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The Role of Protease Inhibitors in the Pathogenesis of HIV-Associated Lipodystrophy: Cellular Mechanisms and Clinical Implications

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Abstract

Metabolic complications associated with HIV infection and treatment frequently present as a relative lack of peripheral adipose tissue associated with dyslipidemia and insulin resistance. In this review we explain the connection between abnormalities of intermediary metabolism, observed either in vitro or in vivo, and this group of metabolic effects. We review molecular mechanisms by which the HIV protease inhibitor (PI) class of drugs may affect the normal stimulatory effect of insulin on glucose and fat storage. We then propose that both chronic inflammation from HIV infection and treatment with some drugs in this class trigger cellular homeostatic stress responses with adverse effects on intermediary metabolism. The physiologic outcome is such that total adipocyte storage capacity is decreased, and the remaining adipocytes resist further fat storage. The excess circulating and dietary lipid metabolites, normally “absorbed” by adipose tissue, are deposited ectopically in lean (muscle and liver) tissue, where they impair insulin action. This process leads to a pathologic cycle of lipotoxicity and lipoatrophy and a clinical phenotype of body fat distribution with elevated waist-to-hip ratio similar to the metabolic syndrome.

Keywords

protease inhibitors; lipodystrophy; dyslipidemia; insulin resistance; cardiovascular disease; mechanisms of toxicity; molecular pathology; clinical pathology; in vitro toxicology; endocrine system; pharmaceutical development/products

Introduction

Early reports of body composition changes in patients infected with HIV (Grunfeld and Kotler 1992) suggested that AIDS wasting led to loss of lean tissue, but that abdominal fat may have been to some extent protected. However, a later study of body composition using DEXA measurements did not confirm these early impressions of a protected abdominal fat compartment (Mulligan et al. 1997). Body composition changes were again reported with the advent of HIV protease inhibitor containing highly active antiretroviral therapy (HAART). These changes consisted of an apparent fat redistribution with a relative increase in abdominal fat, and peripheral lipoatrophy (Waters and Nelson 2007), accompanied by insulin resistance and dyslipidemia (Carr et al. 1998; Grinspoon 2005).

The aggregate of biochemical disturbances (Table 1) observed in many patients on PI-containing HAART resembles those of the metabolic syndrome as defined for the general population (Grundy et al. 2004; Grunfeld and Tien 2003) and suggests that cardiovascular (CV) health may be similarly at risk in HIV-infected patients on long-term HAART (Friis-Moller et al. 2003). An increase in all or some of the components of the metabolic syndrome and in adverse cardiovascular events has been reported in the years following initiation of HAART (Falasca et al. 2007; Friis-Moller et al. 2003; Samaras et al. 2007; Wand et al. 2007). More recently, two long-term prospective studies, one of 23,437 patients (Friis-Moller et al. 2007) and the other of 2386 patients (Kaplan et al. 2007), showed that myocardial infarction is directly correlated with the use of PIs, but not with other components of HAART.

Additionally, the interactions between HIV and the host environment introduce multiple layers of complexity, with independent effects on metabolic and body composition outcomes. For example, the atherogenic effects of HIV infection, AIDS, and antiretrovirals (ARVs) are not easily separable from underlying and preexisting CV risk factors such as age, extended life expectancy consequent to successful treatment, changing demographics of the HIV epidemic, as well as genetic predisposition to dyslipidemia, insulin resistance, and abdominal obesity.

Perhaps the most significant difference between fat redistribution in HIV and the metabolic syndrome is that in the latter case, total body fat and visceral fat compartments are uniformly increased, whereas fat redistribution in HIV has no single pattern for regional fat loss or gain (Mulligan et al. 2006). Nevertheless, similar to the metabolic syndrome, regional fat distribution in HIV consistently results in a relative increase in visceral fat as a percentage of total and peripheral fat, regardless of total net fat gain or loss (Safran and Grunfeld 1999). In other words, body fat changes may ultimately be a consequence of long-term changes in metabolic programming that favor a relative increase in the index of central adiposity or the ratio of trunk fat to peripheral fat with a continuum from early metabolic abnormalities to subsequent body composition changes. The concept is supported by emerging data where dyslipidemia may predict fat redistribution (Jacobson et al. 2005; Lee, Rao et al. 2004; Mallon et al. 2003; Noor Dezi et al. 2004), which suggests that drug therapy could act as a trigger for a pathophysiologic cascade.

