Increased VLDL-apoB and IDL-apoB production rates in nonlipodystrophic HIV-infected patients on a protease inhibitor-containing regimen: a stable isotope kinetic study

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Abstract The aim of this study was to identify the first abnormalities of apolipoprotein B (apoB) metabolism in HIV-infected patients treated by antiretroviral therapy (ART) with protease inhibitors (PIs). The influence of ART on the metabolism of apoB in VLDL, IDL, and LDL was investigated in six patients receiving dual nucleoside reverse transcriptase inhibitors (NRTIs) and PI, and in five patients receiving NRTI and nevirapine. None of the patients had lipodystrophy. The study was performed in the fed state. Each subject received an intravenous injection of a 0.7 mg·kg^{-1} bolus of L-[1-^{13}C]leucine, immediately followed by a 16 h constant infusion at 0.7 mg·kg^{-1}·h^{-1}. The VLDL- and IDL-apoB concentrations were significantly higher in PI-treated patients compared to non-PI-treated patients. The VLDL-apoB and IDL-apoB production rates were markedly higher in PI-treated patients compared to non-PI-treated patients (54.5 ± 30.1 vs. 30.9 ± 8.4 mg·kg^{-1}·d^{-1}, $P = 0.04$; and 43.5 ± 20.0 vs. 18.7 ± 7.8 mg·kg^{-1}·d^{-1}, $P = 0.04$, respectively). In conclusion, our study shows that patients receiving ART with PI present altered metabolism of the VLDL-IDL-LDL chain compared with patients treated without PI. These data confirm that PI therapy is associated with a physiopathological mechanism for dyslipidemia in addition to the effect of lipodystrophy on lipid metabolism.—Petit, J. M., M. Duong, E. Florentin, L. Duvillard, P. Chavanet, J. M. Brun, H. Portier, P. Gambert, and B. Vergès. **Increased VLDL-apoB and IDL-apoB production rates in nonlipodystrophic HIV-infected patients on a protease inhibitor-containing regimen: a stable isotope kinetic study. *J. Lipid Res.* 2003.** 44: **1692–1697.**

Supplementary key words HIV protease inhibitor • nucleoside reverse transcriptase inhibitor • antiretroviral therapy • metabolism abnormalities • apolipoprotein B • dyslipidemia • triglyceride • isotope stable • kinetic study

Antiretroviral therapy (ART) leads to a dramatic and sustained reduction in morbidity and mortality associated with HIV infection (1). Several classes of medications are used to treat HIV infection, including protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), and non-nucleoside analogs transcriptase inhibitors. However, ART has been associated with abnormal body fat distribution (also called lipodystrophy), central adiposity, and negative metabolic effects, such as new-onset diabetes mellitus and hyperlipidemia (2–9). Several hypotheses regarding the physiopathology of the ART-associated dyslipidemia have been proposed. Most studies have linked the development of these abnormalities to PIs. However, both PIs and nucleoside analogs have been suggested to be responsible for the pathogenesis of lipodystrophy and metabolic changes (2, 10–13). The mechanism responsible for ART-associated dyslipidemia is not fully elucidated and appears to be mutifactorial. Indeed, several factors, such as the HIV infection itself, the concomitant presence of lipodystrophy, and the coinfection with hepatitis C, are possible influences on lipid parameters (9, 14–16). The respective influences of PI therapy, HIV infection itself, NRTIs, and lipodystrophy have not been completely evaluated. To explore the alterations of apolipoprotein B (apoB) metabolism associated with the direct action of PI therapy, we performed a stable isotope kinetic study of all apoB-containing lipoproteins in HIV-infected patients without lipodystrophy receiving ART with or without HIV PIs.

