Disturbance of Apolipoprotein B100 Containing Lipoprotein Metabolism in Severe Hyperlipidemic and Lipodystrophic HIV Patients on Combined Antiretroviral Therapy: Evidences of Insulin Resistance Effect

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Abstract: The aim was to study the mechanisms involved in the dyslipidemia associated with lipodystrophy in HIV infected patients on antiretroviral therapy (ART).

We investigated the *in vivo* kinetics of apolipoprotein B100 (apoB) containing lipoproteins using a 14 h primed constant infusion of [5,5,5,$^2H_3$] leucine and compartmental modelling in normolipidemic without lipodystrophy (7 patients, NLD) or dyslipidemic with lipodystrophy (7 patients, LD) treated with ART.

Subjects in group LD showed higher plasma triglycerides (5.73±3.58 *vs* 1.29±0.54 g/L, $p<0.005$), total cholesterol (2.98±0.95 *vs* 1.74±0.26 g/L, $p<0.05$), apoB (1.49±1.11 *vs* 0.51±0.11 g/L, $p<0.005$) and apolipoprotein CIII in apoB containing lipoproteins (117.7±42.2 *vs* 22.6±23.9 g/L, $p<0.005$). LD subjects exhibited an insulin resistant as observed by higher HOMA (3.44±1.62 *vs* 1.60±0.61, $p<0.05$). They exhibited an increase in VLDL (1.24±0.33 *vs* 0.80±0.21 mg/kg/h, $p<0.05$), decrease in IDL (0.20±0.10 *vs* 0.48±0.24 mg/kg/h, $p<0.05$) and no difference in LDL (0.38±0.19 *vs* 0.45±0.25 mg/kg/h) production rate. LD subject also showed a dramatic decrease in transformation of VLDL to IDL (0.013±0.010 *vs* 0.258±0.206 h^{-1}, $p<0.005$) and IDL to LDL (0.088±0.093 *vs* 0.366±0.189 h^{-1}, $p<0.05$) and a decrease in fractional catabolic rate (FCR) of VLDL (0.199±0.132 *vs* 0.555±0.398 h^{-1}, $p<0.05$), IDL (0.110±0.08 *vs* 0.523±0.275 h^{-1}, $p<0.05$) and LDL (0.010±0.005 *vs* 0.025±0.014 h^{-1}, $p<0.05$).

These disturbances, overproduction and an overall delayed catabolism of apoB, are similar to those observed using the same protocol in insulin resistant subjects. Our study suggests that metabolic disturbance of apoB100 observed in lipodystrophic HIV in combined antiretroviral therapy are consecutive to insulin resistance induced by the treatment.

Key Words: HIV, dyslipidemia, apolipoprotein B100, lipodystrophy, modelling.

INTRODUCTION

In patients infected with human immunodeficiency virus, dyslipidemia is frequently observed and characterized by elevated serum levels of triglycerides, reduced levels of total cholesterol, low-density lipoprotein and high-density lipoprotein-cholesterol with a predominance of small dense LDL particles [1,2]. Treatments with Protease inhibitors (PI) decrease HIV associated morbidity and mortality but lead to an increased risk of cardiovascular disease in part related to a strong increase of lipoprotein disturbances. A large variety of changes in metabolic status are seen after introducing the effective therapy for HIV infection, associated or not with lipodystrophy [3] and severe hyperlipidemia [4,5]. In the prospective data collection on adverse events of anti-HIV drugs study [6], it has been found after 6 years of exposition to protease inhibitors that there is a 16% relative risk per year for myocardial infarction in this cohort. This dyslipidemia is characterized by an increased plasma TG and total cholesterol mainly in VLDL [7]. Increase in apolipoprotein CIII, an endogenous inhibitor of lipoprotein lipase, was also reported [8]. Increased or unchanged LDL cholesterol have been reported in some studies [9,10]. The mechanisms involved in these effects of combined antiretroviral therapy on lipids are still debated [11-14] and hypothesis of insulin resistance mediated disturbance is frequently evocated [15].

