Human Immunodeficiency Virus Genotype and Hypertriglyceridemia

Soni J. Anderson,* John F. Bradley, Andrea Ferreira-Gonzalez, and Carleton T. Garrett

Department of Pathology, Division of Molecular Diagnostics, Medical College of Virginia, Richmond, Virginia

Many HIV patients develop a progressive syndrome of abnormal body fat distribution accompanied by hypertriglyceridemia. Antiretroviral agents are thought to be etiologic in the syndrome, often termed “highly active antiretroviral therapy (HAART)-associated lipodystrophy.” In the course of clinical HIV genotype testing, we observed that our HIV patients with hypertriglyceridemia had viral genotypes that were more highly mutated than those of our therapy-matched control patients. Hypertriglyceridemia was statistically associated with predicted resistance for three nucleoside reverse transcriptase inhibitors: zidovudine,

abacavir, and stavudine. Statistical analysis of 51 patients in retrospect revealed a strong association of mutations at reverse transcriptase codons M41 and T215 with hypertriglyceridemia (chi-square (χ^2) = 8.375, P = .0038; and χ^2 = 7.445, P = .0064, respectively). This was in contrast to silent mutations, which occurred at equivalent rates in retroviral genotypes of patients with and without hypertriglyceridemia. The findings imply that the HIV genotype itself may be a significant etiologic factor in antiretroviral-associated lipodystrophy. *J. Clin. Lab. Anal.* 16:202–208, 2002. © 2002 Wiley-Liss, Inc.

Key words: lipodystrophy; HAART; resistance; mutation; reverse transcriptase; protease inhibitor; nucleoside analog

INTRODUCTION

Due to its high mutation rate, human immunodeficiency virus (HIV) exists as a complex mixture of genetically related but distinguishable variants. This extreme mutability is attributable to a high replication rate of the virus combined with a high error rate of the viral encoded reverse transcriptase. Wild-type genotypes predominate in the absence of specific selective pressure. Viruses that carry mutations conferring survival traits predominate in selective environments.

Antiretroviral drug therapy usually selects for increasingly resistant HIV variants over the course of treatment. Simultaneous therapy with multiple antiretroviral agents helps prevent selection of drug-resistant HIV variants, and is standard practice. This polytherapy is sometimes called “highly active antiretroviral therapy” (HAART). Currently available antiretroviral agents include three classes of drugs. Two of these drug classes, nucleoside analogs and non-nucleoside analogs, act as inhibitors of the HIV reverse transcriptase. The third class includes various inhibitors of the viral protease (Table 1).

In treated HIV patients, a progressive syndrome of abnormal body fat distribution accompanied by hypertriglyceridemia has been associated with intensive

antiretroviral therapy. Sometimes termed “HAART-associated lipodystrophy,” the syndrome can affect 50% or more of HIV-infected patients on long-term antiretroviral therapy (1). It is associated with a highly atherogenic serum lipid profile that renders patients vulnerable to coronary artery disease and sudden death from myocardial infarction. Multiple factors are considered etiologic contributors to the syndrome. These include age, sex, genetic predisposition of the patients, HIV viral titer, coinfection with other viruses, disease progression, and therapeutic agents (2–9).

Therapy with protease inhibitors is thought to cause elevation in serum triglycerides (10). Within the protease inhibitor class of antiretroviral agents, ritonavir is most frequently associated with hypertriglyceridemia, followed by indinavir, and then saquinavir (11). Cessation of protease inhibitor therapy is not associated with reversal of the dysmorphic aspects of lipodystrophy (12), or the long-term reversal of severe serum lipid

*Correspondence to: Soni J. Anderson, M.D., Ph.D., Department of Pathology, Division of Molecular Diagnostics, Medical College of Virginia, Richmond, VA 23298-0248. E-mail sjanders@hsc.vcu.edu

Received 8 March 2002; Accepted 6 May 2002

DOI 10.1002/jcla.10042

Published online in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Antiretroviral agents

Nucleoside analogs
Zidovudine
Didanosine
Zalcitabine
Lamivudine
Stavudine
Abacavir
Non nucleoside analogs
Nevirapine
Delavirdine
Efavirenz
Protease inhibitors
Saquinavir
Indinavir
Nelfinavir
Amprenavir
Lopinavir
Ritonavir

abnormalities (13). One study of uninfected normal volunteers reports plasma triglyceride elevation following 2 weeks of ritonavir treatment (14). This has been cited in support of the hypothesis that hypertriglyceridemia in HIV-infected patients may be attributed to drug–host interactions.