MATERIALS AND METHODS

Subjects

Five HIV-infected men receiving ART without PI therapy and six HIV-infected men receiving ART with PI therapy were studied (**Table 1**). For each patient, types and duration of ARTs at time of enrollment were assessed (Table 1). Each patient had been receiving the same ART without any modification for at least 1 year at the time of enrollment. In the non-PI group, the patients were

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TABLE 1. Biometric and clinical data of HIV–infected patients

	Age	BMI (kg·m^2)	WHR	HIV Infection (Months)	CD4	HIV Viral Load (Copies·ml)	CE to PI Therapy (Months)	Current Treatment	HOMA
Non-PI-treated									
1	35	27.1	0.97	84	1,071	<7	12	3TC, AZT, NEV	0.50
2	39	20.5	0.73	72	288	<7	36	D4T, 3TC, NEV	0.38
3	37	22.3	0.76	26	535	11,265	13	D4T, 3TC, NEV	1.80
4	37	22.6	0.89	60	425	222	32	D4T, 3TC, NEV	0.40
5	31	17.9	0.87	102	918	9	21	D4T, DDI, NEV	0.21
Mean ± SD	35.9 ± 3.03	22.1 ± 3.3	0.84 ± 0.09	68 ± 20	707 ± 309	2,301 ± 5,011	22 ± 10		0.67 ± 0.66
PI-treated									
1	35	24.1	0.85	24	375	7	23	D4T, EPV, NEL	0.74
2	38	19.8	0.88	161	295	15	24	AZT, DDI, RIT	0.59
3	37	25.6	0.95	64	750	7	46	AZT, EPV, IND	0.18
4	39	30.6	0.94	12	113	5	12	AZT, EPV, NEL	0.92
5	40	23.4	0.97	24	288	14	22	D4T, DDI,NEL	0.62
6	50	21.8	0.86	144	498	39	36	D4T, DDI, IND, RIT	1.29
Mean ± SD	39.6 ± 5.7	24.2 ± 3.6	0.92 ± 0.05	71 ± 65	386 ± 217	14 ± 12	27 ± 11		0.72 ± 0.37
P	0.19	0.20	0.20	0.58	0.12	0.58	0.46		0.36

BMI, body mass index; WHR, waist-to-hip ratio; CE, cumulative exposure; PI, protease inhibitor; HOMA, homeostasis model assessment. Current treatment: reverse transcriptase inhibitors were stavudine (D4T), lamivudine (3TC), zidovudine (AZT), and didanosine (DDI). Nonnucleoside reverse transcriptase inhibitor was nevirapine (NEV). PIs were nelfinavir (NEL), indinavir (IND), and ritonavir (RIT).

not PI naive, but these components had been stopped at least 1 year before the study. None of the patients had a medical or family history of dyslipidemia. The patients were not taking concurrent therapies known to affect carbohydrate or lipid metabolism (hypoglycemic agents, corticoids, hypolipidemic agents, retinoids, cyclosporin, interferon). Patients having active opportunistic infection, malignancy, diabetes mellitus, lipodystrophy syndrome or having noncontrolled HIV infection were excluded. Lipodystrophy was an exclusion criterion. It was defined clinically by physical examination and by patient report of fat wasting in the face, arms, or legs with or without central obesity, and fat accumulation in the abdomen or over the dorsocervical spine (17). The protocol was approved by the Dijon University Hospital ethics committee, and written informed consent was obtained before the study was started.

Experimental protocol

The kinetic study was performed in the fed state. Food intake, a leucine-poor diet [1,700 kcal·day^{-1}, 55% carbohydrates, 39% fats (13% saturated and 26% unsaturated) and 7% proteins], was identical among subjects and was fractionated in small equal portions that were provided every 2 h, starting 6 h prior to the tracer infusion, up to the end of the study, in order to avoid important variations in apolipoprotein plasma concentration. To determine the kinetics of apoB-100, the subjects received an intravenous injection of a 0.7 mg·kg^{-1} bolus of L-[1-^{13}C]leucine (99% ^{13}C, Eurisotop, Saint Aubin, France) immediately followed by a 16 h constant infusion at 0.7 mg·kg^{-1}·h^{-1}. Blood samples (16 ml) were collected at h 0, 0.25, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 16. Blood was collected in tubes without anticoagulant but with a gel separator (Becton Dickinson, Meylan, France). The serum was separated by centrifugation and stored at 4°C. Sodium azide, an inhibitor of bacterial growth, and aprotinin (Sigma), a PI, were added to plasma samples at final concentrations of 500 mg·l and 17 mg·l, respectively.

