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Review

Metabolic complications associated with antiretroviral therapy

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Abstract

Mortality rates in the HIV-infected patient population have decreased with the advent of highly active antiretroviral therapy (HAART) for the treatment of AIDS. Due to the chronic nature of HAART, long-term metabolic complications are associated with therapy, such as hyperlipidemia, fat redistribution and diabetes mellitus. Currently, all of these symptoms are classified as the lipodystrophy (LD) syndrome(s). However, hyperlipidemia and fat redistribution occur independently, indicating there may be multiple syndromes associated with HAART. Although fat gain/loss and dyslipidemia occur in protease inhibitor (PI) naïve patients treated with nucleoside reverse transcriptase inhibitors (NRTIs), combination therapies (PI and NRTI) accelerate the syndrome. Recent clinical trials, cell culture and animal studies indicate that these effects are not drug class specific and select PIs, NRTIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs) can be associated with metabolic complications. Moreover, the effects can vary between various members of the same class of antiretroviral agents (i.e. not all PIs cause the same adverse reactions) and may be influenced by duration of infection, genetics and environmental factors. Although HAART increases the risk of metabolic complications, this does not outweigh the benefits of survival. In this review, we summarize the latest clinical and scientific information on these metabolic complications, examine current hypotheses explaining the syndromes and comment on the existing methods available to manage these metabolic side effects.

Keywords: Metabolic changes; HIV infections; HAART

1. Introduction

In the period from early 1995 to late 1997, HIV-related mortality rates in the United States decreased from 29.4 per 100 person-years in 1995

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to 8.8 per 100 person-years in 1997 (Palella et al., 1998). Reduction in mortality rates are clearly linked to the introduction of highly active antiretroviral therapy (HAART), with the most dramatic reductions coinciding with the introduction of the protease inhibitors (PIs). The goal of current antiretroviral therapy is to suppress HIV replication and restore the patient's immune function. HAART generally involves the combined use of the three drug categories of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) which are summarized in Table 1. The NNRTIs and the NRTIs inhibit HIV reverse transcriptase (RT) and suppress replication of the viral genome (Beach 1998). The PIs inhibit virus maturation, ultimately limiting the infectivity of the virus particles (Beach 1998).

Realistically, eradication of HIV is an unlikely outcome of HAART and consequently, patients must maintain a lifelong commitment to receiving such therapy. This intense therapy has overtime been associated with unusual adverse events, of which subcutaneous fat wasting (lipoatrophy), abdominal obesity (lipomegaly), insulin resistance and hyperlipidemia have caused the most concern. Current terminology being used to describe the metabolic changes observed in HIV-infected subjects include fat redistribution syndrome, lipodystrophy (LD), peripheral LD, lipohypertrophy, lipomegaly, lipoatrophy, fat accumulation, pseudo-Cushings disease, symmetric lipomatosis, metabolic syndrome X and truncal obesity. It is important to keep in mind that HIV infection itself also causes significant metabolic alterations as summarized in Table 2. In this review, we describe the various metabolic complications associated with HAART, examine potential mechanisms and comment on treatment of these metabolic complications.

Table 1
HIV drugs used in HAART

Nucleoside reverse transcriptase inhibitors inhibitors (NRTIs)	Trizivir™/Abacavir + Lamivudine + Zidovudine Ziagen®/Abacavir (ABC) Combivir®/Lamivudine + Zidovudine (COM) Epivir®/Lamivudine (3TC) Retrovir®/Zidovudine (AZT) Hivid®/Zalcitabine (ddC) Videx®/Didanosine (ddI) Zerit®/Stavudine (d4T)
Protease inhibitors (PIs)	Kaletra™/Lopinavir (LPV) Agenerase®/Amprenavir (APV) Crixivan®/Indinavir (IDV) Fortovase®/Invirase®/Saquinavir (SQV) Norvir®/Ritonavir (RTV) Viracept®/Nelfinavir (NFV)
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Rescriptor®/Delavirdine (DLV) Sustiva®/Efavirenz (EFZ) Viramune®/Nevirapine (NVP)

Table 2
Metabolic consequences of HIV/AIDS infection

Complication	Reference
Cachexia/wasting (weight loss, chronic weakness, diarrhea, intermittent or constant fever)	Gendelman et al. (1998), Kotler (1998) Melchior (1997), Strawford and Hellerstein (1998)
↓Plasma cholesterol and LDL	Shor-Posner et al. (1993), Sellmeyer and Grunfeld (1996)
↑Hypertriglyceridemia and VLDL	Grunfeld et al. (1992b), Christeff et al. (1995)
↑TNFα and IFN-α	Grunfeld and Feingold (1992a), Grunfeld et al. (1991), Belec et al. (1994)
↑Hepatic lipogenesis	Hellerstein et al. (1993)
↑sTNFR	Godfried et al. (1995)
↑Osteoporosis	Hernandez Quero et al. (1993), Serrano et al. (1995), Paton et al. (1997)

2. Monitoring metabolic changes associated with HIV infection and HAART

Currently, there is no consensus on the best method for diagnosing and monitoring the metabolic effects of antiretroviral therapy. However, questionnaires, photographs, physical, radiologic examinations, and serum chemistry

evaluations are used often to assess changes in metabolism and fat distribution. Other commonly used indicators of metabolic alterations, are endocrine and cytokine parameters (thyrotropin, cortisol, leptin and interleukin-6), nutritional and inflammatory markers (albumin, transferrin, haptoglobin, orosomucoid, C-reactive protein), lipid disorders and diabetes mellitus related parameters (cholesterol, glucose, insulin, triglycerides, non-esterified fatty acids, lactate, pyruvate, ketone bodies).

Loss of subcutaneous fat from the face or limbs and increased abdominal girth or breast size are the most visible alterations in HIV-infected subjects. Several body composition studies have been done in both uninfected and HIV-infected subjects using computed tomography (CT; Schoen et al., 1998), magnetic resonance imaging (MRI; Engelson et al., 1999a), dual-energy X-ray absorptiometry (DEXA; Kohrt, 1998), and bioelectrical impedance analysis (BIA; Wattanapenpaiboon et al., 1998; Schwenk et al., 1999). These medical imaging techniques may be used together with measurements of skin-fold thickness, waist to hip ratio, (Wang et al., 1992), photography or questionnaires and self reports to assess fat redistribution (Carr et al., 1999; Gervasoni et al., 1999). Although it may be a reliable technique to measure limb fat, DEXA analysis of body composition has a drawback of being a two-dimensional image and does not allow accurate quantification of visceral fat (Safrin and Grunfeld 1999). In particular, DEXA may be misleading because it may not discriminate between simultaneous fat accumulation and wasting in the same area, such as the trunk where there may be visceral fat accumulation and subcutaneous fat loss. MRI or CT scans produce three-dimensional analyses (Engelson et al., 1999a; Saint-Marc et al., 2000a), however, the cost and lack of standardization of data analysis may be an unfavorable factor. For rapid, inexpensive imaging, single slice CT scans (between L4 and mid-thigh) may serve as an alternative to full body MRI, leading to a reduction in the need for complicated data analysis. Standardization and sensitive measures for fat changes will be critical issues in developing the case definition and diagnosis of metabolic complications associated with HAART.