Treatment of HIV infection with nucleoside analogs (nucleoside reverse transcriptase inhibitors, or NRTIs) promotes the development of many adverse effects, including peripheral neuropathy, pancreatitis, and peripheral fat wasting (Barbaro 2007; Flint 1994; Lee et al. 2003). The proposed mechanism is inhibition of mitochondrial DNA synthesis (Brinkman et al. 1999; Kakuda et al. 1999), but many questions remain unresolved. For example, it is unclear why individual NRTIs differ in the tissues they affect, and whether or not factors other than depletion of mitochondrial DNA may be of equal or greater

importance to NRTI toxicity (Lund et al. 2007; Lund and Wallace 2004; Mallon 2007; Villarroya et al. 2005). The adverse effects of NRTIs on their own do not completely explain the syndrome of metabolic disturbances observed in patients on HAART. It is much more likely that a combination of factors involving NRTIs and other drugs used in HAART are involved (Mallal et al. 2000). In this review, we outline known mechanisms for a direct clinical impact on intermediary metabolism of the other major component of HIV therapy, the protease inhibitor class of drugs, and then consider possible pathways that may link glucose and lipid abnormalities to fat redistribution within the context of an HIV-associated inflammatory milieu. This article is not intended to be a comprehensive review of clinical data, nor of clinical practice in treatment of the metabolic syndrome. Several recent reviews cover these topics (Dube 2006; Dube et al. 2003; Wohl et al. 2006).

Insulin Resistance

Although insulin resistance is commonly observed in chronic infectious states, early reports, using euglycemic insulin clamps and calorimetry in untreated, symptomatic HIV-infected patients, suggested an adaptive increase in insulin sensitivity by fat (Hommes et al. 1991a, b). However, following the availability of treatment for HIV, studies of this kind in untreated patients were not repeated or confirmed by other groups.

It is now accepted that HAART itself is accompanied by an increased risk of type 2 diabetes. Data in patients without evidence of AIDS wasting or lipoatrophy reveals a higher prevalence rate of type 2 diabetes in the HIV-infected population compared to an HIV-seronegative cohort independent of HAART, and the highest prevalence in patients on HAART (Brown et al. 2005).

The evidence supporting a direct role for PIs in insulin resistance and type 2 diabetes comes from prospective studies of patients initiating PI-based HAART (Mulligan et al. 2000). More definitive studies in healthy volunteers administered a PI for short periods suggest that insulin resistance is induced after a relatively short term (Noor et al. 2001), and this effect has been observed even after a single therapeutic dose with some PIs (Lee, Mafong et al. 2004; Noor et al. 2001; Noor et al. 2002). Although inhibition of peripheral glucose uptake appears to be acute with some PIs (Lee et al. 2006; Noor et al. 2006; Noor, Parker et al. 2004), changes in hepatic glucose production may only follow more chronic treatment (Haugaard et al. 2005; Lee, Seneviratne et al. 2004), suggesting additional mechanisms (Lee, Rao, Schwarz et al. 2005; Noor et al. 2006; Noor, Parker et al. 2004; van der Valk et al. 2001). Even more likely, it reflects physiologic adaptations in the form of hypertriglyceridemia that occur in response to chronic inhibition of peripheral glucose uptake (Grunfeld et al. 1991). Thus, it appears that both direct and indirect mechanisms may be at play for insulin resistance at different times during the course of therapy with a PI.

Dyslipidemia

Lipid and lipoprotein abnormalities were found in HIV infected patients with or without AIDS prior to the HAART era and linked to the acute phase response and inflammatory mediators such as interferon- α (Grunfeld et al. 1991) or TNF α , which may promote dyslipidemia (Feingold et al. 1993). During the early stages of HIV infection, levels of both low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) are lower than matched controls. With advanced HIV and AIDS, triglycerides are significantly higher and LDL particles become smaller in density and of the more atherogenic variety (Feingold et al. 1993). With initiation of HAART, the plasma lipid profile showed an increase in total and LDL-C, possibly suggesting a return to pre-seroconversion levels (Riddler et al. 2003). Treatment with PIs as a class in HIV-infected

patients is associated with significant increases in total and LDL cholesterol and triglycerides relative to pretreatment baseline levels (Mulligan et al. 2000).

Direct evidence that PIs promote dyslipidemia (hypertriglyceridemia, increase in total and LDL-C and decrease in HDL-C) derives from short-term studies (≤ 4 weeks) with RTV (Purnell et al. 2000), or LPV/RTV (Lee, Rao, and Grunfeld 2005) in HIV-seronegative men. However, the effect of PIs on serum lipid levels appears to be dissociated from their HIV-inhibitory effects, since not all PIs induce dyslipidemia in HIV-negative (Noor et al. 2001) or -positive patients (Jemsek et al. 2006).