Association of nucleoside or non-nucleoside reverse transcriptase inhibitors with hypertriglyceridemia is variable from one study to another, but stavudine and zidovudine treatment may be more often associated with lipodystrophy than other reverse transcriptase inhibitors (15–19). In a large group of protease inhibitor-naïve patients, hypertriglyceridemia is still noted in association with nucleoside analog treatment (15).

One of the effects of antiretroviral therapy is prolongation of life expectancy (20). This implies a longer disease course and extension of the time over which the virus and host interact. Before the wide availability of antiretroviral therapy, hypertriglyceridemia had already been observed in HIV patients. An early study (21) of HIV-associated hypertriglyceridemia, which does not mention antiretroviral therapy, attributes the triglyceride elevation (observed in 50% of patients in the study) to the HIV infection itself, with cytokines as possible mediators. In one report of a clinical trial with zidovudine treatment (22), hypertriglyceridemia was still attributed to the virus and associated with elevated cytokines. Over the last decade, there has been a shift in the literature from attributing lipodystrophy to retroviral–host interactions, to the current emphasis on antiretroviral therapy–host interactions.

To date, there is a dearth of information on HIV genotypes in lipodystrophy patients. In the course of examining patient records in association with clinical HIV genotype testing, we observed a marked trend:

HIV-infected patients with hypertriglyceridemia had an increased number of mutations at zidovudine resistance sites. Statistical analysis was performed retrospectively to test the validity of the observation. It became apparent that patients with hypertriglyceridemia had a higher percentage of resistance mutations in their viral genomes than did patients without hypertriglyceridemia.

MATERIALS AND METHODS

For the purpose of optimizing antiretroviral therapy, patients' plasma specimens with more than 200 copies/mL of HIV-1 RNA were subjected to genotype analysis. The Trugene HIV-1 genotyping kit was used in conjunction with the OpenGene DNA sequencing system (Visible Genetics, Suwanee, GA) to obtain partial sequences for the viral reverse transcriptase and protease genes. Viral RNA was amplified by a single-tube reverse transcription-polymerase chain reaction (RT-PCR). Sequencing of cDNA in both directions was performed using dideoxy termination with two dye-labeled primers for each of the four sequencing reactions. Detection on polyacrylamide gels employed the Long Read tower sequencer (Visible Genetics, Suwanee, GA). Comparison of obtained sequences to wild-type HIV-1 sequences utilized the Gene Librarian module of GeneObjects software (Visible Genetics, Suwanee, GA). In the current group of patients, resistance mutations were detected at reverse transcriptase codons M41, E44, A62, D67, T69, K70, L74, V75, F77, A98, L100, K101, K103, V106, V108, F116, V118, Q151, V179, Y181, M184, Y188, G190, L210, T215, and K219. Resistance mutations were detected at protease codons L10, K20, D30, V32, M36, M46, I47, G48, I54, L63, A71, V77, V82, I84, N88, and L90.

Following chart review, the first 26 HIV-1 infected patients with hypertriglyceridemia were chosen as the study group. Identified by examination of lipid profiles recorded in the computer Laboratory Information System, patients with serum triglyceride levels equal to or greater than 235 mg/dl, and triglyceride levels greater than cholesterol levels, were included. Pediatric patients (less than 18 years old) were excluded.

The control group consisted of 25 HIV-1-infected patients with no abnormal triglyceride or cholesterol readings. In an effort to match as closely as possible the demographics of the hypertriglyceridemia study group, patients were chosen for inclusion in the control group based upon age and number of years since diagnosis of HIV-1 infection. Because treatment with protease inhibitors is considered a risk factor for HAART-associated lipodystrophy, patients with no history of therapy with protease inhibitors were excluded from the control group.

TABLE 2. Patient Demographics

		Hypertriglyceridemia	Control	<i>t</i> -statistic	<i>P</i>
Age	Mean	41	41	0.168	0.8674
Sex	male	81%	76%	0.435	0.6656
	female	19%	24%		
Years since diagnosis	mean (SD)	9.645 (4.69)	8.36 (3.161)	1.151	0.2554
Hepatitis B virus		31%	24%	0.559	0.5785
Hepatitis C virus		35%	24%	0.860	0.3939
HIV titer, average		10,000	10,000	1.295	0.4484
Number of antiretrovirals, average		7	7	0.464	0.6448
Months on protease inhibitors	mean (SD)	29.46 (17)	30.48 (17)	0.214	0.8315

Statistical analysis used the Statistics Calculator software (StatPac, Inc., Minneapolis, MN). The chi-square test was applied to nominal data, and the two-tailed *t*-test to interval data.