3. HAART associated morphological alterations/body shape changes

Many subjects reported weight gain after the introduction of HAART. However, the actual nature of the weight gain may vary. Some reports suggest the weight gain may be due to increased fat mass, despite prior loss of lean body mass due to AIDS-induced cachexia (Strawford and Hellerstein, 1998). Despite increased fat mass, which occurs in the abdominal region, there may be progressive loss of lean body mass (Strawford and Hellerstein, 1998). In this review, we attempt to identify the categories as fat loss (lipoatrophy), fat accumulation (lipohypertrophy) or a mixture of both fat loss and accumulation. Lipoatrophy in HIV-infected patients is characterized by a loss of subcutaneous fat in the face, buttocks and extremities, resulting in the appearance of sunken cheeks, exaggerated musculature, bones, arteries and veins (Safrin and Grunfeld, 1999; Vigouroux et al., 1999a). Fat accumulation is characterized by increased dorso-cervical fat (buffalo hump), abdominal girth and waist-to-hip ratio (primarily due to an increase in intra-abdominal fat; (Dong et al., 1999), breast enlargement and the appearance of nonspecific lipomatous growths (Lo et al., 1998a,b; Dong et al., 1999). A mixed syndrome has also been reported in patients who exhibit simultaneous fat loss and accumulation at distinct locations of their body (Saint-Marc et al., 2000a). However, with the use of multiple combinations of HIV therapies, it is difficult to elucidate the etiology and the exact manifestations of each syndrome (Caan et al., 1994; Safrin and Grunfeld 1999). Preceding treatment history, genetic disposition, diet, CD4 count, duration of infection and therapy, age and gender also confound data analysis.

3.1. Effects associated with PIs

Summarized briefly in Table 3 are reports examining the occurrence of morphological alterations in HIV-infected subjects receiving HAART (Carr et al., 1998b, 1999; Silva et al., 1998; Christeff et al., 1999b; Dong et al., 1999; Gervasoni et al., 1999; Hadigan et al., 1999a,b; Fetter et al.,

Table 3
Occurence of lipodystrophy in subjects predominantly treated with PI therapy

Reference	Mode of LD measurement	Patient population	*n*	Rx	Fat loss[a]	Fat accumulation[a]	Notes/conclusion
Carr et al. (1998a)	PE, DEXA Questionnaire	HIV negative (F)	47	PI naïve	3%		Pts. Receiving RTV+SQV combination had a ↓ body fat and ↑lipids than pts on IDV
		HIV positive (M = 148)	32				0.5 kg/month weight loss reported for pts with LD symptoms
			116	PI (IDV = 77; RTV/SQV = 25; NFV/SQV = 9)	64% ($P = 0.0001$)		
Silva et al. (1998)	BIA, BMI, REE, SR	HIV Positive (M = 34; F = 4)	38	PI (IDV = 29; SQV = 4;RTV = 3; SQV/RTV = 2)		↑BMI (0.5 kg/m^2) ($P < 0.0001$) no change LBM	Observed a weight gain mainly in fat mass.
							Self report of an increase in waist size. ↑ average caloric intake (915.5 kJ/day)
Gervasoni et al. (1999)	DEXA, PE, SR	HIV Positive (F)	162	2 NRTI	7.4%	7.4%	Fat wasting and accumulation reported as a single parameter
			144	NRTIs+PI	13.9%	13.9	Longer therapy duration and a viral load of >10,000 copies/ml predispose pts towards LD.
Dong et al. (1999)	PE, AM, BMI, WHR	HIV positive (F)	116	HAART	↓peripheral (8%)	↑ truncal (16%)	Serum lipid abnormalities are common in women undergoing HAART and are not necessarily associated with the occurrence of LD.
		HIV negative (F)	20			↑breast size (13%)	
						↑ buffalo hump (4%)	

Table 3 (*Continued*)

Reference	Mode of LD measurement	Patient population	*n*	Rx	Fat loss[a]	Fat accumulation[a]	Notes/conclusion
Christeff et al. (1999a)	CT scan, PE	HIV positive (M)	37	NRTI+PI	37% (↓facial fat loss; ↓ peripheral ↓leg fat loss)	37% (↑BMI; ↑ abdominal obesity)	Fat loss and gain reported as one parameter Demonstrate an association b/w lipodystrophy and the serum cortisol:DHEA ratio
Fetter et al. (2000)	PE	HIV positive	245	NRTI+APV	3%	3%	Mean therapy duration of 56.5 weeks
		HIV positive	241	NRTI+IDV	11% ($P<0.001$)	11% ($P<0.001$)	
Carr et al. (1999)	SR, PE, DEXA Questionnaire	HIV positive (M)	41	HAART no PI	No change	No change	Duration of PI therapy and HIV infection were significantly associated with the severity of LD.
			113	HAART including PI (IDV = 76; RTV/SQV = 24; NFV/SQV = 8; NFV = 4;SQV = 1)	75% (peripheral) ($P=0.06$)	65% (abdominal) ($P=0.06$)	RTV and SQV cause a more rapid onset of LD symptoms in comparison to IDV and NFV 98% concordance with the pts self-report of LD and PE by physician
Hadigan et al. (2000)	BMI; DEXA	HIV negative (F)	30				No significant association of PI usage in the development of LD in women
		HIV positive (F)	58	NRTI	↓ peripheral fat (5%)	↑truncal adiposity (17.3%)	Hypertriglyceridemia was not associated with PI usage or truncal obesity
			17	NRTI+PI (IDV = 16; NFV = 1)			
Hadigan et al. (1999a)	BMI; DEXA	HIV negative (M)	20				Significant hyperinsulinemia in HIV-infected patients in absence of PI usage

Table 3 (*Continued*)

Reference	Mode of LD measurement	Patient population	*n*	Rx	Fat loss[a]	Fat accumulation[a]	Notes/conclusion
		HIV positive (M)	12	No treatment	0.86 trunk: extremity	0.40 trunk/fat ratio	Endogenous gonadal steroid levels are related to hyperinsulinemia in HIV-infected men
			18	NRTI only	1.32 trunk: extremity	0.51 trunk/fat ratio	Pts on PI therapy present an exaggeration of LD symptoms already present in patients only on NRTIs
			20	NRTI+PI	1.47 trunk:extremity	0.53 trunk/fat ratio	

[a] Percentages are the number of patients in that treatment category developing fat loss/accumulation symptoms during therapy.
Abbreviations: PE, physical examination; SR, self report; LBM, lean body mass; BMI, body mass index; REE, resting energy expenditure; AM, anthropometric measures; WHR, waist to hip ratio.

2000). Even though the development of the syndrome was recognized after the introduction of PIs, these manifestations, in part, can be affected by HIV infection per se, NRTIs and/or NNRTIs. Nonetheless, the information obtained from these studies pertains predominantly to PI therapy and the associated incidence of LD.

Carr et al. (1998b) examined patients on PI therapy with mean therapy duration of 13.6 months. The authors report that subjects receiving PIs had overall lower fat mass than the HIV seronegative and seropositive subjects in all body regions except for abdominal fat. Subjects with LD (increase in abdominal girth and peripheral fat loss) had significantly higher triglyceride, insulin and C-peptide levels in comparison to those subjects without LD. They report an average of 0.5 kg weight loss per month in the subjects with LD. In the second year of therapy, Carr et al. (1999) continue to observe LD in the PI-treated patients, however the mean fat loss decreased to 0.13 kg per month. Contrary to these results, Silva et al. (1998) report 1.54 kg ($P < 0.0001$) of weight gain after 12.6 months in 38 subjects treated with a PI. However, both studies report increased abdominal girth.

A few studies have attempted to discriminate between the effects of individual PIs on fat redistribution. Carr et al. (1998b) report of patients receiving a RTV/SQV combination had significantly lower body fat, higher lipids and shorter time to LD than the patients receiving IDV. A review of the clinical adverse events and laboratory changes associated with APV or IDV therapy were made primarily by physician examinations in the clinical trial PROAB3006 (Fetter et al., 1999; Pedneault et al., 1999). The incidence of fat redistribution was 4% in the APV-treated subjects and 12% in the IDV-treated subjects ($P < 0.001$; Fetter et al., 2000).

Three studies on the effects of HAART in women (Dong et al., 1999; Gervasoni et al., 1999; Hadigan et al., 1999a) report facial and peripheral fat loss accompanied by increased abdominal girth and breast size. Interestingly, Hadigan et al. (1999a) demonstrated no significant association of PI usage with the development of LD or hypertriglyceridemia in women. Whereas Gervasoni et al. (1999) reported an increased incidence in LD in women on PI containing therapy in comparison to women treated with NRTI therapy alone. The same group later showed that women were five times more likely to develop LD than men when treated with NRTIs alone (Galli et al., 1999).