Fat Redistribution

Invoking a single mechanism as cause for lipodystrophy in HIV remains elusive. One possibility is that there is not one, but several discrete syndromes affecting HIV-infected patients, such as separate syndromes leading to peripheral lipoatrophy versus central lipohypertrophy (Mulligan et al. 2006). An early absolute increase in truncal fat associated with PI treatment (Dube et al. 2005; Mallon et al. 2003) suggests that visceral fat may be relatively resistant to the wasting effect of NRTIs and PIs but may be affected by increases in circulating lipids. There is strong evidence that visceral fat is transcriptionally (Katzmarzyk et al. 1999) and metabolically (DiGirolamo et al. 1992) distinct from subcutaneous fat.

Regardless of the etiology, the pathophysiologic signals that promote body fat changes in HIV promote alterations in cellular metabolism and simultaneously inhibit normal storage in favor of ectopic visceral fat deposition (Unger 2003). The final phenotype, whether lipoatrophic or lipohypertrophic, likely depends on the amount of body fat at baseline, as well as total energy balance. Those with higher total body fat prior to HAART and a positive energy balance may present with further increases in trunk, including visceral, breast, and dorsocervical fat, whereas those with lower total body fat and neutral or negative energy balance may present with decreased limb or facial fat without ectopic fat deposition (i.e., visceral adiposity) (He et al. 2005). Most importantly, however, the final phenotype, regardless of the initial stage (net total fat gain or fat loss), consistently results in relative central adiposity.

This hypothesis is consistent with cohort studies (Bacchetti et al. 2005) and prospective longitudinal studies (Wand et al. 2005), which found that peripheral fat loss and central fat gain result from independent metabolic pathways. The trigger that turns on the metabolic cascade, however, appears to be specific to the drugs, as some body fat changes may ameliorate after switching to newer therapies (Arranz Caso et al. 2005; Haerter et al. 2004; McComsey et al. 2004; Shlay et al. 2005). Thus, there seems to be both a putative mechanism and a differential role for specific drugs in triggering metabolic programs involved in fat formation and distribution.

PIs and Glucose Transport

The association of PI-based therapy with hyperglycemia and insulin resistance in HIV-infected patients is well established (Behrens et al. 1999; Dube et al. 1997; Walli et al. 1998). In animal as well as in clinical studies of non-HIV-infected human subjects, PIs were shown to acutely inhibit insulin-stimulated glucose disposal (Hruz et al. 2002; Lee, Mafong et al. 2004; Noor et al. 2002). The effect on glucose disposal is both rapid and reversible, suggesting a direct blockade of glucose uptake through the glucose transporter isoform 4 (GLUT4, SLC2A4) (Hertel et al. 2004; Noor et al. 2006). Additional effects on insulin signaling (Cammalleri and Germinario 2003; Rudich et al. 2005; Schutt et al. 2000), SREBP

processing (Caron et al. 2001), and adipokine secretion (Xu et al. 2004) have also been proposed as mechanisms for the inhibition of glucose disposal.

A direct effect on glucose uptake by IDV, RTV, and APV was demonstrated by Murata and colleagues in 3T3-L1 adipocytes. Blockade of glucose uptake was rapid and concentration dependent, but the PIs tested did not impair either insulin action or the translocation of GLUT4 to the plasma membrane (Murata et al. 2000). Inhibition was specific for the GLUT4 isoform, with no inhibition of GLUT1 and minimal inhibition of GLUT2 (Murata et al. 2000, 2002). Thus, direct inhibition of GLUT4 represents the earliest identified direct effect of PIs, or any other drug used in treatment of HIV, on a metabolic target (Figure 1).

PIs as a class, however, do not equally inhibit glucose disposal. In primary rat adipocytes, APV (amprenavir), RTV (ritonavir), or the more therapeutically appropriate combination of LPV (lopinavir) with RTV all inhibited glucose uptake, whereas ATV (atazanavir) displayed no inhibition compared to controls at therapeutic concentrations under euglycemic, hyperinsulinemic clamp conditions in rats (Yan and Hruz 2005). Therapeutic levels (5–10 μ M) of APV, LPV/RTV, and RTV, acutely inhibited both peripheral glucose disposal and glucose uptake in muscle. In the presence of ATV, glucose disposal in rats was similar to controls even at high levels (25 μ M) of drug (Yan and Hruz 2005).

In one study, an increase in glucose uptake by subcutaneous fat in HIV patients with lipoatrophy who had been treated with a HAART regimen including a protease inhibitor for more than three months was seen only in the presence of lipoatrophy, not in patients similarly treated but without evidence of fat wasting (Hadigan et al. 2006). It is possible that this is a late compensatory mechanism for inhibition of GLUT4 by the PIs. Hadigan and colleagues suggest, but do not demonstrate, an upregulation of GLUT1 expression to account for the late increase in glucose uptake (Hadigan et al. 2006). We propose that inhibition of adipose glucose uptake by protease inhibitors is an early and important factor predisposing patients to lipoatrophy as well as peripheral insulin resistance. Clearly, additional factors are required for lipoatrophy to develop; especially important among these factors is treatment with NRTIs (Buffet et al. 2005; Mallon et al. 2003), but the direct association of PIs with white adipose tissue loss has been confirmed both clinically (Dube et al. 2005; Heath et al. 2002) and preclinically in the mouse (Prot et al. 2006).