Analytical procedures

Analytical procedures were performed as previously described in detail (18, 19).

Isolation and measurement of apolipoproteins

VLDL [density (d) < 1.006 g·ml^{-1}], IDL (1.006 < d < 1.019), and LDL (1.019 < d < 1.63) were isolated from plasma by sequential flotation ultracentrifugation, using a 50.4 rotor in an L7 apparatus (Beckman Instruments, Palo Alto, CA). IDL and LDL fractions were then dialyzed against a 10 mmol·l^{-1} ammonium bicarbonate buffer (pH 8.2) containing 0.01% EDTA and 0.013% sodium azide. VLDL, IDL, and LDL fractions were delipidated for 1 h at −20°C using 10 volumes of diethylether-ethanol (3:1). From each lipoprotein fraction, apoB-100 was isolated by preparative SDS-PAGE: the delipidated apoB-100-containing material was solubilized in 0.05 mmol·l Tris buffer (pH 8.6) containing 3% SDS, 3% mercaptoethanol, and 10% glycerol and applied to a 3 mm-thick vertical slab gel (3% acrylamide). After staining with Coomassie blue R-250, apoB-100 bands were excised from the polyacrylamide gels and hydrolyzed in 6 M HCl at 110°C for 16 h under nitrogen vacuum. Samples were then centrifuged to remove polyacrylamide. Supernatants were lyophilized in a Speed Vac (Savant Instrument, Farmingdale, NY). Lyophilized samples were dissolved in 50% acetic acid and applied to an AG-50W-X8 200-400 mesh cation exchange resin (Bio-Rad, Richmond, CA), and amino acids were recovered by elution with 4N NH_4OH. They were converted to *N*-acetyl-*O*-propyl amino esters prior to analysis by gas chromatograph/combustion/isotope ratio mass spectrometry (GC/C/IRMS).

Determination of leucine enrichment by GC/C/IRMS

A Finnigan Mat Delta C isotope ratio mass spectrometer (Finnigan Mat, Bremen, Germany) coupled to an HP 5890 Series II gas chromatograph (GC) (Hewlett Packard), was used to determine sample isotopic enrichment. The GC was equipped with a split/splitless injector and fitted with a BPX5 capillary column (30 m, 0.32 mm i.d., 25 μm film thickness, S.G.E., Ringwood Vic, Australia) and a 2 m retention gap (RGK-1, S.G.E.). The carrier gas was helium, and the column head pressure was set at 14 PSI. Injector temperature was 250°C for leucine analysis. The splitless mode injection was adopted. The solvent purging valve was opened 0.6 min after injection. The column was held isothermal at 50°C for 1 min after injection, then the temperature was programed at 20°C min^{-1} up to 135°C, at 2°C min^{-1} from 135°C to 150°C, at 15°C min^{-1} from 150°C to 290°C, and was held for 5 min at 290°C. The operation conditions of the ion source were as follows: source chamber pressure, 1.4 × 10^{-6} mbar; ionizing energy, 80 eV; ion accelerating voltage, 3 kV. Isotope abundance was expressed relative to pulse peaks of the ref-

erence gas. Data were analyzed using the supplier software (Finnigan ISODAT).

Modeling

Apolipoprotein kinetics data were expressed as tracer-to-tracee mass ratios, z(t), calculated as follows:

$$z(t) = \frac{e(t)}{e_i - e(t)}$$

where e_i is the tracer enrichment, $e(t) = a(t) - a_N$, and a(t) and a_N are the isotope abundance of the labeled and the unlabeled species, respectively.

ApoB-100 data were analyzed by multicompartmental modeling with the program SAAM II (SAAM Institute, Inc., Seattle, WA). The model chosen to describe the data is shown in **Fig. 1**. A forcing function, corresponding to the VLDL-apoB-100 enrichment at plateau, was used to drive the appearance of leucine tracer into the different lipoprotein fractions. A delay compartment was included in the model to account for the time required for the synthesis and secretion of apoB into the plasma. Plasma VLDL, IDL, and LDL-apoB kinetic data are represented, respectively, by compartments 1, 11, and 21. As the experiment was performed in the steady state, the fractional synthetic rate equalled the fractional catabolic rate (FCR).

