of 1.1 and 2.1 μmol/l, respectively. The uptake of 2-deoxyglucose in MIN6 cells was similarly inhibited (IC_{50} of 2.0 μmol/l), whereas glucokinase activity was unaffected at drug levels as high as 1 mmol/l. Glucose utilization was also impaired at comparable drug levels. Insulin secretagogues acting downstream of glucose transport mostly reversed the indinavir-mediated inhibition of insulin release in MIN6 cells. Intravenous infusion of indinavir during hyperglycemic clamps on rats significantly suppressed the first-phase insulin response. These data suggest that therapeutic levels of PIs are sufficient to impair glucose sensing by β-cells. Thus, together with peripheral insulin resistance, β-cell dysfunction likely contributes to altered glucose homeostasis associated with highly active antiretroviral therapy. Diabetes 52: 1695-1700, 2003

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reatment of HIV-infected patients with HIV-1 protease inhibitors (PIs) as part of highly active antiretroviral therapy (HAART) has contributed to significant reductions in HIV viral load and increased CD4^{+} lymphocyte numbers, thereby slowing disease progression and improving patient survival (1). However, despite this clinical success, it is now recognized that PI-based therapy is correlated with a number of significant metabolic complications, including lipodystrophy, hyperlipidemia, and insulin resistance (2). As flux through GLUT4 is rate-limiting in whole-body glucose disposal, it has been proposed that acute inhibition of the high-capacity GLUT4 transporter contributes to the early stages of PI-associated insulin resistance by decreasing peripheral glucose disposal (6). In a rat model of insulin resistance, we have also shown that PIs cause acute and reversible changes in peripheral insulin sensitivity (7). Acute peripheral insulin resistance (i.e., after a single dose of indinavir) has recently been demonstrated in healthy human volunteers, validating the clinical relevance of these in vitro and animal models (8).

Although peripheral insulin resistance is a major component of the abnormalities in glucose homeostasis observed in PI-treated patients, minimal modeling predictions of glucose disposal during oral glucose tolerance testing in HIV-positive patients suggest that impaired β-cell sensitivity also contributes to altered glucose disposal (9,10). In the pancreatic β-cell, flux through facilitative glucose transporters (GLUT1, -2, and/or -3) initiates glucose sensing in which a metabolic signal from glucose oxidation is converted to a transient rise in intracellular calcium, which in turn stimulates insulin vesicle exocytosis. Given our previous observation that the pancreatic glucose transporters GLUT2 and GLUT3 are sensitive to acute inhibition by indinavir in Xenopus oocytes that heterologously express these transporter isoforms (11), we investigated the possibility that PIs may acutely inhibit glucose transport into the β-cell and impair insulin secretion. In the present report, we describe an acute PI-induced inhibition of glucose sensing in a clonal β-cell line, in isolated rodent pancreatic islets and in PI-naïve rats challenged with a hyperglycemic load. This inhibitory effect occurs at therapeutic concentrations of the PI indinavir, is correlated with a direct block of glucose uptake and diminished glucose utilization, and can be partially reversed by washing out the PI or by stimulating insulin release downstream of glucose uptake with nonglucidic insulin secretagogues. Taken together, these results suggest that in addition to impaired glucose disposal in the periphery, PI-induced β-cell dysfunction likely contributes to glucose intolerance.
and should be considered as a potential contributing mechanism for the metabolic complication associated with HAART.

**RESEARCH DESIGN AND METHODS**

**Materials.** Indinavir, ritonavir, nefilavir, and amprenavir were obtained from Merck, Abbott, GlaxoSmithKline, respectively. Rodents were purchased from Charles River Labs (Wilmington, MA). Blood glucose levels were determined using a Glucose Meter Elite XL (Bayer, Tarrytown, NY). BCA protein assay reagents were obtained from Pierce (Rockford, IL). Rat insulin was determined using a Glucometer Elite XL (Bayer, Tarrytown, NY). BCA protein assay reagents were obtained from Pierce (Rockford, IL). Rat insulin was determined using a Glucometer Elite XL (Bayer, Tarrytown, NY). BCA protein assay reagents were obtained from Pierce (Rockford, IL). Rat insulin was determined using a Glucometer Elite XL (Bayer, Tarrytown, NY). BCA protein assay reagents were obtained from Pierce (Rockford, IL). Rat insulin was determined using a Glucometer Elite XL (Bayer, Tarrytown, NY). BCA protein assay reagents were obtained from Pierce (Rockford, IL). Rat insulin was determined using a Glucometer Elite XL (Bayer, Tarrytown, NY). BCA protein assay reagents were obtained from Pierce (Rockford, IL). Rat insulin was determined using a Glucometer Elite XL (Bayer, Tarrytown, NY).