Recent studies have begun to elucidate the relationship between PI structure and the inhibition of GLUT4-mediated glucose transport. All currently available PIs share a similar phenylalanine-like core structure flanked by hydrophobic moieties (Figure 2). Hertel and colleagues screened a panel of di- and tripeptides for their ability to inhibit glucose uptake in primary rat adipocytes (Hertel et al. 2004). The peptides that inhibited GLUT4 all contained a highly aromatic core peptide flanked by hydrophobic moieties similar to the structure contained within the PIs (Figure 2). The successful affinity labeling of GLUT4 with a photactivatable derivative peptide, together with the demonstration that IDV protects against labeling, suggest that the peptide binds to the same site on GLUT4 as some PIs (Hertel et al. 2004).

The identification of GLUT4 as an early and direct molecular target of these drugs provides a clear opportunity for improved PI drug design. Further elucidation of the structure and function relationship between PIs and GLUT4, including the identification of the specific region(s) of GLUT4 bound by PIs, may facilitate the development of a newer generation of drugs that retain efficacy against the HIV protease without affecting GLUT4 transport activity. A question raised by these structure and function studies is why ATV, which contains a peptidomimetic core, does not affect insulin sensitivity in vitro or in vivo (Noor, Parker et al. 2004; Yan and Hruz 2005). One of the explanations for the lack of GLUT4

binding inhibition by ATV is the possibility of steric inhibition by an additional pyridine ring attached to the phenylalanine-like residue as well as differences in hydrophobicity, which prevent binding to the insulin-sensitive glucose transporter. Further studies are needed to explore these differences among individual PIs.

Effects of PIs on Pancreatic β -Cell Function

In addition to their effects on peripheral insulin resistance through inhibition of GLUT4, PIs have also been implicated in glucose intolerance through impairment of β -cell function. In vitro as well as in HIV-infected patients, significant decreases in β -cell function and first-phase insulin release are found following exposure to PIs (Koster et al. 2003; Woerle et al. 2003).

Recently we quantified insulin secretion in MIN-6 murine pancreatic β -cells treated with various PIs at therapeutic levels (10–20 μ M) and demonstrated differential effects (Flint et al. 2005). Lopinavir and RTV dramatically reduced glucose-stimulated insulin secretion, whereas IDV and ATV had little or no effect. PIs also inhibit amino acid-stimulated insulin secretion in vitro; this effect varied widely among the PIs tested, with a rank order of potency similar to that observed for glucose-stimulated insulin secretion (Flint et al. 2005). Our findings were consistent with other in vitro studies showing a concentration-dependent effect by RTV or NFV (nelfinavir), but not by IDV (indinavir) or APV (Dufer et al. 2004; Zhou et al. 2006).

The exact mechanism by which PIs inhibit insulin secretion remains unknown (Figure 3). Glucose uptake through GLUT2 in these cells was not affected, and β -cells do not express GLUT4. The hypothesis supports a mechanism independent from glucose sensing or uptake. Other indirect mechanisms such as PI-induced elevation in triglycerides and fatty acids may also adversely affect pancreatic β -cell function in vivo and after extended treatment (Dubois et al. 2004).

These findings in pancreatic β cells and adipocytes point to a multifactorial etiology for the effect of PIs on glucose metabolism. Possibly the most important event is GLUT4 blockade in peripheral tissues, followed by direct effects on insulin secretion by pancreatic β -cells. Both pathways are affected by individual PIs to varying degrees, but there is a clear pattern indicating that certain PIs (e.g., IDV, LPV, and RTV) are more potent than others (e.g., ATV, APV, and SQV).

PIs and Lipid Metabolism: The Unfolded Protein Response

Several mechanisms for PI-induced dyslipidemia have been proposed. They include but are not limited to decreases in hepatic and lipoprotein lipase (LPL) activity (Purnell et al. 2000), fractional catabolic rate of VLDL-triglyceride (Shahmanesh et al. 2005), or fat clearance owing to impaired LPL-mediated clearance of triglyceride-rich lipoproteins (Sekhar et al. 2005).