The direct FCR of VLDL-apoB and the FCR from VLDL to IDL or LDL, expressed in pool·h^{-1}, were calculated as follows:

$$\text{direct } FCR_{VLDL} = k(0,1)$$

$$FCR_{VLDL \rightarrow IDL \rightarrow LDL} = k(11,1)$$

where k(i,j) is the fractional transfer coefficient from compartments j to i.

Total apoB VLDL FCR is the sum of direct FCR_{VLDL} and $FCR_{VLDL \rightarrow IDL}$.

The direct FCR of IDL-apoB and the FCR from IDL to LDL were calculated as follows:

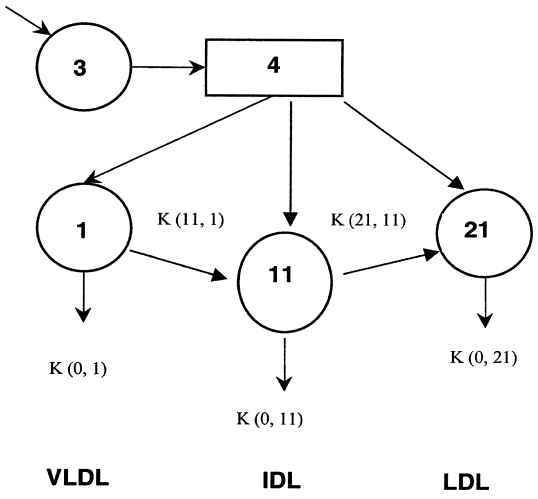

Fig. 1. Multicompartmental model for kinetic analysis of apolipoprotein B-100 (apoB-100) metabolism. A forcing function, corresponding to the VLDL-apoB-100 plateau enrichment, was used to drive the appearance of leucine tracer into the different lipoprotein fractions (compartment 3). Compartment 4 is the delay compartment, representing the time required for the synthesis and secretion of apoB-100 into the plasma. Compartment 1 represents plasma VLDL-apoB-100. Compartment 11 represents plasma intermediate-density lipoprotein (IDL)-apoB-100. Compartment 21 represents plasma low-density lipoprotein (LDL)-apoB-100.

$$\text{direct } FCR_{IDL} = k(0,11)$$

$$FCR_{IDL \rightarrow LDL} = k(21,11)$$

Total apoB-IDL FCR is the sum of direct FCR_{IDL} and $FCR_{IDL \rightarrow LDL}$.

The FCR of LDL-apoB was calculated as follows:

$$FCR_{LDL} = k(0,21)$$

The apoB-100 production rate in one lipoprotein fraction was calculated as the products of the total apoB FCR and pool sizes of that fraction divided by body weight. Pool size was determined as the product of plasma volume and apoB concentration of the lipoprotein fraction. Plasma volume was calculated as 4.5% of body weight.

Apolipoprotein and lipid assays

Concentrations of apoB-100 were measured by immunonephelemetry (Boehringer Mannheim). The apoB masses in each lipoprotein fraction were corrected for experimental losses by comparing the cholesterol value in the three lipoprotein fractions (VLDL, IDL, LDL) with the non-HDL cholesterol value determined in native plasma

All chemical assays were performed on a Cobas-Fara Centrifugal Analyser (Hoffmann-La Roche). Total cholesterol and unesterified cholesterol concentrations were measured by enzymatic methods using Boehringer Mannheim reagents. Triglyceride concentration was measured by enzymatic methods using Roche reagents.

Insulin resistance evaluation

The insulin resistance level was estimated by using the homeostasis model assessment (HOMA) method (20).

The HOMA was calculated with the following formula (20):

$$\text{HOMA} = \frac{[\text{glucose}] \times [\text{insulin}]}{22.5}$$

where [glucose] is the fasting glucose concentration expressed in mmol.l and [insulin] is the fasting insulin concentration expressed in mU·l.

Plasma insulin was measured by radioimmunoassay (CIS Bio International, Gif sur Yvette, France).