Two studies examined the relationship of alterations in serum hormone levels in men infected with HIV on HAART and the development of LD (Christeff et al., 1999a,b; Hadigan et al., 1999b). Christeff et al. (1999b) report an inverse correlation between the serum dihydyroepiandrosterone (DHEA) levels and LD in their patients, which in turn raised the cortisol: DHEA ratios in the LD patients. Hadigan et al. (1999b) also illustrate an inverse correlation between lower serum androgen levels and hyperinsulinemia in HIV-infected male subjects treated with HAART.

3.2. NRTI and NNRTI studies

Fat redistribution has been described in PI naïve subjects, suggesting NRTIs are associated with LD independent of PI therapy (Boufassa et al., 2000; Carr et al., 2000; Galli et al., 1999; Lichtenstein et al., 2000; Mallal et al., 2000; Molina et al., 2000; Saint-Marc et al., 1999, 2000b; Saint-Marc and Touraine 1999b). Summarized in Table 4 are studies focusing on the development of morphological changes associated with NRTI therapy. Most studies of LD in patients naïve to PI therapy describe a presentation similar to that reported by Saint-Marc et al. (1999), where the term LD refers to the loss of subcutaneous fat from the facial fat pads (bichat or preauricular), arms and legs. Mallal and colleagues provide evidence supporting an interaction between NRTIs and PIs in the development of subcutaneous fat loss (Mallal et al., 2000). In a multivariate model adjusted for other confounding factors, the addition of PIs to dual NRTI therapy increased the risk of developing peripheral fat wasting by 13-fold. Carr et al. (2000) observed that subjects treated only with NRTIs had different LD symptoms than subjects treated with NRTIs + PI. The NRTIs + PI group presented symptoms of peripheral fat loss and abdominal distension whereas the NRTI group was

Table 4
Occurrence of lipodystrophy in subjects predominantly treated with NRTI therapy

Study	*n*	Rx	LD assessment	Overall LD prevalence	Analysis	Factors associated with increased risk of LD
Boufassa et al. (2000) Liposud	646	NRTI+PI	Physician assessment	Any LD: 374/646 (58%) LD (PI group): 316/498 (64%) LD (NRTI group): 58/148 (39%)	Unadjusted comparison of LD+ with LD−	Duration of treatment, d4T therapy
Galli et al. (1999)	188	Only NRTI	Physician and patient assessment	Any LD: 31/188 (16%) Any LD (F): 25/96 (26%) Any LD (M): 6/92 (7%)	Multivariate analysis	Female gender, d4T
Saint-Marc et al. (1999) LIPOCO study	154	NRTI+PI	Physician assessment, BIA, CT	Any LD: 82/154 (53%) FL (PI group): 58/100 (58%) FL (NRTI group): 15/39 (38%) FA (PI group): 45/100 (45%) FA (NRTI group): 3/39 (8%)	Multivariate logistic regression	d4T therapy
Carr et al. (2000)	220	NRTI+PI	Physician and patient assessment, DEXA, CT	N/A-case control study 116 LD cases, 104 controls	Multivariate analysis	FL: age, NRTI exposure, d4T therapy, elevated lactate FA: age, PI duration, 3TC
Mallal et al. (2000) W. Australia cohort	277	NRTI+PI	Physician assessment, longitudinal DEXA	Overall FL: 121/277 (44%) FL (PI group): 112/201 (54%) FL (NRTI gp.): 9/71 (13%)	Cox proportional hazards, multivariate linear regression	FL: age, white race, therapy duration
Molina et al. (2000) ALBI study	120	NRTI+PI	Physician assessment	Overall LD: 37/120 (31%) LD (NRTIs alone): 20/61 (33%)	Randomized study in therapy naïve patients	d4T/ddI greater risk than ZDV/3TC

F, female; M, male; FL, fat loss; FA, fat accumulation; BIA, bioelectrical impedance assay; CT, computer tomography.

associated solely with lipoatrophy. The NRTI-lipoatrophy subjects had additional symptoms of recent onset of weight loss, higher lactate and alanine aminotransferase, lower albumin, cholesterol, triglyceride and insulin levels (Carr et al., 2000).

Molina et al. (2000) examined the occurrence of LD in a long-term (30 month) follow-up study of a randomized trial comparing various NRTI-containing regimens (ALBI study). The HIV-infected subjects were antiretroviral therapy-naïve upon enrollment for a 6-month treatment period. The study consisted of three arms, d4T/ddI, ZDV/3TC, or d4T/ddI followed by ZDV/3TC (alternating group). Prevalence of LD was 52, 28 and 27% in each arm, respectively ($P = 0.02$). The overall occurrence of LD was twice as high in the group of subjects randomized in the d4T/ddI arm as compared with the other randomized arms of the study (Molina et al., 2000). Consistent with this observation, all the cohort studies cited in Table 4 have reported an increased risk of development of LD in patients treated with d4T compared with other NRTIs (Carr et al., 2000; Lichtenstein et al., 2000; Mallal et al., 2000; Saint-Marc and Touraine 1999b; Saint-Marc et al., 2000b). Multivariate analyses of these studies have also identified other factors including age and overall duration of therapy as independent risk factors for the development of LD. Saint-Marc and colleagues (Saint-Marc and Touraine 1999b) examined 59 HIV-infected HAART-treated subjects with peripheral fat loss in which d4T therapy was switched to ABC or ZDV. After 12 months, a significant improvement of subcutaneous fat loss ($P < 0.05$) was observed by CT scan measurements in a majority of patients from both the PI-naïve ($n = 18$) and the PI-treated groups ($n = 41$). Despite the increased LD risk described with PI and d4T therapy, it remains clear that this syndrome has a multifactoral etiology and as yet unidentified factors may play a role. Hence, further studies are needed to understand the link between other factors and any one specific drug or drug class in the development of this syndrome.

Limited information is available on the risk of LD associated with NNRTI therapy. A retrospective study evaluated the potential role of NNRTIs in the development of LD in 58 HIV-infected PI-naïve subjects treated with nevirapine (NVP; an NNRTI) and two NRTIs (Aldeen et al., 1999). Patient self-reporting revealed symptoms of central obesity and peripheral fat loss. Nine subjects (16%) developed LD (therapy duration of 6 months) and all cases were associated with undetectable HIV-1 RNA levels. Stryker and colleagues reported on Study 006 (Stryker et al., 1999), in which 3TC-, NNRTI-, and PI-naïve subjects were randomized to ZDV/3TC/IDV or EFV/IDV or IDV/3TC/ZDV, respectively. LD was observed in 0.9, 2.8 and 2.2% of patients in the ZDV/3TC/EFV, EFV/IDV and ZDV/3TC/IDV arms, respectively (Stryker et al., 1999). Although NRTIs may contribute to the development of LD, the role of NNRTIs in the development of this syndrome is not yet clear.

3.3. Reversibility of LD upon switching therapy

A number of studies have tested switching patients from one drug class to another as a means to reduce the metabolic complications associated with HAART. Few studies have tested switching patients from drugs belonging to the same class of antiretroviral agents (Khanlou et al., 2000; Saint-Marc and Touraine 1999b; Saint-Marc et al., 2000b). However, most of these studies were open label, non-randomized, for short duration of time, uncontrolled for earlier drug use, and included individual patient reporting of body fat redistribution measurements. Depending upon the particular change in therapy, metabolic parameters can also fail to improve or worsen. In several studies, the subjects reported a subjective improvement in body shape or other metabolic abnormalities but not a full restoration (Martinez et al., 1999; Khanlou et al., 2000).