RESULTS

Patient Demographics

Using a two-tailed *t*-test for statistical comparison, the patients' ages and years since diagnosis of HIV infection were similar for the hypertriglyceridemia group and the control group (Table 2), indicating that our effort to match the patient groups for these parameters was successful. Also statistically similar were the sex of patients, percent of patients with hepatitis B or C virus coinfection, HIV titer at time of genotyping, the average number per patient of antiretroviral agents ever prescribed, and the mean number of months on protease inhibitor therapy (Table 2).

Treatment History

An examination of treatment history for individual agents revealed similar duration and rates of treatment

in the hypertriglyceridemia and control groups for zidovudine, didanosine, stavudine, nevirapine, delavirdine, efavirenz, abacavir, saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, and lopinavir (Table 3).

The control group had a higher treatment rate for lamivudine (100%) than did the hypertriglyceridemia group (77%). The difference was statistically significant (*t*-statistic = 2.552, *P*-value = .0139). The hypertriglyceridemia group had a higher treatment rate for zalcitabine (38%) than did the control group (8%). The difference was statistically significant (*t*-statistic = 2.534, *P*-value = .0145).

Genotype Indicating Drug Resistance

The mean number of drugs per patient for which the viral genotype indicated resistance was 8.5 antiretroviral agents for the hypertriglyceridemia group compared to 5.48 for the control group. The *t*-test for comparison of independent groups yielded a statistic of 3.181, with a two-tailed *P*-value of .006. Thus the number of antiretroviral agents for which the viral genotype indicated resistance was significantly higher in the hypertriglyceridemia group than in the control group.

TABLE 3. Antiretroviral history

Agent	% usage total	Hypertriglyceridemia	Control	<i>t</i> -statistic	<i>P</i>
Zidovudine	90	84	96	1.421	0.1618
Stavudine	90	85	96	1.333	0.1888
Lamivudine	88	77	100	2.552	0.0139
Nelfinavir	66	61	72	0.831	0.4098
Ritonavir	65	65	64	0.075	0.9408
Didanosine	62	69	56	0.959	0.3421
Nevirapine	57	57	56	0.072	0.9429
Indinavir	53	50	56	0.429	0.6697
Saquinavir	43	46	40	0.433	0.6672
Efavirenz	33	38	28	0.759	0.4517
Abacavir	29	35	24	0.860	0.3939
Zalcitabine	23	38	8	2.534	0.0145
Lopinavir	18	23	12	1.031	0.3076
Amprenavir	14	19	8	1.145	0.2576
Delavirdine	2	0	4	1.030	0.3081

Statistical analysis of predicted resistance for individual antiretroviral agents based upon the viral genotype was performed (Table 4). Hypertriglyceridemia was associated with predicted resistance for three individual antiretroviral agents, all of them nucleoside reverse transcriptase inhibitors. The associated antiretroviral agents were zidovudine (Yate's corrected χ^2 statistic = 19.496, P -value = <.0001), stavudine (Yate's corrected χ^2 statistic = 7.099, P -value = .0077), and abacavir (Yate's corrected χ^2 statistic = 8.653, P -value = .0033).