Statistical analysis

Data are reported as mean ± SD. The Mann-Whitney U-test was used to compare clinical, biological, and kinetic characteristics between patients and controls. The Spearman correlation coefficients were calculated in correlation analyses. A two-tailed probability level of 0.05 was accepted as statistically significant.

RESULTS

Apolipoprotein and lipid concentrations

Clinical and metabolic characteristics of the subjects are presented in Table 1. The two groups (patients with and without PIs) were well-matched for age, sex, body mass index, waist-to-hip ratio, and waist circumference (Table 1). Data for fasting plasma lipoprotein concentrations measured before the kinetic study are presented in **Table 2**. Plasma apoB concentrations in each lipoprotein fraction had been studied in function of time during the kinetic experiment for one individual with our methodology. The coefficients of variation throughout the study were, respectively, 4.8, 9.2, and 5.1% for VLDL-, IDL-, and

TABLE 2. Lipid and apolipoprotein concentrations of study subjects

Concentrations	PI-treated	Non-PI-treated	*P*
Screening values (in fasting state)			
Plasma cholesterol (mmol·l)	5.79 ± 1.02	5.22 ± 0.81	0.36
Plasma triglycerides (mmol·l)	1.93 ± 0.42	1.40 ± 0.39	0.04
HDL cholesterol (mmol·l)	1.03 ± 0.15	1.24 ± 0.42	0.67
LDL cholesterol (mmol·l)	3.88 ± 0.85	3.23 ± 0.94	0.14
Experimental values (in fed state)			
Plasma cholesterol (mmol·l)	5.85 ± 0.67	5.08 ± 1.29	0.27
ApoB (g·l)	1.39 ± 0.13	1.00 ± 0.30	0.01

ApoB, apolipoprotein B; PI, protease inhibitor. Values are mean ± SD.

LDL-apoB. Moreover, no significant variation was observed between measurements at four different infusion times in the studied patients. Thus, all subjects were considered to be in steady state throughout the study. The means of plasma total cholesterol, HDL-cholesterol and LDL-cholesterol levels were not significantly different between the two groups (Table 2). Fasting triglyceride levels and apoB concentrations in the fed state were significantly increased in PI-treated patients compared with non-PI-treated patients (Table 2).

Kinetic parameters

Kinetic parameters of apoB-100 are shown in **Table 3**. PI-treated patients had significantly higher VLDL-apoB and IDL-apoB concentrations than did non-PI-treated patients (Table 3). Compared with non-PI-treated patients, VLDL-apoB and IDL-apoB production rates were significantly higher (54.5 ± 30.1 vs. 30.9 ± 8.4 mg·kg^{-1}·d^{-1}, P = 0.04 and 43.5 ± 20.0 vs. 18.7 ± 7.8 mg·kg^{-1}·d^{-1}, P = 0.04, respectively) in PI-treated infected patients, corresponding to a 76% increase in the VLDL-apoB production rate. The LDL-apoB production rate was increased in PI-treated patients compared with non-PI-treated patients (19.2 ± 2.4 vs. 15.3 ± 4.7 mg·kg^{-1}·d^{-1}), but the difference was borderline significant (P = 0.06). We found a significant correlation between VLDL-apoB production rate and IDL-apoB production rate (r = 0.77; P = 0.01) and a borderline significant correlation between VLDL-apoB production rate and LDL-apoB production rate (r = 0.52; P = 0.09). The VLDL-apoB transfer rate to IDL, the IDL-apoB transfer rate to LDL, and the LDL-apoB fractional catabolic rate were not significantly different in PI-treated patients compared with non-PI-treated patients.

DISCUSSION

Our results show important alterations of apoB metabolism in patients treated with PIs, with a significantly higher apoB production rate in the VLDL and IDL lipoprotein fractions, leading to increased VLDL-apoB and IDL-apoB plasma pools. These data suggest a direct action of PI therapy on lipid metabolism. We have chosen to study the metabolism of apoB in the postprandial state, because it represents the most frequent state in humans over a 24 h period. Moreover, many data suggest that atherosclerosis could be a postprandial phenomenon (21). The daily food intake was divided into small equal portions taken by the subjects every 2 h, as previously performed by several groups (22, 23). According to this protocol, plasma lipid and apolipoprotein values remained constant throughout the kinetic study.