Isolation and culturing of pancreatic islets and β-cells. Anesthetized mice (C57BI/6 x CBA, aged 4–12 months) were killed by cervical dislocation. Pancreata were immediately removed and injected with Hank’s solution containing type XI collagenase (138 mMol/l NaCl, 5.6 mMol/l KCl, 2.2 mMol/l NaHCO3, 1.3 mMol/l CaCl2, 0.44 mMol/l KH2PO4, 0.3 mMol/l NaHPO4, 1 mMol/l EDTA, 1 mg/ml BSA, 5.6 mMol/l d(+)-glucose, and 1 mg/ml collagenase [pH 7.4]). Pancreata were digested for 7 min at 37°C and then hand-shaken and washed three times in ice-cold Hank’s solution. Islets were isolated by hand under dissecting microscope and pooled. Cells were maintained at 37°C in a humidified incubator in RPMI medium (11 mMol/l glucose) supplemented with 10% calf serum and penicillin (100 units/ml) and streptomycin (100 μg/ml). Insulinoma MIN6 cells were cultured in complete RPMI medium (10% FBS). MIN6 cells used in the present study were harvested at passages 30–40.

**Insulin release experiments.** Batch incubation was performed in duplicate on pancreatic islets (10/well) or MIN6 cells (1 x 10⁶ cells/well) incubated in glucose-free DMEM supplemented with d(+)-glucose (1, 7, or 16.7 mMol/l). Pls were added immediately before the glucose stimulus. Control cells were incubated with appropriate water or ethanol vehicle. Cells were incubated for 1 h at 37°C unless indicated, and medium was assayed and assayed for insulin content by radioimmunoassay. For reversibility experiments, islets were assayed for insulin release as described but with the addition of either the sulfonylurea glibenclamide (1 μMol/l) or a mix of glutamine and leucine (10 mMol/l each).

2-Deoxyglucose uptake experiments. MIN6 cells grown to 50–70% confluency were washed three times with Krebs-Ringer phosphate buffer, pH 7.0 (KRP; 100 mMol/l NaCl, 5 mMol/l KCl, 1 mMol/l MgCl2, 1 mMol/l Na2HPO4, and 1 mMol/l CaCl2). The cells were then incubated in the presence of [3H]-2-deoxyglucose (50 μMol/l) for 6 min at 37°C. Reactions were terminated by washing the cells three times with ice-cold KRP buffer containing 0.1 mMol/l phloretin. After air drying for 15 min, the cells were permeabilized with 1% Triton X-100 and aliquots were exposed for radioactivity determination by liquid scintillation counting and protein determination (BCA protein assay). Pls were added to the reaction 2 min before initiation of the transport assay. Nonspecific uptake was measured in the presence of 40 μMol/l cytochalasin B and subtracted from the experimental values. Uptake in this assay was determined to be linear over the 6-min assay interval. Data were analyzed as picomoles of 2-deoxyglucose incorporated per milligram of protein. All experiments were performed in triplicate with the data reported as a percentage of the uptake from control plates incubated in the absence of PI.

**Glucokinase activity.** Glucokinase activity in MIN6 cell extracts was assayed using a spectrophotometric enzyme-coupled assay as described previously (13). Briefly, MIN6 cells were harvested in 20 mMol/l phosphate buffer, pH 7.4, containing 0.02% BSA, 0.5 mMol/l EDTA, 5 mMol/l d-mercaptoethanol, 250 μg/ml, and 0.5% Triton X-100. The cells were homogenized in a glass-Teflon homogenizer, and glucose phosphorylating activity was determined by adding the cell extract to a cuvette containing 100 mMol/l Tris-HCl, 7.5 mMol/l MgCl2, 5 mMol/l ATP, 1 mMol/l NAD+, pH 7.4, with glucose-6-phosphate dehydrogenase and indinavir as noted. The rate of NADH formation was monitored by measuring the change in absorbance at 340 nm. Hexokinase and glucokinase activities were determined including 0.5 mMol/l and 16.7 mMol/l glucose, respectively. Glucokinase activity (activity at 20 mMol/l glucose minus hexokinase activity) represented ~75% of all glucose phosphorylating activity in MIN6 cell extracts.