PIs also have a direct effect on lipoprotein production under the regulation of the sterol regulatory element-binding proteins (SREBPs) (Riddle et al. 2001), which function as intracellular lipid sensors (Horton et al. 2002). SREBPs are activated when intracellular lipid levels drop; they are then transported from the ER to the nucleus, where they up-regulate the expression of genes involved in cholesterol synthesis, transport, and triglyceride and fatty acid biogenesis. In the nucleus, the SREBPs are degraded by the proteasomes (Hirano et al. 2001). Several PIs, including IDV and RTV, have been shown to activate SREBP-1 and SREBP-2 (Riddle et al. 2001; Williams et al. 2004) in hepatocytes. Some PIs may also

affect SREBP translocation into the nucleus facilitated by lamins A and C and resulting in SREBP-1 mislocalization (Caron et al. 2001; Caron et al. 2003; Coffinier et al. 2007).

A consequence of SREBP activation is an excessive accumulation of intracellular cholesterol in the ER membranes, which is detrimental for maintaining intracellular homeostasis and can trigger an intracellular mechanism for sensing stress, regulating cell growth, differentiation, and apoptosis that is known as the unfolded protein response (UPR) (Xu et al. 2005; Zhang and Kaufman 2004). The concentration of misfolded or unfolded proteins in the ER increases and triggers cellular signaling pathways to restore homeostasis. This process involves translational attenuation, up-regulation of ER chaperones, and degradation of unfolded proteins. Although these pathways are aimed at restoring homeostasis, in the extreme, they ultimately result in apoptosis.

Proteasomes are intracellular factors responsible for preventing excessive accumulation of unfolded proteins, managing the interaction of protein synthesis, folding, and trafficking (Kaufman et al. 2002; Travers et al. 2000). The proteasome is also linked with lipid metabolism through several mechanisms including apolipoprotein B degradation (Liang et al. 2001), SREBP degradation in the nucleus (Hirano et al. 2001), and gene activation or repression (Nawaz and O'Malley 2004). At least three PIs—NFV, RTV, and SQV—have been shown to inhibit the proteasomal 20S subunit (De Barros et al. 2007; Hamel et al. 2006; Pajonk et al. 2002; Parker et al. 2005; Schmidtke et al. 1999). In general, PIs differentially inhibit proteasome chymotryptic activity in adipocytes and hepatocytes (Parker et al. 2005).

Proteasome inhibition and the resultant activation of UPR would therefore seem to be a common link between the effects of PIs in adipocytes and hepatocytes, cells with an important role in the metabolic effects of these drugs. However, the acute effects of the PIs are strikingly different in adipocytes and hepatocytes. PIs inhibit triglyceride synthesis and glucose transport in adipocytes; conversely, they increase triglyceride biosynthesis in hepatocytes without affecting glucose uptake. Lipogenic gene expression is also differentially altered (Parker et al. 2005). In hepatocytes, less than 1% of profiled RNA sequences were increased or decreased by more than two-fold, and nearly four times as many genes were induced than repressed. A detailed analysis revealed induction of multiple genes involved in lipid metabolism and gluconeogenesis. Similar *in vivo* effects on hepatic lipogenic gene expression following RTV treatment in the rat have been reported (Lum et al. 2007). The opposite effect was observed in adipocytes, with more genes repressed than induced. Key lipogenic transcription factors (PPAR- β and SREBP-1c) and multiple enzymes involved in lipid biosynthesis were down-regulated in the adipocyte. Differences between the effects of PIs were apparent in both adipocytes and hepatocytes, with some PIs such as ATV having the least impact in both cell types (Parker et al. 2005).

The contrasting effect on lipid synthesis in the two cell types is particularly important. In adipocytes, suppression of glucose uptake, and thereby energy storage, is associated with diminished lipid synthesis (Figure 1). Inhibition of the proteasome in hepatocytes extends the longevity of the key SREBPs involved in regulating lipid synthesis (Figure 4). Thus, at the cellular level, these results suggest a metabolic shift in the fat energy storage from the adipocyte to the hepatocyte, and possibly other nonadipose tissues.

It is interesting to note that intracellular events mirror the clinical manifestations of fat redistribution where lipoatrophy, in the form of depleted adipocyte triglyceride stores, is frequently observed at the same time as hyperlipidemia and increased hepatic lipid secretion. Taken together, these observations suggest that both proteasome inhibition and activation of UPR by stress signals such as chronic HIV infection or by drugs such as PIs can

independently affect the normal regulation of cellular fuel sensing and storage. Long-term disturbance in cellular fuel sensing may underlie the pathophysiology of fat redistribution.