One limitation of our study is that only a clinical diagnosis of lipodystrophy was used. We did not use objective measurements of regional fat, such as dual X-ray absorptiometry or abdominal computed tomography. However, such measurements are not standardized, and these are not included in the definition of lipodystrophy based both on reports from patients and on doctor confirmation (17, 24). In addition, we cannot exclude a direct effect of NRTIs on the lipid abnormalities observed in our patients. In fact, NRTIs can inhibit mitochondrial DNA polymerase-γ,

TABLE 3. Kinetic parameters of VLDL-, IDL-, and LDL-apoB-100 in study subjects

	VLDL-apoB				IDL-apoB				LDL-apoB		
			FCR				FCR				
	apoB	Production	Direct Catabolism	Transfer to IDL/LDL	apoB	Production	Direct Catabolism	Transfer to LDL	apoB	Production	FCR
	mg·l^{-1}	*mg·kg^{-1}·d^{-1}*	*pool·h^{-1}*		*mg·l^{-1}*	*mg·kg^{-1}·d^{-1}*	*pool·h^{-1}*		*mg·l^{-1}*	*mg·kg^{-1}·d^{-1}*	*pool·h^{-1}*
PI-treated men											
1	159	19.20	0.03	0.07	222	15.58	0	0.06	1,007	17.40	0.016
2	320	67.73	0.006	0.19	320	65.66	0.13	0.05	960	21.77	0.021
3	148	47.95	0.16	0.13	181	24.82	0.01	0.11	1,062	22.93	0.020
4	369	107.6	0.22	0.05	315	40.48	0.06	0.05	537	18.55	0.032
5	154	43.90	0	0.26	377	54.15	0.09	0.03	851	17.46	0.019
6	188	40.20	0	0.19	386	60.03	0.10	0.03	804	17.36	0.020
Mean ±SD	223 ± 96	54.5 ± 30.1	0.07 ± 0.09	0.14 ± 0.08	300 ± 82	43.5 ± 20.0	0.06 ± 0.05	0.06 ± 0.03	870 ± 189	19.2 ± 2.4	0.021 ± 0.006
Non-PI-treated men											
1	128	40.09	0.18	0.10	159	23.69	0.07	0.06	630	11.56	0.016
2	78	19.37	0	0.23	220	23.76	0.02	0.07	1,020	17.62	0.016
3	117	37.40	0.14	0.15	72	18.19	0	0.23	630	21.09	0.031
4	83	25.72	0.22	0.06	48	5.18	0	0.10	520	9.54	0.017
5	169	31.94	0.06	0.11	150	22.68	0.03	0.10	1,001	17.29	0.016
Mean ± SD	115 ± 37	30.9 ± 8.4	0.12 ± 0.09	0.13 ± 0.06	129 ± 69	18.7 ± 7.8	0.02 ± 0.03	0.11 ± 0.07	757 ± 229	15.3 ± 4.7	0.019 ± 0.007
P	0.02	0.04	0.40	0.71	0.01	0.04	0.19	0.08	0.36	0.06	0.19

FCR, fractional catabolic rate.

and it has been suggested that lipodystrophy and metabolic disorders could be a manifestation of mitochondrial damage attributable to NRTI therapy (13). Finally, we observed a trend to higher CD4 cell count in the nevirapine-treated group, which, however, was not statistically significant. Although we didn't find any correlation between VLDL-apoB production rate and CD4 cell count in the whole population, we cannot totally exclude an influence of this difference on our results.