3-O-methylglucose uptake experiments. The uptake of 3-O-methylglucose (3-MG) into MIN6 cells was measured using the oil stop-method described by Johnsen et al. MIN6 cells were cultured with PBS containing 3-O-methylglucose 14C-labeled urea (2 mMol/l, 0.5 mCi/mMol) as an intracellular space marker at 37°C for 20 min, followed by incubation at 37°C for 10 min. Assay tubes were prepared by placing a reaction stop mixture containing 0.1% SDS, 1 mMol/l glucose, and 10 mMol/l EDTA into microcentrifuge tubes. This was overlaid with a 4:1 mixture of dibutyl phthalate and dioctyl phthalate. Assays were performed in PBS containing 14C-labeled urea, and H2-labeled 3-MG (20 mMol/l) was placed above the oil layer. Reactions were initiated by adding aliquots of cell suspension ± 20 μMol/l indinavir to the assay tube and terminated by sedimentation through oil layer. Tritiated sugar incorporation was calculated as micromoles of 3-MG per liter of islet cell space, determined from 14C radioactivity, and expressed as a ratio of the radioactivity at equilibrium. Incorporation of nontransportable i-glucose under identical conditions is shown as negative control and represents background counts.

**Glucose utilization.** Glucose utilization in MIN-6 cells was assessed using the method of Ishihara et al. (12). Briefly, MIN6 cells were harvested with PBS containing 0.5 mMol/l EDTA for 5 min and incubated with glucose-free DMEM supplemented with 16.7 mMol/l glucose. Indinavir was added to a 1.5-mmol/l microtube containing 250 μl (1 x 10⁶ cells) 5 min before the addition of [5-H]-glucose. Aliquots of the assay mixture for insulin assay determination were removed immediately before the termination of the 1-h incubation by addition of 3 N HCl ethanol (1:4 [vol/vol]). The unstoppered tubes were incubated in sealed 20-ml glass scintillation vials that contained 500 μl of unlabeled water at 37°C for 16 h, and the radioactivity of the equilibrated water in the scintillation vial was determined by liquid scintillation counting. Background radioactivity was determined in identically treated samples incubated in the absence of MIN6 cells (representing <10% of total counts) and was subtracted from control and indinavir-treated samples. From titrated water controls, the efficiency of water equilibration was determined to be ~90% under these conditions.

**Animal procedures.** All animal procedures were approved by the animal studies committee at Washington University School of Medicine. Rats and mice were housed in the animal facility at Washington University and fed a standard diet and water ad libitum. In clamp experiments (outlined below), catheters were inserted into the left internal carotid artery and right jugular vein under methohexital anesthesia as previously described (15). Rats were allowed to recover from surgery for at least 4 days before experiments were performed, and rats were fasted overnight before each experiment.

**Hyperglycemic clamp experiments.** Clamps were performed on 200–300-g male Wistar rats as previously described (7) with noted differences. A constant infusion of indinavir (0.5 μMol/l kg⁻¹ min⁻¹) or water (for control animals) was initiated through the venous catheter 30 min before the start of the clamp procedure. Blood indinavir reached 25–30 μMol/l during the period of drug infusion, as measured by HPLC assay (16). After determination of fasting glucose concentrations, dextrose (50%) was infused through the venous catheter at a rate sufficient to maintain a plasma glucose level of 200 mg/dl during the assay period. Blood (20 μl) was sampled directly from the arterial catheter at times shown for determination of both blood glucose and insulin levels.