In vivo studies to explain the mechanisms of dyslipidemia in lipodystrophic HIV infected and treated patients are scarce and available data vary according to the studied population, HIV infection status, as well as combination of drugs received by the subjects. In no lipodystrophic HIV patients, higher VLDL production [7,16] and low VLDL and IDL [16] catabolism were reported to explain patients hypertriglyceridemia. LDL kinetic data from patients with no lipodystrophy showed no change [7,16]. In lipodystrophic HIV patients, while Shahmanesh *et al.* [17] reported no change in VLDL apoB100 production, Reeds *et al.* [17] observed a higher production of VLDL triglycerides. In these two studies, FCR of VLDL [17,18], IDL [18] and LDL [19] were decreased. These apparently nonconcordant results are due to the fact that the profile of dyslipidemia in HIV is very variable. The objective of our study was to define the metabolic bases of severe dyslipidemia in lipodystrophic insulin resistant HIV patients on combined retroviral therapy com-

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paring to treated HIV patients with no lipodystrophy and then to compare disturbances with those observed during insulin resistance.

METHODS

Patients

The study was performed on 14 HIV positive men who were taking combined antiretroviral therapy (ART). Patients were distributed in 2 groups according presence or absence of lipid disturbance consecutively to treatment. One group (7 patients, group NLD) had normal fasting lipidemia with no lipodystrophy and the other group (7 patients, group LD) was severely hyperlipidemic and lipodystrophic. Combination antiretroviral therapy was defined as any antiretroviral drug regimen containing three or more drugs, one of which was a PI and a nucleoside reverse-transcriptase inhibitor. The treatments were given in Table **1**. None of the patients had a personal history of diabetes, hypothyroidism, obstructive liver disease, or chronic renal failure. The patients had not been taking any medication for their dyslipidemia. The patients underwent a kinetic study and also anthropometric and biochemical determinations. The experimental protocol was approved by the ethical committee of Nantes University Hospital, and written informed consent was obtained before the study was started. The same protocol was previously applied to control and insulin resistant subject with type 2 diabetes and parameters of apoB100 containing lipoprotein were already published [20].

Morphological Determinations

Presence of morphological changes was evaluated by physician examination, and anthropometric measurements. Patients were asked to assess body fat distribution according to several pre-selected clinical items and evaluation was based on a bidirectional response (increased/decreased). Signs of peripheral atrophy included the following: fat wasting in the face, upper limbs, lower limbs, prominent limb vein and buttock atrophy. Signs of fat accumulation included the following: accumulation of fat in the face or breasts, "buffalo hump," or increased waist size. Symptoms were pooled to design isolated peripheral atrophy, isolated fat accumulation, mixed syndrome (i.e., symptoms of peripheral atrophy and fat accumulation), and absence of signs (i.e., no peripheral

Table 1. Biometric and Clinical Characteristics in Non Lipodystrophic (NLD) and Lipodystrophic (LD) Patients

Subjects	Age years	Weight kg	BMI kg/m^2	HOMA	W/H	Triceps^2 cm	CD4+ cells/mm^3	HIV-1 RNA copies/ml	HIV-positive (months)	Treatments	Treatment with ART (months)
NLD1	28	65	21.7	1.48	0.88	11.2	945	<80	108	I, VI, IX	28
NLD2	40	67	21.4	1.68	0.95	13	200	<80	76	II,III, IX,	13
NLD3	52	77	24.9	2.69	0.91	18.2	374	<400	104	II, VII, IX	20
NLD4	36	72	22.7	1.98	0.96	15	471	1400	94	I, VIII, IX, X	16
NLD5	35	72	20.2	0.76	0.96	10.5	495	4190	96	I, VI, VII	26
NLD6	49	60	18.9	1.24	0.89	11.5	ND[1]	<200	111	II, IV, VII,	67
NLD7	26	69	22.8	1.38	0.86	6	832	<200	97	VIII, IX, X II, IX, X	66
Mean	38	68.9	21.8	1.60	0.92	12.20	552.83		98		33.7
SD	9.81	5.52	1.9	0.61	0.04	3.81	282.22		11.6		23.0
LD1	37	63	20.6	2.52	0.9	3.05	572	<200	96		34
LD2	51	55	16.4	3.22	0.89	4.2	433	<200	132	I, II, VIII, IX, X	42
LD3	41	67	24.0	2.02	0.95	11	311	<200	188		25
LD4	43	74	21.4	2.31	0.96	7.4	920	<200	180	II, III, VIII, XI	54
LD5	42	88.8	26.5	6.76	0.96	10.4	717	<200	120	II, VIII	13
LD6	42	64	22.4	3.14	1	4.25	ND	<200	94	II, VI, V, IX	72
LD7	42	81.5	24.9	4.11	0.95	6.5	170	<200	180	IV, VII, XI	48
Mean	42.6	70.5	22.3	3.44	0.94	6.69	520.50		141.4	II, III, IX, X, XII	41.1
SD	4.2	11.68	3.3	1.62	0.04	3.12	274.0		40.8	II, VI, IX, XI	19.5