The mechanism by which ART alters plasma lipoprotein remains unclear. Hyperlipidemia arising from ART is probably related to several factors, including the direct action of PIs, but also to changes in body composition or insulin sensitivity and improvement in HIV status. Numerous studies have suggested a direct etiologic role of PIs in the development of hyperlipidemia arising from ART (2, 15, 25, 26). Using a stable isotope kinetic analysis, Schmitz et al. showed an increased total apoB synthesis and a reduced rate of VLDL transfer into denser lipoproteins in five HIV-infected patients on a PI-containing regimen (including two patients with lipodystrophy) compared with non-HIV-infected subjects (27). However, the respective influences of PI therapy, lipodystrophy, and HIV infection itself on hyperlipemia could not be demonstrated in this study. By contrast, we evaluated only patients without lipodystrophy, who were similar in terms of immunological and virological status. Thus, our data suggest that PI therapy is associated with a direct physiopathological mechanism for dyslipidemia. Our work is consistent with the data published by Riddle et al. in ritonavir-treated mice. Their study showed a hyperlipidemic effect of PIs that was a direct result of an increased hepatic lipoprotein production, without any effects on lipoprotein clearance from circulation (28). In the same way, Negredo et al. observed an improvement of lipid metabolism when PI-treated patients with moderate hyperlipemia switched from nevirapine to PIs (29). According to our data, this positive effect could be related to a decreased production of VLDL-apoB following the substitution of nevirapine for PIs. However, the absence of a kinetic study in a nontreated HIV-group before the introduction of PIs or nevirapine is a limitation of our study. Our study cannot determine whether nevirapine itself, independently of the absence of PIs, has a favorable effect on lipid parameters. Ritonavir has been reported to increase the risk of hypertriglyceridemia and hypercholesterolemia compared with other PIs (8). In the same way, most of the studies of metabolic effects of PIs in vitro or in vivo have used ritonavir (25, 28). Unfortunately, our study with three different PIs in a small group of patients is unable to determine whether ritonavir has a different effect on VLDL production from other PIs.

Different hypotheses about the pathogenesis of the alterations of apoB metabolism observed in our study could be put forward. Insulin resistance may be responsible for the increased apoB secretion. Indeed, insulin has been shown to inhibit VLDL-apoB production. The increased VLDL-apoB production rate observed in insulin-resistant subjects could be explained by hepatic resistance to the inhibitory effect of insulin on VLDL-apoB production (30). However, the lack of a significant difference in insulin resistance between the two groups does not suggest that increased apoB-VLDL production in our PI-treated patients could be a consequence of a modification in insulin sensitivity. Our data are in accordance with previous studies, suggesting that PI-associated dyslipidemia may occur independently of glucose metabolism abnormalities (7, 31).

It has also been reported recently that PIs inhibit proteasomal degradation of nascent apoB, the principal protein component of triglyceride and cholesterol-rich plasma lipoproteins (32). This study, conducted in vitro with a cultured-cells model for lipoprotein production, implicates the inhibition of proteasomal degradation as a physiologically relevant molecular mechanism by which PI therapy may cause hyperlipidemia. Thus, the inhibition of presecretory apoB degradation caused by PIs may increase the assembly and secretion of VLDL. Finally, the direct inhibition of proteasomal degradation by PIs could be an explanation for the increased VLDL-apoB production observed in our study. Although the interpretation of IDL- and LDL-apoB production in a kinetic study must be cautious (33), the increase in the IDL-apoB production rate found in our work may be a consequence of the increase in the VLDL-apoB production rate, as suggested by the significant correlation observed between VLDL- and IDL-apo B production rates.

In our study, we did not observe an effect of PI therapy on the clearance of triglyceride-rich lipoproteins from circulation. These observations are consistent with a recent study demonstrating no abnormality in the clearance of lipoproteins in ritonavir-treated mice (28). However, ART was reported to decrease VLDL lipolysis in HIV-infected individuals compared with non-HIV-infected subjects (27). The discrepancy may be due to the influence of HIV infection itself on VLDL triglyceride clearance (28). Indeed, in the context of HIV infection before the use of ART, Grunfeld et al. observed that triglyceride clearance was significantly decreased in HIV-infected patients compared with non-HIV-infected controls (34). In summary, our results suggest that PI therapy has a direct effect on lipid metabolism and leads to an increase in the production of VLDL-apoB and IDL-apoB. These data have been confirmed in HIV-infected subjects receiving ART, and in previous results from animal studies. These abnormalities may promote atherosclerosis in these patients and confirm the direct effect of PI therapy on lipid metabolism in addition to the influence of lipodystrophy.

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