**RESULTS**

**Effects of HIV PIs on glucose-induced insulin release.** Purified mouse islets were exposed to varying concentrations of glucose, and insulin release was assayed after a 10-min incubation period. As shown in Fig. 1A, addition of the HIV PI indinavir (20 μMol/l) acutely inhibits insulin release at stimulatory glucose concentrations (7 and 16.7 mMol/l glucose) but has little effect on basal insulin release (1 mMol/l glucose). At high glucose (16.7 mMol/l), the half-maximal inhibitory concentration (IC₅₀) was calculated at ~2 μMol/l (Fig. 1B), a concentration within the reported therapeutic range of the drug (maximum concentration 12 μMol/l) (17). At indinavir concentrations as high as 100 μMol/l, this inhibitory effect plateaued at 35–40% of maximal insulin secretion. In addition to indinavir, significant block of glucose-induced insulin release was observed with other HIV PIs: amprenavir, nelfinavir, and ritonavir (20 μMol/l; Fig. 1C). Moreover, the acute inhibitory effect of the PI did not require preincubation with the drug and was observed in isolated rat islets acutely treated with indinavir (20 μMol/l; data not shown).

**Reversibility of indinavir-induced inhibition of insulin release.** Previously, HIV PIs have been shown accurately
and reversibly to block glucose uptake in cultured adipocytes and in heterologously expressing Xenopus oocytes and reversibly to block glucose uptake in cultured adipocytes and in heterologously expressing Xenopus oocytes and reversibly to block glucose uptake in cultured adipocytes and in heterologously expressing Xenopus oocytes.

and reversibly to block glucose uptake in cultured adipocytes and in heterologously expressing Xenopus oocytes.
as compared with control by unpaired Student’s t test. 

Cose (2-DOG) uptake in MIN6 cells was assayed in the presence of 5 or 10 μmol/l indinavir, and [3H]2-deoxyglucose uptake was assayed during a 6-min incubation period. In addition, insulin release and glucose utilization were assayed separately in MIN6 cells after a 1-h incubation with varying [indinavir]. Each symbol represents the mean ± SE of three separate experiments performed in triplicate. IC50 values of 1.1, 2.0, and 2.2 μmol/l were calculated for insulin release, glucose uptake, and glucose utilization, respectively, using a least-squares fit of the Hill equation as described in Fig. 1. Offsets of 18, 37, and 69% and Hill coefficients of 0.9, 1.8, and 0.9 were calculated for glucose uptake, insulin release, and glucose utilization, respectively. Inset: For comparison of only the indinavir-sensitive components, offsets were subtracted from data in A and replotted on a logarithmic scale, with the Hill coefficients and IC50 values the same as above. B: [3H]2-deoxyglucose (2-DOG) uptake in MIN6 cells was assayed in the presence of 5 or 20 μmol/l of the indicated PIs. Bars represent mean ± SE of three separate experiments performed in triplicate. *P < 0.05 and **P < 0.01 as compared with control by unpaired Student’s t test.

FIG. 3. Effects of indinavir on 2-deoxyglucose uptake in MIN6 cells. A: MIN6 cells were preincubated for 2 min with varying concentrations of indinavir, and [3H]2-deoxyglucose uptake was assayed during a 6-min incubation period. In addition, insulin release and glucose utilization were assayed separately in MIN6 cells after a 1-h incubation with varying [indinavir]. Each symbol represents the mean ± SE of three separate experiments performed in duplicate. IC50 values of 1.1, 2.0, and 2.2 μmol/l were calculated for insulin release, glucose uptake, and glucose utilization, respectively, using a least-squares fit of the Hill equation as described in Fig. 1. Offsets of 18, 37, and 69% and Hill coefficients of 0.9, 1.8, and 0.9 were calculated for glucose uptake, insulin release, and glucose utilization, respectively. Inset: For comparison of only the indinavir-sensitive components, offsets were subtracted from data in A and replotted on a logarithmic scale, with the Hill coefficients and IC50 values the same as above. B: [3H]2-deoxyglucose (2-DOG) uptake in MIN6 cells was assayed in the presence of 5 or 20 μmol/l of the indicated PIs. Bars represent mean ± SE of three separate experiments performed in triplicate. *P < 0.05 and **P < 0.01 as compared with control by unpaired Student’s t test.