[1]ND: Not done; [2]triceps circumference

*p<0.05, **p<0.005.

Protease inhibitors (Nelfinavir (I), Ritonavir(II), Indinavir (III), Lopinavir (IV), Saquinavir (V))

NRTI (Stavudine (VI), Didanosine (VII), Abacavir (VIII), Lamivudine (IX), Zidovudine (X))

NNRTI (Efavirenz (XI), Nevirapine (XII))

atrophy or fat accumulation). The term "lipodystrophy" was defined to include all physical signs of altered body fat distribution, regardless of different biological patterns.

Measurements of Glucose, Insulin, Lipids, ApoB100 and apoCIII

The plasma insulin concentration (microinternational units per mL) was measured by radioimmunometric assay (Sanofi Pharmaceuticals, Inc., Marnes-La-Coquette, France). The fasting blood glucose concentration (micromoles per mL) was evaluated using a glucose oxidase enzymatic assay (BioMérieux, Marcy-l'Etoile, France). The insulin resistance was assessed by insulin sensitivity index (homeostasis model assessment; HOMA) [21]. Cholesterol and TG levels were measured using commercially available enzymatic kits (Boehringer Mannheim GmbH, Mannheim, Germany). Free fatty acids were measured enzymatically (Wako Chemicals, Neuss, Germany). ApoB100 concentration was obtained in VLDL, IDL, and LDL by combining selective precipitation and mass spectrometry [22] and by immunonephelometry (Biomérieux, Marcy l'Etoile, France).

Total serum apoCIII concentrations were measured by immunoturbidimetric assay (K. Assay apoCIII®, Kamiya Biomedical Company, Seattle, USA) on ABX Mira® analyser (Horiba ABX, Montpellier, France). Serum HDL-apoCIII concentrations was determined after precipitation of VLDL and LDL lipoproteins with HDL precipitant FS® (Diasys Europe Ltd, Workingham Berskshire, England). Non HDL-apoCIII was calculated as the difference between total serum apoCIII and HDL-apoCIII.

Kinetic Study

The endogenous labeling of apoB100 was carried out by constant infusion of [5,5,5,$^2H_3$] leucine in subjects fasted overnight for 12 h prior to the study and remained fasting during the entire procedure. The full kinetic protocol has been previously described in details [20].

Analytical Procedures

Isolation of lipoproteins and measurement of leucine enrichment in apoB100 have been described previously [20]. Briefly, lipoproteins were separated by ultracentrifugation and apoB100 was isolated by gel electrophoresis. Apolipoprotein bands were hydrolyzed and the amino acids were purified by cation exchange chromatography, then esterified and derivatized. Electron-impact gas chromatography-mass spectrometry was performed on a 5891 A gas chromatograph connected with a 5971 A quadrupole mass spectrometer. Calculations of apoB100 enrichment were based on the tracer-to-tracee mass ratio [23].