TABLE 4. Predicted resistance to antiretroviral agents based upon genotype

	Hypertriglyceridemia		Control
Zidovudine	Yes	24	7
	No	2	18
Yate's corrected chi-square statistic = 19.496		$P < 0.0001$	
Didanosine	Yes	19	14
	No	7	11
Yate's corrected chi-square statistic = 0.966		$P = 0.3258$	
Zalcitabine	Yes	19	13
	No	7	12
Yate's corrected chi-square statistic = 1.604		$P = 0.2053$	
Lamivudine	Yes	12	11
	No	14	14
Yate's corrected chi-square statistic = 0.016		$P = 0.8990$	
Stavudine	Yes	18	7
	No	8	18
Yate's corrected chi-square statistic = 7.099		$P = 0.0077$	
Abacavir	Yes	20	8
	No	6	17
Yate's corrected chi-square statistic = 8.653		$P = 0.0033$	
Nevirapine	Yes	14	10
	No	12	15
Yate's corrected chi-square statistic = 0.504		$P = 0.4779$	
Delavirdine	Yes	13	8
	No	13	17
Yate's corrected chi-square statistic = 1.043		$P = 0.3072$	
Efavirenz	Yes	13	10
	No	13	15
Yate's corrected chi-square statistic = 0.190		$P = 0.6628$	
Saquinavir	Yes	9	8
	No	17	17
Yate's corrected chi-square statistic = 0.010		$P = 0.9211$	
Indinavir	Yes	15	12
	No	11	13
Yate's corrected chi-square statistic = 0.170		$P = 0.6799$	
Ritonavir	Yes	14	10
	No	12	15
Yate's corrected chi-square statistic = 0.504		$P = 0.5040$	
Nelfinavir	Yes	15	16
	No	11	9
Yate's corrected chi-square statistic = 0.030		$P = 0.8616$	
Ampranavir	Yes	10	3
	No	16	22
Yate's corrected chi-square statistic = 3.409		$P = 0.0648$	
Lopinavir/ritonavir	Yes	8	4
	No	18	21
Yate's corrected chi-square statistic = 0.833		$P = 0.3613$	

The association of hypertriglyceridemia with predicted resistance to two of these nucleoside inhibitors was statistically very strong. To reduce the possibility of falsely associating specific drug resistance with hypertriglyceridemia, we applied Bonferroni's theorem for adjusting P -values. Starting with an alpha level of .05, and dividing by the number of agents for which drug resistance was tested, 15, a stringent alpha level of .0033 was obtained. The P -values for both zidovudine and abacavir were less than or equal to this value, indicating the very strong association of hypertriglyceridemia with these drug-resistant HIV genotypes.

There was no association of hypertriglyceridemia with predicted resistance for didanosine, zalcitabine, lamivudine, nevirapine, delavirdine, efavirenz, saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, or lopinavir/ritonavir combination therapy. It should be noted here that for almost every antiretroviral agent, the rate of predicted resistance in the hypertriglyceridemia group was greater than that in the control group, although for these individual agents the difference was not statistically significant. However, this higher rate of predicted resistance was statistically significant overall (see above).

Specific Mutations

Statistical analysis for the association of hypertriglyceridemia with mutations at specific drug resistance sites was performed. Mutations at two sites, reverse transcriptase codons M41 and T215, were associated with hypertriglyceridemia (Table 5). The association was statistically significant (χ^2 statistic = 8.375, P -value = .0038, and χ^2 statistic = 7.445, P -value = .0064, respectively).

The hypertriglyceridemia group had 18.63% mutations of all the resistance sites in the reverse transcriptase gene compared to 8.92% mutations for the control group. The difference was statistically significant (t -statistic = 5.114, two-tailed = P -value < .001).

The hypertriglyceridemia group had 24.03% mutations of total resistance sites in the protease gene compared to 17.75% mutations for the control group.

TABLE 5. Mutations at specific sites

	Mutation	Wild type
Reverse transcriptase codon M41		
Hypertriglyceridemia	42% (11/26)	58% (15/26)
Control	4% (1/25)	96% (24/25)
Yate's corrected chi-square statistic = 8.375		$P = .0038$
Reverse transcriptase codon T215		
Hypertriglyceridemia	62% (16/26)	38% (10/26)
Control	20% (5/25)	80% (20/25)
Yate's corrected chi-square statistic = 7.445		$P = .0064$

The difference was statistically significant (t -statistic = 2.204, two-tailed P -value = .0278).

Both the hypertriglyceridemia group and the control group had an average rate of silent mutations (no change in amino acid specified) of 27% in the protease gene. The difference in percent silent mutations in the reverse transcriptase gene (12.5% for the hypertriglyceridemia group vs. 16% for the control group) was not statistically significant (t -statistic = 0.401, two-tailed P -value = .6902).

Polymorphism rates (mutations not at known drug resistance sites) were also similar between the hypertriglyceridemia group (16%) and the control group (13%) (t -statistic = 0.555, two-tailed P -value = .5793).

DISCUSSION

The number of variables linked to the etiology of HAART-associated lipodystrophy makes an understanding of the pathologic process difficult to achieve. In the scientific literature, protease inhibitors have been implicated. But if therapy with protease inhibitors is a causative factor, why do only some of the patients on these agents get lipodystrophy? And why doesn't cessation of protease inhibitor therapy result in reversal of lipodystrophy? A partial answer may lie in our current findings.