Martinez et al. (1999) reported on the effects of switching patients from a PI- to a NVP-containing regimen. This study did not discriminate between individual PI usage prior to switching therapy. Of the 23 subjects enrolled in the study, 17 had increased abdominal girth before switching therapy. They examined serum chemistry after 8 months of NVP treatment and reported a 22%

decrease in fasting serum cholesterol levels, a 57% decrease in triglyceride levels and a 45% decrease in the insulin sensitivity index (I_S). Although no changes in anthropometric measurements occurred, 91% of the subjects reported subjective improvement in body shape 6 months after switching therapy. In a clinical trial of 57 HIV-infected subjects (Negredo et al., 1999), switching from a PI- to NVP-containing regimen decreased fat loss in the face and arms and reduced fasting serum lipids by 57%. In contrast, in a clinical trial of 27 HIV-infected subjects (Moyle et al., 1999), switching from a PI- to EFV-containing regimen, increased fasting serum triglyceride (42%), cholesterol levels, and peripheral weight, although central obesity was reduced. These reports demonstrate that the substitution of certain NNRTIs (e.g. EFV) for PIs may not alleviate lipid abnormalities and in some cases may exacerbate them. However, these studies did not control for the PI used before initiating the PI-sparing regimen and thus, no conclusions can be made with regard to the earlier use of individual PIs.

Saint-Marc and Touraine (Saint-Marc and Touraine 1999b; Saint-Marc et al., 2000b) surveyed the reversibility of peripheral fat wasting associated with d4T therapy by switching patients from d4T to either ZDV or abacavir (ABC). The study population included both PI-naïve ($n = 18$) and PI-experienced patients ($n = 41$). Switching from d4T to either ZDV or ABC was shown to improve peripheral fat loss in the majority of patients as assessed by CT and BIA analysis. In addition, significant reductions in serum triglyceride and lactate levels were observed (Saint-Marc et al., 2000b). Preliminary results reported from the CNA30017 clinical trial of 200 HIV-infected subjects randomly switched from 2 NRTIs/PI to 2 NRTIs/ABC (Rozenbaum et al., 2000) demonstrate an improvement in LD upon switching. The term LD in this study refers to abnormal fat deposits, insulin resistance, hyperlipidemia and diabetes mellitus. After 48 weeks, subjects treated with ABC demonstrated a stabilization of serum triglyceride levels and an improvement in glucose metabolism in comparison to subjects maintained on the PI regimen.

4. Alterations in serum chemistries associated with HAART

4.1. Hyperlipidemia

Metabolic changes associated with HAART include various lipid and glucose complications such as hyperlipidemia (Carr et al., 1998b; Behrens et al., 1999; Berthold et al., 1999), insulin resistance (Walli et al., 1998; Behrens et al., 1999; Yarasheski et al., 1999), and diabetes mellitus (Dube et al., 1997; Carr et al., 1999; Rodriguez-Rosado et al., 1999; Vigouroux et al., 1999b). The glucose and lipid changes appear to precede morphologic alterations and can occur with or without weight loss and fat redistribution and hyperlipidemia (Safrin and Grunfeld 1999; Viard and Rakotoambinina 1999; Mulligan et al., 2000).

Summarized in Table 5 are recent reports on the prevalence of hyperlipidemia during HAART (Walli et al., 1998; Berthold et al., 1999; Periard et al., 1999; Schmidt et al., 1999; Segerer et al., 1999; Sadler et al., 2000). Walli and colleagues (Walli et al., 1998) evaluated fasting lipid parameters in treatment naïve and experienced (including a PI) HIV-infected subjects. They observed a median increase of 1.76 mmol/l (113% increase; $P = 0.007$) in serum triglyceride levels and a concomitant increase in total cholesterol (0.068 mmol/l; $P < 0.001$) levels in the PI-treated subjects. Furthermore, they found median total cholesterol was greatest in NFV-treated patients and the highest triglyceride level was in a patient treated with RTV. Results from the Swiss Cohort study (Periard et al., 1999) report fasting total cholesterol levels increased 190% in RTV-treated patients. Likewise, cholesterol increased 110 and 70% in NFV and IDV-treated subjects, respectively, compared with PI-naïve subjects. HDL-cholesterol levels remained unchanged in PI-treated subjects. Periard et al. (1999) and Koppel et al. (2000) also examined the levels of lipoprotein (LPLa), a carrier protein for LDL as a risk factor for the development of cardiovascular disease. Both studies report significant increases of lipoprotein (a) levels in HIV subjects treated with HAART upon comparison with therapy naïve HIV-infected subjects.

Table 5
Alterations in serum chemistries in subjects treated with HAART

Reference	Patient population	*n*	Rx	Cholesterol measurements			Serum TGs (mmol/l)	Notes/conclusions
				Total (mmol/l)	HDL (mmol/l)	LDL (mmol/l)		
Walli et al. (1998)	HIV negative	18						Therapy duration of 8 months
	HIV positive	13	Therapy naïve	4.57			1.56	61% of PI treated patients exhibited insulin resistance
	HIV positive (M = 63; F = 4)	67	HAART + PI (IDV = 29; SQV = 10; NFV = 9)	5.25 ($P = 0.021$)			3.32 ($P = 0.007$)	Treatment with PI lead to significant increases in TGs and total chol and decrease in I_s
Periard et al. (1999)	HIV positive	28	HAART no PI	↑0.1	↓0.2	↑0.3	↓0.1	Mean therapy duration of 15 months
	HIV positive	93	HAART + PI:					Parameters are compared to baseline values of each group
		46	RTV	↑2.0 ($P < 0.001$)	No Δ	↑1.4 ($P \leq 0.001$)	↑1.83 ($P = 0.002$)	RTV administration was associated with a marked increase in plasma TGs and LDL levels
		26	IDV	↑0.8	No Δ	↑0.8 ($P = 0.03$)	↓0.14	Similar changes were observed in 6 children receiving RTV
		21	NFV	↑1.2 ($P < 0.01$)	No Δ	↑ 1.0 ($P \leq 0.05$)	↑0.12	
Schmidt et al. (1999)	HIV positive (M = 20; F = 3)	20	HAART no PI	4.12	0.93	2.7	1.42	Mean therapy duration of 18 months
	HIV positive (M = 78; F = 20)	98	HAART + PI	5.87 ($P \leq 0.00001$)	0.81 ($P = 0.3$)	3.77 ($P = 0.0006$)	2.85 ($P = 0.0003$)	57% of PI treated pts had overt hyperlipidemia High levels of ApoB and ApoE in the PI treated group
Segerer et al. (1999)	HIV positive (M)	72	2 NRTI only					
	HIV positive (F)	19	2 NRTI only	↓0.11			↓0.02	Parameters were compared to baseline values in each group after 6 months

Table 5 (*Continued*)

Reference	Patient population	*n*	Rx	Cholesterol measurements			Serum TGs (mmol/l)	Notes/conclusions
				Total (mmol/l)	HDL (mmol/l)	LDL (mmol/l)		
	HIV positive (M)	130	2 NRTI+PI					The pts in the IDV group had a ↑13% in their cholesterol compared to no change in the SQV group
	HIV positive (F)	18	2 NRTI+PI (IDV = 55; SQV = 51;NFV = 36; RTV+5)	↑0.71 ($P<0.001$)			↑0.42 ($P<0.013$)	
Berthold et al. (1999)	HIV positive (M)	19	2 NRTI+PI	↑ 0.72 ($P=0.01$)	No Δ		↑1.08 ($P=0.014$)	Median therapy duration of 22 weeks Increases are based on comparison with baseline values of the group ↑ In triglyceride-containing lipoproteins
Sadler et al. (2000)	HIV negative (M = 10; F = 8)	18						
		9	APV	↓0.04			↑0.53	Day 7 information ON D7, RTV added to APV therapy and APV added to RTV therapy
		9	RTV	↑0.02			↑1.15 ($P=0.05$)	D14 of combination

Carr et al. (1998b) report subjects receiving RTV/SQV have higher lipids than subjects receiving IDV. Consistent with this observation, a patient on RTV therapy had a 14.5-fold elevation in serum triglycerides and 4.5-fold increase in serum cholesterol (Sullivan et al., 1998). Upon switching therapy to IDV, these effects were reversed. Similarly, Schmidt et al. (1999) observed a significantly higher prevalence of hyperlipidemia, (however, not an exacerbation) in the subjects on two PIs (RTV + SQV). LPL activity decreased and LD (defined as abnormal fat accumulation or loss) increased in PI-treated subjects with exacerbated hyperlipidemia. Genetic analysis demonstrated a higher frequency of the apolipoprotein E2 and E4 alleles in the hyperlipidemic PI-treated subjects.