Modeling

Kinetic analysis of tracer-to-tracee ratios was achieved using computer software for simulation, analysis and modeling (SAAMII). The model used (Fig. **1**) and measurement of kinetic parameters were previously described [20]. This model took into account heterogeneity in VLDL, e.g. large (VLDL1) and small VLDL (VLDL2), VLDL remnants (VLDLR) and shunt between VLDL and LDL. In this model a forcing function, corresponding to the time course of plasma leucine enrichment, was used to drive the appearance of leucine tracer into apoB100 during synthesis process. Methods provided identified values ± standard deviation obtained by iterative least squares fitting for individual kinetic parameters. Standard deviation were less than 30% for most of the parameters (data not shown).The use of more complex models did not provide significant improvement in the fitting from F test and Akaike information criterion [24].

The apoB100 production rate in mg/kg/h represented the product of FCR and pool size of apoB100 in lipoprotein fractions.

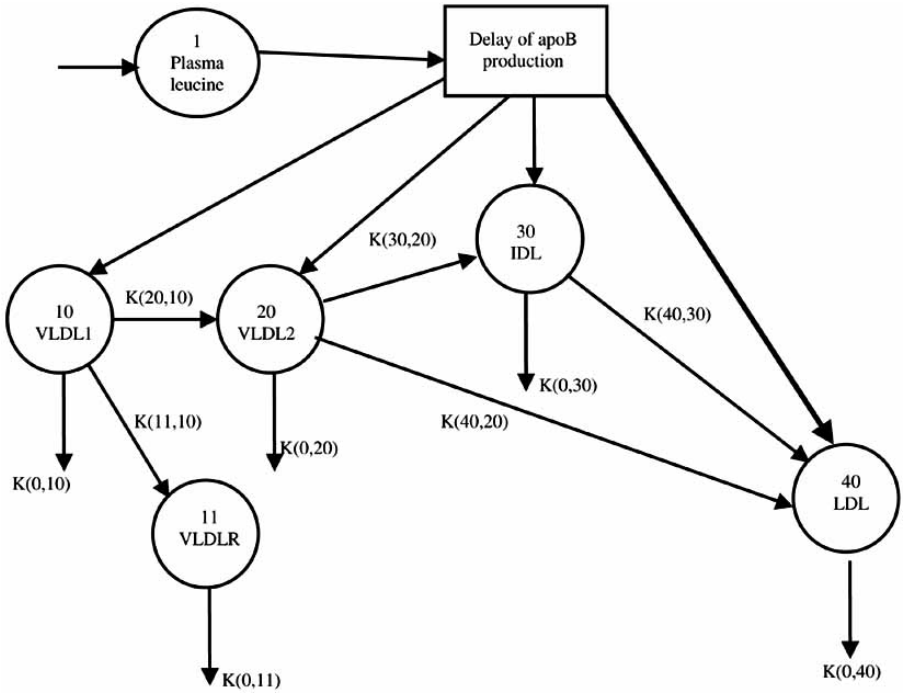

Fig. (1). Model of apolipoprotein B100-containing lipoprotein metabolism. Details are described under *Materials and Methods*.

Statistical Analysis

Results are reported as mean ± standard deviation. The Mann-Whitney-test, performed with Statview F-4.5 (Abacus Concept, Berkeley, CA, USA) was used to compare results for lipodystrophic and no lipodystrophic patients. A two-tailed probability level of 0.05 or less was accepted as statistically significant.

RESULTS

Clinical and anthropometric characteristics are shown in Table **1**. There was no significant difference between groups in age, weight, HbA1C, BMI. Waist to hip ratio, an indirect measure of visceral fat was not different between the two groups. Triceps muscle circumference was significantly reduced in the lipodystrophic group ($p<0.05$). Compared to group NLD, patients of group LD exhibited higher resistance to insulin as measured by HOMA (3.44±1.62 *vs* 1.60±0.61, $p<0.05$).