Lipotoxicity: From Insulin Resistance and Dyslipidemia to Fat Redistribution

The hypothesis of sequential injury, from cellular to multi-organ level, offers a pathophysiological pathway for the clustering of metabolic disturbances observed in HIV-infected patients on PI-based ARV therapy. It is postulated that multiple factors including HIV infection; the subsequent inflammatory response to chronic infection; PI effects on glucose, lipid, and amino acid metabolism; the host's genetic predisposition; and dietary/lifestyle factors interact both sequentially and in concert to effect morphological changes in body composition (Nolte et al. 2001; Shikuma et al. 2005; Summers and Nelson 2005; Unger 2003). At the cellular level, the initial signal is triggered by an induction of ER stress, which triggers a response specific to the cell, an inhibition of glucose uptake in adipocytes, inhibition of insulin release by pancreatic β -cells, and dysregulation of SREBP activity in the hepatocyte (Figure 5). As a consequence of alteration in protein expression, homeostasis in the oxidation of energy substrates is impaired, with a shift toward increased lipolysis (Reeds, Cade et al. 2006). The resulting imbalance in the intracellular rate of lipogenesis versus lipolysis can affect the adipocyte and the hepatocyte, with consequences for total body fat mass and distribution. Ultimately, this represents a failure in fuel sensing and regulation that can in turn further affect both lipid and glucose metabolism, triggering a vicious metabolic cycle.

The initial mitochondrial dysfunction (Shikuma et al. 2005)—either directly by HIV drugs (Reeds, Yarasheski et al. 2006), such as the NRTIs (Pinti et al. 2006), or via glucose uptake inhibition in adipocytes and muscle—impairs intracellular lipid homeostasis that normally protects against accumulation of fatty acids and prevents lipotoxicity in the organs. Elevated cytokines and inflammatory mediators, such as IL-8 (Reeds, Cade et al. 2006) or TNF- α observed in chronic HIV infection, also induce ceramide production and promote intracellular palmitoyl-CoA accumulation (Summers and Nelson 2005), with independent effects on promoting abnormal lipid metabolism. Once triggered, the system would no longer be able to confine dietary energy sources or excess lipid to the adipocytes specifically designed to store calories for the long term.

Lipotoxicity is a consequence of excess deposition of free fatty acids (FFAs), in particular palmitic acid and ceramides, in insulin responsive tissues (adipose, skeletal muscle, liver, heart) and impairs insulin signaling in these tissues (Summers and Nelson 2005; Unger 2003). Intramyocellular lipid (IMCL) content, a common form of ectopic lipid deposition, has been closely associated with insulin resistance after acute elevation of FFA, palmitoyl CoA, and ceramides (Nolte et al. 2001; Straczowski et al. 2004; Summers and Nelson 2005). Similarly, lipotoxicity in the form of ectopic fat deposition in the liver (i.e., hepatic steatosis) has been associated with increased hepatic glucose output in HIV-infected people (Hadigan et al. 2006).

Though insulin resistance is an integral part of HIV dyslipidemia, it may not always be detectable by standard clinical studies, such as fasting blood glucose concentration. Provocative testing such as the hyperinsulinemic euglycemic clamp procedure may be required to detect early evidence of multi-organ insulin resistance (Reeds, Yarasheski et al. 2006). The results of such studies suggest that most subjects with HIV-lipodystrophy who present with dyslipidemia also have evidence of impaired insulin action and, therefore, are at increased risk of developing diabetes. Thus, increased triglyceride and FFA in an HIV-infected individual may be an important early clinical marker of multi-organ lipotoxicity.

Adipocytokines may play a facilitative role in the long-term partitioning of lipids ectopically in the liver and skeletal muscle (Staiger et al. 2003). Adiponectin, the most abundant protein secreted by adipocytes, increases fatty acid oxidation and insulin-mediated suppression of hepatic glucose production (Berg et al. 2001). Low plasma adiponectin concentrations are associated with insulin resistance and impaired glucose tolerance in subjects with lipodystrophy and metabolic disorders in HIV-infected men receiving antiretroviral therapy (Vigouroux et al. 2003) or without HIV infection (Weyer et al. 2001). In one study, whole body glucose disposal was directly related to adiponectin, which was itself directly related to the area of visceral adipose fat, suggesting perhaps that this fat depot may have a critical role in regulating glucose metabolism via the action of adipokines (Hadigan et al. 2006). The exact mechanisms are unknown.

Clinical Implications

The mechanisms of metabolic dysfunction in HIV-infected patients resulting from PI-based ARV therapy discussed in this review (GLUT4 blockade and endoplasmic reticulum stress/unfolded protein response) are presumed to operate concurrently at the cellular level in various organs and systems throughout the body (Carr et al. 1998). Although the functional links between these mechanisms and the observed metabolic alterations are not fully understood, they provide a unifying explanation for the diverse set of metabolic manifestations in HIV-infected patients treated with PIs (Figure 5). As a clinical matter, observations at the level of the organs and systems that are initially affected directly by the PIs and the physiological pathway(s) to the ultimate target organ or overall metabolic dysregulation are of value in the development of diagnosis and treatment strategies. Shown in Table 1 are the organs and systems that, in order of priority, are the key sites of HIV- and PI-related metabolic dysfunction, with physiological consequences and clinical end points associated with each.