A 5-year historical cohort study in a population of 221 HIV-infected patients examined non-fasting serum parameters of patients on PI containing HAART therapy (Tsiodras et al., 2000). The cumulative incidence of new-onset hyperglycemia, hypercholesterolemia and hypertriglyceridemia was 5, 24 and 19%, respectively. They observed that NRTIs also caused weak elevations of cholesterol and triglycerides and reported significant association between RTV therapy and the occurrence of hypertriglyceridemia. In addition, studies in non-infected volunteers show RTV increases serum triglycerides and cholesterol levels in 2 weeks of therapy (Purnell et al., 2000).

4.2. Glucose tolerance and insulin resistance

Non-insulin dependent diabetes mellitus (NIDDM) is associated with impaired glucose tolerance, insulin resistance, high fasting plasma insulin and C-peptide levels and elevated proinsulin and insulin (ProIns:Ins) ratio. Since 1997, a number of subjects developed NIDDM after beginning HAART including a PI therapy (Dube et al., 1997; Carr et al., 1999; Rodriguez-Rosado et al., 1999; Vigouroux et al., 1999b). This prompted the FDA to require PI-class labeling revisions indicating exacerbation of hyperglycemia in some patients. However, results from recent clinical trials and preclinical research indicate not all PIs act similarly and suggest drug-specific effects, rather than class-specific effects on developing NIDDM.

Walli et al. (1998) examined insulin sensitivity (I_S) and oral glucose tolerance in patients receiving HAART (see Table 5 for details of patient population). They observed that I_S was lowest in subjects receiving IDV (72%; median of 59 μmol/l per min), followed by those receiving NFV (66%; median of 75 μmol/l per min) and SQV (50%; median 105 μmol/l per min). Likewise, the Swiss Cohort study (Periard et al., 1999) reports insulin resistance was greater for IDV than NFV or RTV-treated subjects. Behrens et al. (1999) examined glucose tolerance, β-cell function and lipid metabolism in HIV-infected subjects on HAART; 38 subjects used a PI and 17 were PI naïve. Although 39% of the subjects treated with a PI received IDV, this study did not examine the effects of individual PIs or NRTIs on metabolism. Of the PI-treated subjects, 46% demonstrated impaired glucose tolerance (compared with 24% in the PI naïve group). Fasting concentrations of insulin, proinsulin and C-peptide and their secretion response to glucose ingestion increased significantly in the PI-treated subjects. Increased proinsulin may be an indicator of β-cell dysfunction whereas increased insulin and glucose suggest peripheral insulin resistance. Another study examined the effects of IDV in vivo and in vitro on proinsulin to insulin conversion and insulin and C-peptide secretion, respectively (Yarasheski et al., 1999). This study included 10 PI naïve HAART-treated male subjects, 15 HIV-infected subjects on IDV containing HAART and eight HIV-seronegative males. IDV-treated subjects with a body mass index > 28 kg/m^2 had a 45% average increase in their ProIns/Ins ratio and a 16% average increase in their fasting glucose level compared with the HIV-seronegative control group. In contrast, in vitro studies using rat pancreatic β-cells, IDV did not inhibit proinsulin to insulin conversion or impair glucose-induced insulin secretion of insulin or C-peptide. Although this suggest that IDV may not have a direct effect on rat pancreatic β-cells, it is possible that other factors (e.g. incretins, vitamins, retinoids) were missing from the in vitro assay that are normally present in vivo.

Alterations of glucose tolerance have been noted with NRTIs. For example, ddI is reported

to cause nonketonic diabetes (Martin and Fonseca, 1994). Recently, a case report suggested a single patient developed diabetes after treatment with ABC (Modest and Fuller, 2001). However, retrospective analysis of 28 000 patients treated with ABC did not indicate an association of ABC and diabetes (Hetherington et al., 2001), indicating the case report was a rare event.

5. Treatment of metabolic complications

Treatment of fat and carbohydrate metabolism disorders includes nutritional therapy and exercise. For some HIV-infected subjects, changes in behavioral patterns, such as smoking, may also facilitate successful long-term therapy, particularly to improve metabolic disorders. Diet and exercise may help maintain optimal serum glucose and lipid levels, provide adequate calories to sustain desired body weight, control hypertension, prevent complications associated with hyperlipidemia and diabetes mellitus and reduce visceral fat mass. Although exercise training may reduce trunk and subcutaneous fat mass, there is no change or, in fact, an increase in lean body mass or bone density is observed in HIV-positive individuals (Roubenoff et al., 1999; Tenzif et al., 1999). Moreover, a low fat diet can reduce hypertriglyceridemia in humans (Fischer et al., 1998) and mice (Lenhard et al., 2000b) treated with antiretroviral agents. Although diet and exercise diminishes body fat and improves metabolic control (Melroe et al., 1999), these approaches may not reverse the subcutaneous fat loss observed in HIV-infected subjects.

The cardiovascular disease focus group, under the auspices of the Complications Research Agenda Committee of the Adult AIDS Clinical Trials Group (AACTG) developed preliminary guidelines for clinicians to address lipid abnormalities for subjects on HAART (Dube et al., 2000). The NIH consensus statement classifies subjects as having with hypertriglyceridemia if triglyceride levels are greater than 500 mg/dl and borderline hypertriglyceridemia if triglycerides are between 250 and 500 mg/dl. Blood cholesterol levels of 240 mg/dl are classified as high, and 200–239 mg/dl as borderline. HDL cholesterol (HDL-C) should be monitored in subjects whose blood cholesterol is higher than 200 mg/dl, or who have evidence of heart disease or two other cardiovascular risk factors (one of which could be male gender). Concerning HDL-C, the NIH consensus statement selected levels under 35 mg/dl as the cut-off point for identifying individuals at very high risk of cardiovascular complications. There is general agreement that LDL-C is an essential determinant of cardiovascular risk, and those individuals whose LDL-C higher than 150 mg/dl may require treatment for their condition.

When individuals are unable to improve metabolic control with diet and exercise, pharmacological agents may be needed as summarized in Table 6. Statins may be used to treat hypertriglyceridemia and hypercholesterolemia. Most PIs are metabolized by cytochrome p450 3A4 (CYP3A4), which will lead to increase in statin levels. Increases statin levels may result in skeletal muscle toxicity (Dube et al., 2000).

PPARγ agonists, such as troglitazone, pioglitazone or rosiglitazone, can reduce serum glucose levels and related metabolic complications (Goebel and Walli, 1999; Rose et al., 1999). A recent study examined the effects of troglitazone administration to six HAART subjects with LD (Walli et al., 2000). The authors report an increase in I_S and lean body mass. However, since these agents promote anabolism in fat cells and weight gain (Lenhard et al., 1997, 1999), they may be also be useful for treating lipoatrophy (Goebel and Walli, 1999; Lenhard and Cobb 1999). In addition, in vitro studies suggest a decrease in mitochondrial metabolic activity in cells treated with troglitazone associated with an increase in mitochondrial mass (Lenhard et al., 1997), which suggests the glitazones may reverse the mitochondrial defects associated with HAART.

Growth hormone increases lean body mass and decreases serum triglyceride levels and visceral fat in HIV-infected subjects (Engelson et al., 1999b; Mauss et al., 1999) and may alleviate some of the fat redistribution symptoms (Van Loan et al., 1999; Wanke et al., 1999). Yet, it may promote insulin resistance and will not prevent loss of body fat (Johnston et al., 1999). Treatment of the

HIV wasting syndrome includes agents such as megestrol acetate, dronabinol, or anabolic steroids (Balog et al., 1998). Mitochondrial dysfunction resulting in elevation of lactate/pyruvate and beta-hydroxybutyrate/acetoacetate ratios may be reversed by antioxidants (Delaasuncion et al., 1998). However, further investigations are needed on the effectiveness of dietary supplements such as carnitine, thiamin, quinones (co-enzyme Q), tocopherols (vitamins E and K), riboflavin, ascorbate and biotin in subjects using HAART.