Rescue of insulin release by downstream insulin secretogogues. The implied model of PI-induced β-cell dysfunction predicts that stimulation of insulin release downstream of glucose transport and metabolism should circumvent the inhibitory effect of PIs and restore insulin secretion. As described above, incubation of mouse islets with indinavir at nonstimulatory glucose concentration (1 mmol/l) does not suppress basal insulin secretion, although release at stimulatory glucose levels is significantly inhibited. A combination of the nonglucidic nutrients leucine and glutamine (10 mmol/l each), which serve as substrates for the Krebs cycle, stimulate insulin release at basal glucose levels (1 mmol/l). As shown in Fig. 4A, this stimulatory effect is mostly maintained in the presence of indinavir (20 μmol/l). Similarly, the insulinitropic sulfonylurea drugs act downstream of glucose transport and stimulate insulin secretion by inhibiting ATP-sensitive K+ channels, leading to a rise in the [Ca2+]i stimulus. Stimulation of insulin release with the sulfonylurea glibenclamide (1 μmol/l) is not significantly affected by 20 μmol/l indinavir at either basal (1 mmol/l) or stimulatory (16.7 mmol/l) glucose levels, consistent with the sulfonylurea acting downstream of PI block (Fig. 4B).

Characterization of the in vivo effect of indinavir on β-cell response. To assay the effect of sustained indinavir levels on β-cell function in vivo, we maintained catheterized Wistar rats under a hyperglycemic clamp and assessed the β-cell response to the glycemic challenge by measuring blood insulin levels. During the clamp, glucose infusion rate was allowed to vary to maintain a continuous blood glucose level of 200 mg/dl (Fig. 5A). Concurrently, rats were infused with either indinavir or water vehicle at a constant infusion rate of 0.5 mg·kg−1·min−1 (sustained indinavir level of ~25 μmol/l during the 120-min clamp experiment). A lower glucose infusion rate was needed to maintain the hyperglycemic clamp in PI-treated rats (data not shown), consistent with a PI-associated reduction in peripheral glucose disposal (7). It is interesting that the β-cell response to the glucose challenge is significantly impaired in the PI-treated animals with a considerable suppression of the first phase of insulin secretion (0–10 min; Fig. 5A, inset). A similar reduction in first-phase C-peptide levels in indinavir-treated animals was also observed (data not shown). However, despite the suppressed first-phase response, the second phase of insulin secretion (10–120 min) is maintained and slightly augmented in PI-treated rats. First-phase insulin release could be restored to near baseline levels within 2 h after a 30-min intravenous infusion of indinavir (data not shown).

DISCUSSION

The demonstration that PIs cause acute impairment of glucose-stimulated insulin secretion in rodent islet cells provides a novel mechanism for the development of altered glucose homeostasis during HAART. Although overt type 2 diabetes in treated human patients is much less common than insulin resistance, increases in fasting blood glucose levels have been clearly demonstrated (3). Because of the acuteness and reversibility of the changes in insulin sensitivity and β-cell function produced by PIs, failure to account for serum drug levels at the time of glucose tolerance testing may result in an underestimate of the proportion of patients who develop clinically significant changes in glucose homeostasis. Furthermore, type 2 diabetes is a polygenic disorder in which several minor perturbations in glucose sensing and insulin sensitivity contribute additively or synergistically. The same heterogeneous background that leads to the development of diabetes in the general population is present within patients who receive HAART. Therefore, although the acute changes in islet function observed in this study are unlikely to produce β-cell failure alone, these PI-induced changes may significantly contribute to the development of hyperglycemia in genetically predisposed individuals.

Although the observed inhibition of glucose transport function provides a potential mechanism for the acute effects on glucose-stimulated insulin release, the definitive role of impaired glucose transport remains to be deter-
Glucose sensing within the β-cell is generally believed to be the rate-limiting step in glucose-stimulated insulin secretion (21), and mutations in GLUT2 that result in impaired transporter function have been linked to the development of type 2 diabetes in humans (22). Recently, the critical role of GLUT2 in glucose-stimulated insulin secretion has been more firmly established by the generation of transgenic mice with homozygous deficiency of this transporter isoform. GLUT2-null mice are hyperglycemic with hypoinsulinemia and die within the first 3 weeks of life (23). Transgenic reexpression of GLUT2 restores normal glucose-stimulated insulin secretion (24). Thus, under conditions in which GLUT2 expression and/or activity is reduced, glucose transport may become rate-limiting, contributing to impaired glucose-stimulated insulin release. The reduction in first-phase insulin secretion in rats that are treated with indinavir under hyperglycemic clamp conditions is consistent with the effects observed in GLUT2-null mice (23). Importantly, loss of first-phase insulin secretion is an early clinical marker for the development of diabetes (25). The mechanism that accounts for the increased second-phase response is unclear but may be reflective of impaired insulin clearance in the indinavir-treated animals.