Plasma lipids and apolipoproteins levels are shown in Table **2**. Plasma triglycerides and cholesterol were significantly higher in group LD (5.73±3.58 *vs* 1.29±0.54, $p<0.005$ and 2.98±0.95 *vs* 1.74±0.26 g/L, $p<0.05$ respectively) as well as plasma apoB100 (1.49±1.11 *vs* 0.51±0.11 g/L, $p<0.005$). ApoCIII content of apoB100 containing lipoprotein was significantly ($p<0.005$) increased in group LD (117.7±42.2 *vs* 22.6±23.9 mg/L) as compared to group NLD. Patients in group LD showed higher content of cholesterol in VLDL (36±4 *vs* 26±4 %, $p<0.005$) and IDL (47±5 *vs* 26±6 %, $p<0.005$) and triglycerides in LDL (13±5 *vs* 5±1 %, $p<0.05$) than patients from group NLD. ApoB100 concentration was significantly increased in VLDL (5.0 fold, $p<0.005$), IDL (2.7 fold, $p<0.05$) and LDL (2.7 fold, $p<0.005$) in group LD.

Table 2. Lipids and Apolipoprotein Levels in NLD and LD Patients

	Plasma					VLDL				IDL				LDL			
Subjects	**CHT (g/l)**	**TG (g/l)**	**ApoB (g/l)**	**NonCIII-HDL (mg/l)**	**FFA (mmol/L)**	**TG (%)**	**TC (%)**	**PL (%)**	**ApoB (mg/L)**	**TG (%)**	**TC (%)**	**PL (%)**	**ApoB (mg/L)**	**TG (%)**	**TC (%)**	**PL (%)**	**apoB (mg/L)**
NLD1	1.78	0.68	0.47	0	0.174	30	24	19	33.3	14	24	14	14.9	5	44	33	421.7
NLD2	2.01	2.09	0.41	9	0.223	37	22	17	50.7	21	23	19	42.1	5	29	26	315.8
NLD3	2.00	1.69	0.50	33	0.293	38	30	9	26.3	17	25	20	36.7	3	60	19	432.7
NLD4	1.63	1.36	0.37	2	0.242	47	21	13	20.1	22	36	13	20.5	4	52	19	330.1
NLD5	1.91	0.55	0.68	7	0.319	30	24	14	58.8	22	32	8	20.6	4	53	27	602.8
NLD6	1.36	1.38	0.55	49	0.285	42	26	14	51.6	19	23	32	10.2	7	43	26	492.0
NLD7	1.47	1.30	0.60	58	0.296	28	32	17	51.7	20	20	14	26.1	6	49	25	524.2
Mean	1.74	1.29	0.51	22.6	0.26	36	26	15	42	19	26	17	24	5	47	25	446
SD	0.26	0.54	0.11	23.9	0.05	7	4	3	15	3	6	8	11	1	10	5	103
LD1	3.81	3.48	1.09	112	0.177	26	43	8	77.9	16	45	21	16.7	7	50	27	997.1
LD2	3.83	11.24	3.98	105	0.384	30	37	12	308.8	22	48	10	139.1	17	36	29	3527.6
LD3	1.93	5.0	0.80	43	0.369	24	39	14	171.9	20	46	10	27.9	14	39	30	600.6
LD4	1.93	3.7	1.23	138	0.241	34	34	11	147.2	24	39	10	63.6	17	39	27	1019.0
LD5	3.22	4.73	0.88	170	0.204	29	36	16	154.8	17	55	9	83.4	15	43	26	640.3
LD6	3.42	10.2	1.26	156	0.396	37	35	11	298.7	18	53	6	38.3	14	41	27	920.5
LD7	1.66	1.73	1.20	100	0.237	43	29	12	356.2	21	45	11	84.4	4	37	37	760.8
Mean	2.98*	5.73**	1.49**	117.7**	0.29	32	36**	12	216**	20	47**	11	65*	13*	41	29	1209**
SD	0.95	3.58	1.11	42.2	0.09	7	4	3	104	3	5	5	42	5	5	4	1036

TC; Total cholesterol, TG; Triglycerides; PL; Phospholipids, FFA; Free fatty acids. *$p<0.05$, **$p<0.005$.