Conflicting reports suggest HAART may be associated with decreases (Tebas et al., 2000) or increases in bone mineral density (Nolan et al., 2001). HIV infection and pre-HAART body mass index (Nolan et al., 2001) itself may predispose an individual to osteoporosis (Hernandez Quero et al., 1993; Serrano et al., 1995; Paton et al., 1997; Rademaker et al., 1997; Teichmann et al., 2000). However, there is a debate as to whether HAART therapy actually is accelerating osteoporosis (Weiel and Lenhard 2000). More detailed studies are needed to understand the effects HAART has on bone metabolism and how to treat these bone disorders.

6. Pathogenesis of PI-associated metabolic complications

6.1. Animal studies

Investigations on the effects of dietary fat intake and PIs on metabolism demonstrate that alteration in fat and carbohydrate in the diet influences the effects of PIs on metabolism in AKR/J (obesity susceptible) mice (Lenhard et al., 2000b). In mice fed high fat and low carbohydrate diets, IDV and NFV-treated mice had greater blood urea nitrogen and triglyceride levels whereas SQV-treated mice had greater cholesterol levels than placebo-treated mice. In mice fed low fat high carbohydrate diets, IDV and NFV-treated mice had greater serum glucose levels, weight gain and fat accumulation; IDV-treated mice had lower insulin; and SQV-treated mice had lower cholesterol compared with placebo-treated mice. APV treatment had little effect on these serum measurements. These data suggest that high fat, low carbohydrate diet increases PI-associated hyperlipidemia while low fat, high carbohydrate diet increases PI-mediated hyperglycemia in

Table 6
HAART associated metabolic complications and current therapies used for treatment

Complication	Drug therapy	Drug mode of action	Reference
↑Abdominal fat	Metformin growth hormone	Stimulate catabolism; weight loss	Walli et al. (2000)
↓Lean body mass/wasting	Growth hormone anabolic steroids Glitazones	Stimulate anabolism; weight gain	Engelson et al. (1999b), Mauss et al. (1999), Van Loan et al. (1999), Wanke et al. (1999)
Hypertriglyceridemia	Fibrates (Fenofibrate; Gemfibrozil)	Activation of PPARα	Henry et al. (1998), Hewitt et al. (1999), Thomas et al. (2000)
Hypercholesterolemia	Statins (Atorvastatin)	Inhibition of HMG-CoA reductase	Henry et al. (1998), Melroe et al. (1999)
Type II diabetes	Insulin	Promotes glucose utilization	Dube et al. (1997)
	Sulphonylureas	Stimulation of insulin secretion	
	Metformin	Insulin sensitizer/promotes hepatic gluconeogenesis	Saint-Marc et al. (1999), Hadigan et al. (2000)
	Glitazones	Promotes insulin action/PPARγ agonist	Goebel and Walli (1999), Walli et al. (2000)
Lactic acidemia/acidosis	Antioxidants	Reversal in lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios; improved mitochondrial function	Delaasuncion et al. (1998)
Osteoporosis/osteosis	No reports on use of bisphosphonates		Tebas et al. (2000), Nolan et al. (2001)

mice. However, other PI-associated effects were unaffected by diet. For example, IDV and NFV-treated mice had greater fatty acids and pancreatic lipase and IDV-treated mice had greater bilirubin and alkaline phosphatase than control mice fed either diet (Lenhard et al., 2000b).

Pancreatic lipase is an enzyme that hydrolyzes diglycerides in the gut and contributes to increased dietary fat absorption. Inhibitors (e.g. Orlistat) of pancreatic lipase reduce body weight, fat mass and serum lipid levels. Interestingly, treatment of AKR/J mice fed a low fat, high carbohydrate diet with IDV or NFV results in increased pancreatic lipase activity, body weight, fat mass and serum-free fatty acid levels (Lenhard et al., 2000b). Thus, one possibility is that PIs cause fat accumulation and hyperlipidemia by increasing pancreatic lipase activity and absorption of dietary fat. However, the effect of PIs on pancreatic lipase activity or Orlistat on LD remains to be determined in HIV-infected subjects.

There is limited data on the effects of PIs on lipid metabolism using other species of rodents. Although some PIs, such as RTV, may cause hyperlipidemia in humans (Periard et al., 1999) see Section 4.1 for details), RTV treatment decreased serum triglyceride levels in Wistar rats (Ye et al., 1998). Also in contrast to clinical results, SQV and NFV treatment decreased triglyceride levels in obesity resistant SWR/J mice fed a low fat diet (Weiel et al., 1999; Lenhard et al., 2000b). One possibility for these contradictory observations is that environmental factors, such as HIV infection or dietary fat or carbohydrate, influence the effects of PIs on hyperlipidemia and these factors are not equivalent in rodent and human studies. Alternatively, these differences may be due to genetic or species-specific effects. Consistent with the latter hypothesis, NFV treatment elevated serum fatty acid levels in obesity prone AKR/J mice but reduced serum fatty acid levels in obesity resistant SWR/J mice (Weiel et al., 1999; Lenhard et al., 2000b). These observations suggest that susceptibility to obesity may increase the risk of developing hyperlipidemia after treatment with NFV. Moreover, the observation that APV, IDV, NFV and SQV differ in their effects in mice (Lenhard et al., 2000b) and in the clinic, indicates that each PI has unique pharmacological properties.

In rodents, the PIs have multiple effects on glycemia, which may be dependent on the PI, diet, species and strain of the animal studied. For example, RTV treatment of Wistar rats had no significant effect on serum insulin or glucose levels (Ye et al., 1998). However, treatment of obesity resistant SWR/J mice fed a low fat, high carbohydrate diet (obesity resistant) with IDV or NFV for 2 weeks decreased serum glucose levels (Weiel et al., 1999; Lenhard et al., 2000b). In contrast, treatment of obesity prone AKR/J mice fed a low fat, high carbohydrate diet with IDV or NFV significantly increased serum glucose levels and decreased serum insulin levels (Lenhard et al., 2000b). APV and SQV had minimal effects on serum glucose levels in AKR/J or SWR/J mice. Taken together, these observations suggest that genetic background influences the effects of PIs on carbohydrate metabolism (Weiel et al., 1999).

6.2. In vitro studies

The in vitro effects of PIs on adipogenesis have been examined (Gagnon et al., 1998; Zhang et al., 1999; Dowell et al., 2000; Lenhard et al., 2000c,d) using the murine cell lines 3T3-L1 and C3H10T1/2. While 3T3-L1 cells express a phenotype similar to white adipose tissue (Rosen et al., 1979), C3H10T1/2 cells express a phenotype similar to brown adipose tissue (Paulik and Lenhard 1997). In 3T3-L1 cells, Gagnon et al. (1998) showed that RTV (Nguyen et al., 2000) and IDV stimulate adipogenesis as much as 10–40% whereas Zhang et al. (1999) reported that NFV and RTV inhibit lipid accumulation. Similarly, IDV and SQV inhibited differentiation of human preadipocytes into adipocytes by 20 and 40%, respectively (Wentworth et al., 2000). Likewise, in C3H10T1/2 stem cells the PIs, NFV, SQV and RTV inhibited adipogenesis as measured by reduced triglyceride accumulation, lipogenesis and expression of the adipose markers, aP2 and LPL (Lenhard et al., 2000c). Moreover, these three PIs increased acute lipolysis in mature adipocytes. In contrast, APV and IDV had little effect on lipolysis, lipogenesis or expression of aP2 and LPL (Lenhard et al.,

2000c). Taken together, these results suggest the effects of PIs on fat metabolism may vary with the PI, cell type and fat depot.

Control of adipogenesis occurs through the binding of nuclear receptor heterodimers of peroxisome proliferator activated receptors (PPARs) and retinoid X receptors (RXR) to response elements present in adipogenic genes (Rose et al., 1999). IDV and SQV did not impair the activity of the PPARγ/RXR heterodimer in transfected cells (Wentworth et al., 2000). Although, SQV inhibited ligand binding to PPARγ (IC_{50} of 12.7 μM), none of the other PIs bound to the nuclear receptors RXRα or PPARγ (Lenhard et al., 2000c). Thus, inhibition of adipogenesis is not due to antagonism of ligand binding to RXRα or PPAR.