We started with the fact that hypertriglyceridemia is a defining factor in HAART-associated lipodystrophy. In the current study, our patients' serum triglyceride values were readily available from the computer Laboratory Information System. Likewise, viral genotype data were generated in our own laboratory and were uniformly available for each patient. Our study also benefited from multiple prior reports in the literature. This information allowed us to match the hypertriglyceridemia and control groups for various parameters that may be considered etiologic contributors to lipodystrophy. In fact, we had only to match the two groups for number of years of HIV infection, patient age, and therapy with protease inhibitors, and most of the other variables were then similar between the two groups.

The finding that was most striking was the proportion of our patients with hypertriglyceridemia and zidovudine resistant viral genotype (Table 4). Twenty-four out of 26 patients with hypertriglyceridemia had HIV genotypes interpreted as indicating zidovudine resistance. It is interesting to note that the two patients with hypertriglyceridemia and non-zidovudine-resistant HIV both had hepatitis C virus coinfection. Hepatitis C viral infection is an independent risk factor for serum lipid disorders (9).

It has been known for many years that HIV infection itself is associated with hypertriglyceridemia. This may

be mediated through complex interactions between the virus and the host's cytokines modulating immune responses, including tumor necrosis factor- α , interleukin 2, and alpha-interferon (21–23). Much of our early understanding of HIV as a cause of lipodystrophy came before highly active antiretroviral therapy was widely available for patient treatment. The intervention of a decade's experience with intensive antiretroviral therapy should not prevent perception of the importance of the virus itself as an etiologic agent in this syndrome.

The association of antiretroviral therapeutic agents and lipodystrophy is frequently noted in the current literature. However, demonstration of a cause and effect relationship is lacking. Researchers and clinicians must invoke the existence of other unknown factors (2). An explanation in light of our current data may be helpful.

Historically, as the protease inhibitors first became available for patient therapy, clinicians were most likely to prescribe them for patients whose reverse transcriptase inhibitors were less effective. Those with more highly mutated viruses likely represented a large proportion of patients who were not benefiting from reverse transcriptase inhibitors. The switch to protease inhibitor therapy then was temporally associated with the onset of hypertriglyceridemia for a proportion of these patients, and was attributed to the protease inhibitors. Furthermore, the change to protease inhibitor therapy, or even the initiation of new protease inhibitors in therapy-naïve patients, clearly represents a selective pressure on the HIV. Our patients with hypertriglyceridemia had more mutations in the protease gene than did patients without hypertriglyceridemia, and the difference in percent mutated sites was statistically significant. In other words, a cause-and-effect relationship between protease inhibitor therapy and hypertriglyceridemia may actually exist, but with the mutated HIV as mediator.

It is clear from our current data that many patients are able to take protease inhibitors long term without developing hypertriglyceridemia. Our group of control patients had a mean of 30 months on protease inhibitors without developing hypertriglyceridemia. The chief difference that could be identified between the group of control patients and those with hypertriglyceridemia lay in the nature of the HIV genotype itself.

Our patients with hypertriglyceridemia had a lower lamivudine treatment rate than did the control patients (77% vs. 100%), and a higher treatment rate with zalcitabine (38% vs. 8%). One might be tempted to draw simple conclusions—that lamivudine is less likely to induce hypertriglyceridemia, and is a drug of choice for HIV patients; and that zalcitabine is more likely to induce hypertriglyceridemia, and should be avoided. However, if the information on HIV genotyping is taken

into account, there may be a better interpretation. Our patients with hypertriglyceridemia have a higher level of resistance to lamivudine. Therefore, their physicians prescribe it less frequently for them. A similar explanation may account for the higher rate of zalcitabine treatment. This agent is not often prescribed for those patients with normal lipid profiles. For the hypertriglyceridemia group, it seems likely that the search for antiretroviral agents to which the more mutated HIV is still susceptible led to a higher prescription rate for this less commonly prescribed agent.