In adipose tissue, decreased postprandial fat clearance, increased FFA release, and lipodystrophy are the consequences of decreased glucose uptake, mitochondrial damage, and inhibition of triglyceride synthesis. Circulating FFAs deposit with toxic effects in various organs—liver (steatosis), skeletal muscle (increased intramyocellular lipid), and pancreas (α cell toxicity)—which promotes insulin resistance in multiple organ systems. Increased lipid and glucose production in the liver promotes dyslipidemia and proatherogenic effects in the vascular endothelium and increases the rate of atherosclerosis and arteriosclerosis. Postprandial glucose and insulin concentrations increase as a consequence of impaired glucose transport via GLUT4 and the accumulation of intramyocellular lipids, which promotes the development of diabetes in otherwise susceptible subjects. Decreased insulin secretion by the pancreas in the setting of overall increased resistance to insulin action also promotes hyperglycemia. Cytokine production in the macrophages of the cellular immune system increases foam cell activity with proatherogenic effects (Charo and Taubman 2004). The exact outcome varies considerably owing to multiple other modifiers that include genetic predisposition, diet and lifestyle, existence of comorbid conditions, and CVD risk-related behaviors.

In summary, insulin resistance, dyslipidemia, and fat redistribution are common clinical complications in the current HAART era. Many factors, which include, but are not limited to, antiretroviral agents, contribute to their development. The metabolic and body composition changes ultimately contribute to accelerated cardiovascular disease. Some features may be avoided by careful selection of antiviral agents and can be treated effectively with early detection. The key questions are the extent of cardiovascular risk associated with the morphologic and metabolic alterations, and the challenge of effective new HAART regimens that minimize or eliminate drug-related metabolic complications.

Abbreviations

AIDS	acquired immunodeficiency syndrome
APV	amprenavir
ARV	antiretroviral
ATV	atazanavir
CV	cardiovascular
DEXA	dual-energy x-ray absorptiometry
FFA	free fatty acids
HAART	highly active antiretroviral therapy
HDL-C	high-density lipoprotein cholesterol
HIV	human immunodeficiency virus
IDV	indinavir
LDL	low-density lipoprotein
LPV	lopinavir
NRTI	nucleoside reverse transcriptase inhibitor
PI	protease inhibitor
RTV	ritonavir

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Figure 1.

Protease inhibitors inhibit adipocyte glucose uptake. Insulin binds to the adipocyte insulin receptor and induces the mobilization of transporters for glucose (GLUT4) and fatty acids* (FATP1) from the cytoplasm to the cell membrane. The major effect of PIs on adipocytes is direct inhibition of adipocyte glucose uptake, resulting in a significant reduction in triglyceride (TG) synthesis (Parker et al. 2005). The graph shows the inhibition by increasing concentrations of ATV and LPV of insulin-stimulated glucose uptake (ten minutes of exposure to drug) and triglyceride synthesis (sixteen hours of exposure to drug) in cultured, fully differentiated human subcutaneous adipocytes, using previously described methods (Noor et al. 2006; Parker et al. 2005). Reduced triglyceride synthesis contributes to adipocyte shrinkage and lipoatrophy. Proteasome inhibition in adipocytes (Parker et al. 2005) will additionally cause the accumulation of unfolded proteins and induce the ER stress response (Ron and Walter 2007), which, together with glucose deprivation, may be the dominating mechanism for adipocyte degeneration and lipoatrophy. (*Note: fatty acids metabolized by adipocytes are mainly derived from free fatty acids bound to albumin or lipoprotein lipase [LPL]-mediated hydrolysis of triacylglycerol-rich lipoprotein particles such as chylomicrons or very-low-density lipoproteins [VLDL] [Large et al. 2004]).

Figure 2.
Shared structural features of the HIV protease inhibitors. Squares represent the core peptidomimetic structure found within all HIV PIs.

Figure 3.

Protease inhibitors inhibit pancreatic β-cell glucose-stimulated insulin release. Glucose uptake via the GLUT2 transporter is the key driver of insulin release by the pancreatic β cell. Unlike the GLUT4 glucose transporter in adipocytes and muscle, the GLUT2 transporter is not inhibited by PIs in our studies with the MIN6 insulinoma cell line, but insulin release is nevertheless inhibited (Flint et al. 2005). In the β cell, elevated glucose increases the ATP:ADP ratio, closing the ATP-dependent potassium channel, depolarizing the cell membrane, and leading to the opening of voltage-dependent calcium channels (VDCC). Increasing intracellular concentrations of calcium trigger the release of insulin. Protease inhibitors, like ritonavir, may block the calcium channel (Flint et al. unpublished data), inhibiting the signaling cascade that leads to the release of insulin.