Time courses of enrichment in VLDL, IDL and LDL apoB100 for a representative subject from group NLD and group LD are shown in Fig. (**2**). Model fitted lines and experimental points showed close agreement. Kinetic labeling of apoB100 in VLDL, IDL and LDL was slower in group LD compared to group NLD. As noted in Table **3**, total apoB100 production was higher in group LD due to a higher production rate in VLDL (1.24±0.33 *vs* 0.80±0.21 $mg.kg^{-1}.h^{-1}$, $p<0.05$) but total production rate of IDL was significantly lower (0.20±0.10 *vs* 0.48±0.24 $mg.kg^{-1}.h^{-1}$, $p<0.05$) related to direct production (0.050±0.030 *vs* 0.104±0.050 $mg.kg^{-1}.h^{-1}$, $p<0.05$). There was a lower fractional catabolic rate of VLDL (0.199±0.132 *vs* 0.555±0.398 h^{-1} $p<0.05$), IDL (0.110± 0.080 *vs* 0.523±0.275 h^{-1}, $p<0.05$) and LDL (0.010±0.005 *vs* 0.025±0.014 h^{-1},$p<0.05$) in group LD. A significant and dramatic decrease in VLDL (0.013±0.010 *vs* 0.258±0.206 h^{-1}, $p<0.005$) and IDL (0.088±0.093 *vs* 0.366±0.189 h^{-1}, $p<0.05$) conversion rate to IDL and LDL respectively was also found in group LD.

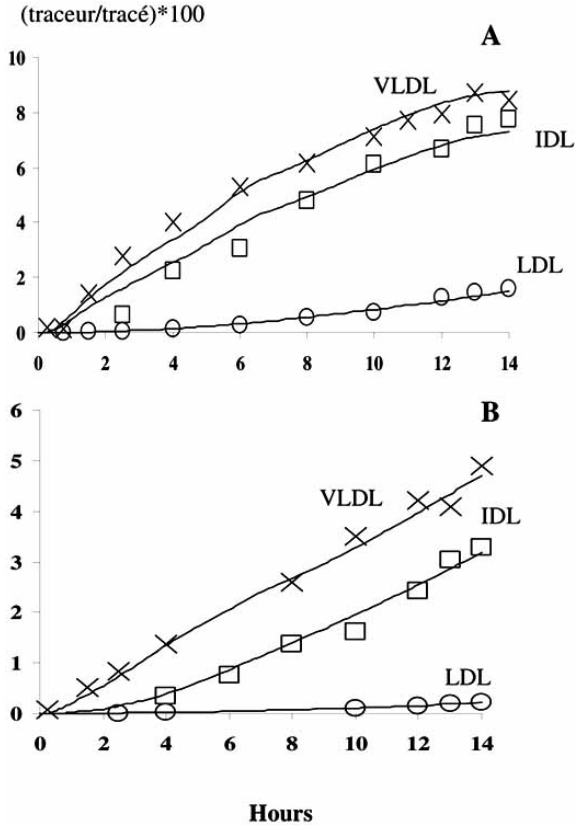

Fig. (2). Time course of enrichment of VLDL (X), IDL (□) and LDL (O) apolipoprotein B100 in representative patient from non lipodystrophic and lipodystrophic patients.

DISCUSSION

The aim of this study was to compare apoB100 containing lipoprotein metabolism in lipodystrophic and severe hypertriglyceridemic HIV infected patients to normolipidemic HIV infected patients on ART treatment. This study showed that hyperlipidemia is a consequence of increased ApoB production rate in VLDL and delayed catabolism of VLDL, IDL and LDL. The conversion rate of VLDL and IDL was also significantly decreased in hyperlipidemic lipodystrophic patients.

Major plasma lipid disturbance observed in lipodystrophic patients are similar to that noted in insulin resistant state associated to type-2 diabetes (20). Contrarily to this last pathological situation, the insulin resistance, in our patients, was not associated to FFA increase in plasma. As elevated FFA concentration are clearly involved in the pathogenesis of insulin resistance [25], our results suggest that the main mechanism resulting in insulin resistance in lipodystrophic subjects could be direct effect of PI on Glut4 activity [26].