Our data may shed some light on the findings of other investigators who have found a significant association of stavudine and zidovudine therapy with aspects of lipodystrophy (16–19). In our study, genotypic changes indicated that zidovudine and stavudine resistance, as well as abacavir resistance, were significantly more frequent in our hypertriglyceridemia patients' HIV than in the control patients' virus. This difference existed in spite of similar treatment histories for the three drugs between our two patient groups. Possibly the other studies, which did not address the topic of viral genotype, would have found similar genotypic changes related to the selective pressure of the reverse transcriptase inhibitor therapy that they noted as significant.

Two specific mutation sites, reverse transcriptase codons M41 and T215, were significantly associated with hypertriglyceridemia. M41 is a codon located in the reverse transcriptase "fingers" domain, which is responsible for holding the template strand during the reverse transcription process. The T215 codon is located in the "palm" domain, which functions in positioning the incorporated nucleotide (24). Further research on these mutations should address what selective advantage the mutated HIV virus could gain in the setting of hypertriglyceridemia, and its accompanying changes in the human inflammatory response.

The percent of resistance mutations to the reverse transcriptase and protease genes was significantly greater in the HIV genotype of our hypertriglyceridemia group than in the control group. This was in contrast to silent mutations, which occurred at similar frequencies in the viral genotypes of both patient groups. The silent mutations served as an internal control, and indicated that the HIV in the hypertriglyceridemia group was not undergoing a higher rate of mutation. Rather, selective pressure resulted in the observed differences between the viral genotypes of the two patient groups.

For HIV genes other than the reverse transcriptase and protease genes, mutational differences may exist which we have not yet studied. Possibly the increased number of mutations in these two genes represents a process that occurs in other HIV genes as well. This possibility is suggested by the finding of "hot spots" for

mutation, such as M41 and T215, whose existence suggests advantages not pertaining to drug resistance. Otherwise, mutations perhaps should be distributed among all possible zidovudine/abacavir/stavudine resistance sites rather than concentrated at these. Many drug resistance mutations represent decreases in replicative fitness for HIV, but may represent survival mechanisms that are selected for within the context of the infected host's defenses against the virus (25–27). Serum lipids are thought to influence HIV activity (28). This may be through membrane interactions. Nearly every step of viral infection and maturation depends on altered portions of the host's cell membranes, from the nuclear membrane to the cell surface.

The hypertriglyceridemia seen in HAART-associated lipodystrophy possibly represents part of a host defense against the more mutated virus that we detected. Alternatively, the hypertriglyceridemia may be part of a condition that selects for the more mutated virus.

In terms of patient care, more frequent HIV genotyping may be indicated for patients with hypertriglyceridemia. Patients with drug-resistant HIV, especially zidovudine-resistant virus, may need closer monitoring of their lipid profiles. Attempts to prevent lipodystrophy in patients receiving intensive antiretroviral therapy should include measures to prevent the selection of mutant genotypes.

In terms of research, our results suggest that future studies should include consideration of the HIV genotype in patients with lipodystrophy syndrome.

ACKNOWLEDGMENTS

The authors thank Dr. E.R. Anderson for a critical reading of the manuscript, and Ms L.R. Barker for computer assistance. The Virginia Commonwealth University–Medical College of Virginia Institutional Review Board approved the research protocol described in this work.

REFERENCES

1. Anderson SJ, Anderson FP, McPherson RA. Antiviral therapy-associated lipodystrophy syndrome. *Clin Chem* 2000;40:68–82.
2. Boufassa F, Dulioust A, Lascaux AS. Lipodystrophy in 685 HIV-1 treated patients: influence of antiretroviral treatment and immunovirological response. *HIV Clin Trials* 2001;2:339–345.
3. Thiebaut R, Dequae-Mechadou L, Ekouevi DK. Incidence and risk factors of severe hypertriglyceridaemia in the era of highly active antiretroviral therapy: the Aquitaine Cohort, France, 1996–99. *HIV Med* 2001;2:84–88.
4. Pernerstorfer-Schoen H, Jilma B, Perschler A. Sex differences in HAART-associated dyslipidaemia. *AIDS* 2001;15:725–734.
5. White AJ. Mitochondrial toxicity and HIV therapy. *Sex Transm Infect* 2001;77:158–173.