Figure 4.

Protease inhibitors stimulate hepatic lipid synthesis by maintaining the nuclear activity of the SREBP regulatory transcription factors. GLUT2 is the major hepatocyte glucose transporter, but unlike GLUT4 in the adipocyte, GLUT2 appears to be unaffected by the PIs (Flint et al. 2005). There is also no evidence that PIs affect the transport of fatty acids by FATP2 or FATP5. Instead, PIs affect hepatocyte metabolism by extending the activity of transcription factors involved in regulating lipid synthesis (Parker et al. 2005). The most important of these factors are the sterol response element binding proteins (SREBPs) that, in the resting state, remain in the endoplasmic reticulum membrane in close association with SCAP, the sterol-sensing escort protein (Horton et al. 2002). When sterols are depleted, however, the SREBP-SCAP complex moves from the endoplasmic reticulum into the Golgi, where Site 1 and 2 proteases (S1P, S2P) clip the active basic helix-loop-helix (bHLH) domain of SREBP out of the membrane. The SREBP transcription factor then enters the nucleus and binds to response elements of lipogenic genes, inducing their transcription and ultimately increasing the synthesis of cholesterol and triglycerides. SREBP half-life in the nucleus is determined by its rate of degradation by nuclear proteasomes (Hirano et al. 2001). PIs inhibit proteasome proteolysis, extending the half-life of SREBP, thus further increasing the synthesis of hepatic lipids (Parker et al. 2005; Riddle et al. 2001).

Figure 5.

Early direct effects of HIV protease inhibitors on intermediary metabolism. Glucose uptake inhibition leading to reduced metabolism dominates the effect in myocytes and adipocytes, but stimulation of lipogenesis is the direct and dominant effect in hepatocytes. The acquired insulin insensitivity in peripheral tissues is exacerbated by reduced pancreatic β -cell function. Once triggered, the cascade is also likely to be further aggravated by inflammatory mediators produced in the presence of chronic HIV infection. The result is hyperlipidemia, coupled with lipo-toxicity in hepatocytes and lipoatrophy/insulin resistance in fat tissues. These processes together can completely explain the lipodystrophy and “metabolic syndrome” observed in HIV patients treated with PIs. However, treatment normally combines drugs from several antiviral classes, including the NRTIs, NNRTIs, and more recently, drugs targeting novel mechanisms such as HIV attachment. It is unfortunate that several of the NRTIs, especially the thymidine analogs, can also inhibit the differentiation of new adipocytes from preadipocytes (Caron et al. 2004; Roche et al. 2002), though there is no evidence that they affect metabolism in the mature adipocyte. It is possible that this mechanism is responsible for reports of lipoatrophy in patients who have not been treated with PIs (Mallal et al. 2000). The combined effect of NRTIs and PIs may be responsible for the reported high prevalence of lipodystrophy, and especially lipoatrophy, in HIV patients (Estrada et al. 2006; Miller et al. 2003; Samaras et al. 2007).

TABLE 1

Metabolic effects of HIV protease inhibitors acutely and with possible consequences targeting the end organs or disease.

Target organ	Primary defect	Possible consequence	End organ damage/disease
Adipose Tissue	↓ Glucose uptake	Adipocyte apoptosis and lipolysis	Lipoatrophy
	↓ Triglyceride synthesis	↑ circulating FFA Alterations in adipocytokines	Muscle (↑ IMCL), Visceral fat deposition, Lipotoxicity
Liver	↑ apoB	↑ Lipid	Liver (steatosis), arteriosclerosis
	↑ VLDL	↑ Triglyceride	Pancreatitis Atherosclerosis
	↑ HGP	↑ Fasting glucose	Type 2 Diabetes
	↑ SREBP-1c	↑ Triglyceride, ↑ Cholesterol	Insulin resistance, Atherosclerosis
Muscle	↓ Glucose uptake	↑ Postprandial glucose	Glucose Intolerance and Type 2 Diabetes
	↑ IMCL	↑ Insulin resistance	Increased Central/Visceral Adiposity
Pancreas	↓ Insulin secretion	Insulin insufficiency (↑ cell toxicity)	Glucose Intolerance and Type 2 Diabetes

Abbreviations: FFA, free fatty acid; HGP, hepatic glucose production; IMCL, intramyocellular lipid levels.