It remains possible that additional targets of PIs are responsible, independently or in conjunction with impaired glucose transport, to the abnormalities in insulin secretion observed with acute indinavir administration. If it is established that PIs selectively inhibit glucose transport in β-cells without producing significant effects on other cellular processes, then these compounds may prove useful in the investigation of normal β-cell function. Although transgenic studies have significantly contributed to the current understanding of the role of the pancreatic glucose transporter in insulin secretion, compensatory changes in protein expression in knockout animals are possible. The rapidity and reversibility with which changes in transporter function can be induced by PIs would obviate these concerns. Regardless of the molecular mechanisms involved, the current data demonstrate that PIs are capable of acutely inducing impaired β-cell glucose sensitivity in rodents, both in vitro and in vivo.

Future clinical studies will be required to determine

**FIG. 4.** Effect of PIs on insulin release by downstream insulin secretagogues. A: Insulin release from isolated mouse islets (10/well) was assayed in 1 mmol/l glucose with indinavir (20 μmol/l) or a glutamine/leucine mix (10 mmol/l each) alone or in combination. Bars represent mean ± SE of at least three separate experiments. For comparison, the inhibitory effect of indinavir (20 μmol/l) on glucose-induced insulin release (16.7 mmol/l) is shown. Data are representative of three independent experiments. Bars indicate mean ± SE of at least three separate experiments. P values by Student’s t test. B: Insulin release from MIN6 cells was assayed in 16.7 mmol/l glucose with indinavir (20 μmol/l) or glibenclamide (1 μmol/l) alone or in combination. Bars represent mean ± SE of three separate experiments. P values by paired Student’s t test.

**FIG. 5.** Effects of indinavir on insulin secretion during hyperglycemic clamps. Catheters were inserted into carotid arteries and jugular veins of 200- to 300-g male Wistar rats 4 days before experiments. A constant intravenous infusion of indinavir or water vehicle was started at a rate of 0.5 mg·kg⁻¹·min⁻¹ 30 min before each clamp. Indinavir levels reached levels of 25–30 μmol/l during the period of drug infusion. Hyperglycemic clamps were performed using dextrose (50%) infused through the venous catheter at a rate sufficient to maintain a plasma glucose level of 200 mg/dl during the assay period. During the hyperglycemic clamp (2 h), blood samples were taken every 2 min during the first 10 min and every 10 min thereafter and assayed for blood glucose (A) and insulin (B) levels. B: Inset: first-phase insulin response (0–10 min) in PI-treated compared with control animals. Control rats: n = 8; PI-treated rats: n = 8. *P < 0.05 by unpaired Student’s t test.
whether the effects of PIs on rodent β-cell function can be translated to human patients. Significant differences have been noted between rodent and human islets. For example, unlike human and rat islets, mouse islets typically do not produce a significant second-phase insulin response to hyperglycemic challenge (23,26). Second, the expression of glucose transporters may not be identical in human and rodent β-cells (27,28). Although the relative levels of GLUT2 versus GLUT3 expression are lower in human islets, both transporter isoforms seem to be sensitive to inhibition by PIs when expressed in Xenopus oocytes, albeit at concentrations above those typically achieved during HAART. Because we have observed significant differences in the IC_{50} values for PI-induced inhibition of glucose transport in mammalian cells compared with Xenopus oocytes (11), significant effects on GLUT3 may occur in human islets at physiologically relevant drug levels.

Taken together, the current data suggest that PIs may significantly contribute to the development of overt diabetes in patients who receive HAART over prolonged periods. A better understanding of the molecular targets of PIs that result in altered glucose homeostasis may assist attempts to design newer generations of antiretroviral agents that maintain their clinical efficacy without causing adverse metabolic effects.

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