6. Mercie P, Tchamgoue S, Thiebaut R. Atherogenic lipid profile in HIV-1-infected patients with lipodystrophy syndrome. *Eur J Intern Med* 2000;11:257–263.
7. Fauvel J, Bonnet E, Ruidavets JB. An interaction between apo C-III variants and protease inhibitors contributes to high triglyceride/low HDL levels in treated HIV patients. *AIDS* 2001;15:2397–2406.
8. Miserez AR, Muller PY, Barella L. A single nucleotide polymorphism in the sterol regulatory element binding protein 1c gene is predictive of HIV related hyperlipoproteinaemia. *AIDS* 2001;15:2045–2049.
9. Duong M, Petit JM, Piroth L. Association between insulin resistance and hepatitis C virus chronic infection in HIV-hepatitis C virus-coinfected patients undergoing antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001;27:245–250.
10. Berthold HK, Parhofer KG, Ritter MM. Influence of protease inhibitor therapy on lipoprotein metabolism. *J Intern Med* 1999;246:567–575.
11. Manfredi R, Chiodo F. Disorders of lipid metabolism in patients with HIV disease treated with antiretroviral agents: frequency, relationship with administered drugs, and role of hypolipidaemic therapy with bezafibrate. *J Infect* 2001;42:181–188.
12. Hatano H, Miller KD, Yoder CP. Metabolic and anthropometric consequences of interruption of highly active antiretroviral therapy. *AIDS* 2000;14:1935–1942.
13. Barreiro P, Soriano V, Blanco F, Casimiro C, de la Cruz JJ, Gonzalez-Lahoz J. Risks and benefits of replacing protease inhibitors by nevirapine in HIV-infected subjects under long-term successful triple combination therapy. *AIDS* 2000;14:807–812.
14. Purnell JQ, Zambon A, Knopp RH. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. *AIDS* 2000;14:51–57.
15. Galli M, Ridolfo AL, Adorni F. Body habitus changes and metabolic alterations in protease inhibitor-naïve HIV-1-infected patients treated with two nucleoside reverse transcriptase inhibitors. *J Acquir Immune Defic Syndr* 2002;29:21–31.
16. Saint-Marc T, Partisani M, Poizot-Martin I. A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. *AIDS* 1999;13:1659–1667.
17. van der Valk M, Gisolf EH, Reiss P. Increased risk of lipodystrophy when nucleoside analogue reverse transcriptase inhibitors are included with protease inhibitors in the treatment of HIV-1 infection. *AIDS* 2001;15:847–855.
18. Carter VM, Hoy JF, Bailey M, Colman PG, Nyulasi I, Mijch AM. The prevalence of lipodystrophy in an ambulant HIV-infected population: it all depends on the definition. *HIV Med* 2001;2:174–180.
19. Lichtenstein KA, Ward DJ, Moorman AC. Clinical assessment of HIV-associated lipodystrophy in an ambulatory population. *AIDS* 2001;15:1389–1398.
20. Prins M, Sabin CA, Lee CA, Devereux H, Coutinho RA. Pre-AIDS mortality and its association with HIV disease progression in haemophilic men, injecting drug users and homosexual men. *AIDS* 2000;14:1829–1837.
21. Grunfeld C, Kotler DP, Hamadeh R, Tierney A, Wang J, Pierson RN. Hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am J Med* 1989;86:27–31.
22. Mildvan D, Machado SG, Wilets I, Grossberg SE. Endogenous interferon and triglyceride concentrations to assess response to zidovudine in AIDS and advanced AIDS-related complex. *Lancet* 1992;339:453–456.
23. Ledru E, Christeff N, Patey O, de Truchis P, Melchior JC, Gougeon ML. Alteration of tumor necrosis factor- α T-cell homeostasis following potent antiretroviral therapy: contribution to the development of human immunodeficiency virus-associated lipodystrophy syndrome. *Blood* 2000;95:3191–3198.
24. Huang H, Chopra R, Verdine GL, Harrison SC. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science* 1998;282:1669–1675.
25. Hance AJ, Lemiale V, Izopet J. Changes in human immunodeficiency virus type 1 populations after treatment interruption in patients failing antiretroviral therapy. *J Virol* 2001;75:6410–6417.
26. Devereux HL, Emery VC, Johnson MA, Loveday C. Replicative fitness in vivo of HIV-1 variants with multiple drug resistance-associated mutations. *J Med Virol* 2001;65:218–224.
27. Kaufmann D, Munoz M, Bleiber G. Virological and immunological characteristics of HIV treatment failure. *AIDS* 2000;14:1767–1774.
28. Raulin J. Human immunodeficiency virus and host cell lipids. Interesting pathways in research for a new HIV therapy. *Prog Lipid Res* 2002;41:27–65.