The choice of drug concentration in vitro is important for generating relevant results. Maximum serum concentrations for protease inhibitors in the clinic are 1–10 μM (Balani et al., 1996; Danner et al., 1995; Pai and Nahata, 1999). The use of very high concentrations (>50 μM) of drugs is potentially toxic to cultured cells because the PIs are hydrophobic compounds with limited solubility (<10 μM for RTV, SQV and NFV) in aqueous solutions (Vonderhelm 1996; Kempf et al., 1998). Since, PIs are highly protein bound in serum ($>95\%$ for RTV, SQV and NFV; the use of serum-free media may substantially alter the in vitro properties of PIs. Murata et al. (Murata et al., 2000) reported that PIs inhibit insulin-stimulated glucose uptake and GLUT4 transport activity in 3T3-L1 cells, using concentrations as high as 100 μM of a PI in serum-free media. Caution should be used when interpreting these results, since the PIs were not bound to serum proteins and may be insoluble under these assay conditions.

6.3. Hypotheses

6.3.1. Retinoid toxicity

Activation of the RXR/PPAR heterodimer increases adipogenesis whereas activation of the RAR/RXR (retinoic acid receptor/RXR) heterodimer inhibits adipogenesis. RAR and RXR are activated through the binding of all-*trans*-retinoic acid (vitamin A/retinol) or its isomer 9-*cis*-retinoic acid. These retinoids may also bind to cytoplasmic retinoic acid binding proteins (CRABP) or other retinoid binding proteins (RBP) present within a cell.

Since there are multiple causes and syndromes associated with the metabolic complications in patients receiving HAART, it is likely that several theories may explain the molecular mechanisms underlying these metabolic complications. One hypothesis is that PIs may bind to CRABP and/or other RBP and interfere with the cytochrome P450 (CYP450) mediated synthesis of 9-*cis*-retinoic acid (9-*cis*-RA). Decreased 9-*cis*-RA levels would lead to decreased activation of RXR and adipogenesis resulting in hyperlipidemia and lipoatrophy (Carr et al., 1998a). Several observations are at odds with this hypothesis. First, Standeven et al. (1996) showed that treatment of rodents with 9-*cis*-RA causes hyperlipidemia. Second, select PIs inhibit in vitro adipogenesis in the presence of RXR agonists (Lenhard et al., 2000c). Finally, Stevens et al. (1999) demonstrated that, troleandomycin (a CYP inhibitor) has no effect on the formation of 9-*cis*-RA in vitro.

An alternative hypothesis is that select PIs displace ATRA from RBP, and the displaced ATRA binds and activates the RXR/RAR heterodimer, resulting in RXR/RAR-mediated adverse reactions. This proposal is substantiated, in part, by the demonstration that IDV, but not other PIs, increases the inhibitory effects of ATRA on adipogenesis in vitro (Lenhard et al., 2000d). Furthermore, the RAR-selective antagonist (AGN 193109) reduces ATRA-mediated hypertriglyceridemia in rats (Standeven et al., 1996) and IDV effects in vitro (Lenhard et al., 2000d). Interestingly, while IDV inhibits in vitro lipid accumulation in the presence of low concentrations of ATRA (conditions that activate RAR) IDV stimulates in vitro lipid accumulation in the presence of high concentrations ATRA (conditions that activate RXR; (Lenhard et al., 2000d). This is expected because activation of RAR inhibits fat formation and activation of RXR leads to fat accumulation in vitro. These observations offer a potential explanation for RAR-mediated lipoatrophy and RXR-mediated lipohypertrophy seen in the clinic.

In the clinic, IDV and retinoid therapies cause similar adverse reactions. Drug interactions have been reported between IDV and 13-*cis*-retinoic acid (Kaul et al., 1999; Padberg et al., 1999), suggesting that IDV and retinoids may exert their effects through similar molecular mechanisms (Lenhard et al., 2000d). Moreover, signs of chronic hypervitaminosis A were reported to be more frequent ($P < 0.001$) in subjects treated with IDV (14% dry skin, 9% xerostomia, 7% alopecia) than with APV (3% dry skin, 1% xerostomia, $< 1\%$ alopecia). Likewise, a positive correlation ($R = 0.73$, $P < 0.005$) was found between IDV and ATRA-induced adverse reactions in AKR/J mice (Lenhard et al., 2000a). These observations support the proposal that altered retinoid signaling promotes some of the adverse reactions associated with IDV therapy (Lenhard et al., 2000d).

Retinoids (Kindmark et al., 1998; Melhus et al., 1998) and HAART are both associated with alterations in bone and fat metabolism (Aukrust et al., 1999; Tebas et al., 2000). As osteoblasts and adipocytes share common progenitor cells and may be affected by retinoids and HAART, it is possible that changes in bone mineral density are related to altered fat metabolism and retinoid signaling (Lenhard et al., 2000d; Nolan et al., 2001). This is consistent with the fact that IDV stimulates ATRA-dependent osteoblastic alkaline phosphatase (ALP) activity and inhibits adipogenesis in vitro (Lenhard et al., 2000d). Likewise, IDV increases serum alkaline phosphatase activity in mice (Lenhard et al., 2000a) and an increased bone mineral density and osteocalcin in humans (Nolan et al., 2001). In contrast, APV, NFV, RTV and SQV do not stimulate ALP activity in vitro or in mice, revealing variable effects of PIs on metabolism. Limited clinical data is available that compares the effects of the individual drugs on bone metabolism (Nolan et al., 2001) and hence the effects of drugs on bone disorders in HIV-infected subjects remains unclear (Weiel and Lenhard, 2000).

6.3.2. Hypocomplementemia

Although the cause of PI-associated LD is unknown, several groups have suggested hypocomplementemia may cause partial LD (Sissons et al., 1976; Ipp et al., 1977; Levy et al., 1998). Both human and murine adipocytes express the complement components C3, factor B and factor D (adipsin; Peake et al., 1997). The combined activity of these components generates a protein, termed acylation-stimulating protein, which stimulates lipogenesis (Baldo et al., 1993). The observation that select PIs inhibit in vitro adipogenesis and lipogenesis, (Zhang et al., 1999; Lenhard et al., 2000c,d; Wentworth et al., 2000) raises the possibility that PIs decrease production of complement factors by adipocytes, causing LD. Consistent with this hypothesis, NFV and RTV were found to decrease expression of complement factor D (adipsin) in vitro (Lenhard et al., 2000c). If this hypothesis is correct, future clinical studies of PIs may include evaluation of their effects on complement levels and on production of acylation-stimulating proteins by adipocytes.

6.3.3. Depot-specific adipocyte apoptosis

It has also been suggested that depot-specific apoptosis occurs in adipocytes, which leads to fat wasting. Biopsy samples from 14 HIV-infected subjects on IDV-therapy and subjects with untreated melanoma were examined for apoptosis in subcutaneous fat tissue using the TUNEL (d(UTP)-digoxigenin nick end-labeling) method (Domingo et al., 1999). The TUNEL assay detects DNA strands breaks in tissue sections and allows quantification of apopotic cells by light microscopy. All the HIV-infected IDV treated subjects exhibited abnormal lipid profiles and had at least one of the clinical characteristics of LD, such as fat loss from the face, buttocks or upper trunk or central obesity. The antero-lateral aspect of the right leg had subcutaneous fat atrophy. Of the 10 assessable biopsy samples, six biopsies showed focally positive apopotic cells and the four demonstrated moderate changes. Controversy exists on the reliability of the TUNEL assay, as it is prone to false positive or negative findings depending on the staining kinetics and the reagent concentration (Saraste 1999). Measurement of cellular markers of apoptosis such as bcl-2 and Bax would have strengthened the observations in the study. However, the report sheds light on the possibility that IDV treatment causes depot-specific apoptosis.