The higher production of total apoB100 measured in this study was related to an increase of VLDL production. To explain the increase of VLDL production in HIV infected patient several hypotheses can be advanced. Hepatic fatty acid availability is the major regulator of VLDL-TG secretion [27] and as fatty acids are frequently increased in HIV patients they probably contribute to the elevated VLDL production. In our study FFA concentration did not differ between groups, suggesting that other factors are involved. Lipodystrophy is often associated to insulin resistance [4]. In our patients, although subjects from group LD were normoglycemic and normoinsulinemic, they exhibit higher HOMA index. The increase of VLDL production in insulin resistant state is well documented [20,28]. An other mechanism to explain the increase of apoB production is the possible PI inhibiting effect of proteosomal apoB degradation [29], mechanism which control the hepatic VLDL production [30]. Other data suggest that PI may directly stimulate hepatic triglyceride synthesis and then VLDL by upregulating mRNA of key enzyme in hepatic cells [31]. But, this overproduction was not observed in our treated normolipidemic patients with no lipodystrophy supporting a possible heterogeneity in HIV patients [32,33].

VLDL production was reported in other studies on HIV subjects with hyperlipidemia [16-18]. However, some studies showed an increase in VLDL triglycerides synthesis in severe hyperlipidemic PI-treated patients with lipodystrophy [17] while other reported no change in VLDL production in lipodystrophic subjects [18]. These differences may be related to hyperlipidemia of studied subjects, or different sensitivity to insulin. In fact, as assessed by HOMA index, patients in the study [18], showing no increase in VLDL production, had no insulin resistance. Increase of HOMA index is frequent in HIV patients with lipodystrophy, as illustrated by our results. VLDL overproduction was a major disturbance noted for insulin resistant subjects in apoB100 containing lipoprotein metabolism assayed by similar protocol [20].

The VLDL and IDL conversion to IDL and LDL respectively were decreased in group LD compared to group NLD. These findings suggest a low lipoprotein lipase (LPL) and hepatic lipase (HL) activity which contributed in part to HIV-associated dyslipidemia. Our data are in accordance with *in vitro* [34,35] and *in vivo* studies [7,16,18]. Insulin resistance of lipodystrophic subjects may result in impairment in LPL activity, as in diabetic subjects [20]. Decrease of triglyceride lipolysis in HIV patients has also been associated with HIV viremia which reduces LPL and HL activities

Table 3. Kinetic Data of apoB100 Containing Lipoproteins in NLD and LD Patients

	VLDL			IDL				LDL		
Subjects	**TPR**	**FCR**	**CR**	**TPR**	**PRd**	**FCR**	**CR**	**TPR**	**PRd**	**FCR**
NLD1	0.58	0.403	0.403	0.650	0.070	1.016	0.410	0.340	0.080	0.019
NLD2	0.91	0.424	0.021	0.230	0.190	0.132	0.012	0.240	0.220	0.018
NLD3	0.67	0.615	0.613	0.800	0.140	0.529	0.527	0.960	0.160	0.054
NLD4	1.18	1.428	0.278	0.300	0.060	0.348	0.348	0.370	0.070	0.027
NLD5	0.92	0.374	0.165	0.500	0.100	0.586	0.586	0.620	0.110	0.025
NLD6	0.65	0.298	0.053	0.180	0.060	0.417	0.417	0.250	0.070	0.012
NLD7	0.71	0.340	0.270	0.670	0.100	0.632	0.265	0.400	0.120	0.019
Mean	0.80	0.555	0.258	0.48	0.104	0.523	0.366	0.45	0.120	0.025
SD	0.21	0.398	0.206	0.24	0.050	0.275	0.189	0.25	0.050	0.014
LD1	1.41	0.441	0.018	0.160	0.050	0.230	0.230	0.720	0.560	0.018
LD2	0.71	0.096	0.000	0.080	0.080	0.000	0.000	0.210	0.210	0.002
LD3	1.48	0.215	0.009	0.120	0.030	0.020	0.020	0.180	0.160	0.007
LD4	1.58	0.265	0.001	0.160	0.080	0.063	0.063	0.530	0.370	0.013
LD5	1.48	0.228	0.021	0.240	0.050	0.024	0.024	0.300	0.220	0.011
LD6	0.97	0.078	0.026	0.330	0.010	0.207	0.206	0.360	0.030	0.009
LD7	1.06	0.073	0.015	0.320	0.050	0.070	0.070	0.370	0.130	0.012
Mean	1.24*	0.199*	0.013**	0.20*	0.050*	0.110*	0.088*	0.38	0.240	0.010*
SD	0.33	0.132	0.010	0.10	0.030	0.080	0.093	0.19	0.180	0.005

TPR: total production rate in mg/kg/h, FCR : Fractional catabolic rate in h^{-1}, CR : Conversion rate in h^{-1}.

PRd: direct production rate in mg/kg/h. *p<0.05; **p<0.005.

[36,37], a mechanism which cannot be advanced in our study, since most of the patients had undetectable plasma HIV RNA. Increase in apolipoprotein CIII, in apoB100 containing lipoproteins, as observed in our lipodystrophic subjects, could be involved [8]. It has been also observed that polymorphism of apoCIII gene, which particularly disturbed insulin regulation of apoCIII expression [38], could be involved in differential response of PI treatment on lipid profile [39]. Treatment with PI has been also associated with delayed LPL activity. In fact a lower mRNA expression of lipoprotein lipase in lipodystrophic subjects was reported [35] and the proposed mechanism could be an inhibitory effect of SREBP-1c pathway [35,40]. Other possible mechanism involves the inhibition of LRP by PI [4]. LRP is a protein bound to lipoprotein lipase on capillary endothelium and cleaves triglycerides in triglyceride rich lipoproteins. But, the effect of PI on hypertriglyceridemia and LPL activity is still controversial because some HIV-infected studied subjects who were naive to all antiviral medications have low LPL and HL activities [41] and treatment with ritonavir in the absence of HIV infection or changes in body composition results in hypertriglyceridemia with no change in post heparin lipoprotein lipase activity [42]. Otherwise, our normolipidemic patient, although on PI therapy, had a normal VLDL and IDL conversion rate. These data suggest again that genetic background could be involved in protease-inhibitor-associated hyperlipoproteinaemia and lipodystrophy in patients with HIV [5,32,33,38]. VLDL and IDL lipolysis reduction was also a major disturbance noted for insulin resistant subjects in apoB100 containing lipoprotein metabolism assayed by similar protocol [20].

The delayed VLDL, IDL and LDL uptake in subjects with lipodystrophy suggests a decrease in LDL receptor activity [19,43]. A lower LDL-R expression in HIV patients with lipodystrophy was reported by Petit JM *et al.* [43]. This lower LDL receptor activity could be a result of the effect of PIs treatment. An alteration of the processing of the transcription factor SREBP which regulates the receptor LDL activity was reported and varies according to the PI used [44]. However, it is not clear to determine if these altered metabolic processes are secondary to lipodystrophy or somehow involved early in the pathogenesis. The association between length of therapy and development of lipodystrophy is also discussed. It was reported in cohort of HIV patients that some developed lipodystrophy a few months (5.7 months) after starting PI therapy while others did never even several years after [3] which suggest again a genetic predisposition for lipodystrophy occurrence [14]. Reduction of LDL catabolism was also a major disturbance noted for insulin resis-

tant subjects in apoB100 containing lipoprotein metabolism [20].

In conclusion this study showed that dyslipidemia associated with lipodystrophy in HIV infected subject is related both to an overproduction and delayed catabolism of apoB100 (both lipolysis and catabolic pathways). Metabolic abnormalities of apoB100 containing lipoprotein are also observed in insulin resistance associated to type-2 diabetes. Our study suggests that disturbance on apoB100 containing lipoprotein metabolism observed in lipodystrophic HIV in combined antiretroviral therapy are consecutive to insulin resistance induced by the treatment.

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