7. Pathogenesis of NRTI-related metabolic complications

7.1. Mitochondrial toxicity

Some authors propose that LD associated with NRTI therapy may be related to mitochondrial toxicity (Brinkman et al., 1999; Kakuda 1999). This is thought to occur via incorporation of NRTI monophosphate (MP) into mitochondrial DNA (mtDNA). This leads to chain termination of DNA synthesis, unless excised by repair mechanisms. DNA polymerase-γ (pol-γ) is responsible for synthesis of mtDNA, so its inhibition would affect mitochondrial function considerably. Inhibition of DNA pol-γ could lead to a progressive depletion of mitochondrial DNA, which eventually leads to altered transcription, faulty protein synthesis and loss of oxidative phosphorylation (OXPHOS) function. Pol-γ is expressed and translated in the absence of mtDNA, suggesting it may also play other, yet unidentified, roles in the cell (Davis et al., 1996). In vivo and in vitro evidence suggests that NRTI-triphosphates (TPs) are more potent inhibitors of pol-γ than the other cellular DNA polymerases (α, β, δ, ε; Huang et al., 1990; Wright and Brown, 1990). The in vitro potency to inhibit DNA pol-γ varies among NRTIs (ddC > ddI > d4T > 3TC > ZDV > ABC; Martin et al., 1994).

The presence of both normal and mutant mitochondrial DNA is known as heteroplasmy. Once the mutant mtDNA accumulates above a critical level or threshold, a defect in cellular OXPHOS develops and is likely to result in clinical symptoms associated with mitochondrial diseases (Brinkman et al., 1998). The clinical presentation of many inherited mitochondrial diseases such as the rare genetic disease of multiple symmetric lipomatosis (MSL; Madelung's disease or Launios–Bensaude syndrome; Hengel et al., 1997) is similar to that of certain toxicities associated with NRTI therapy, and studies of these toxicities have suggested that some may have a mitochondrial pathophysiology.

Carr et al. reported elevated lactate levels in their patients with NRTI-associated LD (Carr et al., 2000) and Saint-Marc demonstrated reductions in lactate levels in those patients whose LD improved after switching from d4T therapy (Saint-Marc et al., 2000b). This may reflect the increased risk of hyperlactatemia with d4T reported by others (Harris et al., 1999; Boubaker et al., 2000) rather than a mitochondrial link to LD. Mallal and colleagues have recently described mitochondrial alterations in light and electron microscopy of fat biopsies from treated HIV patients with LD (Mallal and Nolan 2000). Should evidence of mitochondrial DNA depletion be found in the future, a mitochondrial pathophysiology for LD may be reappraised. While NRTI-associated mitochondrial dysfunction may underlie lactic acidosis, hepatic steatosis, myopathy and neuropathy, the evidence for a role of mitochondrial dysfunction in HAART associated hyperlipidemia and LD is currently limited.

8. Summary and future directions

Because levels of plasma HIV-1 RNA and CD4 count correlate with HIV disease progression, the focus of HIV therapy for the last few years has been reduction of viral RNA and preservation and reconstitution of the immune system. While this strategy has reduced morbidity and mortality, the continuing use of HAART has resulted in increased exposure to treatment regimens with potentially lifelong complications. Four important issues influence future directions for HIV therapy. First, the factors to be considered when selecting an initial antiretroviral regimen have expanded from potency, adherence and resistance, to include pharmacokinetic factors and the potential for long-term complications. All other factors being equal, the first regimen is generally considered the most effective and the most durable (Fischl 1999), therefore most likely to have long-term effects (Mallal et al., 2000). Second, because maintenance of viral RNA reductions with the currently available agents is of paramount importance to prevent disease progression, adequate treatment options for adverse events occurring as a consequence of antiretroviral therapy are essential. Third, we need to understand the mechanism by which individual agents within HAART regi-

mens cause these complications, by utilizing in vitro assays, animal models and pharmacogenetics in clinical trial populations and cohort studies. When the basis of these complications is understood, surrogate marker assays may be developed (for example in vitro adipogenesis assays, Lenhard et al., 2000c) that can be used to discover new agents that have reduced potential to cause these complications. Fourth, a common terminology is needed to discuss these metabolic complications and a case definition of the syndrome(s).

There are not sufficient prospective clinical trials comparing various combination regimens to absolutely conclude what agents are the least likely to cause metabolic complications. It is clear that both nucleosides and PIs contribute to metabolic complications (Mallal et al., 2000). While the two drug classes may have some overlapping adverse effects (e.g. fat maldistribution), evidence suggests that some of these effects are specific to each class. NRTIs may cause lactic acidosis but PIs do not appear to influence the development of this syndrome. PIs and NNRTIs may cause hypercholesterolemia, the latter has not been associated with NRTI therapy (Morris and Carr, 1999). Furthermore, adverse effects can differ within a drug class. Several reports suggest that IDV or d4T may cause more LD than SQV or ZDV (Carr et al., 1998a, 1999; Saint-Marc and Touraine 1999b) and RTV may increase serum triglycerides more than other PIs (Falutz and Turcot 1999). Therefore, it cannot be concluded that nucleoside-based, PI-sparing regimens will not cause toxicity, nor can it be assumed that metabolic adverse events will be identical between individual drugs from a drug class. Although HAART regimens using PIs combined with NNRTIs are likely to lead to hyperlipidemia, it remains to be seen whether these regimens are safer than a PI combined with certain NRTIs. In addition, it is now common to administer a low-dose of RTV in combination with other PIs. The low-dose RTV decreases clearance and enhances the efficacy of other PIs. While RTV raises lipids at higher doses (400–600 mg BID), RTV also increases serum lipids at lower doses (300 and 100 mg BID; (Sadler et al., 2000), albeit to a lesser degree. To fully assess the impact of low dose RTV on lipid profiles requires long-term clinical data for such PI combinations.

Understanding the mechanism of HAART-induced metabolic complications is of paramount importance in the discovery of new antiretroviral agents with less potential for long-term metabolic side effects. This may also lead to the identification of safer regimens using currently available agents and allow for the development of surrogate assays to predict complications associated with HAART. It may also be possible to predict when two or more agents may be synergistic or additive in their toxicity. For example, if NRTIs inhibit DNA pol-γ, then the addition of hydroxyurea may increase mitochondrial toxicity by enhancing the ratio of ddNTP to endogenous dNTP. This is consistent with recent studies that show the addition of hydroxyurea to d4T/ddI therapy increases the risk of developing mitochondrial toxicity (McCarthy et al., 2000; Moore et al., 2000).

Some alleles predispose individuals to metabolic diseases (Bouchard 1997). Thus, it may be possible to correlate the probability of HAART-induced complications with specific genetic polymorphisms. For example, metabolism of PIs involves p450 CYP3A4 (Sommadossi 1999) and 3A4 polymorphisms may influence the pharmacokinetic properties of PIs. Exposure to drug (AUC and time on therapy) may correlate with the probability of developing metabolic complications. Therefore, pharmacogenetic analysis that predicts increasing drug exposure may correlate with the development of metabolic complications. It may not be possible to design new antiretroviral agents in the current drug classes that do not cause metabolic complications, especially if the mechanisms are unknown. Thus, new agents in clinical and pre-clinical development that are of a different mechanistic class may be better in this regard. For example, the fusion inhibitors (e.g. T-20; Pilcher et al., 1999) and HIV integrase inhibitors (Lin et al., 1999) might provide alternatives with less metabolic complications, assuming they can demonstrate sufficient antiviral activity in vivo.

Finally, the term LD has been used to describe everything from the loss or absence of subcutaneous adipose tissue to fat accumulation or to the

whole array of metabolic complications induced by HAART. The medical definition of LD is defective metabolism of fat and is characterized by almost complete lack of subcutaneous fat. In the future, it will be critical to develop common definition(s) that describe the syndrome(s) associated with HAART. Reliable and sensitive measures for fat changes and standardization of reporting data will be critical issues in developing the case definition(s) for the LD syndrome.

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